TECHNICAL REPORTS SERIES No. 61

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

JOINT FAO/IAEA DIVISION OF ATOMIC ENERGY IN AGRICULTURE

INTERNATIONAL ATOMIC ENERGY AGENCY, VIENNA, 1966

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

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Printed by the IAEA in Austria

August 1966

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A JOINT UNDERTAKING BY THE
FOOD AND AGRICULTURE ORGANIZATION
OF THE UNITED NATIONS AND THE
INTERNATIONAL ATOMIC ENERGY AGENCY

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VIENNA, 1966

International Atomic Energy Agency.
Laboratory training manual on the use of isotopes and radiation in entomology. A joint undertaking by the FAO and the IAEA. Vienna, the Agency, 1966.
144 p. (IAEA, Technical reports series no. 61)

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OF ISOTOPES AND RADIATION IN ENTOMOLOGY
IAEA, VIENNA, 1966
STI/DOC/10/61

FOREWORD

The Food and Agriculture Organization of the United Nations (FAO), and the International Atomic Energy Agency (IAEA), in cooperation with the United States Department of Agriculture and the Department of Entomology of the University of Florida, Gainesville, Fla., USA, have jointly sponsored two international training courses on the use of isotopes and radiation in entomology. Scientific authorities from several countries have contributed lectures and, together with the IAEA Secretariat, devised laboratory exercises embracing many and varied entomological techniques.

The present manual consists of two parts: a basic part containing general information and laboratory exercises on the properties of radiation and the principles of use of radioactive tracers, and an applied part consisting of detailed laboratory exercises in the use of isotopes and radiation in entomology. A special team of scientists prepared the basic part as an introduction to all training manuals published by IAEA and FAO on the use of isotopes and radiation in agricultural sciences.

The present manual follows a similar manual (Technical Reports Series No. 29) on isotopes and radiation in soil-plant relations research, and in their programme of disseminating information on the use of isotopes and radiation in food and agriculture, FAO and IAEA intend to publish jointly additional manuals in this series, on agricultural biochemistry, animal research and plant pathology.

The present manual should be useful not only to the personnel of FAO and IAEA in conducting future training programmes on the use of radiation and isotopes in entomology, but also to governments and institutes wishing to hold similar courses, and to research and development scientists using new information in agricultural science.

FAO and IAEA would like to thank the scientists who contributed to the success of the training courses in entomology, in particular to Dr. W. G. Eden of the University of Florida and Dr. A. Lindquist, formerly of the United States Department of Agriculture, for preparing most of the applied part of this manual. Thanks are also due to all scientists contributing to the basic part.

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APPLIED PART

EXPERIMENTS

SOME BASIC SYMBOLS AND UNITS FREQUENTLY USED IN THIS MANUAL

Time (in general)	t
Counting time (duration of)	T
Sum count (accumulated during T)	S
Count rate of sample plus background	$R = S/T$
Count rate of background (blank sample)	r
Activity = amount of radioactive test substance expressed, e.g., as net count rate	A^*, B^*, C^*
Amount, or "pool", of test substance	A, B, C
Specific activity (of substance "A")	$s^* = \frac{A^*}{A}$, e.g.: $\frac{R - r}{A \text{ in sample}}$
Activity concentration	a^*, b^*, c^*
Test substance concentration	a, b, c
Specific activity (of substance "A")	$s^* = \frac{a^*}{a}$
Number of disintegrations per minute	D^* (dis./min.)
Counting yield (overall)	$Y = \frac{R - r}{D^* \text{ in sample}}$
Number of radioactive atoms	N^*
Decay constant in reciprocal minutes	λ^* ($D^* = \lambda^* N^*$)
Distance	d
Thickness	x
"Approximately equal to"	$\doteq$
Unit of count rate, counts per minute,	cpm
Unit of absolute activity†, curie,	$c = 2.22 \times 10^{12}$ dis./min.
Unit of specific activity, e.g.,	cpm per unit weight of test substance.
Unit of activity concentration	cpm per ml cpm per mg } gross material cpm per cm^3
Unit of concentration	M (mole/l)
Unit of thickness, linear	mm, cm or dm
mass-thickness	mg/cm^2 or g/cm^2

† Absolute activity may also be expressed as D^*, N^* or number of moles of radioactive substance. "Absolute specific activity" (e.g. μc/mmole test substance) and "absolute activity concentration" (e.g. μc/ml) are also used.

BASIC PART

LECTURE MATTER

LABORATORY EXERCISES

MENTAL EXERCISES

APPENDIXES

LECTURE MATTER

1. PROPERTIES OF RADIOISOTOPES AND RADIATIONS

1.1. Atomic model: Definitions

An atom is composed of a positively charged nucleus which is surrounded by shells of negatively charged (orbital) electrons. The nucleus contains protons and neutrons as its major components of mass; the former have a positive charge, and the latter have no charge. The nucleus has a diameter of approximately 10^{-12} cm and contains almost the entire mass of the atom. The atom including the orbital electrons has a diameter of approximately 10^{-8} cm or 1 Ångstrom unit (Å).

The number of protons in the nucleus (Z) is characteristic for a chemical element. The atoms of a particular element may, however, not all have the same number of neutrons (N) in the nucleus. Atom types that have the same Z- but different N-values are called isotopes of the same element. As the neutrons and protons represent the major part of the mass of the atom and each has an atomic weight close to unity, the mass number, which is the sum of protons and neutrons, is close to the atomic weight M.

$$\text{Mass number} = Z + N \doteq M$$

The nuclei of some isotopes are not always stable; they disintegrate spontaneously at a characteristic decay rate. In nature a number of unstable isotopes are known, and nowadays many unstable isotopes are produced artificially in atomic reactors and by particle accelerators. As the disintegration of unstable isotopes is accompanied by the emission of various kinds of radiation, these unstable isotopes are called radioisotopes.

The nuclei of radioisotopes may emit α-, β^+-, β^-- and γ-rays. α-particles are fast-moving He nuclei, each containing two protons and two neutrons. β^+- and β^-- particles are, respectively, positively and negatively charged, high-speed electrons, while γ-rays are electromagnetic wave packets (photons) of very short wave-length compared with visible light, but traveling at the speed of light.

Natural isotopes of low Z (except ordinary hydrogen) have approximately the same number of neutrons as protons ($N \doteq Z$) in their nuclei, and they are usually stable. As the atomic number of the elements increases, the number of neutrons increasingly exceeds the number of protons, which finally results in unstable nuclei. Thus, the majority of unstable isotopes in nature is found for elements of high Z-number with a neutron:proton ratio on the order of $1\frac{1}{2}:1$. The emission of α-particles is characteristic of these elements. The combination of two protons and two neutrons is one of the very stable nuclear forms; and this combined form, the α-particle, is ejected as a single particle from the nucleus of the radioactive atom.

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

of a radioisotope is excessive, the number of protons in the nucleus tends to increase by the ejection of a negative β-particle from the nucleus. This beta particle accompanies the transformation of neutron into proton:

$$n \rightarrow p^+ + \beta^- (+\text{neutrino}).$$

An excess of protons in a nucleus may be counteracted by the ejection of a positron, a positively charged electron (regarding MeV, see section 1.3 below):

$$p^+ + 1.02\ \text{MeV} \rightarrow n + \beta^+ (+\text{anti-neutrino}).$$

Excess of protons in the nucleus may alternatively be reduced by the capture of an orbital (valence) electron (K-capture):

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

This process is accompanied by the emission of a characteristic X-ray, representing the energy difference between L- and K-shell electron in the element formed, since the "hole" in the K-shell is filled by an L-electron.

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

A gamma photon may interact with an orbital electron in the decaying atom, whereby the electron is ejected from the atom at a given velocity and the photon ceases to exist. This process results in the combined emission of a fast electron and a characteristic X-ray and is known as "Internal Conversion".

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

Summarizing, we may say that radioisotopes will emit particles and/or photons of the following nature:

α-particle	- doubly positively charged particle, containing two neutrons and two protons and originating at high speed from the nucleus;
β^--particle	- high-speed electron from the nucleus, negatively charged;
β^+-particle	- high-speed positron from the nucleus, positively charged;
γ-ray photon	- electromagnetic energy packet coming from the nucleus at the speed of light;
X-ray photon	- electromagnetic energy packet coming from an electron shell at the speed of light, following K-capture or Internal Conversion;

I.C. electron - (Internal Conversion electron) electron emitted as a result of the interaction between a γ-ray and a valence electron;

Neutron - particle with no charge and a mass close to that of a proton.

1.2. Radioactive decay and "specific activity"

The number of disintegrations per unit increment of time is a constant fraction of the number of radioactive atoms present at that time. Mathematically this can be expressed as

$$D^* = -\frac{dN^*}{dt} = \lambda^* N^*, \tag{1}$$

where D^* is the disintegration rate (expressed per minute) at time t,
N^* is the number of radioactive atoms present at time t, and
λ^* is the decay constant expressed in reciprocal minutes.

The minus sign indicates that the number of radioactive atoms decreases with time t. Integrating the differential equation (1) and calling the number of radioactive atoms present at beginning time N_0, one obtains

$$N^* = N_0^* e^{-\lambda^* t} \text{ or } D^* = D_0^* e^{-\lambda^* t} \tag{2}$$

It follows from equation (2) that the time required for one-half of the original activity to decay is independent of the beginning number of atoms. Designating the time required for half decrease of original activity as $t_{\frac{1}{2}}$, one obtains

$$\tfrac{1}{2} D_0^* = D_0^* e^{-\lambda^* t_{\frac{1}{2}}} \; ; \text{ i.e. } \lambda^* t_{\frac{1}{2}} = \ln 2 = 0.693,$$

where $t_{\frac{1}{2}}$ is the "half-life" of the isotope expressed in minutes. It is seen that the product of decay constant and half-life of any isotope is 0.693, which is useful for conversion of $t_{\frac{1}{2}}$ to λ^*. The decay constant, having the dimension of reciprocal time and being generally a small number, is inconvenient for many purposes. Instead, half-life ($t_{\frac{1}{2}}$ in, e.g., days or years) is often used as the decay characteristic of a radioisotope.

The practical unit of absolute (radio)activity is the curie, equal to 3.70×10^{10} disintegrations per second (approximately equal to the disintegration rate of 1 g of radium). One curie (c) is thus equal to 2.22×10^{12} dis./min., one millicurie (mc), one microcurie (μc) and one picocurie (pc), 2.22×10^9, 2.22×10^6 and 2.22 dis./min., respectively.

If one has g* grams of a radioisotope with a decay constant λ^* and an atomic weight of M, the radioactivity expressed in curies will be as follows (N^0 is Avogadro's number):

$\frac{g^*}{M} \times N^0$ = Total number of radioactive atoms (N^*)

$\lambda^* \times \frac{g^*}{M} \times N^0$ = Total disintegrations per minute (D^*)

$$\lambda^* \times \frac{g^*}{M} \times \frac{N^0}{2.22 \times 10^{12}} = \text{Total activity in curies.}$$

The decay constant or the half-life of an isotope can be graphically determined if the half-life is within a measurable range. It appears from equation (2) that, if the measured activity, $A^* = YD^*$ (where Y is the constant counting yield), is plotted against time on semi-log paper, a straight line will be observed. The half-life or decay constant can easily be found directly (see Fig.1) or from the slope s, which is equal to $-\lambda^*/2.3$. For isotopes of very long half-life, one has to apply the method of absolute measurement for half-life determination.

When two radioisotopes, "A" and "B", are present simultaneously, the observed activity is

$$A_0^* e^{-\lambda_A^* t} + B_0^* e^{-\lambda_B^* t}.$$

If this activity is plotted on semi-log paper, one obtains a composite curve, such as appears in Fig. 2. With the assumption that the half-lives are sufficiently different (e.g. a factor of 10), the curve can be resolved graphically by subtraction of the extrapolated straight line resulting from the long-lived component (B) from the sum curve observed. The two straight lines then yield the two half-lives.

In practice, a radioisotope will be accompanied by a variable quantity of stable isotopes of the same element. The stable form is called "carrier". To specify the concentration of radioisotope in one element or compound, the term specific activity is introduced. This is generally expressed as radioactivity per unit amount of specified test substance. (See the list of symbols and units at the beginning of this manual.)

By some procedures radioisotopes can be prepared virtually free from carrier, in which case they are called "carrier-free".

1.3. Energy of radiation

The energy unit commonly used with regard to radiation is the electron volt (eV). This is equivalent to the kinetic energy acquired by an electron on being accelerated through a potential difference of one volt. 1 KeV and 1 MeV are 10^3 eV and 10^6 eV, respectively; 1 MeV is equal to 1.6×10^{-6} erg.

The kinetic and total energies, respectively, of the particles and photons emitted by radioisotopes have characteristical values, which are usually indicated for each isotope on nuclear charts. Any energy spectrum of the alpha particles, gamma photons or characteristic X-ray photons emitted by a radioisotope is discrete, showing one or a few monoenergetic ("monochromatic") lines. On the other hand, the energy of beta particles ejected by a given isotope varies from zero up to a certain maximum energy (E_{max}) that is at the disposal of the beta particle. This is because a variable part of E_{max} is taken away by a neutrino or an anti-neutrino, neither of which is observable in ordinary counting (they have no charge and practically no mass). As a consequence, the beta particles show a continuous spectrum of energies from zero up to the characteristic E_{max}. The beta energies given in a table or chart of isotopes are E_{max}-values; the average beta-particle

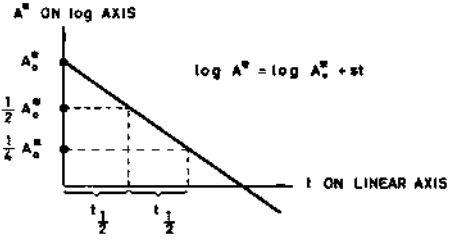

Fig. 1

Decay curve of a single radioisotope

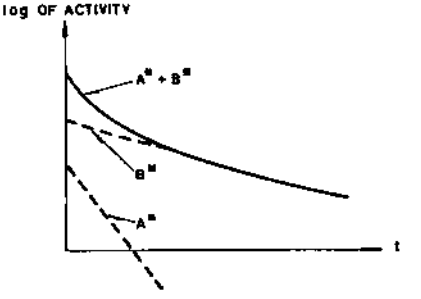

Fig. 2

Decay curve of two radioisotopes, A and B, simultaneously present in a sample

energy is usually about one third E_{max}. The continuous beta spectrum may sometimes be overlapped by one or two monoenergetic lines from I.C. electrons.

The characteristic radiations and energies for a given radioisotope are often shown in the form of decay schemes (for example, see Fig. 3).

A knowledge of decay characteristics is important in considerations on protection against, and measurement of, radioisotopes.

1.4. Interaction of radiation with matter

1.4.1. Absorption of alpha particles

The alpha particles ejected from any particular radioisotope are monoenergetic. In passing through matter and interacting with the atoms thereof, the kinetic energy of the alpha particle will be spent in (1) exciting outer-shell electrons to higher-energy orbits, and (2) ejecting electrons out of their orbits. Since alpha particles are doubly charged and the mass is relatively large (atomic weight 4), a dense track of ion pairs (i.e. ejected electrons and positively charged atom residues) is formed along the path of an alpha particle. As the alpha particle dissipates its energy along its path, the velocity of the particle decreases and finally the particle acquires two electrons from its surroundings and becomes a helium atom. The range,

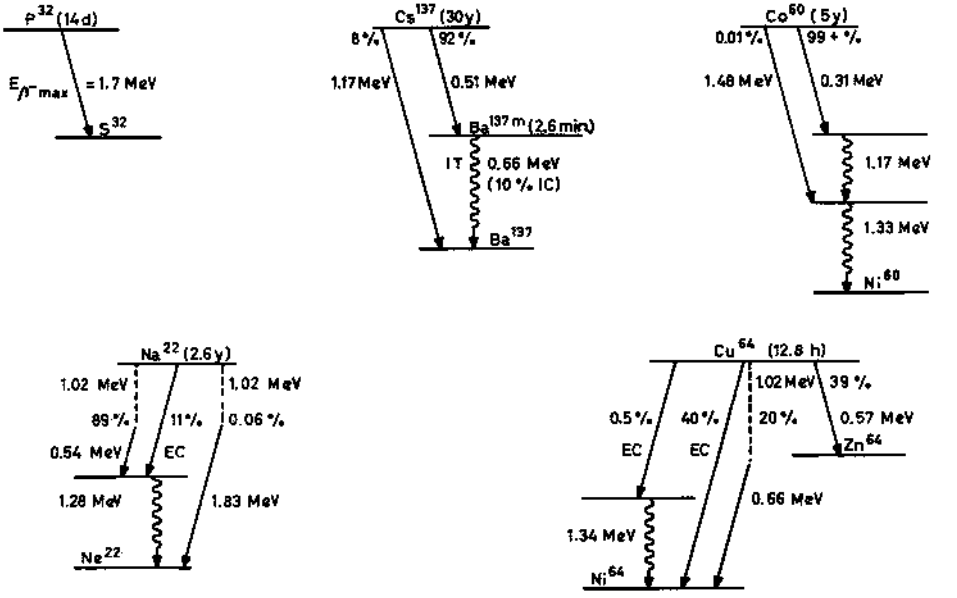

Fig. 3

Disintegration schemes showing characteristic radiations and energies of five different radioisotopes

Key

↘	β^--particle
↙	β^+-particle
↙ EC	electron capture (K-capture)
↓	γ-photon
h	hour(s)
d	day(s)
y	year(s)
Ba^{137m}	excited Ba^{137}, called "metastable" because the emission of the γ-ray is not instantaneous
IT	Isomeric Transition. Different types of the same isotope are called isomers.
I.C.	Internal Conversion

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 the alpha particle is generally small and amounts to several centimeters in air and several microns (10^{-3} mm) in aluminium for energies on the order of 1 – 10 MeV. As the energy of an alpha particle is lost in a relatively thin layer of absorber, it is evident that the number of ion pairs per centimeter of track, the specific ionization, is very high.

1.4.2. Absorption and scattering of beta particles

Beta particles cause excitations and ionizations in matter just as do alpha particles, but the mass of the beta particle is only 1/7000 of the mass of the alpha particle and beta particles have half the charge per particle. They will therefore scatter more, penetrate relatively deeper into matter and have a lower specific ionization. As does the alpha particle, the beta particle has a "range" (i.e. a maximum penetration depth into an absorber) which is characteristic of the initial energy of the particle and the density of the absorber, but this range is not so well defined because of the zig-zag path (scattering) of the electron as compared with the straight path of the helium nucleus.

Because of the fact that beta particles have a continuous spectrum of energies up to an E_{max}, their absorption in matter is at best only approximately exponential and obeys the following equation only crudely:

$$A^* = A_0^* e^{-\mu x}$$

where A_0^* is the activity (intensity) of the incident radiation,
A^* is the activity (intensity) of the transmitted radiation,
μ is the β-absorption coefficient of the absorber, and
x is the thickness of the absorber.

Therefore, when the radiation transmitted by the absorber is plotted as a function of the absorber thickness on semi-log paper, a fairly straight line is obtained over a portion of the curve (Fig. 4).

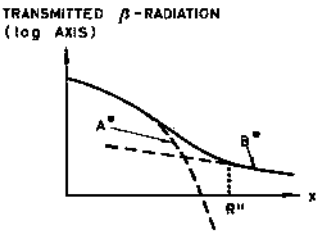

Fig. 4

Curve demonstrating the transmitted β-radiation as a function of absorber thickness

The curve becomes practically horizontal at "R", the "range" for beta particles with E_{max}. Although all the beta rays are stopped at this absorber thickness, one still finds some transmission of radiation, because, particularly at low velocities, the beta particles interact with the atoms of the absorber, giving rise to (non-characteristic) X-rays, the so-called "bremsstrahlung" (B*). By subtraction of B* from the composite curve, the pure beta transmission curve (A*) is obtained.

Positron energy absorption takes place in the same manner as for negative beta radiation. However, when the kinetics energy of the positron be-

comes very low, the positron is annihilated together with an electron, giving rise to two characteristic photons of 0.51 MeV each: $e^+ + e^- \rightarrow 2$ photons.

Absorption and scattering of beta particles is important in the measurement of beta-active samples. Absorption and scattering will occur in a sample cover or a detector window as well as in intervening air. Side-scattering (into the detector) from a counter shield and/or back-scattering from a sample support will also occur. These effects will all influence the counting rate one way or the other. Finally, unless the sample is "infinitely" thin, self-scattering (into and away from the detector) and self-absorption will all take place in the material of the sample itself, and this will cause an overall self-weakening effect, which is largest for thick samples and small (even slightly negative) for very thin samples. The counting rate from samples of increasing thickness at first increases because of greater total activity and then becomes constant (at "infinite" thickness) because the contribution of beta activity from the lower layers of the sample is entirely absorbed in the upper ones.

1.4.3. Attenuation of gamma and X-rays

In passing through matter, the energy of gamma and X-ray photons is attenuated by three important interactions: (1) photoelectric effect, (2) Compton scattering and (3) pair-production.

(1) When the photon energy is below about 0.5 MeV, the photoelectric effect is predominant. The total energy (i.e. the entire photon) is used up in the ejection of an electron at high speed from an atom shell. Subsequently, this fast electron causes many excitations and ionizations just as does a beta particle. The photoelectric effect is particularly important when the atoms of the absorber have a high Z-number.

(2) Compton scattering arises predominantly when gamma photons in the energy range 0.5 – 5 MeV collide with free or loosely bound electrons in the absorber. Part of the photon energy is transferred to the electron as kinetic energy in such a collision, and the reduced photon is deflected (slightly or up to 180°) from its original direction. This effect is important for absorber atoms of high Z-number.

(3) When a photon has an energy of at least 1.02 MeV or higher, it may become extinct in the proximity of an atomic nucleus of the absorber, giving rise to an electron-positron pair. Any photon energy above the required 1.02 MeV is imparted to the e^- and the e^+ as kinetic energy.

Theoretically, gamma or X-radiation is never completely stopped by matter although the transmitted radiation may be reduced to an insignificant value. For a collimated beam of monoenergetic photons, attenuation by absorption and scattering can be described mathematically as follows:

$$I = I_0 e^{-\mu x}$$

where I_0 is the initial intensity of collimated monoenergetic photons,
I is the intensity after passing x cm of the absorber, and
μ is the attenuation coefficient for the photon energy and the material concerned.

This is the well-known Lambert-Beer law for visible light photons. The derivation of the equation from the basic assumption that

$$-\frac{dI}{dx} = \mu I$$

is analogous to the derivation of the radioactive decay law $N^* = N_0^* e^{-\lambda^* t}$ (see section 1.2). The thickness at which I_0 is reduced to half its intensity is called the "half-thickness" (analogous to half-life). If the half-thickness is expressed as mass-thickness (g/cm^2), its value is a function of the energy of the gamma photons but, for 0.5-5 MeV photons, largely independent of the type of material.

An understanding of photon interaction with matter is useful in considerations on shielding, body dose and measurement.

1.4.4. Scattering and absorption of neutrons

Neutrons, being without charge, lose energy only by direct contact with nuclei of matter. The processes may be of the following four types:

(1) Of an elastic nature, like billiard-ball collisions. Ion pairs are produced by these collisions, the hit nucleus loosing one or more of its orbital electrons. Neutrons of high initial energy (fast neutrons) gradually lose their energy by this interaction until they have been moderated to "slow" or "thermal" neutrons. Light elements, especially H, have the best neutron moderating qualities.

(2) Of a type in which the neutron is absorbed by nuclei with resultant nuclear reaction. This occurs predominantly with slow neutrons, e.g.

$$B^{10} + n \rightarrow (B^{11}) \rightarrow Li^7 + \alpha + \gamma .$$

(3) When the nuclei of certain elements of high atomic number are hit by neutrons of appropriate energy, fission results (the nuclear pile).

(4) Finally, free neutrons decay spontaneously, with a half-life of 12 min., to protons and beta particles, which thereupon excite and ionize atoms of matter.

2. RADIATION DETECTION

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.

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-ultraviolet region) form the basis of detection.

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

A 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.

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

A 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 sum count.

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.

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

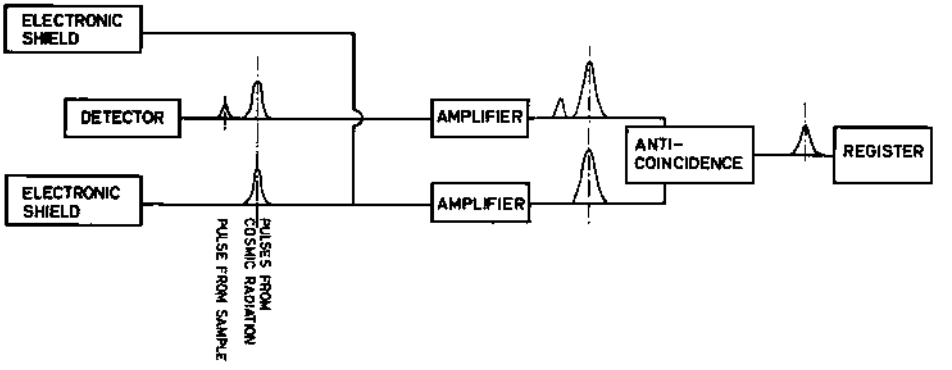

Fig. 5

Block diagram illustrating an anti-coincidence unit used as an electronic shielding against cosmic radiation

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

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

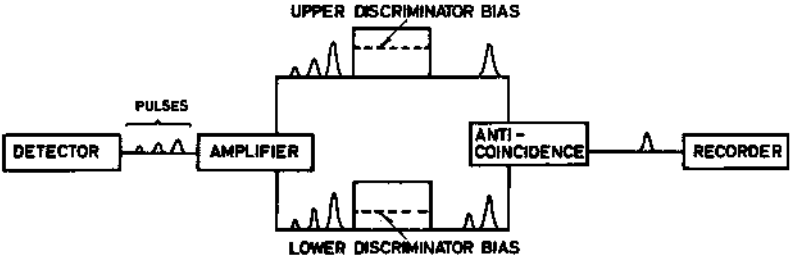

Fig. 6

Block diagram illustrating a pulse-height analyser

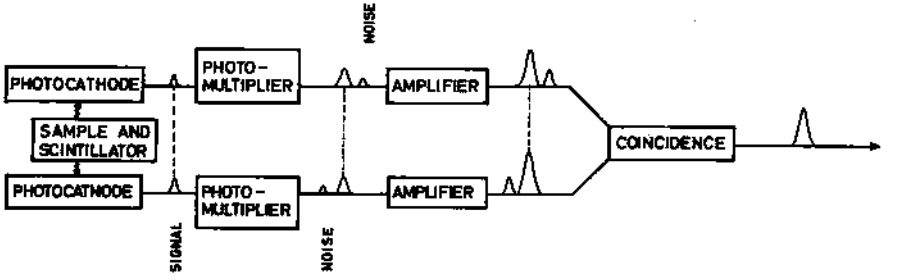

Fig. 7

Block diagram illustrating a coincidence unit

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

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

2.1. Autoradiography

Ionizing radiations affect the silver halide in photographic emulsions. When radioactive material is placed on 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 at a given place will be a function of the exposure time and the amount of activity in the sample at that place. It further depends on the specific ionization (see section 2.2 below) of the radiations. γ-rays with their very low specific ionization will produce hardly any blackening. On the other hand, α-rays and soft β-rays, which have a high specific ionization, are very effective (H^3, C^{14}, S^{35}, Ca^{45}). Hard β-radiation produces more diffuse radiograms on account of the relatively long tracks that these particles travel in the emulsion. The properties of the photographic 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. For example, a thin histological section containing about 100 dis./min. per cm^2 will require several weeks exposure to show sufficient darkening or blackening. For more detailed information, refer to the introduction to the experiment on autoradiography (Applied Part B, section 4.1).

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. The interpretation of autoradiograms of biological material is therefore not always straightforward.

Autoradiography is frequently applied to the determination of the components of a paper chromatogram (see Applied Part B, section 2.1).

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.

2.2. Detection by ionization

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.

α- and β-particles and I.C. electrons (e) have a high specific ionization, i.e. produce a great number of ion pairs per unit length of track. γ- 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

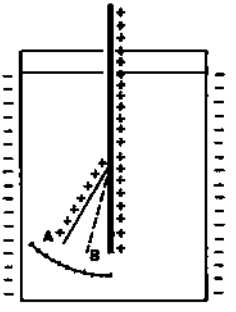

Fig. 8

Electroscope

(or pair production if the energy is very high), and these fast electrons will ionize just as do β-particles. Neutrons may also produce ions, directly (collision) or indirectly (following nuclear absorption), as described in section 1.4.4 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 (electrons) will start moving and by hitting the positive electrode (anode) discharge. Likewise the positive ions will move toward the cathode.

Four different types of ionization instrument will now be described.

2.2.1. Electroscope

In the electroscope or simple electrometer (see Fig.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 maximal (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 dosimeter" and gives a measure of the accumulated dose of external radiation (γ-, X- and hard β-radiation) to which a worker has been exposed during a certain period.

2.2.2. Ionization chamber

Not all the ions will discharge on the electrodes of an electroscope. A certain number will recombine before they have reached the electrodes. If the voltage applied to the electrodes is steadily increased, the losses resulting from recombination will decrease, and eventually all the ions will discharge on the electrodes of the detector. If the voltage difference between the electrodes is further increased up to a certain limit, the number of ion pairs that discharge will remain constant. Each ionizing particle or photon will thus give rise to an electric pulse on the electrodes. A radiation intensity (i.e. a constant stream of particles or photons) gives rise to a continuous series of pulses; and if these are allowed to merge, they form a weak electric current, which may be amplified and registered by an electronic circuit. 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 (e.g. the so-called "cutie pie").

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.

2.2.3. Proportional counter

If the voltage difference between the anode and the wall of the counter is increased above a certain limit, another phenomenon, known as "secondary ionization", will 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 C^{14}, may be counted effectively ("windowless" counting), provided suitable amplification precedes the register.

2.2.4. Geiger-Müller (G-M) 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 (G-M 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). G-M tubes operate at a reduced gas pressure (about one-tenth atmosphere), containing a certain amount of "quenching" gas. Usually the closure of a G-M 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 each discharge with alcohol. Therefore, the quantity of quenching gas in the G-M 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; and the life of the tube is therefore determined by other effects, such as corrosion and leakage.

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

The fact that some time is required for each discharge of electrons (100-300 μsec.) implies that during this time no other particle or photon can be detected by the G-M tube. This time is called the dead time of the G-M

counter; and, particularly for higher count rates, a correction for this dead time must be made.

Let R be the observed count rate and τ the dead time of the counter in min.

During one minute the counter will have been ineffective for $R\tau$ min. Therefore, R counts have been registered in $1-R\tau$ min. The corrected count rate R^+ in cpm will therefore be

$$R^+ = R/(1 - R\tau).$$

When the dead time of the counter tube is known, the correction for high count rates can then be made with the aid of the above expression for R^+. However, this expression is approximate and should not be used to give corrections above 10%, when it is better to dilute or count at a distance from the detector.

Sometimes the dead time of a G–M tube will be fixed electronically at 300 or 400 μsec. so that a correction table can be used. Correction is normally not necessary unless the count rate exceeds about 2000 cpm.

Numerical example: τ = 300 μsec.
= 5 μmin. corr. = $2\frac{1}{2}$%
R = 5000 cpm

G–M counters are used most widely for the detection and measurement of β–particles. For γ–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 β–particles on glassware, benches or trays, monitors are used. A monitor consists of a G–M 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.

Normally, for the assaying of activity in samples, the G–M tube will be connected to a voltage source, an amplifier, a register and a timing unit.

2.3. Detection by excitation

2.3.1. Solid scintillator counting

Solid scintillators are particularly suited for the detection of γ–rays and X–rays because of the high stopping power of the solid. Their operation is based on the following principle:

When a γ–photon interacts with a crystal, e.g. of thallium–activated NaI, at least one fast electron is liberated (see section 1.4.3), and a constant fraction of the electron's kinetic energy is spent on excitation of orbital electrons in atoms of the crystal. On de–excitation these give rise to the emission of a light flash consisting of a number of photons. The number of light photons will be proportional to the energy dissipated in the crystal by the γ–photon.

The light photons reach the photocathode of a photomultiplier, where photoelectrons are released. The number of photoelectrons, being a con–

stant fraction of the number of light photons, is therefore proportional to the energy originally dissipated by the γ-photon. The photocathode is connected with a series of dynodes, i.e. positive electrodes of increasing potential. When a photoelectron hits a dynode, secondary electrons are produced which will, in turn, hit the next dynode. In this way, the photomultiplier will, all in all, produce a large number of electrons (a pulse), proportional to the energy originally dissipated by the γ-photon in the crystal. This final pulse will be amplified linearly and registered.

As opposed to a G-M tube, the scintillation tube thus provides an output pulse that is proportional to the input energy. The scintillation tube is therefore a suitable detector for γ-ray spectrometry (see section 2.5.2). A further advantage of the scintillation counter is its small dead time, of only a few μsec. This enables high count rates to be determined (up to at least 100 000 cpm) without the necessity for application of a correction for dead time.

For the measurement of β-particles, special plastic scintillators (as well as anthracene and naphthalene) which have a much higher efficiency than NaI crystals have been devised. An effective scintillator for alpha particles is a thin layer of silver-activated ZnS.

2.3.2. Liquid scintillation counting

For the counting of very low-energy and low-energy beta particles such as H^3 (0.018 MeV) and C^{14} (0.155 MeV), a method of detection called "liquid scintillation counting" is often employed. In this technique, the sample to be counted is placed in solution with the scintillator so that each radioactive atom or molecule is surrounded by molecules of the scintillator. By this method absorption is reduced, and hence counting yield increases.

The scintillator system contains a solvent which is usually an organic compound, such as toluene or dioxane, and a solute which is the actual scintillator. The solvent absorbs the energy and transfers it to the solute, which then emits the light flash. Often a secondary solute which acts as a wavelength shifter is added; i.e. it increases the wave length of the light flash emitted to one for which the photomultiplier tube is more sensitive, thus increasing the counting yield.

In practice, two photomultiplier tubes are often used facing each other across the counting chamber. A coincidence circuit is employed, and only those events witnessed by both tubes are counted. This increases the signal-to-noise ratio, as previously described above at the beginning of section 2 under "Pulse input sensitivity".

Variable discriminators can be applied to this system; and since the pulse height is proportional to the input energy, pulse-height analysis is possible.

2.4. Yield and statistics

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 one radioactive atom disintegrates, often more than one particle or photon is emitted. For example, a Co^{60} nucleus emits either one β-particle and two γ-photons or, occasionally, one of each (see decay scheme, section 1.3). However, metastable states excepted, a disintegration including the emission of particle(s) and/or photon(s) takes about 10^{-10} sec. or less, whereas even fast counters have a dead time of about 10^{-6} sec., i.e. at least a thousand times longer dead time than the total duration of a disintegration. From this it is apparent that no counter will register more than one count per disintegration (assuming no spurious electronic pulses), even if more than one particle or photon is emitted.

In special cases it is possible to count as much as one count per disintegration. This would be the case if a "carrier-free" β-active source were suspended on an "infinitely thin" support in the middle of the active volume of a detector, which had a β-detection efficiency of 100% (see Fig. 9). In this case absolute activity, D* dis./min., would be measured.

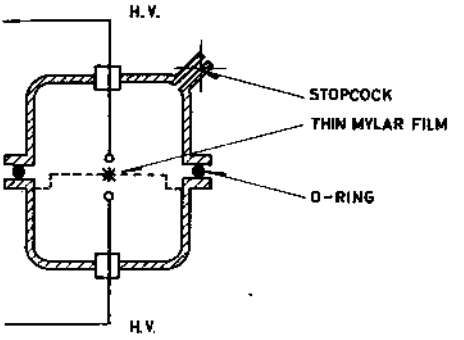

Fig. 9

G-M-assembly of approximately 100% efficiency (4π geometry)

Normally, only a fraction of the disintegrations will be counted, and this fraction is termed the (overall) counting yield Y. This yield is, of course, dependent on the specific counting conditions concerned. It is possible to determine the value of Y under a given set of conditions by purchasing a calibrated standard† of the isotope in question. We then have

$$Y = \frac{R - r}{D^*} \quad \frac{\text{cpm}}{\text{dis./min.}},$$

where R - r is the measured net count rate of the source, and D* is the known disintegration rate taking place in the source. However, very often an experimenter only wishes to know the activities of his samples relative to one another (comparative measurement), and then a determination of Y is not necessary.

† This will be accurate to within 1 - 5%, depending on the isotope and the price.

In comparative measurement of a set of samples the (dead-time corrected) net count rate of the radioactive test substance in each sample is taken as the activity of that sample. The validity of this practice stands and falls with the constancy of the (unknown) yield Y for all samples. As a check of the constancy of the counter itself, one of the samples (e.g. a known fraction of the dose administered) may be used as a reference standard, by counting of it at regular intervals during the measurement of the other samples. The net count rate (i.e. activity) of each sample can then be corrected in accordance with any significant variations in the net count rate of the reference standard sample – when the reference standard, for example, is 1% of dose, this also automatically takes care of any measurable radioactive decay between the counting of different samples.

Finally, one finds that identical samples counted under identical conditions still show variations in count rate. These variations are statistical in nature (binomial distribution), and they result from the unpredictability of just when any one particular radioactive nucleus will disintegrate, in just which direction the particle or photon will be emitted and just which particles or photons will be registered (when the intrinsic counter efficiency is not 100%).

2.4.1. Counting yield

In practice the count rate of a sample is usually determined by the counting of the sample plus the unavoidable background radiation for a certain period of time T. During this counting time a sum count S is accumulated, and then the count rate of sample plus background is $R = S/T$. The radioactive sample is then replaced by a blank sample, and the background count rate, $r = s/t$, is determined in the same way as is R. The activity of the sample, taken as the net count rate (corrected if necessary for dead time and reference variation), is then

$$A^* = (R^+ - r)_{corr}.$$

During the assay of a sample only a fraction of the disintegrations are counted; this fraction is the counting yield, i.e.

$$R - r = YD^*.$$

The fraction Y can be broken down into a number of factors such as geometry, absorption of radiation before it enters the detector, scattering and the intrinsic counter efficiency for those particles or photons that reach or penetrate the active volume of the detector.

The geometry factor is the solid angle that is subtended by the active volume of the detector at the sample, divided by 4π ("all directions"). For a small source close to a detector window the solid angle is about 2π and the geometry factor about 50%.

Because of absorption in the counter window, a fraction of the radiation will not enter the detector. If the particles have a small range, as is the case for α-particles and very soft β-particles, the air between source and window will already greatly reduce or even stop the radiation.

α- and β-particles, and to a much lesser extent γ-rays, are partly absorbed by the material in which the isotopes are contained and a significant fraction of α- and β-activity will be lost. The lower the energy of the particle and the higher the mass-thickness (mg/cm^2) of the material, the greater self-absorption will be. In consequence, the radiation transmitted by a given radioactive material will not increase in proportion to the thickness of the sample. Analysis will show that with increasing thickness it reaches a constant value; the sample is then said to have "infinite thickness" (see the end of section 1.4.2 above and Fig.10).

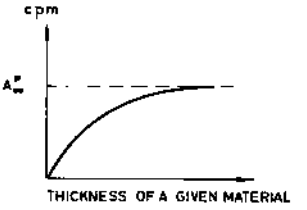

Fig.10

Recorded β-activity as a function of sample thickness

Any further additions of that particular radioactive material to the counting tray will not alter the count rate, except as geometry with relation to the detector window is altered. At infinite thickness the count rate of a sample (A^*_∞) is proportional to activity concentration (i.e. specific activity, cpm per unit weight of sample material) rather than to total activity of the sample.

Energetic particles or photons may reach the detector after collision with the walls of the lead shield or with the sample holders (side-scattering). The amount of side-scattering will be dependent on the energy of the particles and the nature of the material surrounding the source. Radiation may also be scattered back from the sample support. The degree to which radiation is back-scattered depends on the electron density of the backing material. The higher the electron density, the greater the scattering. Back-scattering becomes constant at a certain thickness of the backing (saturation back-scattering). In thick samples self-scattering will take place.

2.4.2. Counting statistics

When a particular sample is counted several times under identical conditions, deviations of the count rate from the mean value will be noticed. These deviations result from random variations in emission and detection inherent to the nature of radioactivity. This we shall call natural uncertainty.

As a consequence of the Poisson probability distribution, the natural standard deviation of a number of registered counts is equal to the square root of this number (as long as this number, i.e. the sum count, is much less than N^*_0, i.e. N^* at $T = 0$). Table I gives the standard deviation for the natural uncertainty of some sum counts. Notice that these deviations may be calculated without actual counting.

TABLE I

STANDARD DEVIATION FOR NATURAL UNCERTAINTY OF SUM COUNTS

The sum count S	Natural standard deviation $\sigma_{nat} = S^{\frac{1}{2}}$	Percentage N.S.D. $\% \sigma_{nat} = \frac{S^{\frac{1}{2}}}{S} 100\%$
100	10	10 %
1000	31.6	3.2%
10 000	100	1.0%
100 000	316	0.3%
1 000 000	1 000	0.1%

In consequence, with increasing sum counts the natural standard deviation (N.S.D.) increases but the percentage natural standard deviation decreases. It also follows that samples of varying radioactivity may be counted with the same accuracy with regard to natural uncertainty, provided sufficient time is allowed for the same sum count to be registered for each sample

The background of a particular counting set depends on the number of cosmic rays which penetrate the counter, the amount of natural radioisotopes in the environment and the "electronic noise" of the equipment. The background will be determined by registration of the number of counts with a blank sample in place.

If a sample (plus background) is counted for T min. and the sum count is S, the natural standard deviation of this sum count is $\sigma_{nat,S} = S^{\frac{1}{2}}$, and the count rate of sample plus background is

$$R = S/T,$$

so the natural standard deviation of the count rate must be

$$\sigma_{nat,R} = S^{\frac{1}{2}}/T$$

(since the counting time has no "natural" uncertainty)

$$\text{or } \sigma_{nat,R} = (RT)^{\frac{1}{2}}/T = (R/T)^{\frac{1}{2}}.$$

We see that, for a given counting rate R, $\sigma_{nat,R}$ is inversely proportional to the square root of the counting time.

If the background is counted for t min. and gives a sum count s, then the activity of the sample, expressed as net count rate, is

$$A^* = S/T - s/t = R - r.$$

The natural standard deviation (N.S.D.) of the sample activity will then be

$$\sigma_{nat,A^*} = \sigma_{nat,R-r} = (\sigma^2_{nat,R} + \sigma^2_{nat,r})^{\frac{1}{2}} = (R/T + r/t)^{\frac{1}{2}},$$

since (see above) $\sigma^2_{nat,R} = R/T$ and, analogously, $\sigma^2_{nat,r} = r/t$.

A useful rule in counting states that the <u>percentage</u> N.S.D. of count rate is equal to the percentage N.S.D. of sum count. The derivation of this rule is as follows:

$$\%\,\sigma_{nat,R} = \frac{\sigma_{nat,R}}{R}\,100\% = \frac{S^{\frac{1}{2}}}{RT}\,100\% = \frac{S^{\frac{1}{2}}}{S}\,100\% = \%\,\sigma_{nat,S}\ .$$

Thus, if one accumulates a sum count of 10 000, the N.S.D. of the count rate is 1%.

The percentage N.S.D. of the sample activity is in general

$$\%\,\sigma_{nat,A^*} = \frac{\sigma_{nat,R-r}}{R-r}\,100\% = \left[\frac{R}{T} + \frac{r}{t}\right]^{\frac{1}{2}} \times \frac{100\%}{R-r}\ . \qquad (1)$$

The use of this formula is only important when the sample count rate is of the same order of magnitude as, or less than, the background count rate. If sample plus background and background alone are each counted for the same length of time T, equation (1) reduces to

$$\%\,\sigma_{nat,A^*} = \frac{[(R+r)/T]^{\frac{1}{2}}}{R-r}\ 100\%. \qquad (2)$$

Regarding the derivation of the following approximative equation:

$$\%\,\sigma_{nat,A^*} \doteq \frac{100}{2T\,(R^{\frac{1}{2}} - r^{\frac{1}{2}})}\ \%, \qquad (3)$$

see Appendix IV (equation (3)).

If a set of n duplicate samples is prepared and counted, one is likely to find that the total S.D. calculated according to the usual formula

$$\sigma_{tot,S} = [\Sigma(S_i - \bar{S})^2/(n-1)]^{\frac{1}{2}}$$

is significantly greater than $\sigma_{nat,S} = \sqrt{S}$. If no real errors have been made, this increase results from <u>technical uncertainty</u>, such as random variation in sample materials, sample preparation, sample placement, intrinsic counter efficiency, electronic noise etc. Natural and technical uncertainty add up geometrically; i.e.

$$\sigma^2_{tot} = \sigma^2_{nat} + \sigma^2_{tech}$$

or

$$\sigma_{tot} = \sqrt{\sigma^2_{nat} + \sigma^2_{tech}}\ .$$ (see Fig. 11.)

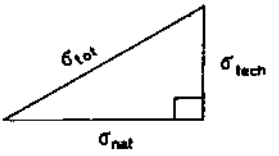

Fig. 11

Vector diagram showing the relationship between natural and technical uncertainties

2.5. Specialized detection techniques

2.5.1. Low-activity measurement

The measurement of samples of low activity, i.e. containing picocurie quantities of radioactive material, constitutes a special problem (1 pc ≙ 2 dis./min.).

Even in the absence of radioactivity, a counting tube will still give a count rate, resulting from the background, i.e. cosmic rays, natural radioisotopes in the instrument itself and electronic noise. Usually the counting tubes are shielded by lead (a "lead castle") for reduction of the background from secondary ionization showers initiated by cosmic rays.

Even so, some hard cosmic rays and natural radioactive material in the shield will be responsible for a background count. When the activity of the sample to be assayed is of the same magnitude as, or less than, the background, an accurate determination is very time-consuming.

For reduction of the count rate from background, an anti-coincidence circuit may be used (see introduction to section 2 above). The G-M counter tube is surrounded by a great number of G-M tubes or by one specially designed, spherical G-M tube. Any pulse from the surrounding tube(s) which coincides with a pulse from the inside tube is not registered. In this way the background count of the inner tube may be reduced to an extremely low level (≙ 0.1 cpm) and will enable the determination of low count rates. (It is assumed that the sample's radiation does not penetrate the inner tube and reach the outer tube(s).)

Regarding the natural uncertainty in low activity counting, see section 2.4.2 above and, in particular, equations (1) to (3).

2.5.2. Gamma-ray spectrometry

Before going into γ-spectrometry, let us say that these rather expensive techniques are not needed in general for radioactive tracer work in biological research. However, for activation analysis γ-ray spectrometry is necessary.

The size of the outcoming pulse from a scintillation detector is proportional to the energy absorbed in the detector from the incident particle or photon. If, therefore, the subsequent reinforcement of the pulse is performed by a linear amplifier, then the pulse height (P.H.) of the final pulse, which is ready for registering, is also proportional to the energy absorbed in the detector from the incident particle or photon.

In P.H. analysis, each final pulse has to pass an electronic selecting system preceding the register; if rejected, the pulse is not registered.

The selecting system in a differential, single-channel, P.H. analyser consists of two discriminators and an anti-coincidence circuit. The bias setting of the lower discriminator constitutes the "threshold" voltage, and the difference between the voltage setting of the upper discriminator and the threshold constitutes the voltage channel or "window". To a certain limit the channel width determines the resolution of the instrument.

If the P.H. of a pulse is below the threshold voltage, the pulse is rejected directly. If the P.H. of a pulse is above the threshold-plus-channel voltage, the pulse passes both discriminators and is rejected in the anti-coincidence circuit. Thus, only pulses with a P.H. within the channel are registered.

A P.H. spectrum, i.e. the number of pulses as a function of P.H., is obtained by the increasing of the threshold in small increments from zero to a voltage above that of the largest pulse, a count being taken at each threshold value.

The energy absorbed by a scintillation crystal from an incident γ-photon will be somewhere between zero and the total energy of the photon.

A P.H. spectrum of the pulses from a large number of incident photons of equal energy, for instance 1 MeV, is really a frequency spectrum of the various interactions of the photons with the crystal. For example, a spectral line, known as the photopeak, will appear at 1 MeV; and this represents the number of photons that are completely absorbed in the crystal, either by a photoelectric interaction alone or, for instance, by a Compton interaction followed by a photoelectric interaction. For photons of another energy, e.g. 0.5 MeV, the photopeak will, of course, be at this other energy, 0.5 MeV.

A photon which undergoes Compton scattering and then escapes from the crystal will give a pulse with a P.H. somewhere in the interval between zero and a certain maximum value (lower than the photopeak value), depending on the magnitude of the photon deflection caused by the Compton scattering in the crystal (see Fig. 12, Compton region).

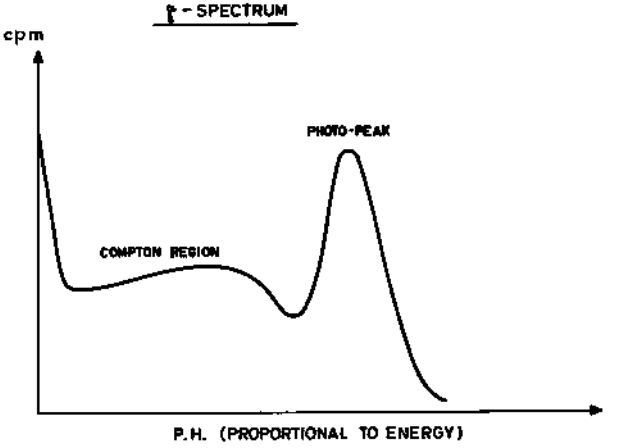

Fig. 12

A pulse-height spectrum of a gamma emitter

Calibration of the threshold values (i.e. P.H.) in terms of MeV is done with the help of radioisotopes emitting monoenergetic γ-photons of known energy, for instance Cr^{51}, Cs^{137}, Mn^{54} or Zn^{65}, with photon energies of 0.32, 0.66, 0.84 and 1.12 MeV, respectively.

In the multichannel system, the pulses are sorted electronically according to energy into the channel with the appropriate energy upper and lower limits, so in this system every pulse received is sorted whereas in the single-moving-channel system only those pulses which fall in the range defined by the channel at the particular threshold position which it has reached are recorded. Pulses of higher or lower energy will be recorded only when the window threshold moves to the appropriate P.H. position. The advantages of the single-channel system are relative simplicity and cheapness; that of the multichannel system is speed, since all pulses are analysed simultaneously. Multichannel analysers are available commercially in 100, 200 and up to several thousand channel models.

2.5.3. Neutron detection

Neutron detectors are similar in design to proportional or G-M tubes but contain BF_3 as a gas or as a boron coating inside the tube. When a slow neutron hits the nucleus of a boron atom, an α-particle is ejected.

$$n + B^{10} \rightarrow Li^{7} + \alpha$$

The α-particle then causes ionization and is counted in the normal way. Fast neutrons have to be slowed down before they can be counted. The moderation can be done by a layer of paraffin wax around the counter tube.

When hydrogen is used as the gas in a neutron counter tube, fast neutrons will collide elastically with the hydrogen atom, whereby protons are released. The ionization caused by the protons can again be counted in the usual way.

Detection of slow neutrons is used in the determination of moisture content in materials (e.g. soils). A source containing a mixture of Ra and Be (or Po and Be) provides fast neutrons, which are slowed down and scattered, predominantly by hydrogen atoms of water.

An end-window G-M tube with a piece of silver foil across the window may be used (if nothing better is at hand) to monitor neutron irradiation. The neutrons rapidly activate, in particular, Ag^{109} atoms, and subsequently Ag^{110} atoms (with a half-life of about 20 sec.) eject β-particles, of which many enter the G-M tube.

3. HEALTH PHYSICS

3.1. Units: Basic considerations

A health hazard is involved when human tissues are subjected to ionizing radiation. The nature and the degree of the damage that is caused depend on the degree to which the particular radiation is able to penetrate the tissues,

its specific ionization, i.e. whether a small or a great number of ion pairs are produced per unit length of track, and the type of tissue being irradiated.

Usually radiation tissue damage will increase with the degree of cell reproduction and decrease with the degree of differentiation.

A commonly used unit of electromagnetic radiation is the röntgen (r), which is essentially defined as that dose of γ- or X-radiation which produces ion pairs carrying 1 Electrostatic Unit (esu) of Charge (of each sign) per cm^3 of standard air surrounded by air. As the numerical charge of an electron is 4.8×10^{-10} esu, $1/(4.8 \times 10^{-10}) \doteq 2.1 \times 10^9$ ion pairs per cm^3 are formed during the penetration of air by 1 r.

For the formation of one ion pair in air, about 33 eV is required on the average. One röntgen will therefore be equivalent to $2.1 \times 10^9 \times 33$ eV = 6.9×10^4 MeV = 0.11 erg of radiation energy absorbed per cm^3 of air.

As 1 cm^3 air has a weight of 0.0013 g, one röntgen will dissipate $0.11/0.0013 \doteq 85$ erg/g air.

A unit of absorbed dose is the RAD, one RAD being equal to an absorbed dose of 100 erg/g of irradiated material. The RAD-dosage absorbed during the exposure of a material to a given dose (e.g. 1 r) of radiation is different for different materials, depending primarily on the scattering power (electron density) of the constituent atoms.

The RAD unit as such is independent of the nature of the radiation.

It is obvious, however, that radiation dissipating 1 RAD with a high specific ionization will have a greater biological effect than will radiation dissipating 1 RAD with a low specific ionization. For combination of the biological effect of various kinds of radiation, a standard of comparison, X-rays of 200 KeV, has been adopted. Based on comparison with the standard, a concept has been defined: the R.B.E. (Relative Biological Effect).

$$\text{R.B.E.} = \frac{\text{dose in RAD from 200 KeV X-rays causing a specific effect}}{\text{dose in RAD from radiation causing the same effect}}$$

R.B.E. values, as they stand today, are given in Table II.

For a combination of the effect of doses of different kinds of radiation, the RAD and the R.B.E. have been combined. The product of R.B.E. and the dose in RAD units is called the dose in REM units (Röntgen Equivalent Mammal or Man).

$$\text{Dose in REM} = (\text{Dose in RAD}) \times \text{R.B.E.}$$

1 REM of β-radiation will per definition have the same biological effect as 1 REM of γ- or neutron radiation. Therefore, doses expressed in REM units may be added in evaluation of the sum effect of a mixture of different kinds of radiation.

It is of importance that, before beginning any work with appreciable amounts of radioisotopes, the operator should know how great the electromagnetic radiation intensity from the source will be. Before the use of a dose-rate meter, which gives the number of röntgen per unit of time, the dose rate at a particular distance from the source in question should be estimated from the K_γ^*-value when the nature of the radioisotope is known (see Table III).

TABLE II

RELATIVE BIOLOGICAL EFFECT VALUES

R.B.E.	Kind of radiation
1	X, γ, β
10	fact neutrons
3-4	slow neutrons
10	α
20	nuclear fragments

A "point" source of C* curies of an isotope, which emits on the average a γ-ray energy of $\overline{E}$ MeV per disintegration, will generate an energy flux of

$$(3.7\times10^{10}\times C^*\times\overline{E})/4\pi d^2 \text{ MeV/sec. per cm}^2$$

at d cm from the source. If μ is the absorption coefficient per cm of air, $(3.7\times10^{10}\times C^*\times\overline{E}\times\mu)/4\pi d^2$ MeV/sec. will be absorbed per cm^3 or air (at d cm from the source). As 1 röntgen is equivalent to 6.9×10^4 MeV absorbed per cm^3 of air (see fourth paragraph in this section), the dose rate at d cm will be $(3.7\times10^{10}\times C^*\times\overline{E}\times\mu)/(4\pi d^2\times 6.9\times10^4)$ r/sec. or about $1.5\times10^8\times(C^*\times\overline{E}\times\mu)/d^2$ r/h.

The above equation can be simplified when C* is taken as 1 c, d as 100 cm (1 m), and the fraction, μ, of γ-photons absorbed per cm^3 of air as about 33×10^{-6} for all photon energies in the range 0.1 - 3 MeV. Then the specific dose rate, for γ-energies in the range 0.1 - 3 MeV, is $K_\gamma^* \doteq \frac{1}{2}\overline{E}$ r/h at 1 m from 1 c "point" source. $\overline{E}$ may be evaluated for a particular radioisotope by a study of the energies of the photons and the branching ratios in the decay scheme of that isotope. Table III lists K_γ^*-values, together with the predominant γ-photon energies.

3.2. Radiation hazard

Two kinds of hazard may be distinguished:

(1) External irradiation from a source outside the body, and

(2) Internal irradiation from isotopes which have entered the body.

With regard to total body irradiation (external plus internal), the International Commission on Radiological Protection (ICRP) has fixed the accumulated dose that may be received by occupational workers.

The maximum permissible accumulated total body dose up to age N is D = 5(N-18), in which D = accumulated doses of radiation expressed in REM, and N is the age of the person in years. (Persons under 18 y therefore should not be occupationally exposed to ionizing radiations.)

Based on the above criteria, it is advisable that the average yearly dose to be received by a worker should not exceed 5 REM, and the average weekly dose should remain below 0.1 REM. The accumulated dose over any con-

TABLE III

$K_\gamma^{\cdot}$ VALUES AT VARIOUS γ-PHOTON ENERGIES

	Approx. $K_\gamma^{\cdot}$ - value (r/h at 1 m from 1 c)	Predominant γ-photon energy (MeV)
Na^{22}	1.2	1.3 and 0.5†
Na^{24}	2	2.8 and 1.4
Mg^{28} (+ equil. Al^{28})	1.6	1.8 and 1.4
K^{42}	0.15	1.5
Cr^{51}	0.02	0.3
Mn^{54}	0.5	0.8
Co^{58}	0.6	0.8 and 0.5†
Fe^{59}	0.6	1.3 and 1.1
Co^{60}	1.3	1.3 and 1.2
Cu^{64}	0.1	0.5†
Zn^{65}	0.3	1.1
Br^{82}	1.5	1.5 - 0.6
Rb^{86}	0.05	1.1
Zr^{95}	0.4	0.8 and 0.7
I^{131}	0.2	0.4
Cs^{137} (+ equil. Ba^{137m})	0.3	0.7
Ta^{182}	0.7	1.2 and 0.2
Au^{198}	0.3	0.4
Ra^{226} (+ equil. decay chain) with 0.5-mm Pt cover	0.825	many different

† Annihilation photons following β^+.

secutive 13 weeks shall be less than 3 REM. These criteria pertain to exposure of the eye lenses, gonads or blood-forming organs as well as to total body radiation.

When only hands are subjected to radiation, the maximum permissible levels are higher and amount to 20 REM per 13 weeks or 75 REM per year.

This means that for work with β- and γ-emitters which have an R.B.E. value of 1 the maximum permissible average dose for the entire body, the lenses of the eyes, the blood-forming organs or the gonads should not exceed 0.1 RAD per week. When only hands are subjected to radiation, 1.5 RAD per week is the maximum permissible average dose.

1 r of penetrating γ-radiation (above 0.2 MeV) will dissipate about 1 RAD in body tissue. X- or γ-photons of energy below 0.1 MeV will dissipate 2 - 5 RAD per röntgen in bone tissue. α- and β-emitters become hazardous on entry into the body. The calculation of the number of RAD in such a case can be a difficult and complex task. The maximum permissible body burden of isotopes is given in Appendix I.

The hazard involved when radioisotopes are ingested or inhaled will depend on a number of factors, such as

(1) Half-life and energy of the isotope;
(2) Biological half-life, i.e. the time required for the elimination of half of the ingested material from the human body;
(3) The accumulation of isotopes in critical organs; and
(4) Formation of toxic by-products as a result of (a) splitting of molecules by radiation or (b) reactions of free radicals.

A number of isotopes and a classification of their danger when ingested by the human body are listed in the Table IV. The highly toxic elements such as Sr^{90}, Ca^{45} and Sr^{89} accumulate in bones and produce damage to the blood-producing cells. I^{131} accumulates in the thyroid gland. The moderately toxic elements do not accumulate to such high degrees in critical organs and have a relatively short biological half-life. Tritium and C^{14} are usually only slightly toxic because of their rapid biological turnover. However, H^3 and C^{14} can be very toxic under conditions of slow turnover, (e.g. in nucleic acids), or, for example, as $BaC^{14}O_3$ dust lodged in the lungs.

TABLE IV

DANGER OF ISOTOPES INGESTED BY THE HUMAN BODY

	Isotope
Very highly toxic	Sr^{90}
Highly toxic	Ca^{45}, Sr^{89}, Ba^{140}, I^{131}
Moderately toxic	Na^{22}, Na^{24}, P^{32}, S^{35}, Cl^{36}, K^{42}, Mn^{52}, Mn^{54}, Mn^{56}, Fe^{55}, Co^{58}, Co^{60}, Co^{64}, Zn^{65}, Br^{82}, Rb^{86}, Mo^{99}, Cs^{137}, Ba^{137}
Slightly toxic	H^3, C^{14}

A complete list of maximum permissible concentrations of radioactive isotopes in air and drinking water, together with the critical organs of accumulation, may be found in Appendix I.

3.3. Safety procedures and precautions

Protection against external radiation is obtained by three different means:

(a) distance,
(b) short exposure time,
(c) shielding.

If a small source (point source) is considered, the intensity $I_d^{\cdot}$ at a distance of d cm from the source will be $I_d^{\cdot} = I_1^{\cdot} / d^2$, in which $I_1^{\cdot}$ is (mathematically) the intensity of the "point" source at 1 cm distance.

Even relatively weak sources should therefore be handled with tweezers, as the inverse-square law shows the intensity of radiation will increase considerably between for example 1 cm and 1 mm.

If a source gave a dose of 1 millirŏntgen per hour (mr/h) at 10 cm distance, any manipulations with the source by means of long tweezers would be harmless. If this were done without tweezers, for instance with rubber gloves as the only protection, the radiation dose at 1 mm distance would then be 10 000 mr/h = 10 r/h to the finger tips.

For all kinds of manipulations with radioactive sources remote handling equipment has been designed. Usually a quick check with a dose-rate meter will tell the operator at what distance the manipulations can be done safely. Important in this respect is the time required for the work. If, once a week for a few months, a concentrated radioactive stock solution giving 1 r/h at 30 cm has to be tapped, this could be done without undue harm from a distance of 30 cm if the operation lasted only about one minute. Approximately 15 - 20 mr per week would then be received by parts of the body (e.g. the lenses of the eyes), which is below the maximum permissible dose of 100 mr/week. On the other hand, if such exposure over longer periods were involved, shielding would be strongly advisable, because in connection with maximum permissible doses it is recommended by ICRP "that all doses be kept as low as practicable, and that any unnecessary exposure be avoided".

With α-emitters, shielding against external radiation would not be required, because the wall of the vessel or a few cm of air will absorb all particles. Also soft β-emitters, such as S^{35}, Ca^{45} or C^{14}, will not require shielding as the walls of the containers are adequate for this purpose. With strong sources of hard β-emitters, such as P^{32}, shielding may be necessary. In this case a plastic sheet of 0.5 - 1 cm thickness may be used; a lead sheet is not advisable because bremsstrahlung increases when the Z-value is high.

Lead bricks may be used when γ-rays are dealt with. The thickness of lead that is required to halve the dose rate from a given γ-emitter will vary 50 - 100% according to the geometry of the situation (broad divergent beam or collimated beam). However, see Table V. To obtain the approximate half-thickness of water, the corresponding half-thickness of lead may be multiplied by 10. (The density of lead is about ten times that of water.) 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 attenuation factor F and the number of half-thicknesses n are related as follows:

$$F = 2^n, \quad \text{or}$$

$$n = \log_{10} F / 0.3 .$$

As soon as the shield has been erected, the calculated dose rate should be checked by a dose-rate meter, and preferably rechecked by a second dose-rate meter - "triple safeguard philosophy".

TABLE V

APPROXIMATE MEAN MAGNITUDE FOR THE HALF-THICKNESS OF LEAD AS A FUNCTION OF γ-PHOTON ENERGY

γ-photon energy (MeV)	Approximate half-thickness of practical lead shielding (cm)
0.5	$\frac{1}{2}$
1.0	1
1.5	$1\frac{1}{2}$
2 - 4	2

Any person dealing with radioactive isotopes (except, e.g., H^3) should wear a film badge on the wrist and/or on the laboratory coat. The blackening produced on development of the film is a measure of the external dose of radiation that has been received during the exposure time. Control films have to be calibrated by means of a standard radiation source and developed together with the film badge. Various types of film badge which permit separate evaluation of the accumulated dose received from β- or γ-rays or neutrons have been designed.

Besides a film badge, and especially in the absence of a film-badge service, a pocket dosimeter should be used.

Frequently it appears that strong β-rays have an effect on the eye lenses even if the levels of radiation are far below the maximum permissible dose. It is therefore desirable to wear plastic (or normal) glasses which will protect the operator against any damage of the eyes from β-radiation.

With regard to the field of tracer applications, the levels of external radiation are usually low. However, when stocks are received from the supplier and dilutions have to be made, or when relatively large levels are necessary, shielding may be required. In some cases, samples which have accumulated radioactive material to a high extent should be kept behind a shield.

Contamination of laboratory, benches, glassware and operator by radioactive material should be avoided for two reasons:

(a) Experimental results may become doubtful;

(b) A health hazard, in particular an internal health hazard, may be involved.

A number of rules should therefore be strictly adhered to:

(1) Each person in a radioisotope laboratory should wear a laboratory coat, closed to the neck. This laboratory coat should be worn only in the laboratory space where the experiments with isotopes are done but preferably not in the counting rooms. Furthermore, a plastic apron should be put on when stock is dealt with; this apron should remain in the stock room.

(2) When there is a risk that the hands become contaminated, thin surgical gloves of rubber or plastic should be worn. These gloves have to

be put on in such a way that the inside never touches the outside for prevention of direct contamination of the skin. A detailed description of the procedure for putting on or removing gloves is given in Appendix II.

As soon as the risk for contamination of the skin is no longer present, the gloves should be removed, because they constitute a source of contamination of glassware and equipment.

A thin layer of "barrier cream" spread on the skin of the hands is used by a number of workers in situations calling for something in between rubber gloves and bare hands.

(3) To prevent contamination of gloves, hands or equipment, paper tissues are always used as an intermediate. After use, these tissues have to be disposed of in (foot-operated) waste bins or large drums.

(4) For avoidance of the spreading of radioactivity by means of shoes, linen or plastic shoe covers or special shoes may be used in the radioisotope laboratory.

(5) Any equipment or glassware which requires operation by mouth may not be used in a radioisotope laboratory. Specially designed equipment and glassware have to be used. Suction is usually applied by means of a vacuum, a pumpet or a plunger pipette.

(6) It is obvious that eating, drinking and smoking are strictly prohibited.

(7) For checking of gloves, hands, laboratory coats and equipment, frequent monitoring is most essential. Any contamination that is observed should be removed in the appropriate way (see section 3.4, Decontamination). Be careful not to allow wet glassware etc. to drip on the detector.

(8) For prevention of contamination of benches and floors, all manipulations with strong radioisotope solutions should be carried out in trays. The bottoms of the trays should be covered with absorbent paper.

(9) Avoid cross-contamination by using glassware, can openers, tweezers etc. for one particular isotope only.

(10) When work at a μc level is being done, sources of mc level should not be admitted to that laboratory. When this is unavoidable, the use of highly active sources should be restricted to a limited space only (fume cupboard).

(11) Radioactive waste should be controlled and disposed of according to the recommendations of the International Commission on Radiological Protection (see Appendix III). Generally, liquid waste is stored in polythene containers and not disposed of through laboratory sinks. Solid waste should be put in foot-operated bins. Under no circumstances may isotopes of greatly different half-lives be mixed.

(12) Radioactive waste should be stored and disposed of by ordinary means only when the activity has decayed or when the waste is diluted to permissible concentration. Long-lived isotopes cannot be disposed of and should be stored. In some countries a central organization is in charge of collection, storage and/or burial of radioactive materials.

(13) Each container, beaker, vessel or bottle that contains a radioactive solution should be marked "radioactive", and the activity concentration in the solution as well as the nature of the isotope and the date should be mentioned. Each container of activity should be placed inside a larger, non-breakable container and should be surrounded by tissue paper; contamination will then be reduced to a minimum in case of an accident.

(14) Any operation in which radioactive dust may arise should be carried out in a glove-box in which a slight under-pressure can be maintained. In the exhaust system a dust filter must be present to collect radioactive particles. These precautions are particularly important in the case of α-activity.

(15) Before leaving the laboratory, check hands, clothes and shoe soles with a suitable monitor.

3.4. Decontamination

Decontamination of the skin is done by washing with soft soap, possibly with a soft brush. Care should be taken to avoid damaging the skin by excessive washing. If contamination with a substance of high specific activity or carrier-free material has occurred, washing with an excess of carrier solution may reduce the contamination by exchange of radioactive isotope with the stable element. It is obvious that such a procedure is only successful if the carrier solution has no damaging effect on the skin.

Generally, the decontamination of glassware, metal surfaces or painted surfaces with radioisotopes 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.

Glass	may be effectively decontaminated with 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	Treatment with 4 N hydrochloric acid until the reaction starts. Wash well with a dilute alkaline solution, and finish with water.
Linoleum	Remove wax surface with xylol or trichlorethylene.
Painted surfaces	Wash with spreading agent and ammonium citrate or ammonium bifluoride.
Wood and concrete	are difficult to decontaminate. Partial or complete removal of the contaminated material will usually be the only effective method.

In general, it will be necessary to maintain a regular check on laboratories in which work with radioisotopes is done. Benches and floors must be monitored frequently. A vacuum cleaner will be useful for removal of radioactive dust that may lodge on floors and benches, and air samples may be tested for radioactive dust by means of an oiled filter paper placed across the inlet of a vacuum cleaner.

3.5. Special features of isotope laboratories

A laboratory in which work with radioisotopes is done should have facilities that

(a) prevent or reduce contamination to the greatest extent, and

(b) make possible rapid decontamination.

These facilities are further determined by the nature of the work which is going to be carried out. Three types of laboratory may accordingly be distinguished (see Table VI, taken from IAEA Safety Series No.1, Vienna, 1962). Usually, an "A" laboratory will be found near reactor sites or waste processing plants. For biological research, "B" or "C" laboratories will generally be adequate.

TABLE VI

FACILITIES FOR RADIOISOTOPE WORK

Radiotoxicity of isotopes	Minimum significant toxic quantity	Type of laboratory or working place required		
		Type C Good chemical laboratory	Type B Radioisotope laboratory	Type A High-level laboratory
Very high	0.1 μc	10 μc or less	10 μc - 10 mc	10 mc or more
High	1.0 μc	100 μc or less	100 μc - 100 mc	100 mc or more
Moderate	10 μc	1 mc or less	1 mc - 1 c	1 c or more
Slight	100 μc	10 mc or less	10 mc - 10 c	10 c or more

A "C" laboratory may be any ordinary laboratory that has a good ventilating system and a fume cupboard. Floors and benches should have a surface which can be cleaned easily. It will, however, be essential to provide a class "B" laboratory when larger quantities of isotopes are to be dealt with. For instance, the dilution of stocks or the preparation of compounds of high specific activity will require the facilities of a "B" laboratory.

The characteristics of a "B" laboratory may be described as follows:

(a) Each isotope is confined to a particular place for prevention of cross-contamination.

(b) Counting rooms are separated from the laboratory room.

(c) Ventilation of the laboratory room should proceed at a rate of at least 12 times the laboratory volume per hour; the air should flow from the least active to the highly active areas. Recirculation of air should be prevented.

(d) Fume cupboards should have a filter for the collection of radioactive dust in the outlet. If more than one fume cupboard is present, it should not be possible to start the suction on one without simultaneously starting it on the other(s).

(e) All operations involving the production of radioactive dust (grinding of samples) should be carried out in a glove box or fume cupboard (e.g. grinding plant samples in mill).

(f) For facilitation of decontamination, floors and benches should be covered with plastic or linoleum, preferably without seams. Under no circumstances should uncovered wooden or concrete floors and bench tops be allowed.

(g) Taps should be of a foot- or elbow-operated design for prevention of contamination from gloves or hands.

(h) Outlets should be present in the floor for the drainage of water.

(i) Ridges and corners in which dust may accumulate and which are difficult to clean should be absent.

(j) The furniture should be made of a non-porous material, preferably acid-resistant, for facilitation of decontamination.

(k) In the counting rooms, fluorescent tubes which ionize the air should be absent; otherwise, a higher background will be measured if organically quenched G-M tubes or liquid scintillation counters are employed. Any equipment such as röntgen apparatus, particle accelerator or large quantities of emitting sources should not be present in the vicinity of the counting room or in adjacent rooms.

4. SOME UTILIZATIONS IN BASIC PRINCIPLE

By virtue of the sensitivity with which their radiation "signals" (ionizations and excitations) can be detected, radioisotopes are widely used as indicators in tracer work, whenever stable indicators are unobtainable or impractical. Furthermore, neutron rays are used for activation analysis and for moisture determination, gamma rays for measuring bulk density and beta (or alpha) rays for surveying thin (or very thin) layers. Finally, large doses of ionizing radiations may be used to sterilize insects, to prevent sprouting of vegetables or to speed up the natural frequency of mutations (with a view to selective breeding) and very large radiation doses to destroy unwanted organisms or growths. A very special use of a radioisotope is as a clock, e.g. in radiocarbon or tritium dating.

Many of the basic principles underlying the above-mentioned applications have already been treated in the foregoing sections. However, the basic lecture matter includes another five sections, which deal with some supplementary principles involved in localization, isotope dilution, tracer kinetics, activation analysis, neutron scattering and gamma attenuation. Seven principal limitations involved in the use of radioactive tracers are given in the introduction to the "Applied Parts" of this manual, and a great number of practical principles are brought out in the various "Applied Parts".

4.1. Translocation and identification

A radioisotope may be used to trace the place, time or amount of translocation (movement, deposition, uptake or excretion) of a test object. A radiotracer may also be used to identify enzymes, antibodies, residues, precursors, metabolites or degradation products.

When an object such as an intact organism or a portion of sand is to be traced, the radioisotope used may belong to any chemical element. The choice of radioactive "label" will then be governed (1) by the ease of attachment to or incorporation into the test object, (2) by the penetrating power of the radiation (since the test object is likely to be some distance away or covered by a certain thickness of matter), and (3) by half-life, in order to

assure a fairly rapid decay of the radioactivity, which is likely to be spread afield after the experiment.

In certain cases an inverse principle of localization may be useful. Instead of the labelling of the object of investigation, a confined amount of radioisotope is placed at the expected place of arrival of the moving object. The moment the object shows a sign of being radioactive, it has reached its destination. This principle has, for instance, been used in the study of rooting patterns.

When the test object is an organic material or compound, the radioisotope used must belong to one of the few elements in the compound. This often reduces the choice to C^{14} or H^3, plus sometimes P^{32}, S^{35}, Cl^{36} or I^{131}. The label may be built into the test materials through biological growth or chemical synthesis.

If the object to be traced is a mineral nutrient, the label must be an isotope of that element. However, in special cases there may be an exception to this rule; e.g. Rb^{86} has been used as a tracer for K for want of a sufficiently long-lived potassium radioisotope.

When "time-of-arrival" experiments are carried out by the injection of radioactive test substance at one place (I) and the later detection of it at a second place (II), the first sign of activity at II may not be the correct moment, because the activity is likely to have become diffuse along its path from I to II (see Fig.13).

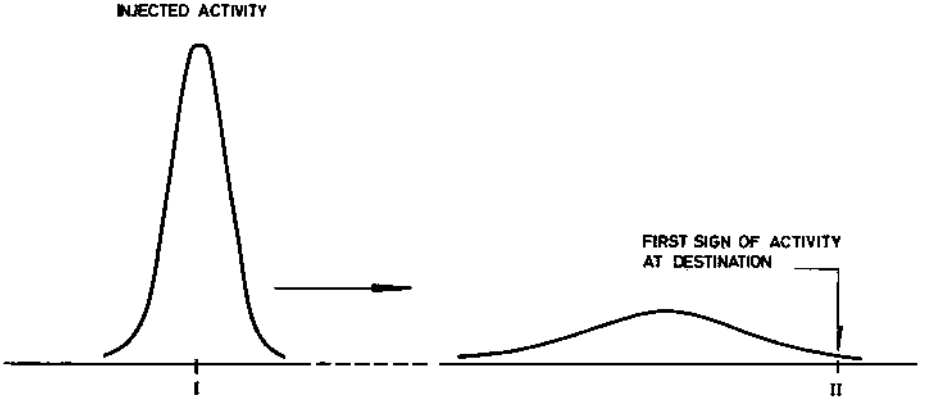

Fig.13

Illustration of the diffuse reappearance at place II after injection of a radioactive test substance at place I

Minute amounts of test substance, not measurable by conventional methods, may be assayed with the aid of a radioactive indicator because of the tremendous sensitivity with which radioisotopes can be detected. For example, when it is judged permissible to assume that the specific activity s* of the test substance has reached the same value at every place of interest in the system under investigation, then the amount A of a test substance "A" in any sample is given by

$$A = \frac{A^*}{s^*} \frac{\text{cpm}}{\text{cpm per unit of "A"}} \qquad (1)$$

where A* is the net count rate of the sample. For determination of the value of s* this method necessitates that there be at least one place in the system where a "big" sample can be obtained with a sufficiently large content of test substance for it to be measured by a chemical or physical method, since we then have

$$s^* = A^*_{big} / A_{big}, \quad (2)$$

where A_{big} and A^*_{big} are the test substance content and activity, respectively, in the "big" sample.

Finally, we shall consider a straightforward case. A particular portion B of a test substance is labelled with a measured specific activity of s* cpm per unit of "B". An unknown amount of B, say b units, translocates to a place of interest, where it may become mixed with an unknown amount of unlabelled test substance. According to the very idea of a tracer, the quantity b can then be identified by measurement of the activity, b* cpm, at the place of interest if we can assume that no b has been diluted en route. It should then be clear that

$$b^*/s^* = b/1 \text{ or } b = b^*/s^*. \quad (3)$$

4.2. Isotope dilution

Principle of the dilution technique: For a given constant amount of radioactivity, the specific activity is inversely proportional to the total amount of test substance present.

This technique, introduced by Hevesy, is particularly useful when quantitative separations are not possible or are too tedious for the systems under study. A basic assumption in this technique is that after "equilibrium" mixing the system is uniform with respect to the specific activity s^*_e of the particular element or compound concerned (the test substance).

Supposing a system contains the unknown amount B g of a test substance "B". To this system is added B_a g of the same substance labelled with a specific activity of s^*_a (cpm per g of "B"). Then at equilibrium, according to the dilution principle,

$$s^*_a/s^*_e = B_e/B_a = (B + B_a)/B_a;$$

therefore

$$B = B_a(s^*_a/s^*_e - 1). \quad (1)$$

Since B_a and s^*_a are known or can be determined, the determination of s^*_e, the specific activity of the test substance after mixing to equilibrium, will enable one to calculate B. In the determination of s^*_e, quantitative isolation of the test substance from the system is not necessary, because the specific activity in either a small or a large sample of the same uniform material is the same. This type of determination is termed the "direct" isotope dilution method.

If B_a^* (cpm) of radioactive test substance of negligible weight, i.e. $B_a \doteq 0$, is added and mixed into a system containing B (mmol) of non-radioactive test substance, then, by the definition of specific activity (see section 1.2 above),

$$s_e^* \underset{=}{\text{def}} B_e^*/B_e \doteq B_a^*/B \text{ (cpm per mmol)}.$$

Hence, instead of equation (1), in this more special case we have

$$B \doteq B_a^*/s_e^*. \tag{2}$$

A variation of the direct isotope dilution technique, called the "inverse dilution technique", enables one to determine the amount of radioactive test substance in a system by the addition of a known amount of unlabelled test substance. Designating the unknown amount of test substance in the system by B, the amount of added test substance by B_a and the specific activities of the test substance, before and after equilibrium mixing with the added test substance, by s* and s_e^*, respectively, one gets from the dilution principle

$$s^*/s_e^* = B_c/B = (B + B_a)/B, \text{ or}$$

$$B = B_a/(s^*/s_e^* - 1). \tag{3}$$

Therefore, the determination of the specific activities in samples taken from the system "before and after" the addition of non-radioactive test substance enables one to calculate the amount of test substance originally present in the system. A modification of this inverse dilution technique is often used in activation analysis.

Example (1)
Liquid volume determination using "carrier-free" isotope (Fig. 14)

An activity of A_a^* cpm of a dissolvable isotope, "A", is added to an unknown volume, V ml, of a non-radioactive liquid. In this case, the liquid is considered to be the test substance. Accordingly, the activity concentration a* could also be considered to be the specific activity s* defined with

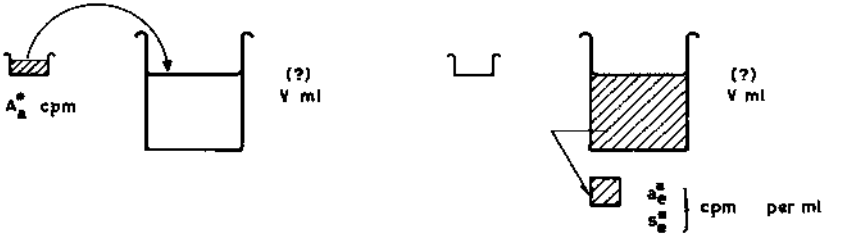

Fig. 14

Block diagram illustrating the isotopic dilution principle in liquid volume determination with "carrier-free" isotopes

respect to the amount of liquid test substance. At equilibrium the isotope is assumed to be uniformly distributed, and a sample of the equilibrium mixture is found to have an activity concentration of a_e^* cpm per ml for a specific activity of s_e^* cpm per ml. The volume of the test substance can then be calculated as follows (cf. equation (2)):

$$V = A_a^* / a_e^* = A_a^* / s_e^* \text{ ml.}$$

This equation simply expresses that the total number of milliliters equals the total activity divided by the amount of activity per milliliter.

Example (2)
Liquid volume determination using labelled liquid†

V_a ml with a specific activity of s_a^* is added to an unknown non-radioactive volume, V ml. The specific activity of the equilibrium mixture is found to be s_e^*. The (unknown) volume of the mixture (see Fig. 15) is then

$$V_e = \frac{A_a^*}{s_e^*} \text{ (ml),}$$

where A_a^* is the total activity added and hence the total activity in the mixture. The amount of this activity added may be calculated as follows:

$$A_a^* = V_a s_a^* \text{ (cpm),}$$

which simply expresses that the total added activity equals the total added volume times the amount of activity per milliliter in the added liquid. Combining the two above equations, we find the original unknown volume as follows:

$$V = V_e - V_a = V_a s_a^* / s_e^* - V_a = V_a (s_a^* / s_e^* - 1),$$

which is analogous to equation (1).

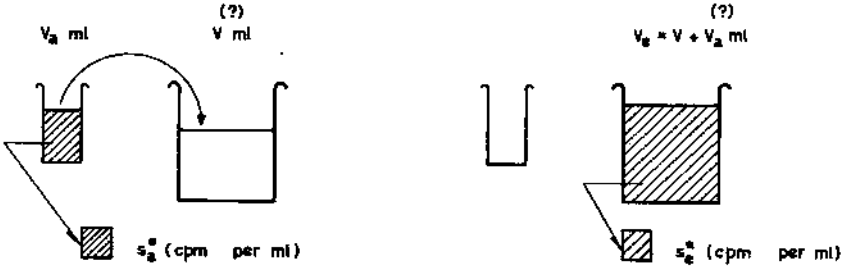

Fig. 15

Block diagram illustrating the isotopic dilution principle in liquid volume determination with labelled liquid of finite volume

† Here, as in Example (1), the liquid of the system is considered to be the test substance, so a_e^* and s_e^* coincide.

Example (3)
Determination of a "pool" size of a test substance † (Fig. 16)

Let us suppose that the test substance is an unknown amount, A (meq.), of an unlabelled anion "A". In order to determine the amount of "A", we

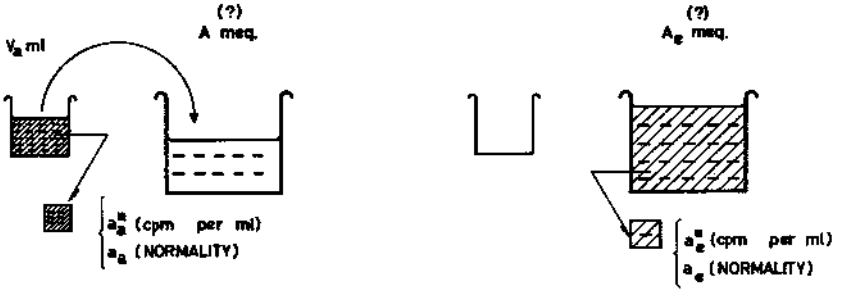

Fig. 16

Block diagram illustrating the isotopic dilution principle in determination of the "pool" size of a test substance with labelled liquid of finite volume and composition

add V_a ml of labelled anion solution of known specific activity and allow it to mix with the unknown amount until equilibrium is reached. The (unknown) total amount of anion A_e in the equilibrium mixture is then

$$A_e = A_a^* / s_e^* \text{ (meq.)},$$

where A_a^* is the total activity added and therefore present in the mixture and s_e^* is the specific activity (cpm per meq.) of the anions in the equilibrium mixture. Neither of these two values is measured directly. Knowing V_a, a_a^* and a_a, and measuring a_e^* and a_e, we have

$$\left.\begin{aligned} A_a^* &= V_a\, a_a^* \\ s_e^* &= a_e^* / a_e \end{aligned}\right\} \quad A_e = V_a (a_a^* / a_e^*) a_e, \text{ and finally}$$

$$A = A_e - V_a a_a.$$

Example (4)
Uneven distribution of test substance. Double "pool"

Even if a test substance is unevenly distributed in the system under investigation, the amount of test substance can still be evaluated by isotope dilution as long as the radioactive label at equilibrium is distributed uniformly over the test substance, i.e. as long as the specific activity of the test substance at equilibrium is the same everywhere in the system.

† Here, and in Example (4), as is more usually the case, activity concentration a^* and specific activity s^* do not coincide (s^* referring to activity per unit of test substance, and a^* per unit of gross sample).

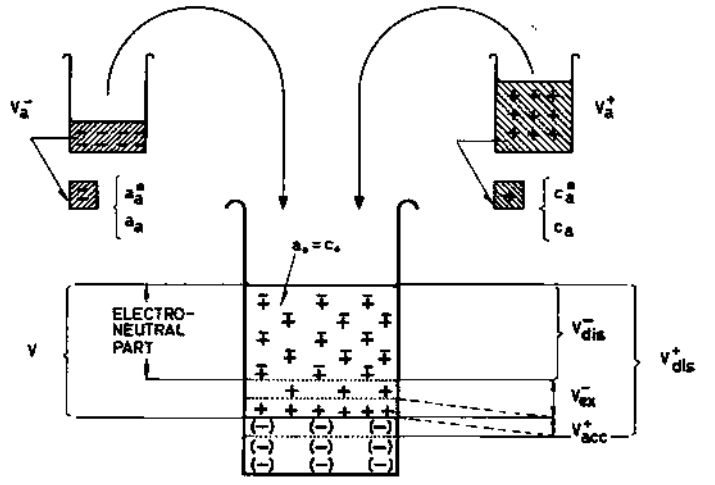

Fig. 17

Block diagram illustrating the isotopic dilution principle in double pool determination of a test substance with labelled liquids of finite volumes and composition

As an example combining the uneven distribution of a test substance with a double pool determination, let us imagine the exaggerated and idealized situation illustrated in Fig. 17. The solution contains a single cation and a single anion. The unknown total amount C of the test cation is greater than the unknown total amount A of the test anion, because some cation is adsorbed at the bottom of the vessel containing the solution (owing to the fact that the non-conducting, thick bottom of the vessel is in possession of a negative charge density). This system may be considered as a schematic version of the situation existing in suspensions of particles exhibiting adsorption properties.

If we define the amount of adsorbed cation and the amount of repelled anion as

$$\gamma^{+}_{ads} \stackrel{def}{=} C - Vc_0$$

$$\gamma^{-}_{rep} \stackrel{def}{=} Va_0 - A = Vc_0 - A,$$

where V is the true volume of the solution (see Fig. 17) and $a_0 = c_0$ is the ion concentration in the electro-neutral part of the solution (not too near the bottom), then the "cation adsorption capacity", γ^{+}, taken as being the sum of γ^{+}_{ads} and γ^{-}_{rep}, may be defined as the surplus of cations over anions as follows:

$$\gamma^{+} = C - A.$$

The two unknowns, C and A, may each be found by isotope dilution in quite the same way as in the previous example. After addition of the two

labelled solutions, the various concentrations and volumes (see Fig. 17) may be significantly altered. If we take a sample of the mixture at equilibrium (i.e. uniform distribution of labels over test substances) and measure the four values: c_e^*, a_e^*, c_e and a_e, it will however be true, as in Example (3), that

$$C_e = V_a^+(c_a^*/c_e^*)\,c_e, \text{ and}$$

$$A_e = V_a^-(a_a^*/a_e^*)\,a_e,$$

where C_e and A_e are the total amounts of test cation and test anion, respectively, in the system after equilibration with the added volumes V_a^+ and V_a^-. Finally C and A, and thereby the concept $\gamma^+ = C - A$, are easily calculated from the following two equations:

$$C = C_e - V_a^+ c_a$$

$$A = A_e - V_a^- a_a,$$

which do not demand any knowledge of c_0(or a_0).

An implicit assumption in this method is that

(1) c* and a* may be determined simultaneously and independently;

(2) The a and c gradients are not too different; and

(3) Exchange of adsorbed c with c* is so slow as to be negligible.

In Fig. 17 the following "volumes" (other than true volumes) are indicated:

$V_{dis}^+ \overset{def}{=} \frac{C}{c_0}$ = apparent volume of even distribution of cation;

$V_{dis}^- \overset{def}{=} \frac{A}{a_0}$ = apparent volume of even distribution of anion;

$V_{acc}^+ \overset{def}{=} V_{dis}^+ - V$ = apparent volume of accumulation of cation;

$V_{exc}^- \overset{def}{=} V - V_{dis}^-$ = apparent volume of exclusion of anion.

Once C and A have been determined by double isotope dilution, as described above, these various apparent volumes can also be calculated if $a_0 = c_0$ was determined. One can then, for instance, write the amount of adsorbed cation in the following way:

$$\gamma_{ads}^+ \overset{def}{=} C - Vc_0 = V_{dis}^+ \times c_0 - Vc_0 = V_{acc}^+ \times c_0.$$

4.3. Tracer kinetics

Many physico-chemical processes in nature have been found to proceed exponentially; i.e. the fractional rate of change is constant. Very often it is possible to see if this is so by injecting a radioisotope and subsequently inspecting the appropriate semi-log plot of count rate as a function of time.

If this turns out to be a straight line, agreement with a simple exponential hypothesis has been found.

When there is a net transfer of test substance (removal, accumulation) the radioisotope label is invaluable for practical reasons, and in the case of no net transfer of test substance (turnover, exchange) a tracer is indispensable if the rate constant is to be found.

Example (1)
Removal. Accumulation

In this case (see Fig. 18) test substance "A" disappears exponentially from phase 1 through two channels with the rate constants, k_1' and k_1''. The

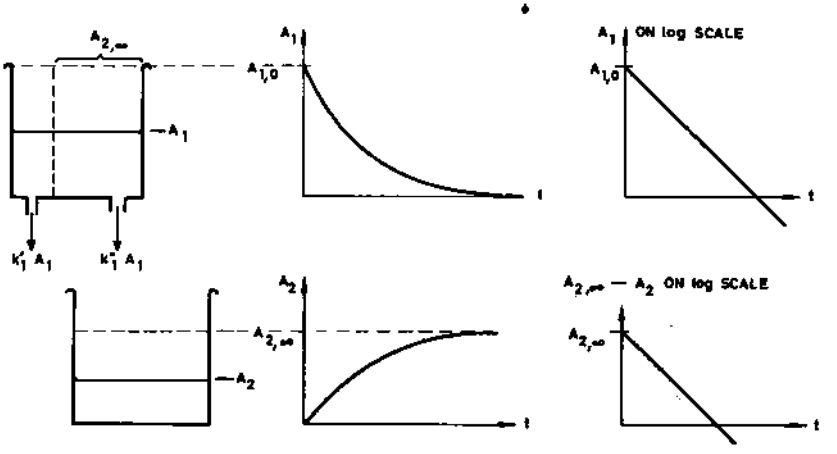

Fig. 18

Illustration of the disappearance of a test substance "A" from phase 1 and appearance in phase 2
Disappearance and appearance are given in linear and semilog plots versus time.

test substance flowing through the second channel is accumulated in phase 2 (whereas that flowing through the first channel is lost). Linear plots, and the appropriate semi-log plots, of amounts plotted against time are also shown in Fig. 18.

Mathematically, the rate of removal from phase 1 is

$$-dA_1/dt = k_1' A_1 + k_1'' A_1 = k_1 A_1, \qquad (1)$$

where $k_1 = k_1' + k_1''$ is the rate constant (fraction per unit time) for overall removal from phase 1. In analogy to radioactive decay (section 1.2), equation (1) may be integrated to give

$$\boxed{A_1 = A_{1,0} e^{-k_1 t}}, \qquad (2)$$

where $A_{1,0}$ is the amount of "A" present in phase 1 at zero time.

Phase 2 is assumed to be empty at zero time. Mathematically at infinite time, or in practice at an equilibrium time long enough for the amount of "A" remaining in phase 1 to fall below the limits of accuracy of measurement, the amount of test substance in phase 2 is

$$A_{2,\infty} = (k_1''/k_1)\, A_{1,0}, \tag{3}$$

which simply states that "A" is dispersed through channel 2 in accordance with the branching ratio of this channel. Likewise, the amount of test substance in phase 2 at any time t is equal to k_1''/k_1 multiplied by the amount of "A" that has disappeared from phase 1 during the time t, i.e.

$$A_2 = (k_1''/k_1)\,(A_{1,0} - A_1),$$

which can be transformed, by the use of equation (3), to read

$$A_2 = A_{2,\infty} - (k_1''/k_1)A_1 .$$

Solving this equation with respect to A_1, one finds

$$A_1 = (k_1/k_1'')(A_{2,\infty} - A_2). \tag{4}$$

The rate of accumulation in phase 2 is given by

$$dA_2/dt = k_1'' A_1,$$

or, by substitution of A_1 from equation (4),

$$dA_2/dt = k_1(A_{2,\infty} - A_2), \quad \text{or} \quad -d(A_{2,\infty} - A_2)/dt = k_1(A_{2,\infty} - A_2).$$

This differential equation (in analogy to equation (1)) may be integrated to give

$$\boxed{A_{2,\infty} - A_2 = A_{2,\infty} e^{-k_1 t}}. \tag{5}$$

Thus, a plot of log $(A_{2,\infty} - A_2)$ against time yields a straight line, and from the slope of this line one may find k_1, which is the rate constant for overall removal from phase 1. The rate constant k_1'' for partial removal into phase 2 may be easily calculated from k_1 by the use of equation (3), i.e.

$$\boxed{k_1'' = k_1 A_{2,\infty}/A_{1,0}}. \tag{6}$$

So far we have considered only unlabelled test substance. If a radioisotope of "A" is mixed with A_1 at zero time, this will label the test substance and enable us to determine the rate constant(s) by the assay of radioactivity. The equations corresponding to (2), (5) and (6) are then simply the following:

$$A_1^* = A_{1,0}^* e^{-k_1 t} \tag{2'}$$

$$A_{2,\infty}^* - A_2^* = A_{2,\infty}^* e^{-k_1 t} \tag{5'}$$

$$k_1'' = k_1 A_{2,\infty}^* / A_{1,0}^*, \tag{6'}$$

where A* stands for activity of "A", e.g. expressed in cpm.

Example (2)
Turnover

In the case of turnover, we assume that a "steady state" prevails; i.e. rate of renewal equals rate of loss. There is no net change in amount of test substance. A is constant (see Fig. 19). We further assume that there is no change in volume of the system.

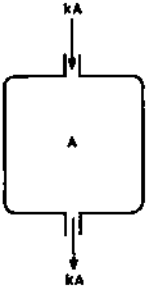

Fig. 19

Schematic model illustrating steady state in an open turnover compartment

If one wishes to determine the turnover rate constant k by investigations confined to the volume containing "A", a tracer, for instance a dye or a radioisotope, is necessary.

Let us suppose that at zero time a radioactive isotope of "A" is mixed into the volume containing the test substance, so that the total amount, A mole, of test substance consists of A° mole stable isotope and A* mole radioisotope (see Fig. 20). In practice, A* will be a very small fraction of A; hence A° will remain practically constant.

The rate of disappearance of the activity (see Fig. 20) is given by

$$-dA^*/dt = kA^*,$$

and by integration this gives

$$\boxed{A^* = A_0^* e^{-kt}}. \tag{7}$$

Thus, plotting of A* (expressed, e.g., as net count rate) as a function of time on semi-log paper will yield the turnover rate constant k. However,

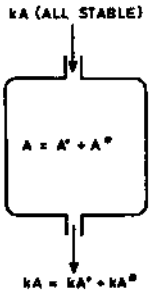

Fig. 20

Schematic model illustrating the determination of the turnover rate constant k by injection of an infinite amount of a radioactive isotope of A into the compartment volume

rather than to assay the total activity A*, it will often be more practical to draw samples from the volume containing the test substance and to determine the activity concentration a* or the specific activity s*. Either of these variables will serve as well as the total activity, since the size of the volume and the amount of test substance are both constant. In other words, the following two equations are valid besides equation (7):

$$a^* = a_0^* e^{-kt} \text{ (cpm per ml)} \tag{7a}$$

$$s^* = s_0^* e^{-kt} \text{ (cpm per mole)} \tag{7s}$$

Often the rate of turnover is stated in terms of "turnover time," $t_t = 1/k$. t_t is the time it takes for an amount equal to A (see Fig. 20) simultaneously to enter and leave. Statistically, about one-third of the A mole that leave during a turnover time period consists of molecules that also entered during this turnover period, and about two-thirds consist of molecules that entered during previous turnover periods.

Example (3)
Exchange

Fig. 21 illustrates (schematically) the process of a typical steady-state exchange system after the injection (and uniform mixing) of radioactive test substance into one of the phases at zero time. The total amount of injected activity A^*_{tot} will at any later time be partially transferred to the second phase; however, since the system as a whole is assumed to be closed, the sum of the amounts of activity in the two phases will remain constant; i.e.

$$A_1^* + A_2^* = A_{tot}^* \,. \tag{8}$$

Since there is no net transfer, A_1 and A_2 (i.e. the total amounts of test substance in the two phases, respectively) are both constant and, therefore, $k_2 A_2 = k_1 A_1$ (see Fig. 21). Hence, the rate constant k_2 for one-way transfer from phase 2 may be related to the rate constant k_1 for one-way transfer

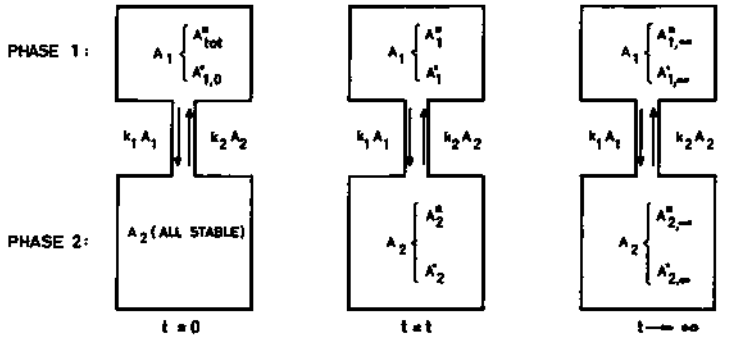

Fig. 21

Schematic models illustrating steady-state exchange in a closed two-compartment system
An infinite amount of a radioactive isotope of A has been injected into phase 1 at zero time.

from phase 1 as follows:

$$k_2 = k_1 A_1/A_2 \ . \tag{9}$$

Mathematically, the (net) disappearance of activity from phase 1 is given by the differential equation

$$-dA^*_1/dt = k_1A^*_1 - k_2A^*_2 \ ;$$

but, by the use of equations (8) and (9), this may be transformed to

$$-dA^*_1/dt = k_1A^*_1 - k_1(A_1/A_2)(A^*_{tot} - A^*_1), \text{ or}$$

$$-dA^*_1/dt = [(A_1+A_2)/A_2]k_1A^*_1 - k_1(A_1/A_2)A^*_{tot} \ , \text{ or}$$

$$-dA^*_1/dt = [(A_1+A_2)/A_2]k_1(A^*_1 - [A_1/(A_1+A_2)]\,A^*_{tot}), \text{ or}$$

$$-\frac{d(A^*_1 - \frac{A_1}{A_1+A_2}A^*_{tot})}{dt} = \frac{A_1+A_2}{A_2}k_1(A^*_1 - \frac{A_1}{A_1+A_2}A^*_{tot}).$$

This differential equation (in analogy to equations (1) and (2)) may be integrated to give

$$A^*_1 - \frac{A}{A_1+A_2}A^*_{tot} = (A^*_{tot} - \frac{A_1}{A_1+A_2}A^*_{tot})e^{-[(A_1+A_2)/A_2]k_1t} \ . \tag{10}$$

At equilibrium (mathematically, $t \to \infty$) the activity will be spread uniformly over the test substance; i.e. the specific activity will be the same everywhere in the system; hence

$$A^*_{1,\infty}/A_1 = A^*_{tot}/(A_1+A_2); \text{ or}$$

$$A_1/(A_1+A_2) = A^*_{1,\infty}/A^*_{tot} \ . \tag{11}$$

Introduction of equation (11) into (10) yields

$$\boxed{A_1^* - A_{1,\infty}^* = (A_{tot}^* - A_{1,\infty}^*)e^{-k_1^* t}}, \qquad (12)$$

where $k_1^* = [(A_1 + A_2)/A_2]k_1$. Equation (12) shows that a plot of log $(A_1^* - A_{1,\infty}^*)$ against time should give a straight line (see Fig. 22), and from the slope of this line one may obtain the rate constant k_1^* for net transfer of radioactivity from phase 1.

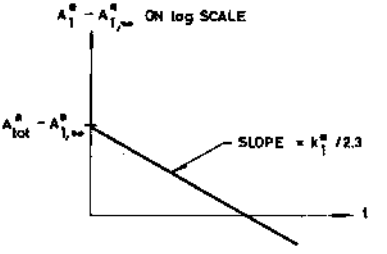

Fig. 22

Illustration of the straight-line relationship between $\log(A_1^* - A_{1,\infty}^*)$ and time

The last step is to calculate the exchange rate constant k_1 for one way transfer of test substance (and activity) from phase 1. The following transformation is then useful:

$$k_1 = [A_2/(A_1 + A_2)]k_1^* = [1 - A_1/(A_1 + A_2)]k_1^* = (1 - A_{1,\infty}^*/A_{tot}^*)k_1^*,$$

where the last equation follows from equation (11), so

$$\boxed{k_1 = [(A_{tot}^* - A_{1,\infty}^*)/A_{tot}^*]k_1^*}. \qquad (13)$$

In words: k_1 is equal to k_1^* multiplied by the ratio of the ordinate intercept (see Fig. 22) to the total amount of activity (which is the ordinate intercept plus $A_{1,\infty}^*$).

As is seen in equations (12) and (13), the exchange rate constant k_1 may be determined by measurement of activity alone; i.e. no chemical measurement is necessary.

In conclusion, it is noted that it might well be more practical in a specific case to assay activity concentration a* or to measure specific activity s* rather than to assay A*. This does not lead to any further problem, because both the phase volume and the phase content of test substance are considered to be constant, so that equations (12) and (13) may equally well be expressed as follows:

$$a_1^* - a_{1,\infty}^* = (a_{1,0}^* - a_{1,\infty}^*)e^{-k_1^* t} \qquad (12a)$$

$$s_1^* - s_{1,\infty}^* = (s_{1,0}^* - s_{1,\infty}^*)e^{-k_1^* t} \qquad (12s)$$

$$k_1 = k_1^*(a_{1,0}^* - a_{1,\infty}^*)/a_{1,0}^* = k_1^*(s_{1,0}^* - s_{1,\infty}^*)/s_{1,0}^*. \qquad (13a \text{ and } 13s)$$

4.4. Activation analysis

A nuclear reaction is a process in which a nucleus reacts with another nucleus, an elementary particle or a photon to produce one or more other nuclei. The notation used for nuclear reactions is analogous to that in chemical reactions; however, a shorthand notation is often used.

The light bombarding particle and the light fragment are written in parentheses between the initial and final nucleus. The production of O^{17} by bombarding N^{14} with α-particles ($_7N^{14} + {}_2He^4 \rightarrow {}_8O^{17} + {}_1p^1$) can, therefore, be written as $N^{14}(\alpha, p)O^{17}$.

The probability of a nuclear process is generally expressed in terms of a cross-section σ, which has the dimensions of an area. This originated from the simple picture that the probability for the nuclear reaction between a nucleus and an impinging particle is proportional to the cross-sectional area of the target nucleus. Although this picture does not hold for every nuclear reaction, the cross-section is a very useful measure of the probability for any nuclear reaction. The cross-section σ is defined by the following equation:

$$-dI = In\sigma dx, \tag{1}$$

where I is the number of incident particles/cm^2 per sec. ("intensity"),
n is the number of target nuclei per cm^3, and
x is the target thickness (cm).

As the equation (1) shows, the fractional attenuation ($-dI/I$) of the incident particles is proportional to the population density of target nuclei and the distance the particle has traversed. The proportionality factor is the cross-section σ. If we assume that σ is constant during the passage of incident particles through the target, the beam intensity after traversing a target thickness of d is, by integration of equation (1),

$$I = I_0 e^{-n\sigma d}, \tag{2}$$

where I_0 is the initial intensity of the beam.

The number of particles (per cm^2 per sec.) that undergo reaction will then be

$$I_0 - I = I_0(I - e^{-n\sigma d}). \tag{3}$$

σ is a characteristic of the reaction and the target nuclei.

Bombarding particles which have been used to effect nuclear reactions are neutrons, protons, deuterons, H^3, He^3, He^4, photons, electrons and others. Among the particles, the neutron is extensively used because of its ready availability from a reactor. The (n,γ) reaction is the only type commonly occurring with slow neutrons. This reaction occurs with very nearly every target. After the emission of γ from the target, the product nuclei may separate from the chemical compound because of rupture of chemical bonds by recoil. Because of this so-called Szilard-Chalmers process, the chemical identity of the product after irradiation may be different from that of the original target.

The breakup of some heavy nuclei into two or more medium-heavy fragments also takes place after bombardment by rays or particles. This process is termed "fission". Some of the fission products are radioactive with relatively long half-lives and are extremely harmful to the human being because of their accumulating character in the human body.

Radioactivation analysis is the method of quantitative analysis of a certain element after conversion of that element into a radioisotope under irradiation of particle beams. The method is capable of high sensitivity and accuracy for many elements which are not conveniently determinable by conventional chemical methods. Analysis with a nuclear reactor is generally used although some other nuclear-irradiation device can be used.

In reactor activation, the (relatively thin) sample to be analysed is placed in a uniformly high flux of slow neutrons for a length of time sufficient to produce a measurable amount of radioisotope of the test element. The disintegration rate for a given isotope at time t from the start of irradiation is given by the following equation:

$$D = NI\sigma(1 - e^{-\lambda t})E,$$

where D is the total number of disintegrations per second,
N is the total number of target nuclei,
σ is the cross-section of the element in cm^2/atom,
λ is the decay constant for radioisotope formed,
t is the time of irradiation,
I is the neutron flux per cm^2 per sec., and
E is the fractional abundance of target isotope in natural element.

Fig. 23 shows the growth of the radioisotope formed during activation and the subsequent decay after activation has ceased. As can be seen, the growth curve is geometrically a mirror image of the decay curve.

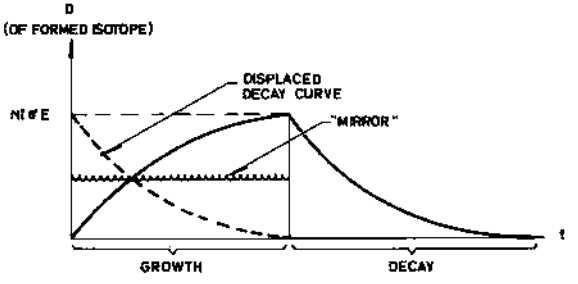

Fig. 23

Growth and decay of radioisotope formed during activation

It is generally convenient to activate for about one half-life of the desired nuclide. Since the neutron flux for reactors is nearly always around $10^{12} \sim 10^{14}$ n/cm^2 per second, the sensitivity of the activation analysis depends in practice on the cross-section of the parent nuclide. In the case of Mn^{55}, which is present in trace amounts in biological tissues, the cross-section for the reaction $Mn^{55}(n,\gamma)Mn^{56}$ is 13.3 barns (1 barn = 10^{-24} cm^2). The resulting specific activity, obtainable in a flux of 10^{14} n/cm^2 per second,

is nearly 195 c/g after irradiation for 2.58 h, which is the half-life of Mn^{56}. Since it is possible to detect about 400 dis./min. with standard counting equipment, the approximate limit of detection of Mn by activation analysis is around 10^{-12} g. This amount is far too small to be detected by ordinary chemical means.

Slow-neutron activation analysis is unsatisfactory for elements lighter than sodium, mainly because the half-lives of the products are very short. For some elements such as S, Ca and Fe the cross-sections are too low.

Proton activation can be used for B, C, O and F, while deuterons are used for N and S.

4.5. Neutron moderation and gamma attenuation

4.5.1. Neutron moderation in relation to moisture determination

Physicists have for many years utilized fast-neutron sources in studying fundamental nuclear reactions. A common source for this purpose is a mixture of radium and finely ground beryllium. Nuclear reactions that occur with this combination are as follows:

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

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

(alpha particle) (fast neutron)

Such a source can be depended upon for a constant neutron flux without the need for source decay correction (the half-life of Ra^{226} is over 1500 y).

Recently soil and plant scientists have become intrigued with fast-neutron sources by reason of the known interaction between fast neutrons and absorbing materials which results in a moderation or slowing-down of the neutrons. Since it has proved possible to correlate the degree of neutron moderation with hydrogen concentration of a medium, it should be possible to extend the principle to measurement of moisture content.

Moisture measurement is based on physical laws which control the moderation of neutrons in matter. Fast neutrons which are released into a given material collide with the atoms of that material and are scattered in all directions in random fashion. With each collision that a certain fast neutron undergoes, however, a fraction of its kinetic energy is given up to the nucleus encountered. The process of collision, scattering and kinetic energy reduction continues until the original fast neutron has been reduced in energy to the level of a slow neutron. There is much greater energy loss of fast neutrons impinging on atoms of low atomic number than in collisions with heavier atoms; and, in particular, half the neutron kinetic energy is lost on the average per collision with hydrogen. Since hydrogen is the primary element of low atomic number of many materials, it is the predominant moderator in such material (e.g. soil) of the fast neutrons introduced, and the number of moderated (slow) neutrons produced per unit volume per unit of time can be correlated with the concentration of hydrogen atoms in the material.

Slow neutrons are readily detected, even in the presence of fast neutrons, by a further nuclear reaction in a neutron counter tube. Most commonly this is a detector filled with B^{10} enriched BF_3 gas, in which the following nuclear reaction occurs:

$$_0n^1 + {}_5B^{10} \rightarrow {}_3Li^7 + {}_2He^4.$$
(slow neutron) (alpha particle)

Absorption of the alpha particle in the detector gas is the ionizing event which by amplification can be caused to trigger a counting mechanism (scaler).

Because of the fact that the major portion of hydrogen atoms in, for example, soil is contained in molecules of free water, the slow-neutron count rate represents a measure of soil moisture content. With a 5 mc source, this measurement embraces a spherical volume of soil ranging from 15 to 60 cm in radius, varying inversely with moisture content.

Other elements than hydrogen are efficient moderators of neutrons. These include cadmium, boron, lithium, clorine and manganese; but fortunately these are rarely present in soil in other than trace amounts. Compared with the many kg of water (and hydrogen) per m^3 of soil with normal moisture content, these elements, usually in concentrations on the order of a gram per m^3, have no influence as a rule. However, boron (19% B^{10}) in small amounts may be significant because of its great affinity for absorbing slow neutrons (as utilized in the neutron detector described above).

4.5.2. Gamma attenuation in relation to density determination

The degree of scattering and absorption is involved, as with neutron moisture measurement, but in this case involving gamma photons. The only nuclear reaction is the initial one involved in the disintegration of the gamma-emitting isotope source. This source is usually Cs^{137}, and the reaction is as follows:

$$_{55}Cs^{137} \rightarrow {}_{56}Ba^{137} + {}_{-1}e^0 + \gamma.$$

The theory of density determination is based on the well-known types of interaction of gamma rays with orbital electrons of atoms and free electrons. When a source is introduced into soil, the gamma photons emitted are scattered randomly in all directions because of Compton collisions between the photons and outer orbital or free electrons in the material. If the probe containing the source is also outfitted with a detector, the number of gamma photons scattered back can be measured.

As the mean electron density of, for example, soil material (and soil bulk density) increases, the scattering probability per cm photon path increases correspondingly. With each Compton scattering the gamma photon transmits some of its energy to the electron hit, whereby the probability per cm path of being absorbed by a photoelectric process is greatly increased. The combined result is difficult to see, but in practice fewer gamma rays return to the probe detector as the surrounding soil increases in density. Thus, the bulk density of the absorbing soil proves inversely correlated with the count rate of the detector.

LABORATORY EXERCISES

1. EXPERIMENTS WITH A G-M COUNTER

1.1. The plateau of a G-M tube

Introduction

Geiger-Müller counter assemblies in normal operation often show an appreciable variation in performance from time to time. 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 y). 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).

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

(1) The threshold or starting potential, and

(2) The length and shape of the plateau.

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

EXPERIMENTAL PROCEDURE

(1) Obtain a source counting about 8000 cpm.

(2) Put the source into the holder (in the lead castle), and increase the high voltage slowly until the first counts are obtained. This voltage 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 increment may be 20 to 50 volts.)

(4) When the count rate does not change appreciably as the high voltage is increased, the G-M tube is operating in the "plateau region". When the count rate starts to increase again, no further high-voltage steps should be applied. Above this voltage V_R the counter will start to race, and damage to the G-M tube is likely to occur.

(5) Calculate the slope as percentage increase in count rate per 100 volts and the plateau length (see Fig. 24), $V_2 - V_1$.
The slope is

$$[(R_2 - R_1)/R_W] / [(V_2 - V_1)/100]\ 100\% \text{ per } 100 \text{ volts},$$

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

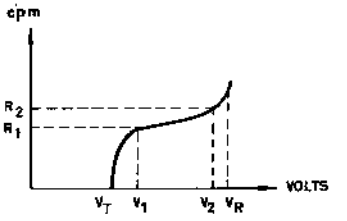

Fig. 24

Characteristic of a G-M tube showing the relationship between count rate and voltage

(6) As the ~~counter~~ tube 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 is less than 150 volts. Occasional checks on the plateau characteristics of a tube are necessary with age (number of counts).

1.2. The dead time of a G-M counter

Introduction

The dead time, i.e. the time after each pulse that the G-M tube is not able to 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 counting rates and the observed count rate, the dead time can be estimated.

Let the true count rate be R^+ cpm and the observed count rate R cpm. If the dead time is τ min., the counter has been inoperative during $R\tau$ min. per minute. R counts have therefore been registered in $1 - R\tau$ effective minute. The corrected count rate R^+ is thus as follows:

$$R^+ = R/(1 - R\tau). \qquad (1)$$

If the R of a radioisotope of known half-life is plotted against time on semi-log paper, R^+ for the highest counting rates can be extrapolated from the R of the lowest counting rates, and τ can then be estimated approximately with the aid of equation (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_1^+, R_2^+, R_{12}^+ and r^+ 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_{12} and r be the corresponding observed count rates. Then by definition

$$R_1^+ + R_2^+ = R_{12}^+ + r^+;$$

and thus from equation (1)

$$R_1/(1-R_1\tau)+R_2/(1-R_2\tau)=R_{12}/(1-R_{12}\tau)+r/(1-r\tau).$$

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

$$R_i/(1-R_i\tau) \doteq R_i+R_i^2\tau, \quad r/(1-r\tau) \doteq r.$$

Therefore, after substituting, we obtain

$$\tau \doteq (R_1+R_2-R_{12}-r)/(R_{12}^2-R_1^2-R_2^2) \tag{2}$$

or

$$\tau \doteq (R_1+R_2-R_{12}-r)/2R_1R_2 \tag{3}$$

since

$$R_{12}^2 \doteq (R_1+R_2)^2.$$

PROCEDURE

(1) Tap about 5 ml (approx. 25 000 cpm) from the Ba^{137m} column used in experiment 2.2; cover the counting dish with a plastic cap of 100 – 150 mg/cm², and immediately start making 6 – 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 Ba^{137m} against time on semi-log paper.
(3) A straight line with its slope corresponding to the "tenth-life" of Ba^{137m} (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 G-M counter, using equation (1).
(4) Select two β-samples (e.g. Tl^{204} or Cl^{36}) of approximately 12 000 cpm.
(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 equation (2) or (3).

1.3. Natural and technical uncertainty (statistics)

Introduction

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 $\sigma_{nat} = S^{\frac{1}{2}}$, where S is the sum-count. This is a measure of the natural uncertainty inherent in radioactive decay, and 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 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 AND REAGENTS

(1) P^{32} solution containing on the order of 0.05 μc/ml.
(2) 1-ml pipette, and a pro-pipette (e.g. rubber bulb).
(3) 25 counting cups.

PROCEDURE

(1) Obtain a bottle of P^{32} solution containing about 10 000 cpm per ml.
(2) Using the 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 $S^{\frac{1}{2}}$ and the total standard deviation according to the following formula:
Let $S_1, S_2, \ldots S_n$ be the sum counts registered in n countings. Calculate $\overline{S} = (\Sigma s)/n$, which is the mean sum count. Calculate $S_1 - \overline{S}$, $S_2 - \overline{S}, \ldots S_n - \overline{S}$.
The total standard deviation of the sum count is then

$$\sigma_{tot} = \{[\Sigma(S - \overline{S})^2]/(n-1)\}^{\frac{1}{2}} \qquad (1)$$

(5) Compare this 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 equation (1), and compare with S^1. Calculate σ_{tech} in per cent, using the equation

$$\sigma^2_{tot} = \sigma^2_{nat} + \sigma^2_{tech} .$$

(8) Calculate the count rate (R) and its total standard deviation in per cent and in cpm (see Lecture Matter, section 2.4.2).

1.4. External absorption of β-particles

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

A transmission curve of P^{32} β-particles through aluminium will be prepared in the present experiment.

PROCEDURE

(1) Pipette 100 μl of a solution containing approximately 0.1 μc P^{32}/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 μc P^{32}/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 cpm 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 strong count rates, and background for weak.) This factor serves to transform weak-source net count rates into strong-source net count rates. Avoid direct exposure of the G-M 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 G-M tube and the air thickness from G-M tube window to sample into consideration when assessing the zero point of the linear scale. (This is particularly important in the case of soft β-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 on the order of 800 mg/cm^2 for P^{32}.

(10) Repeat the exercise, using C^{14} instead of P^{32}. 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 becomes $-\infty$; see Lecture Matter, section 1.4.2).

(12) How well can the top part of the C^{14} transmission curve, corresponding to the first decade or two on the log scale, be approximated by a straight line?

1.5. Self-absorption and self-scattering of β-particles

1.5.1. Introduction

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 β-emitters whose maximal energy is less than about 0.5 MeV. Scattering tends to increase the counting rate and is most noticeable with high-energy beta emitters. (The effect of self-absorption and self-scattering also exists with γ-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 grows 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-β 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^* (or s_1^*/s_2^*, but this again equals a_1^*/a_2^* divided by a_1/a_2), where a_1^* and a_2^* are the activity concentrations of test substance in two samples. If in such a case the tracer is a low-energy β-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 activity concentrations will also suffice, since they are to be used as a ratio only. Correct activity concentrations 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 activity concentration of the

radioisotope used in the experiment, a series of samples covering the thickness range of the experimental samples is prepared. From these reference samples a reference curve is constructed by count rate plotted against weight of sample. This is now (whatever shape the curve may have) a reference of constant activity concentration throughout the length of the curve, so the relative activity concentration 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 weight (thickness) of reference material (see Fig. 25):

$$a^*_{rel} = \frac{\text{net count rate of exp. sample of wt. w}}{\text{net count rate of ref. sample of wt. w}} \qquad (1)$$

When corrections for self-weakening to yield the "true" count rates of β-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 activity concentration (or constant total activity) and from these construct a self-transmission curve, cpm plotted against mg/cm^2; or, on the other hand, one may make mathematical assumptions regarding the nature of the self-weakening effect.

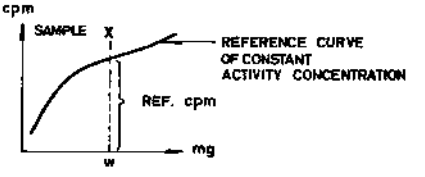

Fig. 25

Reference curve showing a relationship between count rate and weight of samples of constant activity concentration

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 cpm per mg sample (or the total activity in cpm). 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 the approximation, at least through the first decade or two, of the curve obtained in the previous exercise (section 1.4) by a straight line.) Accepting these assumptions, we find that the self-absorption loss is mathematically given by

$$-da^*/dx = \mu a^*,$$

where a_0^* = number of β-particles per cm^3 sample ejected in the direction of the G-M tube,

μ = linear absorption coefficient (characteristic of $E_{\beta max}$ and density of sample material), and

x = sample layer (cm);

i.e. $a^* = a_0^* e^{-\mu x}$, where a^* means transmitted activity (per cm^3 sample) from depth x below the surface of the sample. 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 sample as follows:

$$A^* = \int_0^X a_0^* \ell e^{-\mu x}\, dx = a_0^* \ell (1 - e^{-\mu X})/\mu = (A_0^*/\mu X)(1 - e^{-\mu X}).$$

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

$$A^*/A_0^* = f = (1 - e^{-\mu X})/\mu X, \qquad (2)$$

where μ may be taken as the mass absorption coefficient (characteristic of $E_{\beta max}$ alone) and X as mass-thickness (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 activity concentration will approach (according to equation (2)) asymptotically a constant value A_∞^* as X approaches ∞. In practice A_{obs}^* becomes constant at a thickness equal to about half the "range" of the E_{max} of the β-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 activity concentrations of the samples. This is merely a limiting case of the general equation (1), in which the denominator, having become constant, may be disregarded (with respect to all inifinitely 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 Ca^{45}. The self-weakening will be determined from the series of sources. In the second experiment, a self-transmission curve will be constructed for C^{14} in $CaCO_3$ prepared from $C^{14}O_2$, and the corrected count rates will be calculated from the theoretical equation above, with $\mu = 0.29$ (cm^2/mg).

1.5.2. PROCEDURE FOR $Ca^{45}CO_3$

(1) Prepare 50 ml of a solution (solution A), 0.25 M $CaCl_2$ and 0.1 N HCl, containing 1 μc Ca^{45}.

(2) To prepare solution B, pipette 5 ml of solution A into a 50-ml volumetric flask, and make up with 0.1 N HCl.

(3) Prepare about 50 ml of 5 N ammonium hydroxide and 50 ml of 1 M sodium carbonate solution.

TABLE VII

SOLUTIONS IN $Ca^{45}CO_3$ PROCEDURE

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

(4) Pipette solution A, B, water, ammonia and sodium carbonate in order according to Table VII.

(5) After precipitation, heat the precipitates on a hot-water bath or in a beaker with water on a hot plate. Do not boil.

(6) Decant the supernatant, and wash twice with methanol (CH_3OH). Leave a $CaCO_3$ -methanol slurry.

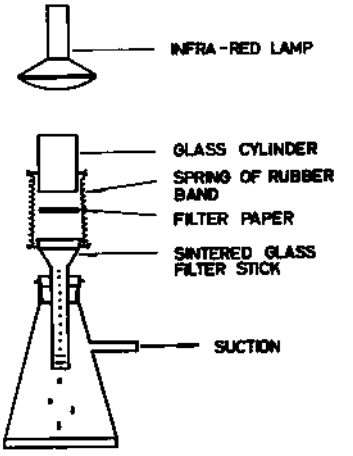

Fig. 26

Exploded view of filtering assembly

(7) Assemble the filtering apparatus (see Fig. 26) after the filter paper has been weighed carefully, and pour the $CaCO_3$ slurry into the glass cylinder. After it has settled for 2–3 min., initiate suction action gently, until the first 2–3 ml methanol has come through. Then gradually apply full suction.

(8) When the filtration is complete, turn on the infra-red lamp, situated 10–15 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.

(10) Plot sample count rate as a function of sample weight (mg).

(11) From this plot determine the correct activity concentration (cpm per mg) 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 = correct activity concentration (cpm per mg) x sample weight (mg).

(12) Finally, determine the "self-weakening factor" (S.W.F.) of each sample. The S.W.F. is defined as the ratio of the correct count rate to the observed count rate. Plot the S.W.F. as a function of sample weight.

(13) This last curve may be used to correct the count rates of Ca^{45}-labelled samples of $CaCO_3$ of known weight and the above cross-sectional area. The formula is simply

$$\text{correct cpm} = \text{observed cpm} \times (\text{S. W. F.})$$

1.5.3. PROCEDURE FOR $CaC^{14}O_3$

This procedure includes the conversion of $BaC^{14}O_3$ to $C^{14}O_2$, which is then precipitated as $CaC^{14}O_3$. (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 $C^{14}O_2$ 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, e.g. 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

$$BaC^{14}O_3 \xrightarrow{H^+} C^{14}O_2 \xrightarrow[H_2O]{Ca^{+2}} CaC^{14}O_3$$

(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. 27).

(2) Put N/10, CO_2-free NaOH in the gas-washing bottle and place in the main flask approximately 200 mg of $BaC^{14}O_3$ containing about 0.2 μc C^{14}. Into the funnel of the reaction vessel place 2 - 3 ml of 10% $HClO_4$ (perchloric acid).

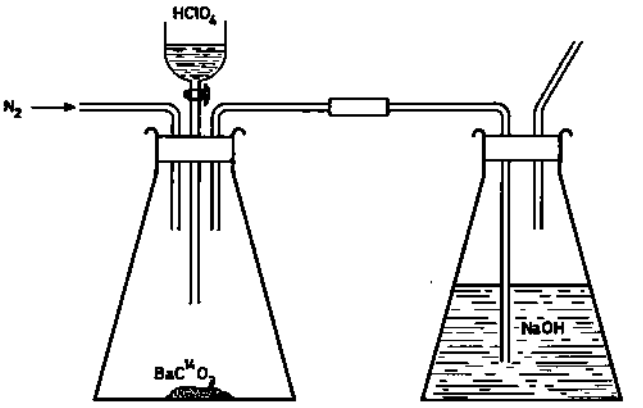

Fig. 27

Open assembly for conversion of $C^{14}O_2$

(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 10 min. beyond the final addition of acid.

(5) Remove the NaOH solution containing the $C^{14}O_3^{2-}$ 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: 2×5 ml, 2×10 ml, 2×20 ml, 30 ml and 50 ml, and place in 100 ml centrifuge tubes. Dilute each portion to 50 ml with CO_2-free water, and precipitate the $CaC^{14}O_3$ 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 - 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 abs. CH_3OH and centrifuge. Pour off the supernatant; suspend in CH_3OH and plate as in experiment 1.5.2.

(7) Count the plates, weigh and plot 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 equation (2) in the introduction to this section, by assuming $\mu = 0.29$. (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.

2. EXPERIMENTS WITH A SCINTILLATION COUNTER

2.1. Crystal scintillation counting

Introduction

When ionizing radiation hits the scintillator of a scintillation tube, a number of light photons (in the visible and ultra-violet range) are liberated. This number is proportional to the energy dissipated by the incident radiation. When these photons hit the photocathode of the photomultiplier, a proportional number of electrons is liberated. Finally, a multiplication of the electrons by a constant factor takes place. The whole sequence, from ionization and on, gives rise to an output pulse which is proportional to the energy dissipated in the crystal by the primary ionizing event.

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

α-radiation: Zinc sulphide crystals spread thinly (10 - 20 mg/cm^2).
β-radiation: Anthracene, or naphthalene containing 0.1% of anthracene, in the form of a large crystal. Recently, plastic scintillators have been developed.
γ-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 G-M tubes for counting γ-photons. These are

(1) Higher counting efficiencies (20 to 40 times);
(2) No significant resolving (dead) time corrections up to 10^5 cpm;
(3) The output pulse height is proportional to the input photon energy.

The γ-crystal is hygroscopic and therefore cased in an air-tight metal can, which is sealed at the open end by plate glass for contact with the evacuated glass tube containing the photomultiplier. Crystal and phototube are housed in a light-tight metal barrel. The end part of the barrel containing the crystal is surrounded by a lead castle. In "well-counting" the crystal is provided with a bore hole sufficiently large to hold a test tube containing a solid or liquid angle of the γ-emitting radioisotope. In spite of lead shielding, a scintillation detector will have a relatively high background of 50 - 500 cpm, partly from "electronic noise", which is reduced at low 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 G-M counter, which is operated at one 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 is less obvious than in the case of the G-M counter. The correct adjustment of the high voltage (and the input bias voltage) will result in a considerable saving of time at low count rates.

Criteria for optimum operating conditions based on natural statistics are deduced in Appendix IV. Sometimes in practice other considerations, e.g. on electronic stability, outweigh those on natural counting statistics.

In the following experiment, the optimal working voltage (according to natural statistics) for a strong and a weak source will be determined at two bias voltages, with the criteria given in Appendix IV.

PROCEDURE

(1) Obtain a source containing about 0.2 μc of a γ-emitter such as I^{131}, Cs^{137} or Co^{60}.
(2) Obtain a second source containing about 0.002 μc 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 (H.V.).
(4) Determine the background at bias 5 V and at 50-V intervals of H.V.
(5) Repeat the sample and background counts for bias 20 V†.
(6) Plot $R^{\frac{1}{2}} - r^{\frac{1}{2}}$ as a function of the H.V., and determine the optimal working voltage for both samples at bias 5 and 20 V, respectively.
(7) Determine the maximum value of $(R - r)^2/r$ for both samples at bias 5 and 20 V.
(8) Calculate the percentage natural standard deviation at which the samples have been counted, both at maximum $R^{\frac{1}{2}} - r^{\frac{1}{2}}$ and maximum $(R - r)^2/r$.

2.2. Rapid radioactive decay

Introduction

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

In 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 D_t^*, the count rate, being proportional to the disintegration rate, may be expressed as

$$A_t^* = Y D_t^*, \qquad (1)$$

where Y is the counting yield.

† Instructor must find out whether 5 or 20 V is relevant to the instrument being used.

The disintegration rate, however, changes with time according to the equation (2):

$$D_t^* = D_0^* e^{-\lambda^* t}, \tag{2}$$

where D_0^* = the disintegration rate at zero time,
λ^* = disintegration constant = $0.693/t_{\frac{1}{2}}$, and
t = time elapsed since zero time.

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

$$D_{t+T}^* = D_0^* e^{-\lambda^*(t+T)} . \tag{3}$$

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. 28.

The observed activity $\bar{A}^*$ is the average over the counting period T. $\bar{A}^*$ and the sample activity at the beginning of the counting period A_t^* are related as follows†:

$$\bar{A}^* = Y\bar{D}^* = Y(N_t^* - N_{t+T}^*)/T = Y[N_t^*(1 - e^{-\lambda^* T})]/T, \text{since (see equations (2) and (3))}$$

$$N_{t+T}^* = N_t^* e^{-\lambda^* T};$$

hence

$$\bar{A}^* = Y(D_t^*/\lambda^* T)(1 - e^{-\lambda^* T}),$$

which follows from $D_t^* = \lambda^* N_t^*$.

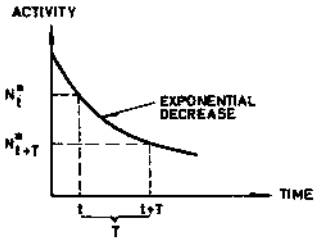

Fig. 28

Curve showing the exponential decay of the activity of a sample as function of time

† If the counting time T is not over $\frac{1}{2}t_{\frac{1}{2}}$, the relation $\bar{A}_t^* \doteq A_{t+\frac{1}{2}T}^*$ (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.

Thus, using equation (1), we find that

$$\bar{A}^* = A_t^*(1 - e^{-\lambda^* T})/\lambda^* T, \qquad (4)$$

where

$$\lambda^* = \frac{0.693}{t_{\frac{1}{2}}} \quad (t_{\frac{1}{2}} \text{ and } T \text{ in min.}).$$

With the aid of equation (4) the ratio of A_t^* to $\bar{A}^*$ 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 $A_t^* : \bar{A}^*$ is constant.

In this experiment, the equation (4) will be used to correct the count rate of Ba^{137m}. Ba^{137m} is the metastable isomer of Ba^{137}, and it emits 0.66-MeV γ-photons.

REAGENTS AND MATERIALS

(1) SO_3-H type cation exchange resin (e.g. Amberlite IR-120 or Dowex 50).
(2) About 200 μc Cs^{137}.
(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 half up with resin. Run 1 l of destilled 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 Cs^{137}. Elute the column repeatedly with EDTA solution at 10-min. intervals until the amount of Ba^{137m} coming through each time no longer increases. At each elution the Ba^{137m} concentration in the effluent will be maximum after about a half or a third 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 Ba^{137m} in a plastic counting container; start counting immediately, using the scintillation (well) 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 removal of the sample container.

(5) At the end of 30 min. from the starting time of counting, the Ba^{137m} remaining in the liquid will be much less than 1% of the original, and most of the count rate observed above empty container background results from some Cs^{137} 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 Ba^{137m}, against time on semi-log paper. Deduce the half-life of Ba^{137m} from this plot.

(7) Use equation (4) 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 semi-log paper.

2.3. Inverse-square law: Attenuation of γ-rays

Introduction

The intensity of the rays emitted from a source of radiation can be reduced in the following two ways: (a) By increase of the distance from the source of radiation; and (b) By increase of the mass-thickness in the path of the rays.

(a) 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 in between. This is usually referred to as the "inverse-square law". The intensity at a distance d from a point source (or other source with small dimensions 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 Matter, section 1.4.3).

In this exercise diminution by distance of γ-rays from a 5 μc Co^{60} source is investigated. The crystal scintillation counter is used.

PROCEDURE

(1) Apply an operating voltage previously set in experiment 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 (A* = R − r) against the 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, with the "inverse-square law".

(b) The attenuation by matter of a collimated beam of monoenergetic γ-photons is exponential. The attenuation of γ-rays from Co^{60} which emits γ-photons of slightly different energy (1.2. and 1.3 MeV) will be investigated.

PROCEDURE

(1) The previous experimental set-up is used except that the distance between the sample and counter is now 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 absorber between source and counter, and keep on repeating with increasing amounts of absorbers.
(4) Remove the source completely, and determine the background (with all absorbers 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 semi-log paper.
(6) Determine the half-thickness of lead for Co^{60} γ-rays and compare with the table value(Lecture Matter, section 3.3).
(7) Explain any discrepancy with simple exponential law (i.e. any lack of straight line on semi-log paper).

2.4. Liquid scintillation counting

Introduction

The principles of solid-crystal scintillation counting of isotopes emitting γ-rays have been dealt with in experiment 2.1.

For the assay of low-energy beta particles, liquid scintillation counting is often employed. This system operates on many of the same principles as solid-crystal counting; however, there are 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. 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 of the electronic components and thus aids in reducing the background.

The photomultiplier tube(s) is (are) connected to electronic equipment in the usual way.

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

One of the main sources of error in liquid scintillation counting 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 part of the light from the scintillations (quenching), which results in a lowered counting efficiency for the procedure. Chemical reactions between the sample and the scintillator may also result in quench-

ing. 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.

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
Cellosolve †	1 part	83	ml	
PPO (2-5 diphenyloxazole)	1% w/v‡	5	g	
Naphthalene	5% w/v	25	g	
POPOP (1-4-bis-2-5-phenyloxazolyl)		0.25	g	
~~Benzene~~ Toluene				

Labelled compounds

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

TABLE VIII

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

† Ethyleneglycol-monoethylether.
‡ Weight per unit volume.

Solutions

A: 10 000 to 12 000 dis./min. or 0.005 μc/ml C^{14} toluene
B: 50 000 to 60 000 dis./min. or 0.025 μc/ml H^3 toluene

Make up counting vials as indicated in Table VIII.

(2) Set lower and upper discriminator biases at 40 and 70 V, respectively, and determine the background rate r as a function of high voltage using intervals of 50 - 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 C^{14} (using vial 1) and H^3 (vial 6), and plot the curves of Y^2/r against H.V.
(4) Count the different bottles at the H.V. that corresponds to maximal Y^2/r† for C^{14} and H^3. Plot the activity curves.
(5) If time permits, repeat steps 2–4 for other bias settings.

3. CONTAMINATION AND DECONTAMINATION

Introduction

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 it.

The methods of decontamination may be divided into physical and chemical. Physical methods include 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 in contamination of several different materials with several isotopes and later decontamination of the materials with some chemical agents.

REAGENTS AND MATERIALS

(1) Radioactive solutions of P^{32}, Ca^{45} and I^{131}.
(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 pH approximately 12, (or e.g. "Radiacwash").
(4) 1% carrier solutions of P, Ca and I.
(5) 2 N HCl.
(6) Acetone.

† See Appendix IV

(7) Kleenex tissue paper.
(8) Barrier cream (e.g. ICI's "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 Kleenex tissue paper, dry the sample, and measure the activity.
(5) For a second series, use "washing solution" as decontaminating material. 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 decontaminating agents upon various substances and radioactive isotopes.
(8) Discuss the results.

4. EXERCISES ON BASIC UTILIZATION PRINCIPLES

4.1. Combustion of carbon compounds (determination of specific activity by persulphate oxidation of compound)

Introduction

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. A convenient one is carbon dioxide, which, for practical purposes, we measure as the barium salt, $BaCO_3$.

Combustion may be performed in two major manners: "dry" combustion and "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 caught in a NaOH gas-washing bottle and converted to $BaCO_3$ for plating.

REAGENTS AND MATERIALS

(1) $CH_3C^{14}OONa$.
(2) 200 mg $K_2S_2O_8$.
(3) 1 ml 5% $AgNO_3$.
(4) 0.1 N NaOH, CO_2-free.
(5) H_2O, CO_2-free.
(6) $BaCl_2$ (saturated aqueous solution).
(7) CH_3OH, abs.
(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 C^{14} source of usual sample area and containing known number of dis./min.).

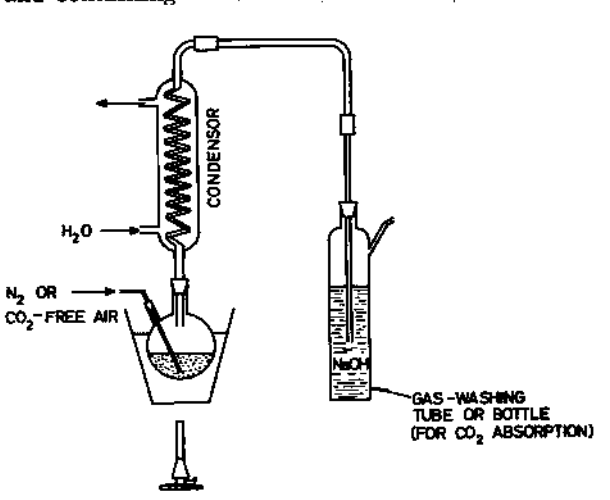

Fig. 29

Assembly for combustion of carbon compounds and conversion of CO_2 produced

PROCEDURE

(1) Arrange the flow apparatus as shown in Fig. 29.
(2) Calculate the amount of CH_3C^*OONa 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.)

(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 $C^{14}O_2$.
(6) Wash the inlet of the gas-washing system with CO_2-free water, combining the washings with the N/10 NaOH.
(7) Add sufficient saturated $BaCl_2$ solution to precipitate all the $C^{14}O_2$.
(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 abs. CH_3OH.
(10) Re-suspend in CH_3OH, and filter onto the filter apparatus.
(11) Place an infra-red lamp 4 - 5 cm above the plating apparatus, and continue drawing warmed air through the apparatus for 10 - 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 (dis./min. per mmole), making corrections for self-absorption and taking the counting yield of the standard into account.

QUESTIONS

(1) State the conditions for preference for persulphate versus Van Slyke oxidation procedures.
(2) What is the fate of the persulphate in the reaction?

4.2. Isotope dilution chemistry

Introduction

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 mmole P/ml solution.
(2) Unknown P solution (on the order of 10^{-1} M).
(3) Solution containing about 0.1 μc P^{32}/ml ("carrier-free" or of known P concentration).

(4) Fiske's reagent (13 g MgO, 175 g citric acid, 330 ml 25% NH_4OH in water to give 1-l 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 0.20 M 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 K-beakers should be roughly in between the amount from 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 weights of precipitate alone.
(8) Count the activity, using a G-M tube.
(9) Express the specific activities of P in the solid samples in cpm per mmole.
(10) Calculate the molarity of the unknown P-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 P conc. if the activities of the samples are expressed as cpm per mg precipitate? Explain.
(3) Can the unknown P conc. be determined from the weights of the precipitates alone?

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

Introduction

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)

$$AX^* + BX^\circ \longrightarrow AX^\circ + BX^*,$$

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

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

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

$$-(1/a)\,dx/dt = k_A\,(x/a)\,[(b-y)/b] - k_A\,[(a-x)/a]\,(y/b) = k_A\,x/a - k_A\,y/b$$

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

$$dx/dt = -[(a+b)/b]\,k_A\,x + (a/b)\,ck_A;$$

and on integrating, we obtain

$$x = C_1 e^{-[(a+b)/b]k_A t} + [a/(a+b)]\,c\,, \qquad (1)$$

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 equation (1) becomes

$$x_\infty = [a/(a+b)]\,c; \text{ and } y_\infty = c - x_\infty = [b/(a+b)]\,c.$$

Hence

$$x_\infty / y_\infty = a/b,$$

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 equation (1), the following final form is obtained:

$$x - x_\infty = (c - x_\infty)\, e^{-[(a+b)/b]k_A t} \quad . \qquad (2)$$

Thus, if the activity concentration of the solution is x* cpm per ml, 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.

$$k^*_A = [(a+b)/b]k_A \, ,$$

may be obtained from the slope of the line. Finally, the exchange-rate constant k_A for one-way transfer of test substance from solution to solid phase may be calculated from the following three activity concepts:

k^*_A, $x^*_0 - x^*_\infty$ and x^*_∞ (see Lecture Matter, section 4.3, example (3)).

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 μc P^{32}.
(4) 10 μc Ca^{45}.
(5) 10^{-3} M KH_2PO_4.
(6) 10^{-3} M $CaCl_2$.
(7) Stop-watch.
(8) 2 pipette tip caps.

PROCEDURE

(1) Measure 2 ml of wet cation and 4 ml of wet anion exchange resin into 150-ml beakers separately.
(2) Add 100 ml each of phosphate and calcium solutions into respectively saturated resin. (Retain about 10 ml of each solution for steps (3) and (4).)
(3) Add to one of the solutions 5 or 10 μc of the radioisotope (diluted in some of the respective solutions) while stirring, and simultaneously start the stop-watch. Then take out 1 ml of the supernatant solution† 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

† It is important to avoid getting any resin particles in 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).

order to eliminate the activity concentration gradient near the resin particles.

(4) Repeat step (3), using the other solution.

(5) Dry the samples under an infra-red lamp, and count the activity x^*, using a thin-mica-window G-M 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) Try to explain the small discrepancy, if any, from a straight line.

(2)† Extrapolate each straight line to zero time; what does the ordinate intercept equal?

(3)† Calculate the exchange rate constant k_A for each solution.

(4)† Calculate the reverse exchange rate constant k_B for each resin.

(5) How many meq. of ion are exchanged per minute per ml of solution in each system?

(6) Same as (5), per ml of wet resin?

(7) How great are the respective ion exchange capacities in meq. per ml of wet resin (after saturation in 10^{-3} M solution)?

4.4. Determination of copper in biological material by activation analysis

Introduction

Because of the low Cu content of biological materials, any chemical means to determine it quantitatively requires large amounts of the material.

Activating the Cu present in biological material with neutrons has the advantage of requiring only a small sample for a quantitative determination.

Cu present in a tissue sample and in a standard is activated with neutrons to form Cu^{64}, which has a half-life of 12.8 h and which emits β- and γ-radiation of 0.57 and 1.34 MeV, respectively. Activation for 13 h at a neutron flux of 10^{12} gives a specific activity of 50 mc/g. Simultaneously Cu^{66} is formed with a half-life of 5.1 min. After tissue digestion, Cu carrier is added and mixed with the active Cu. Cu is then separated (non-quantitatively) by a chemical method, and weighed and the Cu^{64} is counted. The specific activity of the unknown separate is compared with that of the known standard separate, and the content of Cu in the original tissue sample is thereby calculated.

REAGENTS

24N HNO_3	15% w/v Na_2SO_3
16N HNO_3	20% w/v KSCN
N HNO_3	10% w/v $Fe(NO_3)_3$

† On the basis of activity concepts alone.

18 N CH_3CO_2H
15 N NH_3
2 N NH_3 — 10% w/v $NH_4H_2PO_4$
2% w/v Salicylaldoxime in C_2H_5OH
Acetone
SO_2 saturated water.
Cu carrier (20 mg Cu/ml): 6.28 g Cu $(CH_3CO_2)_2 H_2O$ in 100 ml water.

PROCEDURE

Take about 0.05 g of tissue and a 1 μg of Cu standard sealed in polythene. Activate for 13 h.

(1) In a fume chamber transfer tissue and standard to 50-ml centrifuge tubes, and add 10 drops of 24 N 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 16 N HNO_3; add 5 drops of $Fe(NO_3)_3$ and 1 drop of $NH_4H_2PO_4$, then 15 N NH_3 till dark brown. Boil, spin down $Fe(OH)_3$ precipitate and wash it once with 2 N NH_3.

(3) Combine supernatant and washings in a fresh tube, and acidify with CH_3CO_2H till pale blue. Then add 0.5 ml of 16 N 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 16 N HNO_3; add 15 N 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 G-M counter. Correct counts for decay and self-absorption, and check the half-life of the separated copper-64. The chemical steps take about 2 h for eight samples. The chemical yield is about 75%.

CALCULATION

$$\mu g \text{ Cu in sample} = \frac{\text{cpm sample}}{\text{cpm standard}} \times \frac{\text{wt standard Cu-salicylaldoxime}}{\text{wt sample Cu-salicylaldoxime}}.$$

RANGE AND ACCURACY

0.05 – 0.5 μg of Cu is a convenient range for determination within an accuracy of 5%.

INTERFERENCES

Large amounts of zinc might interfere because of the reaction $Zn^{64}(n, p)Cu^{64}$. 1 μg of zinc in the sample yields copper-64 equivalent to 7×10^{-4} μg of copper.

4.5. Determination of phosphorus in biological material by activation analysis

Introduction

Neutron activation of phosphorus gives rise to 14-d phosphorus-32, which emits beta particles of energy 1.71 MeV. Activation for 14 d at $\phi = 10^{12}$ gives a specific activity of 50 mc/g.

REAGENTS

36 N H_2SO_4.
16 N HNO_3.
15 N NH_3.
50% w/v $MgCl_2$.
5% w/v H_2SO_4 in diethyl ether.
Acetone.
Diethyl ether (dried over $CaCl_2$).

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 1 l 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 give pH 9. Cool and add 1 ml of $MgCl_2$; let 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 G-M 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 8 samples, excluding "ashing" time. The chemical yield is about 90%.

CALCULATION

$$\mu g \text{ P in sample} = \frac{10 \times \text{cpm sample}}{\text{cpm standard}} \times \frac{\text{wt standard } MgNH_4PO_4}{\text{wt sample } MgNH_4PO_4}.$$

RANGE AND ACCURACY

This depends on the activation time. With a 15-h activation period, 0.5-10 μg of P can be determined within $\pm 5\%$. If the activation period is extended to 14 d, this can be reduced to 0.02 μg of P.

INTERFERENCES

Large amounts of sulphur can interfere by the $S^{32}(n, p)P^{32}$ reaction. 1 mg of S gives rise to phosphorus-32 equivalent to 55 μg of phosphorus. Chlorine could also interfere by the $Cl^{35}(n,\alpha)P^{32}$ 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.

MENTAL EXERCISES

(1) When the Z-number of all nuclides is plotted against the N-number, the 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

α-particle?
β^--particle (electron)?
β^+-particle (positron)?
γ-ray?
electron capture (K-capture)?
internal conversion?
neutron?

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

(3) Calculate the weight of 10 mc C^{14} and 1 c P^{32} and the number of atoms in each of these quantities of activity.

(4) Calculate the theoretical maximum specific activities of K^{42} and Sr^{90} in c/g.

(5) If the activity fall-off factor F is defined as $F = N^*_0/N^*$, show that $F = 2^{t/t_{\frac{1}{2}}}$.

(6) A radioisotope has lost 15/16 of its original activity in 32 min. Calculate the half-life of the isotope.

(7) Ba^{137m} is formed from Cs^{137}. How many mc of Ba^{137m} can be formed from 100 mc of Cs^{137} in 1, 2 and 20 min.?

(8) Calculate the weight of Ba^{137m} formed from 100 mc of Cs^{137} in 1, 2 and 20 min.

(9) Determine the daily decrement of activity (in per cent) of any P^{32} preparation.

(10) A Na^{24} sample ($t_{\frac{1}{2}} = 14.8$ h) had a counting rate of 24 000 cpm. 100 h later it gave 250 cpm. Roughly estimate the dead time of the G-M counter.

(11) The activity of C^{14} in 8 g of natural carbon sample with background was found to have 10.2 cpm. The background of the counter was 4.5 cpm and the counting yield was 5%. Neglecting the statistical deviation, calculate the C^{14} content. $t_{\frac{1}{2}} = 5600$ y.

(12) A 0.1-mg sample of pure Pu^{239} underwent 1.4×10^7 dis./min. Calculate the half-life of this radioisotope.

(13) A γ-source of 100 mc has to be shielded. What will be the minimum thickness of the lead shield that is required if an operator has to handle the source during half an hour at weekly intervals?

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

(n, p),
(n, γ),
(n, n).

(15) Scandium is to be determined by the activation method. Assuming the lower limit of the determination to be 50 cpm at 10% G-M counting yield, compute the minimum amount of scandium determinable if the sample is subjected to a neutron flux of 10^{12} n/cm^2 per sec. for 2 h. Assume the (n, γ) reaction is the most likely reaction and that the shielding effect is negligible.

(16) What would be the specific activity of phosphorus having a cross-section of $\sigma = 0.2$ b after irradiation by a neutron flux of 10^{12} n/cm^2 per sec. for 1 h, 1 d and 10 d?

APPENDIXES

APPENDIX I

ABRIDGED VERSION OF TABLE OF MAXIMUM PERMISSIBLE* CONCENTRATIONS OF RADIONUCLIDES IN AIR AND IN DRINKING WATER FOR OCCUPATIONAL EXPOSURE, PUBLISHED IN REPORT OF COMMITTEE II ICRP, AND MAXIMUM BODY BURDEN

(From: IAEA Safety Series No.1 (1962) and United States Department of Commerce, National Bureau of Standards, Handbook 69)

Radionuclide	Critical organ**	Maximum permissible concentrations For 40-h week		For 168-h week		Maximum permissible burden in total body (μc)
		(MPC)w (μc/cm³)	(MPC)a (μc/cm³)	(MPC)w (μc/cm³)	(MPC)a (μc/cm³)	
$_1H^3$(HTO or T_2O)*** (sol.)	Body tissue Total body	0.1	5×10^{-6}	0.03	2×10^{-6}	10^3 2×10^3
(T_2) (submersion)	Skin		2×10^{-3}		4×10^{-4}	-
$_4Be^7$ (sol.)	GI (LLI) Total body	0.05	6×10^{-6}	0.02	2×10^{-6}	- 600
(insol.)	Lung GI (LLI)	0.05	10^{-6}	0.02	4×10^{-7}	- -
$_6C^{14}(CO_2)$*** (sol.)	Fat	0.02	4×10^{-6}	8×10^{-3}	10^{-6}	300
(submersion)	Total body		5×10^{-5}		10^{-6}	-
$_9F^{18}$ (sol.)	GI (SI)	0.02	5×10^{-6}	8×10^{-3}	2×10^{-6}	-
(insol.)	GI (ULI)	0.01	3×10^{-6}	5×10^{-3}	9×10^{-7}	-
$_{11}Na^{22}$ (sol.)	Total body	10^{-3}	2×10^{-7}	4×10^{-4}	6×10^{-8}	10
(insol.)	Lung GI (LLI)	9×10^{-4}	9×10^{-9}	3×10^{-4}	3×10^{-9}	- -
$_{11}Na^{24}$ (sol.)	GI (SI)	6×10^{-3}	10^{-7}	2×10^{-3}	4×10^{-7}	-
(insol.)	GI (LLI)	8×10^{-4}	10^{-7}	3×10^{-4}	5×10^{-8}	-
$_{14}Si^{31}$ (sol.)	GI (S)	0.03	6×10^{-6}	9×10^{-3}	2×10^{-6}	-
(insol.)	GI (ULI)	6×10^{-3}	10^{-6}	2×10^{-3}	3×10^{-7}	-

* Subject to your local competent authority

** The abbreviations GI, S, SI, ULI and LLI refer to gastrointestinal tract, stomach, small intestine, upper large intestine and lower large intestine, respectively.

*** See word of caution, section 3.2, Basic Part: Lecture Matter.

Radionuclide		Critical organ**	Maximum permissible concentrations For 40 h-week		For 168-h week		Maximum permissible burden in total body (μc)
			(MPC)w ($\mu c/cm^3$)	(MPC)a ($\mu c/cm^3$)	(MPC)w ($\mu c/cm^3$)	(MPC)a ($\mu c/cm^3$)	
$_{15}P^{32}$	(sol.)	Bone	5×10^{-4}	7×10^{-8}	2×10^{-4}	2×10^{-8}	6
	(insol.)	Lung GI (LLI)	7×10^{-4}	8×10^{-8}	2×10^{-4}	3×10^{-8}	- -
$_{16}S^{35}$	(sol.)	Testis	2×10^{-3}	3×10^{-7}	6×10^{-4}	9×10^{-8}	90
	(insol.)	Lung GI (LLI)	8×10^{-3}	3×10^{-7}	3×10^{-3}	9×10^{-8}	-
$_{17}Cl^{36}$	(sol.)	Total body	2×10^{-3}	4×10^{-7}	8×10^{-4}	10^{-7}	80
	(insol.)	Lung GI (LLI)	2×10^{-3}	2×10^{-8}	6×10^{-4}	8×10^{-9}	- -
$_{17}Cl^{38}$	(sol.)	GI (S)	0.01	3×10^{-6}	4×10^{-3}	9×10^{-7}	-
	(insol.)	GI (S)	0.01	2×10^{-6}	4×10^{-3}	7×10^{-7}	-
$_{18}Ar^{41}$ (submersion)		Total body		2×10^{-6}		4×10^{-7}	- -
$_{19}K^{42}$	(sol.)	GI (S)	9×10^{-3}	2×10^{-6}	3×10^{-3}	7×10^{-7}	-
	(insol.)	GI (LLI)	6×10^{-4}	10^{-7}	2×10^{-4}	4×10^{-8}	-
$_{20}Ca^{45}$	(sol.)	Bone	3×10^{-4}	3×10^{-8}	9×10^{-5}	10^{-8}	30
	(insol.)	Lung GI (LLI)		10^{-7}	2×10^{-4}	4×10^{-8}	- -
$_{20}Ca^{47}$	(sol.)	Bone	10^{-3}	2×10^{-7}	5×10^{-4}	6×10^{-8}	5
	(insol.)	GI (LLI) Lung	10^{-3}	2×10^{-7} 2×10^{-7}	3×10^{-4}	6×10^{-8} 6×10^{-8}	- -
$_{21}Sc^{46}$	(sol.)	GI (LLI) Liver	10^{-3}	2×10^{-7} 2×10^{-7}	4×10^{-4}	8×10^{-8} 8×10^{-8}	- 10
	(insol.)	Lung GI (LLI)	10^{-3}	2×10^{-8}	4×10^{-4}	8×10^{-9}	- -
$_{21}Sc^{47}$	(sol.)	GI (LLI)	3×10^{-3}	6×10^{-7}	9×10^{-4}	2×10^{-7}	-
	(insol.)	GI (LLI)	3×10^{-3}	5×10^{-7}	9×10^{-4}	2×10^{-7}	-

** The abbreviations GI, S, SI, ULI and LLI refer to gastrointestinal tract, stomach, small intestine, upper large intestine and lower large intestine, respectively.

Radionuclide		Critical organ**	Maximum permissible concentrations For 40 h-week		For 168-h week		Maximum permissible burden in total body (μc)
			(MPC)w (μc/cm³)	(MPC)a (μc/cm³)	(MPC)w (μc/cm³)	(MPC)a (μc/cm³)	
$_{21}Sc^{48}$	(sol.)	GI (LLI)	8×10^{-4}	2×10^{-7}	3×10^{-4}	6×10^{-8}	-
	(insol.)	GI (LLI)	8×10^{-4}	10^{-7}	3×10^{-4}	5×10^{-8}	-
$_{23}V^{48}$	(sol.)	GI (LLI)	9×10^{-4}	2×10^{-4}	3×10^{-4}	6×10^{-8}	-
	(insol.)	Lung		6×10^{-8}		2×10^{-8}	-
		GI (LLI)	8×10^{-4}		3×10^{-4}		-
$_{24}Cr^{51}$	(sol.)	GI (LLI)	0.05	10^{-5}	0.02	4×10^{-6}	-
		Total body		10^{-5}		4×10^{-6}	800
	(insol.)	Lung		2×10^{-6}		8×10^{-7}	-
		GI (LLI)	0.05		0.02		-
$_{25}Mn^{52}$	(sol.)	GI (LLI)	10^{-3}	2×10^{-7}	3×10^{-4}	7×10^{-8}	-
	(insol.)	Lung		10^{-7}		5×10^{-8}	-
		GI (LLI)	9×10^{-4}		3×10^{-4}	5×10^{-8}	-
$_{25}Mn^{54}$	(sol.)	GI (LLI)	4×10^{-3}		10^{-3}		-
		Liver		4×10^{-7}		10^{-7}	20
	(insol.)	Lung		4×10^{-9}		10^{-8}	-
		GI (LLI)	3×10^{-3}		10^{-3}		-
$_{26}Fe^{55}$	(sol.)	Spleen	0.02	9×10^{-7}	8×10^{-8}	3×10^{-7}	10^3
	(insol.)	Lung		10^{-6}		3×10^{-7}	-
		GI (LLI)	0.07		0.02		-
$_{26}Fe^{59}$	(sol.)	GI (LLI)	2×10^{-3}		6×10^{-4}		-
		Spleen		10^{-7}		5×10^{-8}	20
	(insol.)	Lung		5×10^{-8}		2×10^{-8}	-
		GI (LLI)	2×10^{-3}		5×10^{-4}		-
$_{27}Co^{60}$	(sol.)	GI (LLI)	10^{-3}	3×10^{-7}	5×10^{-4}	10^{-7}	-
		Total body				10^{-7}	10
	(insol.)	Lung		9×10^{-9}		3×10^{-9}	-
		GI (LLI)	10^{-3}		3×10^{-4}		-
$_{28}Ni^{59}$	(sol.)	Bone	6×10^{-3}	5×10^{-7}	2×10^{-3}	2×10^{-7}	10^3
	(insol.)	Lung		8×10^{-7}		3×10^{-7}	-
		GI (LLI)	0.06		0.02		-

** The abbreviations GI, S, SI, ULI and LLI refer to gastrointestinal tract, stomach, small intestine, upper large intestine and lower large intestine, respectively.

Radionuclide		Critical organ**	Maximum permissible concentrations For 40-h week		For 168-h week		Maximum permissible burden in total body (μc)
			(MPC)w (μc/cm³)	(MPC)a (μc/cm³)	(MPC)w (μc/cm³)	(MPC)a (μc/cm³)	
$_{29}Cu^{64}$	(sol.)	GI (LLI)	0.01	2×10^{-6}	3×10^{-3}	7×10^{-7}	-
	(insol.)	GI (LLI)	6×10^{-3}	10^{-6}	2×10^{-3}	4×10^{-7}	-
$_{30}Zn^{65}$	(sol.)	Total body Prostate Liver	3×10^{-3}	10^{-7} 10^{-7} 10^{-7}	10^{-3} 10^{-3} 10^{-3}	4×10^{-8} 4×10^{-8}	60 70 80
	(insol.)	Lung GI (LLI)	5×10^{-3}	6×10^{-8}	2×10^{-3}	2×10^{-8}	- -
$_{30}Zn^{69}$	(sol.)	GI (S) Prostate	0.05	7×10^{-6}	0.02	2×10^{-6}	- 0.8
	(insol.)	GI (S)	0.05	9×10^{-6}	0.02	3×10^{-6}	-
$_{31}Ga^{72}$	(sol.)	GI (LLI)	10^{-3}	2×10^{-7}	4×10^{-4}	8×10^{-8}	-
	(insol.)	GI (LLI)	10^{-3}	2×10^{-7}	4×10^{-4}	8×10^{-8}	-
$_{32}Ge^{71}$	(sol.)	GI (LLI)	0.05	10^{-5}	0.02	4×10^{-6}	-
	(insol.)	Lung GI (LLI)	0.05	6×10^{-6}	0.02	2×10^{-6}	- -
$_{33}As^{76}$	(sol.)	GI (LLI)	6×10^{-4}	10^{-7}	2×10^{-4}	4×10^{-8}	-
	(insol.)	GI (LLI)	6×10^{-4}	10^{-7}	2×10^{-4}	3×10^{-8}	-
$_{34}Se^{75}$	(sol.)	Kidney Total body	9×10^{-3}	10^{-6} 10^{-6}	3×10^{-3} 3×10^{-3}	4×10^{-7}	90 100
	(insol.)	Lung GI (LLI)	8×10^{-3}	10^{-7}	3×10^{-3}	4×10^{-8}	-
$_{35}Br^{82}$	(sol.)	Total body GI (SI)	8×10^{-3} 8×10^{-3}	10^{-6}	3×10^{-3} 3×10^{-3}	4×10^{-7}	10 -
	(insol.)	GI (LLI)	10^{-3}	2×10^{-7}	4×10^{-4}	6×10^{-8}	-
$_{37}Rb^{86}$	(sol.)	Total body Pancreas Liver	2×10^{-3} 2×10^{-3}	3×10^{-7} 3×10^{-7}	7×10^{-4} 7×10^{-4}	10^{-7} 10^{-7} 10^{-7}	30 30 40
	(insol.)	Lung GI (LLI)	7×10^{-4}	7×10^{-8}	2×10^{-4}	2×10^{-8}	- -

** The abbreviations GI, S, SI, ULI and LLI refer to gastrointestinal tract, stomach, small intestine, upper large intestine and lower large intestine, respectively.

Radionuclide		Critical organ**	Maximum permissible concentrations For 40-h week		For 168-h week		Maximum permissible burden in total body (μc)
			(MPC)w (μc/cm³)	(MPC)a (μc/cm³)	(MPC)w (μc/cm³)	(MPC)a (μc/cm³)	
$_{37}Rb^{87}$	(sol.)	Pancrease Total body Liver	3×10^{-3}	5×10^{-7}	10^{-3}	2×10^{-7} 2×10^{-7} 2×10^{-7}	200 200 200
	(insol.)	Lung GI (LLI)	5×10^{-3}	7×10^{-8}	2×10^{-3}	2×10^{-8}	- -
$_{38}Sr^{85}$	(sol.)	Total body	3×10^{-3}	2×10^{-7}	10^{-3}	8×10^{-8}	60
	(insol.)	Lung GI (LLI)	5×10^{-3}	10^{-7}	2×10^{-3}	4×10^{-8}	- -
$_{38}Sr^{89}$	(sol.)	Bone	3×10^{-4}	3×10^{-8}	10^{-4}	10^{-8}	4
	(insol.)	Lung GI (LLI)	8×10^{-4}	4×10^{-8}	3×10^{-4}	10^{-8}	- -
$_{38}Sr^{90}$	(sol.)	Bone	4×10^{-6}	3×10^{-10}	10^{-6}	10^{-10}	2
	(insol.)	Lung GI (LLI)	10^{-3}	5×10^{-9}	4×10^{-4}	2×10^{-9}	- -
$_{39}Y^{90}$	(sol.)	GI (LLI)	6×10^{-4}	10^{-7}	2×10^{-4}	2×10^{-8}	-
	(insol.)	GI (LLI)	6×10^{-4}	10^{-7}	2×10^{-4}	3×10^{-8}	-
$_{39}Y^{91}$	(sol.)	GI (LLI) Bone	8×10^{-4}	4×10^{-8}	3×10^{-4}	10^{-8}	- 5
	(insol.)	Lung GI (LLI)	8×10^{-4}	3×10^{-8}	3×10^{-4}	10^{-8}	- -
$_{40}Zr^{95}$	(sol.)	GI (LLI) Total body	2×10^{-3}	10^{-7}	6×10^{-4}	4×10^{-8}	- 20
	(insol.)	Lung GI (LLI)	2×10^{-3}	3×10^{-8}	6×10^{-4}	10^{-8}	- -
$_{40}Zr^{97}$	(sol.)	GI (LLI)	5×10^{-4}	10^{-7}	2×10^{-4}	4×10^{-8}	-
	(insol.)	GI (LLI)	5×10^{-4}	9×10^{-8}	2×10^{-4}	3×10^{-8}	-
$_{41}Nb^{95}$	(sol.)	GI (LLI) Total body	3×10^{-3}	5×10^{-7}	10^{-3}	2×10^{-7} 2×10^{-7}	- 40
	(insol.)	Lung GI (LLI)	3×10^{-3}	10^{-7}	10^{-3}	3×10^{-8}	- -

** The abbreviations GI, S, SI, ULI and LLI refer to gastrointestinal tract, stomach, small intestine, upper large intestine and lower large intestine, respectively.

Radionuclide		Critical organ **	Maximum permissible concentrations For 40-h week		For 168-h week		Maximum permissible burden in total body (μc)
			(MPC)w (μc/cm³)	(MPC)a (μc/cm³)	(MPC)w (μc/cm³)	(MPC)a (μc/cm³)	
$_{42}Mo^{99}$	(sol.)	Kidney GI (LLI)	5×10^{-3}	7×10^{-7}	2×10^{-3} 2×10^{-3}	3×10^{-7}	8 -
	(insol.)	GI (LLI)	10^{-3}	2×10^{-7}	4×10^{-4}	7×10^{-8}	-
$_{43}Tc^{96}$	(sol.)	GI (LLI)	3×10^{-3}	6×10^{-7}	10^{-3}	2×10^{-7}	-
	(insol.)	GI (LLI)	10^{-3}	2×10^{-7}	5×10^{-4}	8×10^{-8}	-
$_{44}Ru^{106}$	(sol.)	GI (LLI)	4×10^{-4}	8×10^{-8}	10^{-4}	3×10^{-8}	-
	(insol.)	Lung GI (LLI)	3×10^{-4}	6×10^{-9}	10^{-4}	2×10^{-9}	- -
$_{45}Rh^{105}$	(sol.)	GI (LLI)	4×10^{-3}	8×10^{-7}	10^{-3}	3×10^{-7}	-
	(insol.)	GI (LLI)	3×10^{-3}	5×10^{-7}	10^{-3}	2×10^{-7}	-
$_{46}Pd^{103}$	(sol.)	GI (LLI) Kidney	0.01	10^{-6}	3×10^{-3}	5×10^{-7}	- 20
	(insol.)	Lung GI (LLI)	8×10^{-3}	7×10^{-7}	3×10^{-3}	3×10^{-7}	- -
$_{47}Ag^{105}$	(sol.)	GI (LLI)	3×10^{-3}	6×10^{-7}	10^{-3}	2×10^{-7}	-
	(insol.)	Lung GI (LLI)	3×10^{-3}	8×10^{-8}	10^{-3}	3×10^{-8}	- -
$_{47}Ag^{111}$	(sol.)	GI (LLI)	10^{-3}	3×10^{-7}	4×10^{-4}	10^{-7}	--
	(insol.)	GI (LLI)	10^{-3}	2×10^{-7}	4×10^{-4}	8×10^{-8}	-
$_{48}Cd^{109}$	(sol.)	GI (LLI) Liver Kidney	5×10^{-3}	5×10^{-6}	2×10^{-3}	2×10^{-8} 2×10^{-6}	- 20 20
	(insol.)	Lung GI (LLI)	5×10^{-3}	7×10^{-8}	2×10^{-3}	3×10^{-8}	- -
$_{50}Sn^{113}$	(sol.)	GI (LLI) Bone	2×10^{-3}	4×10^{-7}	9×10^{-4}	10^{-7}	- 30
	(insol.)	Lung GI (LLI)	2×10^{-3}	5×10^{-6}	8×10^{-4}	2×10^{-8}	- -

** The abbreviations GI, S, SI, ULI and LLI refer to gastrointestinal tract, stomach, small intestine, upper large intestine and lower large intestine, respectively.

Radionuclide	Critical organ**	Maximum permissible concentrations For 40-h week		For 168-h week		Maximum permissible burden in total body (μc)
		(MPC)w (μc/cm³)	(MPC)a (μc/cm³)	(MPC)w (μc/cm³)	(MPC)a (μc/cm³)	
$_{51}Sb^{125}$ (sol.)	GI (LLI)	3×10^{-3}	5×10^{-7}	10^{-3}	2×10^{-7}	-
	Lung				2×10^{-7}	40
	Total body				2×10^{-7}	60
	Bone				2×10^{-7}	70
(insol.)	Lung		3×10^{-8}		9×10^{-9}	-
	GI (LLI)	3×10^{-3}		10^{-3}		-
$_{52}Te^{127}$ (sol.)	GI (LLI)	8×10^{-3}	2×10^{-6}	3×10^{-3}	6×10^{-7}	-
(insol.)	GI (LLI)	5×10^{-3}	9×10^{-7}	2×10^{-3}	3×10^{-7}	-
$_{52}Te^{129}$ (sol.)	GI (S)	0.02	5×10^{-6}	8×10^{-3}	2×10^{-6}	-
(insol.)	GI (ULI)	0.02	4×10^{-6}	8×10^{-3}	10^{-6}	-
$_{53}I^{131}$ (sol.)	Thyroid	6×10^{-5}	9×10^{-9}	2×10^{-5}	3×10^{-9}	
(insol.)	GI (LLI)	2×10^{-3}	3×10^{-7}	6×10^{-4}	10^{-7}	-
	Lung		3×10^{-7}		10^{-7}	-
$_{53}I^{132}$ (sol.)	Thyroid	2×10^{-3}	2×10^{-7}	6×10^{-4}	8×10^{-8}	0.3
(insol.)	GI (ULI)	5×10^{-3}	9×10^{-7}	2×10^{-3}	3×10^{-7}	-
$_{53}I^{133}$ (sol.)	Thyroid	2×10^{-4}	3×10^{-8}	7×10^{-5}	10^{-8}	0.3
(insol.)	GI (LLI)	10^{-3}	2×10^{-7}	4×10^{-4}	7×10^{-8}	-
$_{53}I^{134}$ (sol.)	Thyroid	4×10^{-3}	5×10^{-7}	10^{-3}	2×10^{-7}	0.2
(insol.)	GI (S)	0.02	3×10^{-6}	6×10^{-3}	10^{-6}	-
$_{53}I^{135}$ (sol.)	Thyroid	7×10^{-4}	10^{-7}	2×10^{-4}	4×10^{-8}	0.3
(insol.)	GI (LLI)	2×10^{-3}	4×10^{-7}	7×10^{-4}	10^{-7}	-
$_{54}Xe^{133}$ (submersion)	Total body		10^{-5}		3×10^{-6}	-
$_{54}Xe^{135}$ (submersion)	Total body		4×10^{-6}		10^{-6}	-
$_{55}Cs^{137}$ (sol.)	Total body	4×10^{-4}	6×10^{-8}	2×10^{-4}	2×10^{-8}	30
	Liver			2×10^{-4}		40
	Spleen			2×10^{-4}		50
	Muscle			2×10^{-4}		50
(insol.)	Lung		10^{-8}		5×10^{-9}	-
	GI (LLI)	10^{-3}		4×10^{-4}		-

** The abbreviations GI, S, SI, ULI and LLI refer to gastrointestinal tract, stomach, small intestine, upper large intestine and lower large intestine, respectively.

Radionuclide		Critical organ**	Maximum permissible concentrations For 40-h week		For 168-h week		Maximum permissible burden in total body (μc)
			(MPC)w (μc/cm³)	(MPC)a (μc/cm³)	(MPC)w (μc/cm³)	(MPC)a (μc/cm³)	
$_{56}Ba^{140}$	(sol.)	GI (LLI) Bone	8×10^{-4}	10^{-7}	3×10^{-4}	4×10^{-8}	- 4
	(insol.)	Lung GI (LLI)	7×10^{-4}	4×10^{-8}	2×10^{-4}	10^{-8}	-
$_{57}La^{140}$	(sol.)	GI (LLI)	7×10^{-4}	2×10^{-7}	2×10^{-4}	5×10^{-8}	-
	(insol.)	GI (LLI)	7×10^{-4}	10^{-7}	2×10^{-4}	2×10^{-8}	-
$_{59}Pr^{143}$	(sol.)	GI (LLI)	10^{-3}	3×10^{-7}	5×10^{-4}	10^{-7}	-
	(insol.)	Lung GI (LLI)	10^{-3}	2×10^{-7}	5×10^{-4}	6×10^{-8}	- -
$_{61}Pm^{147}$	(sol.)	GI (LLI) Bone	6×10^{-3}	6×10^{-8}	2×10^{-3}	2×10^{-8}	- 60
	(insol.)	Lung GI (LLI)	6×10^{-3}	10^{-7}	2×10^{-3}	3×10^{-8}	- -
$_{62}Sm^{151}$	(sol.)	GI (LLI) Bone	0.01	6×10^{-8}	4×10^{-3}	2×10^{-8}	- 100
	(insol.)	Lung GI (LLI)	0.01	10^{-7}	4×10^{-3}	5×10^{-8}	- -
$_{63}Eu^{152}$ (9.2 h)	(sol.)	GI (LLI)	2×10^{-5}	4×10^{-7}	6×10^{-4}	10^{-7}	-
	(insol.)	GI (LLI)	2×10^{-5}	3×10^{-7}	6×10^{-4}	10^{-7}	-
$_{63}Eu^{152}$ (13 ys)	(sol.)	GI (LLI) Kidney	2×10^{-3}	10^{-8}	8×10^{-4}	4×10^{-9}	- 20
	(insol.)	Lung GI (LLI)	2×10^{-3}	2×10^{-8}	8×10^{-4}	6×10^{-9}	- -
$_{63}Eu^{154}$	(sol.)	GI (LLI) Kidney Bone	6×10^{-4}	4×10^{-9} 4×10^{-9}	2×10^{-4}	10^{-9} 10^{-9}	- 5 5
	(insol.)	Lung GI (LLI)	6×10^{-4}	7×10^{-9}	2×10^{-4}	2×10^{-9}	-

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Radionuclides		Critical organ**	Maximum permissible concentrations For 40-h week		For 168-h week		Maximum permissible burden in total body (μc)
			(MPC)w (μc/cm³)	(MPC)a (μc/cm³)	(MPC)w (μc/cm³)	(MPC)a (μc/cm³)	
$_{67}Ho^{166}$	(sol.)	GI (LLI)	9×10^{-4}	2×10^{-7}	3×10^{-4}	7×10^{-8}	-
	(insol.)	GI (LLI)	9×10^{-4}	2×10^{-7}	3×10^{-4}	6×10^{-8}	-
$_{69}Tm^{170}$	(sol.)	GI (LLI) Bone	10^{-3}	 4×10^{-8}	5×10^{-4}	 10^{-8}	- 9
	(insol.)	Lung GI (LLI)	 10^{-3}	3×10^{-8}	 5×10^{-4}	10^{-8}	- -
$_{71}Lu^{177}$	(sol.)	GI (LLI)	3×10^{-3}	6×10^{-7}	10^{-3}	2×10^{-7}	-
	(insol.)	GI (LLI) Lung	3×10^{-3}	5×10^{-7}	10^{-3}	2×10^{-7} 2×10^{-7}	- -
$_{73}Ta^{182}$	(sol.)	GI (LLI) Liver	10^{-3}	 4×10^{-8}	4×10^{-4}	 10^{-8}	- 7
	(insol.)	Lung GI (LLI)	 10^{-3}	2×10^{-8}	 4×10^{-4}	7×10^{-9}	- -
$_{74}W^{181}$	(sol.)	GI (LLI)	0.01	2×10^{-6}	4×10^{-3}	8×10^{-7}	-
	(insol.)	Lung GI (LLI)	 0.01	10^{-7}	 3×10^{-3}	4×10^{-8}	- -
$_{75}Re^{185}$	(sol.)	GI (LLI) Total body	0.02 0.02	 3×10^{-6}	6×10^{-3}	 9×10^{-7}	- 80
	(insol.)	Lung GI (LLI)	 8×10^{-3}	2×10^{-7}	 3×10^{-3}	5×10^{-8}	- -
$_{77}Ir^{190}$	(sol.)	GI (LLI)	6×10^{-3}	10^{-6}	2×10^{-3}	4×10^{-7}	-
	(insol.)	Lung GI (LLI)	 5×10^{-3}	4×10^{-7}	 2×10^{-3}	10^{-7}	- -
$_{77}Ir^{192}$	(sol.)	GI (LLI) Kidney Spleen	10^{-3}	 10^{-7} 10^{-7}	4×10^{-4}	 4×10^{-8}	- 6 7
	(insol.)	Lung GI (LLI)	 10^{-3}	3×10^{-8}	 4×10^{-4}	9×10^{-9}	- -

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Radionuclides		Critical organ**	Maximum permissible concentration For 40-h week		For 168-h week		Maximum permissible burden in total body (μc)
			(MPC)w (μc/cm³)	(MPC)a (μc/cm³)	(MPC)w (μc/cm³)	(MPC)a (μc/cm³)	
$_{78}Pt^{191}$	(sol.)	GI (LLI)	4×10^{-3}	8×10^{-7}	10^{-3}	3×10^{-7}	-
	(insol.)	GI (LLI)	3×10^{-3}	6×10^{-7}	10^{-3}	2×10^{-7}	-
$_{78}Pt^{193}$	(sol.)	Kidney	0.03	10^{-6}	9×10^{-3}	4×10^{-7}	70
	(insol.)	Lung GI (LLI)	0.05	3×10^{-7}	0.02	10^{-7}	- -
$_{78}Pt^{197}$	(sol.)	GI (LLI)	4×10^{-3}	8×10^{-7}	10^{-3}	3×10^{-7}	-
	(insol.)	GI (LLI)	3×10^{-3}	6×10^{-7}	10^{-3}	2×10^{-7}	-
$_{79}Au^{196}$	(sol.)	GI (LLI)	5×10^{-3}	10^{-6}	2×10^{-3}	4×10^{-7}	-
	(insol.)	Lung GI (LLI)	4×10^{-3}	6×10^{-7}	10^{-3}	2×10^{-7}	-- -
$_{79}Au^{198}$	(sol.)	GI (LLI)	2×10^{-3}	3×10^{-7}	5×10^{-4}	10^{-7}	-
	(insol.)	GI (LLI)	10^{-3}	2×10^{-7}	5×10^{-4}	8×10^{-8}	-
$_{79}Au^{199}$	(sol.)	GI (LLI)	5×10^{-3}	10^{-6}	2×10^{-3}	4×10^{-7}	-
	(insol.)	GI (LLI)	4×10^{-3}	8×10^{-7}	2×10^{-3}	3×10^{-7}	-
$_{80}Hg^{197}$	(sol.)	Kidney	9×10^{-3}	10^{-6}	3×10^{-3}	4×10^{-7}	4
	(insol.)	GI (LLI)	0.01	3×10^{-6}	5×10^{-3}	9×10^{-7}	-
$_{80}Hg^{203}$	(sol.)	Kidney	5×10^{-4}	7×10^{-8}	2×10^{-4}	2×10^{-8}	4
	(insol.)	Lung GI (LLI)	3×10^{-3}	10^{-7}	10^{-3}	4×10^{-8}	- -
$_{81}Tl^{200}$	(sol.)	GI (LLI)	0.01	3×10^{-6}	4×10^{-3}	9×10^{-7}	-
	(insol.)	GI (LLI)	7×10^{-3}	10^{-6}	2×10^{-3}	4×10^{-7}	-
$_{81}Tl^{201}$	(sol.)	GI (LLI)	9×10^{-3}	2×10^{-6}	3×10^{-3}	7×10^{-7}	-
	(insol.)	GI (LLI)	5×10^{-3}	9×10^{-7}	2×10^{-3}	3×10^{-7}	-
$_{81}Tl^{202}$	(sol.)	GI (LLI)	4×10^{-3}	8×10^{-7}	10^{-3}	3×10^{-7}	-
	(insol.)	Lung GI (LLI)	2×10^{-3}	2×10^{-7}	7×10^{-4}	8×10^{-8}	- -

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Radionuclides		Critical organ**	Maximum permissible concentrations For 40-h week		For 168-h week		Maximum permissible burden in total body (μc)
			(MPC)w (μc/cm³)	(MPC)a (μc/cm³)	(MPC)w (μc/cm³)	(MPC)a (μc/cm³)	
$_{81}Tl^{204}$	(sol.)	GI (LLI) Kidney	3×10^{-3}	6×10^{-7}	10^{-3}	2×10^{-7} 2×10^{-7}	- 10
	(insol.)	Lung GI (LLI)	2×10^{-3}	3×10^{-8}	6×10^{-4}	9×10^{-9}	-
$_{82}Pb^{203}$	(sol.)	GI (LLI)	0.01	3×10^{-6}	4×10^{-3}	9×10^{-7}	-
	(insol.)	GI (LLI)	0.01	2×10^{-6}	4×10^{-3}	6×10^{-7}	-
$_{82}Pb^{210}$	(sol.)	Kidney Total body	4×10^{-6} 4×10^{-6}	10^{-10}	10^{-6} 10^{-6}	4×10^{-11}	0.4 .4
	(insol.)	Lung GI (LLI)	5×10^{-3}	2×10^{-10}	2×10^{-3}	8×10^{-11}	- -
$_{84}Po^{210}$	(sol.)	Spleen Kidney	2×10^{-5} 2×10^{-5}	5×10^{-10} 5×10^{-10}	7×10^{-6}	2×10^{-10} 2×10^{-10}	0.03 0.04
	(insol.)	Lung GI (LLI)	8×10^{-4}	2×10^{-10}	3×10^{-4}	7×10^{-11}	- -
$_{85}At^{211}$	(sol.)	Thyroid Ovary	5×10^{-5} 5×10^{-5}	7×10^{-9} 7×10^{-9}	2×10^{-5} 2×10^{-5}	2×10^{-9}	0.02 0.02
	(insol.)	Lung GI (ULI)	2×10^{-3}	3×10^{-8}	7×10^{-4}	10^{-8}	-
$_{86}Rn^{220}$	*	Lung		3×10^{-7}*		10^{-7}*	-
$_{86}Rn^{222}$	*	Lung		3×10^{-8}*		10^{-8}*	-
$_{88}Ra^{226}$	(sol.)	Bone	4×10^{-7}	3×10^{-11}	10^{-7}	10^{-11}	0.1
	(insol.)	GI (LLI)	9×10^{-4}	2×10^{-7}	3×10^{-4}	6×10^{-8}	-
$_{89}Ac^{227}$	(sol.)	Bone	6×10^{-5}	2×10^{-12}	2×10^{-5}	8×10^{-13}	0.03
	(insol.)	Lung GI (LLI)	9×10^{-3}	3×10^{-11}	3×10^{-3}	9×10^{-12}	- -

* The daughter elements of Rn^{220} and Rn^{222} are assumed to be present to the extent that they occur in unfiltered air. For all other isotopes the daughter elements are not considered as part of the intake; and, if present, they must be considered on the basis of the rules for mixtures.

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Radionuclide		Critical organ**	Maximum permissible concentrations For 40-h week		For 168-h week		Maximum permissible burden in total body (μc)
			(MPC)w (μc/cm³)	(MPC)a (μc/cm³)	(MPC)w (μc/cm³)	(MPC)a (μc/cm³)	
$_{90}Th^{234}$	(sol.)	GI (LLI) Bone	5×10^{-4}	6×10^{-8}	2×10^{-4}	2×10^{-6}	- 4
	(insol.)	Lung GI (LLI)	5×10^{-4}	3×10^{-8}	2×10^{-4}	10^{-8}	- -
$_{90}$ Th-nat*	(sol.)	Bone	3×10^{-5}	2×10^{-12}*	10^{-5}	6×10^{-13}*	0.01
		Lung GI (LLI)	3×10^{-4}	4×10^{-12}	10^{-4}	10^{-12}	- -
$_{92}U^{233}$	(sol.)	GI (LLI) Bone	9×10^{-4}	5×10^{-10}	3×10^{-4}	2×10^{-10}	- 0.05
	(insol.)	Lung GI (LLI)	9×10^{-4}	10^{-10}	3×10^{-4}	4×10^{-11}	- -
$_{92}U^{235}$	(sol.)	GI (LLI) Kidney Bone	8×10^{-4}	5×10^{-10}	3×10^{-4}	2×10^{-10} 2×10^{-10}	- 0.03 0.06
	(insol.)	Lung GI (LLI)	8×10^{-4}	10^{-10}	3×10^{-4}	4×10^{-11}	- -
$_{92}$ U-nat	(sol.)	GI (LLI) Kidney	5×10^{-4}	7×10^{-11}	2×10^{-4}	3×10^{-11}	- 5×10^{-3}
	(insol.)	Lung GI (LLI)	5×10^{-4}	6×10^{-11}	2×10^{-4}	2×10^{-11}	-
$_{94}Pu^{239}$	(sol.)	Bone	10^{-4}	2×10^{-12}	5×10^{-5}	6×10^{-13}	0.04
	(insol.)	Lung GI (LLI)	8×10^{-4}	4×10^{-11}	3×10^{-4}	10^{-11}	- -
$_{95}Am^{241}$	(sol.)	Kidney Bone	10^{-4} 10^{-4}	6×10^{-12} 6×10^{-12}	4×10^{-5}	2×10^{-12} 2×10^{-12}	0.1 0.05
	(insol.)	Lung GI (LLI)	8×10^{-4}	10^{-10}	3×10^{-4}	4×10^{-11}	-
$_{96}Cm^{242}$	(sol.)	GI (LLI) Liver	7×10^{-4}	10^{-10}	2×10^{-4}	4×10^{-11}	- 0.05
	(insol.)	Lung GI (LLI)	7×10^{-4}	2×10^{-10}	2×10^{-4}	6×10^{-11}	- -

* Provisional values for Th-nat.

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APPENDIX II

HOW TO PUT ON AND TAKE OFF RUBBER GLOVES*

The technique employed in this procedure is such that the inside of the glove is not touched by the outside, nor is any part of the outside allowed to come into contact with the bare skin.

The procedure is as follows:

(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 by manipulating the fingers inside the fold.
(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, e.g. in colour or texture.

APPENDIX III

RADIOACTIVE WASTE CONTROL AND DISPOSAL**

WASTE COLLECTION

In all working places where radioactive wastes may originate, suitable receptacles should be available.

Solid waste should be deposited in refuse bins with foot-operated lids. The bins should be lined with removable paper or plastic bags to facilitate removal of the waste without contamination.

Liquid waste should, if no other facilities for liquid waste disposal exist, be collected in bottles kept in pails or trays designed to retain all their contents in the event of a breakage.

All receptacles for radioactive wastes should be clearly identified. In general, it will be desirable to classify radioactive wastes according to methods of disposal or of storage and to provide separate containers for the various classifications used. Depending upon the needs of the installation, one or more of the following bases for classification of wastes may be desirable:

Gamma radiation levels (high, low),
Total activity (high, intermediate, low),
Half-life (long, short),
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.

It is generally desirable to maintain an approximate record of quantities of radioactive wastes released to drainage systems, to sewers, or for burial. This may be particularly important in the case of long-lived radioisotopes. For this purpose it is desirable or necessary to maintain a record of estimated quantities of radioactivity deposited in various receptacles, particularly those receiving high levels of activity or long-lived isotopes. 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 wastes should be removed from working places by designated personnel under the supervision of the "radiological health and safety officer".

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* Adapted from "Safety Techniques for Radioactive Tracers", Cambridge (1958).

** Adapted from the International Atomic Energy Agency Safety Series No. 1: Safe Handling of Radioisotopes (1962).

WASTE STORAGE

All wastes which cannot be immediately disposed of in conformity with the requirements of the competent authority have to 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, to permit monitoring of materials of unknown degree of hazard or to await the availability of suitable transport. Indefinite storage in special places has to be provided for the more hazardous wastes for which no ultimate disposal method is available to the particular user.

Storage conditions should meet the safety requirements for storage of sources.

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.

DISPOSAL OF WASTES TO THE ENVIRONMENT

General considerations

Disposal of radioactive wastes to the environment should be carried out in accordance with the 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 for any proposed waste disposal method.

The capacity of any route of disposal to accept wastes 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 waste disposal which will be safe under all circumstances. This usually allows a very considerable safety factor. The real capacity of a particular route of waste disposal can only be found by 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 waste disposal. Such a restrictive safe limit is provided by keeping the level of activity at the point of release into the environment below the permissible levels for non-occupationally exposed persons recommended by the International Commission on Radiological Protection for activity in drinking water or in air and indicated in Appendix I. This rule should be superseded if the competent authority provides any alternative requirements or if local studies by experts provide reasonable justification for other levels.

Disposal to drains and sewers

The release of wastes 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 concentrations of radioactive waste material based on the total available flow of water in the system, averaged over a moderate period (daily or monthly), would not exceed the maximum permissible levels for drinking water recommended by the International Commission on Radiological Protection for individuals occupationally exposed; these are indicated in Appendix I. This would provide a large safety factor since water from drains and sewers is not generally to be considered as drinking water. However, in situations where the contamination affects the public water supply, the final concentrations in the water supply should be to the levels set for non-occupationally exposed persons. Some present studies suggest that if the contamination affects water used for irrigation, the final concentrations in the irrigating water should be lower by a factor of at least ten below the levels set for occupational exposure and the possible build up of activity in the irrigated lands and crops should be carefully surveyed.

Finally, before release of wastes to public drains, sewers and rivers, the competent authorities should be informed and consulted to ascertain that no other radioactive release is carried out in such a way that the accumulation of releases will create a hazardous situation.

Radioactive wastes disposed to drains should be readily soluble or dispersible in water. Account should be taken of the possible changes of pH due to dilution, or other physico-chemical factors which may lead to precipitation or vaporization of diluted materials.

In general, the excreta of persons being treated by radioisotopes do not call for any special consideration. (This, however, does not apply to the unused residues of medical isotope shipments.)

Wastes should be flushed down by a copious stream of water.

The dilution of carrier-free material by the inactive element in the same chemical form is sometimes helpful.

Maintenance work on active drains within an establishment should only be carried out with the knowledge and under the supervision of the "radiological health and safety officer". Special care should be given to the possibility that small sources have been dropped into sinks and retained in traps or catchment basins.

The release of waste to sewers should be done in such a manner as not to require protective measures during maintenance work of the sewers outside the establishment, unless other agreement has been reached with the authority in charge of these sewers. The authority in charge of the sewer system outside the establishment should be informed of the release of radioactive wastes in this system; mutual discussion of the technical aspects of the waste disposal problem is desirable to provide protection without unnecessary anxiety.

Disposal to the atmosphere

Release of radioactive waste in the form of aerosols or gases into the atmosphere should conform with the requirements of the competent authority.

Subject to the competent authority, concentrations of radioactive gases or aerosols at the point of release into the environment should not exceed the accepted maximum permissible levels for non-occupationally exposed persons referred to in Appendix I. If higher levels are required and protection is based on an elevated release point from a stack, such levels can only be set after examination of local conditions by an expert.

Even if activity below permissible levels is achieved at the release point for an aerosol, a hazard or nuisance may still arise from fall-out of coarse particles. Therefore, the need for filtration should be assessed.

Used filters should be handled as solid wastes.

Burial of wastes

Burial of wastes in soil sometimes provides a measure of protection not found if the wastes are released directly into 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 meter) provides economical protection from the external radiation of the accumulated deposit.

A burial site should be under the control of the user with adequate means of excluding the public.

A record should be kept of disposals into the ground.

Incineration of wastes

If solid wastes are incinerated to reduce the bulk to manageable proportions, certain precautions should be taken.

The incineration of active wastes should only be carried out in equipment embodying those features of 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 ordinary active waste.

APPENDIX IV

CRITERIA OF OPTIMUM OPERATING CONDITIONS FOR A PROPORTIONAL COUNTER (e.g. gas-flow or scintillation)

Counting time of sample + background	T
Counting time of background (blank sample)	t
Total counting time, T+t	θ

Count rate of sample + background	R
Count rate of background (blank sample)	r
Fractional natural standard deviation of the net count rate of sample, $\frac{\sigma_{nat.}\ R-r}{R-r}$	f
Counting yield, over-all	Y

According to the Lecture Matter (Part I), section 2.5.1, formula (1), one has

$$f=(R/T+r/t)^{\frac{1}{2}}/(R-r). \tag{1}$$

For a given sample activity, a given operating high voltage, a given input bias voltage and a given total counting time θ, the "best partition" (from the point of view of natural uncertainty) of θ between T and t can be deduced from equation (1) as follows:

$$f=[R/(\theta-t)+r/t]^{\frac{1}{2}}/(R-r)$$

$$df/dt=[R/(\theta-t)^2-r/t^2]/2\,[R/(\theta-t)+r/t]^{\frac{1}{2}}(R-r)=0 \text{ for } t^2R=(\theta-t)^2r \text{ or}$$

$$t/T=(r-R)^{\frac{1}{2}}. \tag{2}$$

The partition of θ (between t and T) in conformity with equation (2) corresponds mathematically to the smallest possible (i.e. "best partition") f-value $f_{b.p.}$ which is obtainable under the given conditions. The two equations $t+T=\theta$ and $t/T=(r/R)^{\frac{1}{2}}$ are equivalent to

$$t=[r^{\frac{1}{2}}/(R^{\frac{1}{2}}+r^{\frac{1}{2}})]\theta \text{ and } T=[R^{\frac{1}{2}}/(R^{\frac{1}{2}}+r^{\frac{1}{2}})]\theta,$$

which, by substitution into equation (1), gives

$$f_{b.p.}=1/\theta(R^{\frac{1}{2}}-r^{\frac{1}{2}}). \tag{3}$$

For a given sample activity in a proportional counter (e.g. gas-flow or scintillation), the values of R and r 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 "optimal setting", $R^{\frac{1}{2}}-r^{\frac{1}{2}}$ will attain its maximum value, and the natural uncertainty (for the best partition of the given θ) will, according to equation (3), attain its minimum value, $f_{b.p.,min}$. For another sample containing a different activity the optimal setting corresponding to $f_{b.p.,min}$ 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 <u>low</u> activity samples ($R \doteq r$) are to be measured. But when R is not much greater than r, the difference $R^{\frac{1}{2}}-r^{\frac{1}{2}}$ may be approximated by $[(R-r)/2]r^{-\frac{1}{2}}$* so that the optimal setting (corresponding to $f_{b.p.,min}$) may be approximated by that for which $[(R-r)/2]r^{-\frac{1}{2}}$ or $\underline{(R-r)^2/r}$ attains its maximum value. Since this approximate optimal criterion for low activities is equivalent to Y^2/r attaining its maximum value, it is <u>independent</u> of sample activity.

In tracer work (non-G-M counter) operating conditions are usually chosen as optimal on the basis of minimum natural uncertainty for very low-activity samples; i.e. the maximum of $(R-r)^2/r$ or Y^2/r 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 $(R-r)^2/r$, which is permissible because this maximum, as mentioned above, is independent of sample activity.

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* $R^{\frac{1}{2}}-r^{\frac{1}{2}}=[r+(R-r)]^{\frac{1}{2}}-r^{\frac{1}{2}}$

$=r^{\frac{1}{2}}\{[1+(R-r)/r]^{\frac{1}{2}}-1\}$; so, for $R \doteq r$,

$R^{\frac{1}{2}}-r^{\frac{1}{2}} \doteq r^{\frac{1}{2}}[1+\frac{1}{2}(R-r)/r-1]$, or

$R^{\frac{1}{2}}-r^{\frac{1}{2}} \doteq (R-r)/2r^{\frac{1}{2}}$.

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APPLIED PART

EXPERIMENTS

1-3. METABOLISM OF C^{14}-MALATHION IN THE HOUSE FLY

INTRODUCTION

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 insects and the ability of insects to defend themselves against the poison, i.e. the rate at which insects detoxify the material. Differences in rates of absorption and degradation of a compound often account for differences in toxicity to different species or strains within a species. Experiments 1, 2 and 3 are closely related and should be done simultaneously. The experiments will require 7 to 9 h of work over a 2-d period.

PURPOSE

The purposes of Experiments 1, 2 and 3 are to study the metabolism of C^{14}-malathion in susceptible and malathion-resistant house flies. The rate of absorption and rates of detoxication of malathion will be measured in vivo and in vitro in flies treated with malathion alone and in flies pretreated with a malathion synergist.

MATERIALS

(1) C^{14}-malathion with high activity.
(2) DEF (S, S, S-tributylphosphorotrithioate), a malathion synergist.
(3) A supply of adult female house flies 2-6 d old of a susceptible and of a resistant strain.
(4) Micrometer-driven syringe (or other dosing device) equipped with 27-30-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) Glassware.
(11) Counting equipment.
(12) Sand washed with acetone.
(13) Filter paper.

1. ABSORPTION OF MALATHION IN THE HOUSE FLY

PROCEDURE

Treat 6 groups of 5 flies each of both strains topically with 1 μg of C^{14}-malathion dissolved in acetone (concentration: 1 μg per μl). Use a 27-gauge

needle. At 0, 5, 10, 20, 40 and 80 min 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.

2. DETOXICATION OF MALATHION IN VIVO

PROCEDURE

A. Treat 25 flies of each of both strains topically with 1 μg of DEF (concentration: 1 μg per μl). One hour later inject 1 μg of C^{14}-malathion into each of the same flies, using a 30-gauge needle. At 0, 5, 10, 20 and 40 min after injection, grind groups of 5 flies in sand with a a mortar and pestle. Pour flies 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 dioxan cocktail, and liquid scintillation counting equipment.

B. Inject 1 μg of C^{14}-malathion into 25 flies of each of both strains. At 0, 5, 10, 20 and 40 min 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.

3. DETOXICATION OF MALATHION IN VITRO

PROCEDURE

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 C^{14}-malathion. Add 5 ml of chloroform and 4 ml of water to one tube after 15 min and to the other after 30 min.

Pipette 2-ml aliquots of each strain into separate centrifuge tubes containing 2 μg of DEF. After 15 min pipette 1 ml from each tube into tubes containing 1 μg of C^{14}-malathion. After 15 min add 4 ml of water and 5 ml of chloroform.

To two centrifuge tubes containing 1 μg of C^{14}-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 1.

QUESTIONS ON EXPERIMENTS 1-3

(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 rates and what is the effect of the synergist? What practical advantage might this have?
(3) Compare detoxication rates in vitro between the strains. Is there a difference? What is the effect of the synergist?

4. METABOLISM OF DDT

INTRODUCTION

Numerous economically important insect pests are resistant to DDT and other insecticides. Studies on the absorption and metabolism of insecticides on various insect species will help in understanding some of the problems related to resistance. The time required to conduct this experiment is 4 to 6 h.

PURPOSE

To study the metabolism of DDT in house flies by means of paper chromatography.

MATERIALS

(1) C^{14}-DDT with high activity.
(2) Acetone.
(3) Paper chromatographic scanner.
(4) Mechanical shaker.
(5) Counting equipment.
(6) Usual laboratory glassware.
(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.
(18) Benzene.
(19) Mortar and pestle.
(20) N, N-Dimethylformamide.
(21) Mineral oil.
(22) Isooctane.
(23) Soxhlet apparatus.
(24) Micrometer-driven syringe.

PROCEDURE

Prepare stock solution of C^{14}-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 one 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 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 h.

Concentrate the extract in a water bath at 50-60°C, and prepare for radio assay and paper chromatography.

Purification of extract is accomplished as follows. For each 1 gm of flies (approximately 50 flies) weigh 5 g of Celite. Mix Celite with 1.5 ml of concentrated H_2SO_4. Add CCl_4 and triturate until a homogeneous slurry is obtained. Place slurry in column in small portions and pack tightly with a tamping rod. Adjust flow rate to approximately 120 drops/min. Gently pour the concentrated extract (in CCl_4) into the column and allow to settle to within 2 mm of the absorbent. Then pour 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.

For paper chromatographic analyses set up the following systems:

	Stationary Phase	Mobile Phase
Solvent System I	4% mineral oil in ether	3:1 acetone - H_2O (by volume)
Solvent System II	35% Dimethylformamide in ether	Redistilled isooctane

Place a small quantity of the radioactive extract on strips or sheets of Whatman No. 1 filter paper. Make spots as small as possible. Allow to dry. Dip paper in stationary phase liquid in such a manner that it will not touch the spots. Allow to dry for a few minutes. Place paper in chromatography chamber and allow mobile phase solvent to move up the paper to within 1-2 cm from the top. Remove paper and allow to dry.

Cut paper into 1-in wide strips and scan in a radioactive scanner with recording attachment. Determine Rf values and compare with known standards treated similarly.

$$\text{Rf value} = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}$$

QUESTION

Why are the extracts purified by passing them through the Celite column before paper chromatography?

5. METABOLISM OF ALDRIN

INTRODUCTION

Numerous economically important insect pests are resistant to DDT and other insecticides. Studies on the absorption and metabolism of insecticides on various insect species will help in understanding some of the problems related to resistance. Time required to conduct this experiment is 4–6 h.

PURPOSE

To study the metabolism of aldrin in house flies by the use of thin layer chromatography.

MATERIALS

(1) C^{14}-aldrin with high activity.
(2) Thin-layer chromatographic glass plates.
(3) Silica-gel G.
(4) Eastman Chromagram polyester sheets (Eastman Type K301R2) may be used instead of 2 and 3 above.
(5) Benzene.
(6) Acetone.
(7) Mortar and pestle.
(8) Mechanical shaker.
(9) Counting equipment.
(10) Usual laboratory glassware.
(11) Potassium hydroxide.
(12) Ethyl alcohol.
(13) Hexane.
(14) Sodium chloride.
(15) Sodium sulphate, anhydrous.
(16) Soxhlet apparatus.
(17) Micrometer-driven syringe.
(18) Magnesite.
(19) Celite 545.
(20) Separatory funnel.

PROCEDURE

Prepare stock solution of C^{14}-aldrin in benzene or acetone, and make proper dilutions as desired.

Apply topically to individual male or female flies the desired amount of insecticide in one μl of solvent.

At predetermined intervals, rinse the flies with three 15-ml portions of acetone to remove the unabsorbed aldrin. Combine the rinses.

Grind 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 h.

Concentrate the extract in a water bath at 50-60°C, and prepare for radio assay and thin layer chromatography.

Purify the aldrin extract as follows:

Method A

Evaporate extract to dryness in a round-bottom flask. Add 3 ml of 50% aqueous KOH and 12 ml of 95% ethanol. Reflux for 1 h. Cool; add 10 ml cold H_2O and 50 ml hexane. Shake in separatory funnel and discard H_2O. Wash hexane with distilled water until neutral, then wash with saturated NaCl or with Na_2SO_4 to dry the hexane. Concentrate hexane and prepare for chromatography.

Method B

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 and 1 part Celite 545. Saturate absorbent with redistilled hexane. Add concentrated extract and elute with 150 ml of redistilled hexane. Evaporate eluate and prepare for chromatography.

Thin-layer chromatography: Prepare glass plates with silica-gel G or obtain commercially prepared Eastman Chromagram polyester sheets (Eastman Type K301R2). Apply spots and develop with purified hexane. Cut strips (if polyester sheets are used) and scan for radioactivity with a scanner with recording attachment.

For scanning glass plates it is necessary to add an attachment to the scanning device or use other means of detection.

QUESTIONS

(1) At what interval did you obtain the greatest penetration?
(2) At what interval did you get the greatest metabolism?
(3) Did dead insects contain as many metabolites as live insects?
(4) Is metabolism of aldrin a function of time?
(5) Is the metabolism curve linear?

6 AND 7. SCINTILLATION DETECTION OF INSECTICIDE PENETRATION THROUGH AND INTO INSECT CUTICLE

INTRODUCTION

Scintillation counters make it possible for the scientist selectively to detect gamma, beta, and alpha radiation and fast neutrons even in the presence of heavy background counts. These instruments also give information on the energy spectrum of the radiation, and will operate effectively at extremely high counting rates. Liquid scintillation counting equipment will be used. These two experiments will require 4 to 6 h each to complete.

PURPOSE

The purposes of these two experiments are to detect the penetration of H_3-labelled insecticide through and into insect cuticle.

MATERIALS

(1) H_3-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) 1.5 M tris buffer (tris(hydroxymethyl)aminomethane).
(11) Colourless concentrated nitric acid.
(12) Forceps and scissors.
(13) Semilog graph paper.
(14) Scintillation counter.

6. PENETRATION THROUGH THE CUTICLE

PROCEDURE

Apply 1 μl of the labelled insecticide in acetone solution to the pronotum of each of 15 cockroaches. Remove the pronota from 3 cockroaches at each of 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 test tube in boiling water on hot plate. When pronotum has dissolved add 9 ml of water and allow to cool. After cooling remove

1 ml and add to 18 ml of dioxane cocktail. To this add 1 ml of 1.5 M tris buffer and count.

For controls (zero time) remove 3 pronota and prepare as above except that 1 μl of H_3-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 1.5 M tris buffer.

When all counts are completed, plot per cent remaining on cuticle on y axis and time on x axis of graph paper.

7. PENETRATION INTO THE CUTICLE

PROCEDURE

Use same dosage of solution of H_3-labelled insecticide, same number of insects and same time intervals as in Experiment 6. Instead of removing pronotum, carefully wash the pronotum into a test tube with 2 ml acetone, then add 18 ml of toluene cocktail and count.

For control, add 1 μl of H_3-labelled insecticide to 2 ml of acetone and 1 ml of toluene cocktail, and count.

For blank add 2 ml of acetone to 18 ml of toluene cocktail and count. Plot on graph paper as in Experiment 6.

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 in 12 and 24 h by extrapolating your graphs?

8. EFFECT OF TEMPERATURE ON THE ABSORPTION OF C^{14}-DDT

INTRODUCTION

It has been known for many years that DDT will knock out and kill house flies faster when the flies 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 h on each of two consecutive days to complete.

PURPOSE

To determine the amount of C^{14}-DDT absorbed when house flies are held at 21°C and 32°C after treatment.

MATERIALS

(1) C^{14}-DDT with high activity.
(2) House flies that require approximately 5 μg of DDT 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 glassware.
(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 point, and prepare an acetone solution of C^{14}-DDT so that each μl contains the LD50 dosage of DDT.

Treat 40 flies with an LD50 dosage of C^{14}-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 C^{14}-DDT solution in each of 3 planchets, dry, and obtain counts per minute.

In 20-24 h separate live and dead flies from the jars held at each temperature. Kill the live flies with chloroform. Keep different lots of flies separate and well marked.

Rinse the external DDT from lots of 5 flies from each of the 4 groups in 2-3 ml of acetone in a vial or test tube. Pour the acetone into planchets, mark, dry, and count. Macerate the 5 flies from each group in a mortar with 5 ml of acetone to extract the C^{14}-DDT. Decant acetone into planchets and count.

Tabulate 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? If so, explain.
(2) How does mortality of flies compare at the two temperatures?

9. EXCISED LEAVES FOR STUDY OF TRANSLOCATION OF A SYSTEMIC INSECTICIDE

INTRODUCTION

This experiment demonstrates how a systemic insecticide may be studied by using radio-labelled material. It has been designed so that an insect bio-assay could be used in conjunction with the radio-assay. 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 h to complete.

PURPOSE

To measure the extent of translocation of a systemic insecticide in a leaf following topical application.

MATERIALS

(1) P^{32}-labelled systemic insecticides such as Bidrin or Phosphamidon, preferably with a specific activity greater than 5 mc/mmole.
(2) Potted plants (beans, peas, cotton, etc.) with fully expanded leaves.
(3) Micropipettes or other apparatus for accurately measuring 5 to 10 μl.
(4) Radio-assay equipment.

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 to 15 μl of the solution or emulsion to a 1 to 2 cm^2 spot between the major leaf veins of a leaf. Treat several leaves. Retain the plant under normal growing conditions for 2 to 4 d.

To estimate the amount of translocation, either an autoradiograph may be prepared or direct radio-assay may be used. For the autoradiograph remove the treated leaf from the plant and expose to X-ray film for several days in a deep freeze. For the direct radio-assay, cut out the treated area of the leaf and several equal size areas round the treated area. Radio-assay these pieces of leaf directly. Draw a picture of the leaf and record on the picture the radioactivity associated with each piece that was radio-assayed.

QUESTIONS

(1) What path has the systemic insecticide followed in the leaf?

(2) How far from the point of application? Assuming proportionality, what is the rate of translocation per unit time?

10. EXCISED LEAVES FOR STUDY OF METABOLISM OF A SYSTEMIC INSECTICIDE

INTRODUCTION

This experiment demonstrates how the metabolism of a plant systemic insecticide may be studied by using radiolabelled material. It has been designed so that an insect bio-assay can be used in conjunction with the radio-assay. It can be expanded to obtain precise metabolism data if techniques are available for separation of metabolites. The experiment requires several hours over a 7-d period to complete.

PURPOSE

To study the metabolism of a plant systemic insecticide in plant tissue.

MATERIALS

(1) P^{32}-labelled systemic insecticides, such as Bidrin or Phosphamidon, preferably with a specific activity greater than 5 mc/mmole.
(2) Radio-assay equipment.
(3) Plants (bean, pea, 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 several of the small vials. With the petioles under water, cut several leaves from the plant. Cut petioles about 2 cm longer than vials. Place the petioles of these leaves in the vials containing the solution of labelled insecticide. Place the leaves under a lamp so that the leaves 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 d after treatment grind up a leaf or two 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 min. Allow water and chloroform to separate. If they do not separate readily, place the liquid in centrifuge tubes and centrifuge until separation is achieved.

Radio-assay 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 due to volatilization may be reduced by adding 10 to 25 mg of cooking oil to each planchet.

Draw a curve showing decomposition of parent compound to non-toxic hydrolytic products.

QUESTIONS

(1) What sort of curve (exponential, linear) is obtained? Explain why.
(2) During what period is the rate of decomposition greatest and if so, to what part of the curve does that period correspond, or is the rate constant?

11. COMPARATIVE ABSORPTION AND TRANSLOCATION OF SYSTEMIC INSECTICIDES BY PLANTS FOLLOWING SOIL APPLICATION

INTRODUCTION

This experiment demonstrates how the absorption and translocation of two or more labelled systemic insecticides may be studied simultaneously in plants. It has been designed so that an insect bio-assay can be used in conjunction with the radio-assay. It can be expanded to obtain precise metabolism data if techniques are available for separation of metabolites. Each part of the experiment can be completed in several hours over a 7-d period.

PURPOSE

To compare the systemic action of two or more systemic insecticides applied as soil treatments.

MATERIALS

(1) Two or more P^{32}-labelled systemic insecticides such as Bidrin and Phosphamidon with specific activities of 1 mc/mmole or greater.
(2) Potted plants (bean, pea, cotton, etc.) of uniform size growing in soil.
(3) Oven.
(4) Laboratory grinder or mortar and pestle.

PROCEDURE

Prepare water solution or emulsions of each labelled insecticide. Either pour the desired amount on top of the soil round 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 d 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 to 50°C for 24 to 48 h. Keep the leaves from each plant separate. Obtain 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 to 125 mg aliquots of each dried, ground leaf tissue sample and radio-assay.

Express results as

(a) cpm/mg dry weight;
(b) cpm/mg fresh weight;
(c) μg-equivalents/mg dry weight;
(d) μg-equivalents/mg fresh weight;
(e) ppm (μg equivalents/g fresh weight);
(f) percentage of applied dose recovered.

QUESTIONS

(1) For any of the insecticides, which is more meaningful from the practical standpoint, cpm/dry weight or cpm/fresh weight and why?
(2) As above: μg equivalents/mg dry weight or μg equivalent/mg 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?

12. DEGRADATION OF SYSTEMIC INSECTICIDES BY INSECTS FEEDING ON TREATED PLANTS

INTRODUCTION

This experiment demonstrates how the degradation of systemic insecticides by insects feeding on treated plants may be studied by the use of radioisotope-labelled compounds. Each part of the experiment requires several hours over a 7-d period to complete.

PURPOSE

To determine the fate of systemic insecticides in insects feeding on treated plants.

MATERIALS

(1) P^{32}-labelled systemic insecticides such as Bidrin or Phosphamidon, preferably with a specific activity greater than 5 mc/mmole.
(2) Radio-assay equipment.
(3) Plant (bean, pea, 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.
(10) Holding containers for insects.
(11) Insects (large lepidopterous larvae that are quite tolerant to the insecticide are excellent test insects).

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 several small vials. With the petioles under water, cut several leaves from the plant. Cut petioles about 2 cm longer than vials. Place the petioles of these leaves in the vials containing the solution of the labelled insecticide. Place the leaves under a lamp so that the leaves 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 intervals of 0, 1, 2, 4, and 7 d after treatment of the leaves, feed the treated leaves to the test insects for 12 or 24 h periods. At the end of the feeding periods, grind and extract the treated leaves, test insects, and the faecal material from the test insects.

At 0, 1, 2, 4 and 7 d after treatment grind up a leaf or two with sand and water in the mortar. Place these homogenates in a separatory funnel with equal volumes of chloroform and water. Shake for 1-2 min. Allow water and chloroform to separate. If they do not separate readily, place the liquid in centrifuge tubes and spin until separation is achieved.

Radio-assay aliquots of water and chloroform phase 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 due to volatilization may be reduced by adding 10 to 25 mg of cooking oil to each planchet.

Calculate the extent of degradation of the toxicant in the leaves, insect and faeces for each interval.

QUESTION

Where does the severest degradation take place; in the plant or in the insect?

13. DETERMINATION OF NECTAR PREFERENCE OF MOSQUITOES

INTRODUCTION

It is well known that most mosquitoes and particularly the males require the nectar or exudates of plants to sustain life. A knowledge of the host preferences for a certain species of mosquito could lead to the control of this mosquito by controlling its host plant. Radioisotopes provide an excellent tool to determine the preference of a certain species of mosquito for certain species of plant.

PURPOSE

To determine the host preference for any locally available species of mosquito.

MATERIALS

(1) Several cages 12 × 12 × 12 in 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) P^{32}-phosphoric acid.
(6) Radiation counter or radiographic film.

PROCEDURE

Twenty-four hours before the start of the actual experiment each species of potential host should be placed in a dilute solution $H_3P^{32}O_4$. Provision should be made to prevent mosquitoes from coming in contact with the P^{32} solution.

After the 24-h 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 h the potential hosts 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 sexed and radioactivity determined for each sex. If no radioactivity is detected, the mosquitoes have not fed on the host.

QUESTIONS

(1) What conclusion can be reached from this experiment?

(2) Were both sexes radioactive?
(3) Were all specimens of either sex radioactive?

14. DETERMINATION OF MOVEMENT OF SUBTERRANEAN INSECTS

INTRODUCTION

Radioisotopes provide the easiest and the most accurate method of detecting and measuring the movement of insects that spend relatively long periods completely below the soil surface. The determination of 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 a mole cricket (or other species such as wireworms, corn rootworms, etc.), and to plot these movements on graph paper.

MATERIAL

(1) One large shallow tray 18 × 30 × 3 in of wood or metal.
(2) Soil with good tilth and moisture content.
(3) Fine Co^{60} wire or a water-soluble Co^{60} salt. Activity of Co^{60} should be sufficient to penetrate 3 in of soil, about 0.05 mc/mm 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 tray with soil and make grid over tray with either string or wire. Place Co^{60} wire into the abdominal cavity of test insect, or put a drop of Co^{60} salt solution on the adhesive side of a small piece of cellulose tape and allow the solvent to evaporate. Place tape under the wing of the mole cricket.

The tagged insect should then be placed at the surface of the soil at any particular location within the grid. The location should be marked on the graph paper containing the same number of grid spaces as the tray.

Periodically the position of the insect should be located with the counter. Active insects should be located at 5-min intervals. The highest activity will be at a location 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 movements could be expected in soil that contained a host of the insect?
(3) How far did the insect used in the experiment travel per unit of time?

15. TAGGING INSECTS WITH RADIOISOTOPES

INTRODUCTION

The tagging of insects with a radioactive marker has been used in studying dispersion, speed of flight, total numbers of a natural insect population in an area and other biological investigations. P^{32} is a useful tagging agent but the short half life of 14.3 d precludes studies extending for more than a few weeks.

PURPOSE

To demonstrate tagging of mosquito larvae with P^{32}.

MATERIALS

(1) Pans or glassware holding from 0.5 to 1 litre of water and suitable for rearing mosquito larvae.
(2) P^{32} as phosphoric acid or a phosphate.
(3) Insect emergence cages.
(4) Pipettes and other laboratory glassware.
(5) Counting equipment.

PROCEDURE

The best way to tag adult mosquitoes is to place the P^{32} in larval water and tag the larvae. The larvae absorb the P^{32} or it is taken up with the food in the water.

Use any species of mosquito larvae that can be reared in the laboratory. Place about 100 late third- and early fourth-instar larvae in a shallow pan of water (0.5 litre). Apply P^{32} at a rate of 10 μc per litre of water. Mix the solution in the water. Add artificial food to the water.

At 24 and 48 h transfer 10 larvae into a beaker of clean water and place them in an emergence cage. Make counts of radioactivity of both males and females. Compare counts of the two sexes.

QUESTIONS

(1) Which sex has the greatest radioactivity?

(2) How does the uptake of radioactivity compare in the 24- and 48-h treatments?

16. DEMONSTRATION OF FOOD EXCHANGE AMONG SOCIAL INSECTS

INTRODUCTION

Social insects like ants, bees and termites have a highly developed behaviour of social food exchange between the different individuals of the community. These problems have been studied with 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 differences between these that have to be taken into consideration in tracer field experiments. This experiment requires from 4 to 8 h after all equipment and ants are assembled.

PURPOSE

To demonstrate uptake and distribution of food from ant to ant or caste to caste.

MATERIALS

(1) Glass containers, 10 × 10 × 5 cm, with covers.
(2) Insect tweezers.
(3) Glass slides and cover slips.
(4) Chloroform or ether.
(5) Honey or sucrose solution (20%).
(6) P^{32} 0.5–1.0 mc in water, with a specific activity of about 0.5–1 mc/ml.
(7) Open-ended glass vials, 12 mm in diameter and 12 mm high, with covers.
(8) Non-radioactive phosphorus of the same concentration as No.(6).
(9) Vials, bottles, ordinary laboratory glassware, plastic squeeze bottles.
(10) Radiation-measuring equipment.

(11) 500 ants from subfamily Camponotinae and 500 from subfamily Myrmicinae.
(12) Decontamination solution: 1% sodium phosphate plus small amount of detergent such as Triton X-100 or Tween 20.

PROCEDURE

Prepare liquid radioactive food for the ants by mixing 2 parts of a 30% solution of honey in water with 1 part of P^{32} solution. The specific activity of the finished food should be 0.5 to 1.0 mc/ml.

Put 10-15 specimens of each species into separate jars. Feed the ants by putting a drop of the prepared radioactive food on coverslips and placing them in the jars. Record starting time, duration of feeding, and air temperature.

Since the ants may be contaminated externally with radioactivity they must be decontaminated before measuring their internal radioactivity. Place the decontamination fluid in a squeeze bottle. Spray the insects with the fluid and finally with pure water. Put the ants on layers of soft absorbent cellulose (Kleenex tissue) and let them run on this material after they have been decontaminated.

Radioactivity of individual ants can be measured by placing them in individual cages. Cages may be made from $\frac{1}{2}$-in sections of glass or plastic tubing. The ends may be closed with Scotch tape. Note and record the activity of each ant. Small gelatin capsules can also be used for this purpose.

Individual ants with the highest radioactivity are now used as donors. Carefully mark the donors with a colour spot and put each donor in a jar containing 30 hungry worker ants (unfed for 2-3 d) of the same species and from the same colony. Prepare 3 jars of each species.

By the use of chloroform or ether kill the ants in one jar after 4 h, those in another after 20 h, and those in the third after 40 h.

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 cpm. Compare food distribution of the two species on a time basis.

QUESTIONS

(1) What was the length of time required for maximum distribution of food to be obtained?
(2) Which subfamily accomplished distribution first?

17. DEMONSTRATION OF TRANSFER OF GLANDULAR SECRETION OF ANTS

INTRODUCTION

In addition to the transfer of food from the crop among ants there is also a transfer of glandular secretions. It is possible to demonstrate this phenomenon in laboratory experiments by the use of radioactive materials. The experiment requires several days to complete but involves only $\frac{1}{2}$ to 1 h of work per day.

PURPOSE

To prove the transfer of glandular secretion by biological clearing of the crops of ants.

MATERIALS

(1) Glass container, 10 × 10 × 5 cm, with covers.
(2) Insect tweezers.
(3) Glass slides and cover slips.
(4) Chloroform or ether.
(5) Honey or sucrose solution (20%).
(6) P^{32} 0.5–1.0 mc in water, with a specific activity of about 0.5–1.0 mc/ml.
(7) Open-ended glass vials, 12 mm in diameter and 12 mm high, with covers.
(8) Non-radioactive phosphorus of the same concentration as No. (6).
(9) Vials, bottles, ordinary laboratory glassware, plastic squeeze bottles.
(10) Radiation-measuring equipment.
(11) 500 ants from subfamily Camponotinae and 500 from subfamily Myrmicinae.
(12) Decontamination solution: 1% sodium phosphate plus small amount of detergent such as Triton X-100 or Tween 20.

PROCEDURE

Prepare liquid radioactive food for the ants by mixing 2 parts of a 30% solution of honey in water with 1 part of P^{32} solution. The specific activity of the finished food should be 0.5 to 1.0 mc/ml.

Put 10 to 15 specimens of each species in separate jars. Feed the ants by putting a drop of the prepared radioactive food on coverslips and placing them in the jars. When feeding is complete, remove the food and decontaminate the ants externally. Put the fluid in a squeeze bottle and spray

the ants extensively. Finally wash the ants with pure water and allow them to dry by placing them on layers of soft absorbent cellulose (Kleenex tissues).

Isolate individual radioactive-fed ants in vials for 48 h. After 48 h allow the ants to empty their crops through repeated regurgitations with hungry workers from the same nests. Then feed the individually caged ants several times with non-radioactive food solution that they are permitted to regurgitate to feed additional hungry workers. By measuring the receptor workers for radioactivity, the point at which the crops of the radioactive fed ants are emptied can be determined.

After some time, especially during the rearing of the sexual brood, the crops of the fed workers acquire secondary radioactivity. This is due to secretions from pharyngeal glands that had stored some of the original radioactivity. Since these glands have no reservoirs and open into the pharynx, the secretions are swallowed and stored in the crop that acts as a reservoir for the pharyngeal glands. By measuring the radioactivity of ants that have been fed regurgitations at a later period, as well as measuring contents from dissected crops, the secondary radioactivity can be shown. The secondary crop content is regurgitated particularly during the early spring as special food for queens and for rearing the sexual brood.

QUESTIONS

(1) What was the length of time required for secondary (pharyngeal secretion) radioactivity to appear?
(2) Was there any difference in this behaviour between species?

18. CUTICULAR EXCRETION OF P^{32}

INTRODUCTION

This experiment is designed to show that P^{32} injected into ants is excreted through the cuticle. The experiment requires 1 to 3 h of work over a period of 1 to 2 d.

PURPOSE

To show that P^{32} is excreted through the cuticle.

MATERIALS

(1) Ordinary laboratory glassware.
(2) $\frac{1}{4}$-ml microsyringe capable of delivering 1 μl through a 27-gauge hypodermic needle.
(3) P^{32}-labelled phosphate.

(4) Large ants.
(5) Smaller workers from the same colony as the large ants.
(6) Device for measuring radioactivity.

PROCEDURE

Queens or extremely large worker ants are injected with 1 μl of P^{32}-labelled phosphate. Cement the mouth and anus of each injected ant with fingernail lacquer to prevent transfer of radioactivity by transfer of crop food or anal secretions.

After 48 h put 10–15 smaller workers in vials containing the injected specimens. After 1 h begin measuring the radioactivity of the individual workers.

QUESTION

Could the transfer of cuticular excretions be proved by regurgitations from worker ants who licked and cleaned injected specimens?

19. MEASUREMENT OF BIOLOGICAL HALF LIFE OF RADIOTRACERS IN INSECTS

INTRODUCTION

Biological half life is the time required for the loss of half the amount of a substance ingested. It is primarily dependent on the rate of excretion. The measurement of biological half life (T_{biol}), however, cannot be obtained directly. Whereas for a radioactive substance it can be computed by measuring the effective half life (T_{eff}) that is the total effective decrease in the impulse rate of a radioactive substance (in a time unit) due to both biological and physical decay of the radioactivity.
This can be expressed

$$\frac{1}{T_{eff}} = \frac{1}{T_{phys}} + \frac{1}{T_{biol}}$$

The effective decrease in the activity of a radioactive substance follows an exponential law, as does the physical and biological decrease in impulse rate. Since the physical half life of a radioactive isotope is known, the biological half life can be calculated after having graphically obtained the effective half life (T_{eff}) from the following formula:

$$T_{biol} = \frac{1}{\frac{1}{T_{eff}} - \frac{1}{T_{phys}}} \quad \text{or} \quad T_{biol} = \frac{T_{eff} \times T_{phys}}{T_{phys} - T_{eff}}$$

PURPOSE

To determine the biological half life of P^{32} and I^{131} in cockroaches.

MATERIALS

(1) Medicine droppers.
(2) Pipettes.
(3) $\frac{1}{4}$-ml syringe.
(4) P^{32}
(5) I^{131}
(6) Petri dishes.
(7) Gelatin capsules to hold cockroaches firmly.
(8) Sucrose solution.
(9) Counting equipment.
(10) Glass slides.
(11) Ordinary laboratory glassware.
(12) Carbon dioxide for anaesthetizing insects.
(13) Sodium phosphate.

PROCEDURE

Anaesthetize cockroaches with CO_2 and strap on to glass slides with the dorsum next to the glass. Scotch tape is satisfactory for strapping. As the cockroaches are recovering from the CO_2 feed them P^{32} or I^{131} in sucrose solution with a medicine dropper with a fine tip. The insects will readily imbibe sufficient amounts of the solutions. The specific activity of both preparations should be about 0.5 mc/ml. The roaches 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 secondly with pure water. Allow the insects to dry.

Measurements of radiation should be taken soon after feeding and repeated 2, 24, 48, 72 and 96 h later. Measure P^{32} with a G-M tube and I^{131} with scintillation counters. Each measurement must be taken under the same geometric and physical conditions. Confining cockroaches in a small gelatine capsule is a good way to maintain the same geometry for different measurements.

Keep cockroaches isolated in Petri dishes. Renew filter paper in the dishes daily and provide food and water. Excrements also have to be measured for radioactivity to obtain values for passage through the alimentary canal. Wipe the pronota after 2–3 d with filter paper and measure for cuticular excretion.

Plot results on semilog paper (log cpm 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 P^{32} and I^{131} solutions?
(2) If there are differences, to what may they be attributed?

20-26. INSECT STERILIZATION BY USE OF Co^{60} AND CHEMOSTERILANTS

INTRODUCTION

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.

These seven experiments require approximately 24 d to complete when starting with insect eggs. When adult insects of the right stage and age are ready, approximately 10 d are required with certain manipulations to be done every day. Any single experiment requires about 10 d to complete but not day-to-day attention. It is essential to organize the programme carefully, and spend a full 4-h laboratory period each day during the 10-d period if all experiments are done at one time.

MATERIALS

The materials needed for all seven experiments are given below, but methods and procedures are given under each experiment.

(1) Housefly and mosquito colonies.
(2) Small cages and all equipment necessary for handling flies.
(3) Cobalt-60 source.
(4) Chemosterilants.
(5) Usual laboratory glassware and other equipment.
(6) Micro applicator.
(7) Housefly saline: 14.52 g NaCl, 0.36 g KCl, 0.29 g $CaCl_2$, 2.5 g dextrose (all g/l) and 25 ml of 0.04 M phosphate buffer of pH 7.0.
(8) Acetone.

20. RADIATION DOSAGE REQUIRED TO PRODUCE STERILITY IN THE HOUSE FLY

PURPOSE

This experiment is conducted to determine which dosage of gamma radiation produces 100 per cent 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. One observes whether the females lay eggs or not, and if they do, whether the eggs hatch.

PROCEDURE

Expose several hundred house fly pupae, between 2 to 3 d old, to each of the following dosages of radiation in a cobalt-60 source:

500 r 1000 r 2000 r 3000 r 6000 r and 9000 r

Place 100 pupae from each dosage level in emergence cages. Allow the adults to emerge and separate the sexes after a period of 12 h. When 3 d old, place 30 irradiated males from each group in cages containing 25 virgin females. Also place 25 irradiated females from each group with 30 3-d-old virgin males. A control cage consists of 30 3-d-old virgin males and 25 3-d-old virgin females. Five additional males are included to ensure that 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 7th day. Float the eggs from each group. Place about 300-400 eggs on moist, black cloth in each Petri dish until all the eggs are plated. Record the number of eggs in each dish. Determine the per cent hatch after 24 h. To be truly precise, the females should be egged individually. This is tedious and the method given above 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 a jeweller's 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[1] on a slide, and the spermathecae and ducts are dissected under a 10-15 X magnification. The spermathecae and duct are then placed in another drop of Ringer's 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 per cent lethality 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 dosage level into emergence cages.

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[1] Ringer's solution: 0.1 g potassium chloride (KCl)
0.0135 g calcium chloride ($CaCl_2$)
0.0120 g sodium bicarbonate ($NaHCO_3$)
0.75 g sodium chloride (NaCl)
Dissolve in sufficient distilled water to make 100 ml of solution.

Day 2 – a.m. Sex the flies and place in separate cages.
Day 4 – Set up the mating cages for each group.
Day 7 – Collect and count 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 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.

21. EFFECT OF RADIATION ON SEXUAL COMPETITIVENESS OF MALE HOUSE FLIES

PURPOSE

To determine whether any deleterious effect is produced in the males by irradiation with 6000 r that would prevent them from being sexually competitive with non-irradiated males.

PROCEDURE

House fly pupae, 65–72 h old, are exposed to a sterilizing dosage level of 6000 r. 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 h after the males.

The adults are allowed to mate and several days later CSMA larval medium is placed in the cages and the females allowed to oviposit. All the

eggs from an individual cage are floated in water and then 100 eggs, randomly selected, are placed on a square of moistened cloth. The cloth is placed in a Petri dish and 24 h later the percentage of hatch determined. The eggs from each cage are evaluated in the following manner.

Schedule

Day 1 a.m. House fly pupae are irradiated at 6000 r. This will be done by the instructor.

Day 2 a.m. All flies that 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 competitive cages with adequate food and water. The females are held for 24 h to allow all males to become oriented in the cage.

Day 3 p.m. Introduce the females into the competitive cages.

Day 8 p.m. Collect and count random sample of 100 eggs from each cage.

Day 9 p.m. Observe the eggs with a dissecting scope to determine the number that have hatched. Calculate the percentage of sterility.

QUESTION

Is a dosage of 6000 r effective for sterilization?

22. THE 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 will be treated in various ways – topically, by injection or through residues, and the efficacy of the various treatments will be compared.

PROCEDURE

A. Topical application

Groups of 30 1-d old unmated males and 25 1-d old unmated females are treated topically by means of a microapplicator at the following concen-

trations of the chemosterilant tepa in an acetone solution: at 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 then 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 cages containing the treated females. When the females are 6 d old, they are allowed to oviposit over a period of 3 h. The checks for egg hatch and for insemination are carried out in the same way as described in Experiment 20. Once the data have been obtained, plot the per cent lethality as indicated by the hatch versus the concentration of the chemosterilant for both sexes.

B. Injection

By means of a micro-applicator the same number 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 the males are injected with the modified saline solution only and the females are also injected with a modified saline solution. The rest of the test is carried out in exactly the same way as in A above.

C. Residue applications

The interiors of pint jars are treated with 5, 10, 25, 50 and 100 mg ft^2 of tepa and allowed to stand for 24 h. Groups of flies as described under A are anaesthetized and 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 h, the flies are permitted to escape from the jars into holding cages containing food and water. On the 3rd day, 30 virgin males and 25 virgin females are introduced to the respective treated cages for the crosses. The rest of the experiment is handled in the same way as in Part A.

QUESTIONS

(1) Compare the percentage of dominant lethals obtained by the different methods of application of the chemosterilant. Explain any differences observed. Which treatment is the most effective? Why?
(2) What effect does the sex of the fly have on the results of the treatment? Why?

23. EFFECT OF A CHEMOSTERILANT ON SEXUAL COMPETITIVENESS OF MALE HOUSE FLIES

PURPOSE

To determine whether any deleterious effect is produced by the chemosterilant in the males as to affect their sexual competitiveness, thereby reducing the sterilant impact of their introduction into a natural population.

PROCEDURE

Newly enclosed adult male flies are offered fly food containing 0.25% tepa for 3 d. The treated males are confined with normal males in ratios of 1:1, 2:1, and 5:1. Five females are introduced into each of the mating cages 1 h later. To determine if the males are sterile, a cross of treated males and normal females is run. Normal males and normal females are run concurrently to establish a natural sterility base line. Two replications of each ratio will be set up using the following number of flies:

Sterile ♂	Normal ♂	Normal ♀
--	5	5
5	–	5
5	5	5
10	5	5
25	5	5

The flies are allowed to mate and three days later moistened CSMA larval medium is placed in the cages and females allowed to oviposit. The eggs from the individual cages are floated in water and 300 eggs selected randomly are placed on moistened black cloth patches. The following day the number of eggs that hatched is determined.

Schedule

Day 1 a.m. Instructor prepares treated diet. Tepa is incorporated in the fly diet as acetone solution, allowed to dry overnight in the hood, then repulverized.

Day 2 a.m. Instructor sexes flies upon emergence to assure that insects have not mated.

Day 2 p.m. Students set up cages with treated or untreated food and introduce sexed flies.

Day 5 p.m. Students remove flies from cages and make proper crosses.

Day 9 a.m. Instructor initiates egging procedure for competitive tests.

Day 9 p.m. Students collect eggs and prepare 100 egg samples from each test cage.

Day 10 p.m. Students check hatch of eggs collected previous day and calculate percentage sterility obtained.

QUESTIONS

(1) What is the effect of increasing the ratio of treated males to normal males?
(2) Is the effect more or less than the theoretical? Explain.

24. EFFECT OF 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 h 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. Normal males and normal females are run concurrently to establish a natural sterility base line. Two replications of each ratio will be set up using the following number of mosquitoes:

Sterile ♂	Normal ♂	Normal ♀
---	25	25
25	---	25
25	25	25
50	25	25
125	25	25

The mosquitoes are allowed to mate for 4 d, after which time guinea pigs are offered to the females for blood feeding. The guinea pigs are placed in the mosquito cage for 1 h. 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, flooded, and the hatching rate determined.

Schedule

Because the experiment is of such a prolonged nature, the instructor will perform the initial phases at the laboratory.

Day 1 a.m. Instructor sets eggs in order to obtain adults of the proper age.

Day 8 and 9 a.m. Instructor separates pupae from larvae and later sexes newly emerged adults, to ensure that all test insects have not mated.

Day 10 a.m. Instructor exposes virgin adults to tepa-treated jars for 4 h.

Day 10 p.m. Instructor makes necessary mating crosses.

Day 14 a.m. Students feed the mosquitoes on a guinea pig since a blood meal is necessary for oviposition.

Day 16 p.m. Students egg the mosquitoes.

Day 23 p.m. Students collect egg samples and check hatching rate.

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 23 with house flies?

25. EFFECT OF STERILIZING TREATMENTS ON REPRODUCTIVE FUNCTIONS OF THE HOUSE FLY

PURPOSE

Often it becomes necessary 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. This information can be derived by conducting the simple experiment described below.

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 jeweller's 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[2] on a depression slide, and the spermathecae and ducts are dissected from the adjoining tissue under 10 to 15 × 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.

26. EFFECT OF STERILIZING TREATMENTS ON CHROMOSOMES OF THE HOUSE FLY

PURPOSE

A simple technique has been developed whereby consistently good chromosome preparations from gonads of adult female house flies can be obtained to determine whether the irradiation or chemosterilant has produced any effect on the chromosomes.

PROCEDURE

(1) Tissue is dissected out and placed in 1.0% hypotonic solution of sodium citrate for 10 min.
(2) Tissue is soaked in modified Carnoy's fixative for 5 min.
(3) Tissue is briefly washed in 45% glacial acetic acid.
(4) Tissue is placed in a drop of 45% glacial acetic acid on a glass slide.
(5) Tissue is covered with a siliconized cover slip and a piece of filter paper.
(6) Gentle pressure is applied to filter paper to squash tissue and then cover slip is gently tapped.
(7) The slide is placed on dry ice for 30 min.
(8) The cover slip is removed with chilled razor blade.
(9) Slide is immersed in 95% ethyl alcohol in a coplin jar for 5 min.
(10) A drop of Gurr's Natural Orcein and fast green stain is placed on the tissue while the slide is still damp.
(11) Tissue is covered with a siliconized cover slip.
(12) Excess stain is removed with filter paper.

---

[2] Ringer's solution: 0.1 g potassium chloride (KCl)
0.0135 g calcium chloride ($CaCl_2$)
0.0120 g sodium bicarbonate ($NaHCO_3$)
0.75 g sodium chloride (NaCl)
Dissolve in sufficient distilled water to make 100 ml of solution.

(13) Mount is now temporary but will last for months if cover slip is ringed with warm balsam–paraffin.

To make the slide permanent, ignore step No. 13 and proceed to step No. 14.

(14) Slide is placed on dry ice again for 1 to 8 h.
(15) Cover slip is removed with a chilled razor blade.
(16) Slide is bathed in 95% ethyl alcohol for 5 min and excess alcohol allowed to drain.
(17) Slide is transferred to absolute ethyl alcohol for 1 min 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) Mount is allowed to dry for 24 h before use.

27. TAGGING ADULT HOUSE FLIES FOR ESTIMATING POPULATION DENSITY BY THE ISOTOPE DILUTION METHOD

INTRODUCTION

Estimations of insect populations in certain areas are important since they allow 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 density is a method by which many problems can be solved.

PURPOSE

Instead of a field experiment that requires much preparation and field work, this experiment uses large numbers of insects and special equipment for training in these techniques by simulated field experiments under laboratory conditions. 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 × 3 ft × 3 ft).
(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 500 newly hatched house flies of mixed sexes but do not count the exact number.
(5) Milk and sucrose solution.
(6) About 1 mc of P^{32} as sodium phosphate.
(7) Tweezers.
(8) Waxed cardboard cups.

(9) Filter paper.
(10) Small fly traps.

PROCEDURE

1. Tagging the house flies

An exact known number of house flies (500) are provided in the small cage (filter paper on the bottom) with radioactive food. Milk, sugar solution and P^{32} (total amount circa 25 ml) are well mixed in the 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 inactive milk or sugar solution to the remainder of the radioactivity. Thus more P^{32} is available to the flies.

Check after 24 h some captured individuals under the scaler for cpm.

Take out of the cage the radioactive-contaminated feeding cup, the filter-paper that might have been contaminated by excrement, etc., of the flies. This has to be done to prevent contamination of the unknown population.

2. Release of the labelled individuals in a population of unknown density

The small cage is introduced totally into the big one containing about 500 newly hatched flies. Check for dead flies, take these out and subtract their number, together with the flies that have been taken out for informal measurements, from the number of individual tagged flies.

Cut with scissors several openings in the cloth of the small cage. Be sure that there is a thorough mixture of the tagged and untagged flies.

3. Recaptures and measurements

The first recaptured samples should be taken using traps or a mechanical aspirator or by hand after 4–5 h, about 200 specimens being in each sample. Check individually for radioactivity.

Final recapture samples (2 × 200 specimens) should be taken after 72 h; check individually for radioactivity.

4. Calculations of population density

Density is calculated using the formula of Bailey (on estimating the size of mobile populations from recapture data)[3] as follows:

[3] Biometrika 38 (1951) 293.

$$N = \frac{T(n+1)}{t+1}$$

N = total number in the whole population
T = the number of introduced tagged individuals
n = the number of recaptured specimens
t = the number of marked individuals in the recapture sample.

(In calculations of the final recapture remember to subtract from T the number of recaptured marked individuals of the first recapture.)

Variance has to be calculated as follows:

$$V = N^2 \frac{n-t}{(n+t)(t+1)}$$

5. Final examination of the method

To obtain an idea of how to use the method, count by hand the population remaining in the cage (after killing with chloroform). Add number of recapture samples.

QUESTIONS

(1) What are some limiting factors in this technique?
(2) What are some advantages of this technique over other marking techniques?
(3) What was the maximum variation between replicates?

28. EFFECT OF ANOXIA ON RADIATION STERILIZATION OF INSECTS

INTRODUCTION

Inadequate supplies of oxygen during radiation sterilization of insects frequently result in incomplete sterilization and 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 occurs cell division in proliferating tissues slows down or comes to a halt, a condition which tends to nullify the sterilizing effects of radiation.

PURPOSE

The purpose of this experiment is to show that insects in the state of anoxia require larger dosages of gamma radiation to cause sterility than insects with an adequate supply of oxygen.

MATERIALS

(1) House fly pupae, one day from emergence (7500).
(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 same size as Petri dishes.
(8) Three binocular microscopes.
(9) Three lengths of rubber tubing for gas.
(10) Plastic bags.
(11) Pressure-reducing valves.

PROCEDURE

Divide the class into three groups, one group for each gas. Divide the fly pupae into 15 lots. Work with one gas at a time. Put fly pupae in unsealed gas-tight containers and put containers in plastic bag. Fill bag with gas (5 litres/min for 5 min, and then 1 litre/min for 25 min). Remove gas hose and close bag opening. Place lids on gas-tight containers without disturbing the gas concentration, and irradiate each of the five lots within a group at 1000, 2000, 3000, 4000 and 5000 r respectively, as follows:

CLASS

Group 1		Group 2		Group 3	
Lot (pupae)	Irradiation (r)	Lot (pupae)	Irradiation (r)	Lot (pupae)	Irradiation (r)
1	1000	1	1000	1	1000
2	2000	2	2000	2	2000
3	3000	3	3000	3	3000
4	4000	4	4000	4	4000
5	5000	5	5000	5	5000

After irradiation place pupae in cages with food and water and a properly prepared label, stating gas used and 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 dosage response linear or exponential with each of the various gases?
(2) Which gas caused the greatest depression of fertility? And which gas caused the least?
(3) What are some ways to prevent anoxia when large numbers of insects are being irradiated?

29. AUTORADIOGRAPHY

INTRODUCTION

Photographic emulsions are acted upon by radiation in a manner similar to light and form 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 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 may be determined by the darkening pattern on the film.

PURPOSE

The purpose of this experiment is to study autoradiography as an entomological technique.

MATERIALS

(1) A water-soluble compound containing a beta-emitting isotope ($H_3P^{32}O_4$ preferred).
(2) Two lots of about 500 adult house flies that have been starved for 10-12 h.
(3) Sucrose solution (about 10%).
(4) X-ray sensitive film (Eastman, no-screen).
(5) Glassware.
(6) Cages.
(7) Cellulose tape.
(8) Glass plates the same size as X-ray film or slightly larger.
(9) Forceps.

PROCEDURE

Add the labelled compound to the sucrose solution in amount to give 10^6 cpm/ml. (Approximately 1 ml for each batch of 100 flies will be required.)

Place radioactive food in cages for 1 h. 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 8 lots each containing 25 flies dorsum down on the sticky surface of the cellulose tape. Arrange the flies so that they will contact the X-ray sensitive film. Tape the flies sternum down to the film. Place glass plate on flies to assure good contact. Do not move film or glass plate.

At the end of 1, 3, 6 and 24 h remove two lots of flies from contact with the X-ray film. Develop film as convenient.

Kill second lot of flies 24 h after exposure, and repeat the exposure technique as above.

QUESTIONS

(1) Which exposure period gave the clearest resolution?
(2) Was the darkening of the film linear or exponential with respect to time?
(3) Had the flies fed uniformly?
(4) Did the flies killed 24 h after feeding on radioactive food have as much radioactivity as those killed immediately? Why? Which lot had the best distribution of radioactivity?

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