Laboratory Training Manual

on the Use of Isotopes and Radiation

in Soil-Plant Relations Research

A JOINT UNDERTAKING BY THE

INTERNATIONAL ATOMIC ENERGY AGENCY AND THE

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS

INTERNATIONAL ATOMIC ENERGY AGENCY, VIENNA, 1964
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The Agency's Statute was approved on 23 October 1956 by the Conference on the Statute of the IAEA held at United Nations Headquarters, New York; it entered into force on 29 July 1957. The Headquarters of the Agency are situated in Vienna. Its principal objective is "to accelerate and enlarge the contribution of atomic energy to peace, health and prosperity throughout the world".

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This publication may be obtained by writing to the International Atomic Energy Agency, Kärntner Ring 11, Vienna 1, Austria.
LABORATORY TRAINING MANUAL
ON THE USE
OF ISOTOPES AND RADIATION
IN SOIL-PLANT RELATIONS RESEARCH

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INTERNATIONAL ATOMIC ENERGY AGENCY
VIENNA, 1964
International Atomic Energy Agency.


166 p. (IAEA, Technical reports series no. 29)

(542.1:541.28) (621.039.8:63)
FOREWORD

The International Atomic Energy Agency (IAEA) and the Food and Agriculture Organization of the United Nations (FAO) in co-operation with local authorities in various countries have jointly sponsored international laboratory training courses on the use of isotopes and radiation in specialized fields of agriculture. Outstanding scientists from various countries have given lectures and devised and conducted the laboratory exercises; research workers from all over the world have attended these courses. In addition, under the United Nations Expanded Programme of Technical Assistance the IAEA in co-operation with host governments has conducted similar regional courses. This laboratory manual is a natural outgrowth of these activities. The contents represents the efforts not only of the IAEA and FAO Secretariats but also of the various instructors who have participated in the courses, a Special Consultant, Victor Middelboe, and a panel of scientists who met in Vienna from 3 to 7 September 1962 and revised the initial version assembled by Hans Broeshart and Chai Moo Cho of the IAEA Secretariat.

Liberal use was made of material developed during these training courses and used in other training courses, such as those sponsored by the United States Atomic Energy Commission at Oak Ridge, Tenn., Instituut voor Toepassing van Atoomenergie in de Landbouw at Wageningen, The Netherlands, and the United Kingdom Atomic Energy Authority at Harwell.

The IAEA and FAO would like to convey their thanks to all scientists who have helped make this venture possible and in particular to the following scientists who have contributed directly to the contents of the manual: S. Aronoff, Department of Biochemistry and Biophysics, Iowa State University, Ames, Iowa, United States of America; G. Bolt, Laboratory of Soils and Fertilizers, Agricultural State University, Wageningen, The Netherlands; H.J.M. Bowen, United Kingdom Atomic Energy Authority, Wantage Research Laboratory, Wantage, Berkshire, United Kingdom; F. Kalifelz, Department of Physical Biology, New York State Veterinary College, Cornell University, Ithaca, New York, United States of America; H. Laudelout, Institute of Agronomy, Catholic University of Louvain, Heverlee, Louvain, Belgium; V. Middelboe, Isotope Laboratory, The Royal Veterinary and Agricultural College, Copenhagen, Denmark; H. E. Oberländer, Federal Experimental Station for Agricultural Chemistry, Vienna, Austria; B. Ulrich, Soil Science Institute of Göttingen University, Faculty of Forestry, Hannoversch-Münden, Federal Republic of Germany; and the following members of the staff of the IAEA: H. Broeshart, C.M. Cho, G. Cook, M. Fried, E. Haunold, C.G. Lamm and R.A. Olson.

The present manual consists of two parts: a basic part which contains general information and laboratory exercises on the properties of radiation and the principles of use of radioactive tracers, and a second part which contains a series of detailed laboratory exercises in the field of soil-plant
relationships. It is intended to publish at least four additional parts on the subjects of the use of isotopes and radiation in animal science, agricultural biochemistry, entomology and plant pathology.

This manual, dealing with an important aspect of the peaceful application and use of atomic energy, should prove helpful not only to those working with the IAEA and FAO training programmes but to other research scientists dealing with the development and use of new information in agricultural science all over the world.
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SOME BASIC SYMBOLS AND UNITS
FREQUENTLY USED IN THIS MANUAL

Time (in general)
Counting time (duration of)
Sum count (accumulated during T)
Count rate of sample plus background
Count rate of background (blank sample)
Activity = amount of radioactive test
    substance expressed, e.g., as
    net count rate
Amount, or "pool", of test substance
Specific activity (of substance "A")
Activity concentration
Test substance concentration
Specific activity (of substance "A")
Number of disintegrations per minute
Counting yield (overall)
Number of radioactive atoms
Decay constant in reciprocal minutes
Distance
Thickness
"Approximately equal to"
Unit of count rate, counts per minute,
Unit of absolute activity†, curie,
Unit of specific activity, e.g.,
Unit of activity concentration
Unit of concentration
Unit of thickness, linear
    mass-thickness

\[ t \quad T \quad S \quad R = S/T \quad R - r \quad A*, B*, C* \quad A, B, C \quad s* = \frac{A*}{A} \quad a*, b*, c* \quad a, b, c \quad s* = \frac{a*}{a} \quad D* (\text{dis./min.}) \quad Y = \frac{R - r}{D* \text{ in sample}} \quad N* \quad \lambda* (D* = \lambda*N*) \quad d \quad x \quad z \quad cpm \quad c = 2.22 \times 10^{12} \text{ dis./min.} \quad cpm \text{ per unit weight of test substance} \quad cpm \text{ per ml} \quad cpm \text{ per mg gross material} \quad cpm \text{ per cm}^2 \quad M \text{ (mole/l)} \quad \text{mm, cm or dm} \quad \text{mg/cm}^2 \text{ 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. \( \mu c/\text{mmole test substance} \)) and "absolute activity concentration" (e.g. \( \mu c/\text{ml} \)) 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 $M$.

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

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 $\alpha$-, $\beta^+$-, $\beta^-$-, and $\gamma$-rays. $\alpha$-particles are fast-moving He nuclei, each containing two protons and two neutrons. $\beta^+$- and $\beta^-$- particles are, respectively, positively and negatively charged, high-speed electrons, while $\gamma$-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 ($N \approx Z$) 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 $\alpha$-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 $\alpha$-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 $\beta$-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 $\text{U}^{235}$ 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:

- $\alpha$-particle - doubly positively charged particle, containing two neutrons and two protons and originating at high speed from the nucleus;
- $\beta^-$-particle - high-speed electron from the nucleus, negatively charged;
- $\beta^+$-particle - high-speed positron from the nucleus, positively charged;
- $\gamma$-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;
1.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

\[ D^* = \frac{dN^*}{dt} = \lambda^* N^* \]

where \( D^* \) is the disintegration rate (expressed per minute) at time \( t \), \( N^* \) is the number of radioactive atoms present at time \( t \), and \( \lambda^* \) 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

\[ N^* = N_0 e^{-\lambda^* t} \quad \text{or} \quad D^* = D_0 e^{-\lambda^* t} \]

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 \( t_1 \), one obtains

\[ \frac{1}{2} D_0 = D_0 e^{-\lambda^* t_1} ; \quad \text{i.e.} \quad \lambda^* t_1 = \ln 2 = 0.693, \]

where \( t_1 \) 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 \( t_1 \) to \( \lambda^* \). The decay constant, having the dimension of reciprocal time and being generally a small number, is inconvenient for many purposes. Instead, half-life (\( t_1 \) 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 \( 3.70 \times 10^{10} \) disintegrations per second (approximately equal to the disintegration rate of 1 g of radium). One curie (c) is thus equal to \( 2.22 \times 10^{12} \) dis./min., one millicurie (mc), one microcurie (µc) and one picocurie (pc), \( 2.22 \times 10^9, 2.22 \times 10^6 \) and \( 2.22 \) dis./min., respectively.

If one has \( g^* \) grams of a radioisotope with a decay constant \( \lambda^* \) and an atomic weight of \( M \), the radioactivity expressed in curies will be as follows (\( N_0 \) is Avogadro's number):

\[ \frac{g^*}{M} \times N_0 = \text{Total number of radioactive atoms (N*)} \]

\[ \lambda^* \times \frac{g^*}{M} \times N_0 = \text{Total disintegrations per minute (D*)} \]
\[ \lambda^* \times \frac{\gamma^*}{M} \times \frac{N^0}{2.22 \times 10^{12}} = \text{Total activity in curies.} \]

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 \( 1.6 \times 10^6 \) erg.

The kinetic and total energies, respectively, of the particles and photons emitted by radioisotopes have characteristic 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 \( (E_{\text{max}}) \) that is at the disposal of the beta particle. This is because a variable part of \( E_{\text{max}} \) 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 \( E_{\text{max}} \). The beta energies given in a table or chart of isotopes are \( E_{\text{max}} \)-values; the average beta-particle
A * ON log AXIS

\[
\log A^* = \log A^*_{\text{eq}} + \text{st}
\]

Fig. 1

Decay curve of a single radioisotope

A * ON log AXIS

\[
\frac{1}{2} A^* \quad \frac{1}{4} A^* \quad \frac{1}{2} \quad \frac{1}{4}
\]

1 ON LINEAR AXIS

Fig. 2

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

Energy is usually about one third \( E_{\text{max}} \). 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
- Beta particle
- Beta particle
- Electron capture (K-capture)
- Gamma photon
- h hour(s)
- d day(s)
- y year(s)
- Ba$^{137m}$ excited Ba$^{137}$, called "metastable" because the emission of the gamma-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 \( E_{\text{max}} \), 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,

\( \mu \) is the \( \beta \)-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](Image of a graph demonstrating the transmitted \( \beta \)-radiation as a function of absorber thickness)

The curve becomes practically horizontal at "\( R \)", the "range" for beta particles with \( E_{\text{max}} \). 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
$\mu$ 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.

![Block diagram illustrating an anti-coincidence unit used as an electronic shielding against cosmic radiation](image)

**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).

A **registering unit.** This may be a scaler, i.e. a set of decades displaying the sum count or a certain fraction thereof, a count-rate meter (visible or audible), a voltmeter reading out accumulated radiation dose, a sensitive electric-current meter displaying dose rate or even a recording potentiometer.
Historically, it might be noted that 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 (H³, C¹⁴, S³⁵, Ca⁴⁵). 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.

$\alpha$- and $\beta$-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. $\gamma$- and X-rays have a much lower primary specific ionization; but at least one fast electron will be released by each photoelectric effect or Compton scattering.
(or pair production if the energy is very high), and these fast electrons will ionize just as do $\beta$-particles. Neutrons may also produce ions, directly (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 ($\alpha$, $\beta$, $e$, $\gamma$, 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 ($\gamma$, X- and hard $\beta$-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 $\beta^-$ from $^{14}$C, 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 $\beta^-$ or $\gamma$- 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 \( \tau \) 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

\[
R^+ = \frac{R}{1-R\tau}.
\]

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 cor-
rections 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 \( \mu \)sec. so that a correction table can be used. Correction is
normally not necessary unless the count rate exceeds about 2000 cpm.

\begin{align*}
\text{Numerical example:} & \quad \tau = 300 \mu\text{sec.} \\
& \quad = 5 \mu\text{min.} \quad \text{corr.} = 2.5\% \\
R = 5000 \text{ cpm}
\end{align*}

G-M counters are used most widely for the detection and measurement
of \( \beta \)-particles. For \( \gamma \)-rays they are not very effective (1-3% efficiency);
because most of the photons will penetrate the gas without any interaction.
For the detection of \( \beta \)-particles on 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 \( \gamma \)-rays
and X-rays because of the high stopping power of the solid. Their operation
is based on the following principle:

When a \( \gamma \)-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 elec-
trons in atoms of the crystal. On de-excitation these give rise to the emis-
sion 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
\( \gamma \)-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 $\gamma$-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 $\gamma$-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 $\gamma$-ray spectrometry (see section 2.5.2). A further advantage of the scintillation counter is its small dead time, of only a few $\mu$sec. 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 $\beta$-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 $^3$H (0.018 MeV) and $^{14}$C (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 wavelength 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 \(\beta\)-particle and two \(\gamma\)-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" \(\beta\)-active source were suspended on an "infinitely thin" support in the middle of the active volume of a detector, which had a \(\beta\)-detection efficiency of 100% (see Fig. 9). In this case absolute activity, \(D^*\) dis./min., would be measured.

![Fig. 9](image)

G-M-assembly of approximately 100% efficiency (4\(\pi\) 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\(^\dagger\) of the isotope in question. We then have

\[
Y = \frac{R - r}{D^*} \text{ cpm per dis./min.}
\]

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.

\(\dagger\) 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 \(R = \frac{S}{T}\). The radioactive sample is then replaced by a blank sample, and the background count rate, \(r = \frac{s}{t}\), 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

\[
A^* = (R + r)_{\text{corr}}.
\]

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 \(4\pi\) ("all directions"). For a small source close to a detector window the solid angle is about \(2\pi\) 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 \(\alpha\)-particles and very soft \(\beta\)-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/cm²) 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](image)

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 \((A^*_\infty)\) 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 III

**STANDARD DEVIATION FOR NATURAL UNCERTAINTY OF SUM COUNTS**

<table>
<thead>
<tr>
<th>The sum count $S$</th>
<th>Natural standard deviation $\sigma_{nat} = S^{1/2}$</th>
<th>Percentage N.S.D. $%\sigma_{nat} = \frac{S^{1/2}}{S} \times 100%$</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>10</td>
<td>10 %</td>
</tr>
<tr>
<td>1000</td>
<td>31.6</td>
<td>3.2%</td>
</tr>
<tr>
<td>10000</td>
<td>100</td>
<td>1.0%</td>
</tr>
<tr>
<td>100000</td>
<td>316</td>
<td>0.3%</td>
</tr>
<tr>
<td>1000000</td>
<td>1050</td>
<td>0.1%</td>
</tr>
</tbody>
</table>

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 $\sigma_{nat,S} = S^{1/2}$, and the count rate of sample plus background is

$$R = \frac{S}{T},$$

so the natural standard deviation of the count rate must be

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

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

or

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

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

$$A^* = \frac{S}{T} - \frac{s}{t} = R - r.$$
The natural standard deviation (N.S.D.) of the sample activity will then be

\[ \sigma_{nat,A^*} = \sigma_{nat,R-r} = (\sigma_{nat,R}^2 + \sigma_{nat,r}^2)\sqrt[4]{1 + \frac{R}{T + r/t}}, \]

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

A useful rule in counting states that the percentage 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:

\[ \frac{\% \sigma_{nat,R}}{\% \sigma_{nat,R}} = \frac{\sigma_{nat,R}}{R} = \frac{100\%}{RT} = \frac{100\%}{S} = \frac{100\%}{\sigma_{nat,S}}. \]

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

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

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

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

Regarding the derivation of the following approximative equation:

\[ \% \sigma_{nat,A^*} \approx \frac{100}{2T(R^4 - r^4)} \% \tag{3} \]

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

\[ \sigma_{tot,S} = \sqrt{\sum(S_i - \overline{S})^2/(n-1)} \]

is significantly greater than \( \sigma_{nat,S} = \sqrt{S} \). If no real errors have been made, this increase results from technical uncertainty, 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.

\[ \sigma_{tot}^2 = \sigma_{nat}^2 + \sigma_{tech}^2 \]

or

\[ \sigma_{tot}^2 = \sqrt{\sigma_{nat}^2 + \sigma_{tech}^2}. \text{ (see Fig. 11.)} \]
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 radio-isotopes 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).

![P-H Spectrum](image-url)
Calibration of the threshold values (i.e. P.H.) in terms of MeV is done with the help of radioisotopes emitting monoenergetic $\gamma$-photons of known energy, for instance Cr$^{51}$, Cs$^{137}$, Mn$^{54}$ or Zn$^{55}$, 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 $\alpha$-particle is ejected.

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

The $\alpha$-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 $\beta$-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 cm³ of standard air surrounded by air. As the numerical charge of an electron is $4.8 \times 10^{-10}$ esu, $\frac{1}{(4.8 \times 10^{-10})} = 2.1 \times 10^9$ ion pairs per cm³ 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 = $6.9 \times 10^4$ MeV = 0.11 erg of radiation energy absorbed per cm³ of air.

As 1 cm³ air has a weight of 0.0013 g, one röntgen will dissipate $\frac{0.11}{0.0013} = 85$ 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).

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

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 $K_\gamma$-value when the nature of the radioisotope is known (see Table III).
A "point" source of $C^*$ curies of an isotope, which emits on the average a $\gamma$-ray energy of $E$ MeV per disintegration, will generate an energy flux of

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

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

The above equation can be simplified when $C^*$ is taken as 1 c, $d$ as 100 cm (1 m), and the fraction, $\mu$, of $\gamma$-photons absorbed per cm$^3$ of air as about $33 \times 10^{-6}$ for all photon energies in the range 0.1-3 MeV. Then the specific dose rate, for $\gamma$-energies in the range 0.1-3 MeV, is $K_\gamma = 2 \times E/3 \text{ r/h}$ at 1 m from 1 c "point" source. $E$ 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 $K_\gamma$-values, together with the predominant $\gamma$-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 $D = \text{accumulated doses of radiation expressed in REM}$, 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

**Kₘ Values at Various γ-Photon Energies**

<table>
<thead>
<tr>
<th></th>
<th>Approx. $K'_γ$ - value (r/h at 1 m from 1 c)</th>
<th>Predominant γ-photon energy (MeV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na²²</td>
<td>1.2</td>
<td>1.3 and 0.5†</td>
</tr>
<tr>
<td>Na²⁴</td>
<td>2</td>
<td>2.8 and 1.4</td>
</tr>
<tr>
<td>Mg²⁸ (+ equil. Al²⁸)</td>
<td>1.6</td>
<td>1.8 and 1.4</td>
</tr>
<tr>
<td>K⁴²</td>
<td>0.15</td>
<td>1.5</td>
</tr>
<tr>
<td>Cr⁵¹</td>
<td>0.02</td>
<td>0.3</td>
</tr>
<tr>
<td>Mn²⁴</td>
<td>0.5</td>
<td>0.8</td>
</tr>
<tr>
<td>Co⁵⁸</td>
<td>0.6</td>
<td>0.8 and 0.5†</td>
</tr>
<tr>
<td>Fe⁵⁹</td>
<td>0.6</td>
<td>1.3 and 1.1</td>
</tr>
<tr>
<td>Co⁶⁰</td>
<td>1.3</td>
<td>1.3 and 1.2</td>
</tr>
<tr>
<td>Cu⁶⁴</td>
<td>0.1</td>
<td>0.5†</td>
</tr>
<tr>
<td>Zn⁶⁵</td>
<td>0.3</td>
<td>1.1</td>
</tr>
<tr>
<td>Br⁸²</td>
<td>1.5</td>
<td>1.5-0.6</td>
</tr>
<tr>
<td>Rb⁸⁶</td>
<td>0.05</td>
<td>1.1</td>
</tr>
<tr>
<td>Zr⁹⁵</td>
<td>0.4</td>
<td>0.8 and 0.7</td>
</tr>
<tr>
<td>I¹³¹</td>
<td>0.2</td>
<td>0.4</td>
</tr>
<tr>
<td>Cs¹³⁷ (+ equil. Ba¹³⁷m)</td>
<td>0.3</td>
<td>0.7</td>
</tr>
<tr>
<td>Ta¹⁸¹</td>
<td>0.7</td>
<td>1.2 and 0.2</td>
</tr>
<tr>
<td>Au¹⁹⁸</td>
<td>0.3</td>
<td>0.4</td>
</tr>
<tr>
<td>Ra²²⁶ (+ equil. decay chain) with 0.5-mm Pt cover</td>
<td>0.825</td>
<td>many different</td>
</tr>
</tbody>
</table>

† 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\textsuperscript{90}, Ca\textsuperscript{45} and Sr\textsuperscript{89} accumulate in bones and produce damage to the blood-producing cells. I\textsuperscript{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\textsuperscript{14} are usually only slightly toxic because of their rapid biological turnover. However, H\textsuperscript{3} and C\textsuperscript{14} can be very toxic under conditions of slow turnover, (e.g. in nucleic acids), or, for example, as BaC\textsuperscript{14}O\textsubscript{3} dust lodged in the lungs.

### Table IV

**DANGER OF ISOTOPES INGESTED BY THE HUMAN BODY**

<table>
<thead>
<tr>
<th>Isotope</th>
<th>Very highly toxic</th>
<th>Highly toxic</th>
<th>Moderately toxic</th>
<th>Slightly toxic</th>
</tr>
</thead>
<tbody>
<tr>
<td>S\textsuperscript{80}</td>
<td>Ca\textsuperscript{45}, Sr\textsuperscript{89}, Ba\textsuperscript{140}, I\textsuperscript{131}</td>
<td>Na\textsuperscript{24}, S\textsuperscript{35}, K\textsuperscript{42}, Mn\textsuperscript{52}, Mn\textsuperscript{54}, Mn\textsuperscript{56}, Fe\textsuperscript{55}, Co\textsuperscript{58}, Co\textsuperscript{60}, Zn\textsuperscript{65}, Br\textsuperscript{85}, Rb\textsuperscript{86}, Mo\textsuperscript{99}, Cs\textsuperscript{137}, Ba\textsuperscript{137}</td>
<td>H\textsuperscript{3}, C\textsuperscript{14}</td>
<td></td>
</tr>
</tbody>
</table>

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 $I_j$ at a distance of $d$ cm from the source will be $I_j = I_0 / d^2$, in which $I_0$ 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 milliröntgen 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 $10000 \text{mr/h} = 10 \text{r/h}$ 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 $15 - 20 \text{mr/week}$ would then be received by parts of the body (e.g. the lenses of the eyes), which is below the maximum permissible dose of $100 \text{mr/week}$. 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 $\alpha$-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 $\beta$-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 $\beta$-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 $\gamma$-rays are dealt with. The thickness of lead that is required to halve the dose rate from a given $\gamma$-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:

$$F = 2^n, \quad \text{or} \quad n = \log_{10} F / 0.3.$$ 

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".
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.
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.

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.
  - 10% nitric acid, sodium metasilicate or sodium metaphosphate.

- **Aluminium**
  - Phosphoric acid plus a spreading agent.

- **Steel**
  - Treatment with 4 N hydrochloric acid until the reaction starts. Wash well with a dilute alkaline solution, and finish with water.

- **Lead**
  - Remove wax surface with xylol or trichlorethylene.

- **Linoleum**
  - Wash with spreading agent and ammonium citrate or ammonium bifluoride.

- **Painted surfaces**
  - 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**

<table>
<thead>
<tr>
<th>Radiotoxicity of isotopes</th>
<th>Minimum significant toxic quantity</th>
<th>Type of laboratory or working place required</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Type C Good chemical laboratory</td>
</tr>
<tr>
<td>Very high</td>
<td>0.1 µc</td>
<td>10 µc or less</td>
</tr>
<tr>
<td>High</td>
<td>1.9 µc</td>
<td>100 µc or less</td>
</tr>
<tr>
<td>Moderate</td>
<td>10 µc</td>
<td>1 mc or less</td>
</tr>
<tr>
<td>Slight</td>
<td>100 µc</td>
<td>10 mc or less</td>
</tr>
</tbody>
</table>

A "C" laboratory may be any ordinary laboratory that has a good ven-
tilating system and a fume cupboard. Floors and benches should have a
surface which can be cleaned easily. It will, however, be essential to pro-
vide 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 cir-
cumstances 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).

\[ A = \frac{A^* \text{ cpm}}{s^* \text{ cpm per unit of } "A"} \]  \hspace{1cm} (1)

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
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

$$s^* = \frac{A^*}{A_{big}^*}, \tag{2}$$

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

$$\frac{b^*}{s^*} = \frac{b}{1} \text{ or } b = \frac{b^*}{s^*}. \tag{3}$$

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 $B_a$ 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,

$$\frac{s_a^*}{s_e^*} = \frac{B_a}{B_e} = \frac{B + B_a}{B_a},$$

therefore

$$B = B_a \left( \frac{s_a^*}{s_e^*} - 1 \right). \tag{1}$$

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

$$s^e \overset{\text{def}}{=} \frac{B^e_*}{B^e_a} = \frac{B^e_*}{B^e_*(\text{cpm per mmol})}.$$  

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

$$B^e_*= B^e_*/s^e.$$  (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 $B^e$, the amount of added test substance by $B^e_a$ and the specific activities of the test substance, before and after equilibrium mixing with the added test substance, by $s^e_*$ and $s^e_*$, respectively, one gets from the dilution principle

$$\frac{s^e_*}{s^e_c} = \frac{B^e_c}{B^e} = \frac{(B^e + B^e_a)/B^e}{(s^e_* - 1)}.$$  (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 $A^e_*(\text{cpm})$ of a dissolvable isotope, "$A^e$", is added to an unknown volume, $V \text{ ml}$, of a non-radioactive liquid. In this case, the liquid is considered to be the test substance. Accordingly, the activity concentration $a^e_*$ could also be considered to be the specific activity $s^e_*$ defined with

![Fig. 14](image_url)

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)):

$$V = \frac{A^*}{a^*_{e}} = \frac{A^*}{s^*_{e}} \text{ ml.}$$

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

$$V_e = \frac{A^*}{s^*_{e}} \text{ (ml),}$$

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:

$$V = V_e - V_a = V_a s^*_{a} / s^*_{e} - V_a = V_a \left(\frac{s^*_{e}}{s^*_{a}} - 1\right),$$

which is analogous to equation (1).

![Fig. 15](image)

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 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

$$A_e = \frac{A^*}{s^*} \text{ (meq.)},$$

where $A^*$ is the total activity added and therefore present in the mixture and $s^*$ 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 $s^*_e$, we have

$$\begin{align*}
A^*_a &= V_a a^*_a \\
\frac{s^*_e}{a^*_e} &= \frac{a^*_e}{a^*_a}
\end{align*}$$

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).
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

$$\gamma_{\text{ads}}^+ \overset{\text{def}}{=} C - Vc_0,$$

$$\gamma_{\text{rep}}^- \overset{\text{def}}{=} Va_0 - A = Vc_0 - A,$$

where $V$ is the true volume of the solution (see Fig. 17) and $c_0 = c_0$ is the ion concentration in the electro-neutral part of the solution (not too near the bottom), then the "cation adsorption capacity", $\gamma^+$, taken as being the sum of $\gamma_{\text{ads}}^+$ and $\gamma_{\text{rep}}^-$, 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: \( c^* \), \( a^* \), \( c_e \) and \( a_e \), it will however be true, as in Example (3), that

\[
C_e = V^+_a (c^*_e / c^*_a) c_e, \quad \text{and} \\
A_e = V^-_a (a^*_a / a^*_e) a_e,
\]

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:

\[
\begin{align*}
V^+_\text{dis} & \overset{\text{def}}{=} \frac{C}{c_0} = \text{apparent volume of even distribution of cation;} \\
V^-_{\text{dis}} & \overset{\text{def}}{=} \frac{A}{a_0} = \text{apparent volume of even distribution of anion;} \\
V^+_{\text{acc}} & \overset{\text{def}}{=} V^+_\text{dis} - V = \text{apparent volume of accumulation of cation;} \\
V^-_{\text{exc}} & \overset{\text{def}}{=} V - V^-_{\text{dis}} = \text{apparent volume of exclusion of anion.}
\end{align*}
\]

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:

\[
\gamma^+_\text{ads} \overset{\text{def}}{=} C - V^-_0 \times c^-_0 - V^+_0 \times c^+_0 = V^+_{\text{acc}} \times c_0.
\]

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 \( k_2 \). The 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

\[
\frac{-dA_1}{dt} = k_1 A_1 + k_2 A_1 = k_1 A_1 \tag{1}
\]

where \( k_1 = k_1 + k_2 \) 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

\[
A_1 = A_{1,0} e^{-k_1 t} \tag{2}
\]

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

\[ A_{2, \infty} = \left( \frac{k_1^*}{k_1} \right) A_{1, 0} \]  

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.

\[ A_2 = \left( \frac{k_1^*/k_1} \right) (A_{1, 0} - A_1) \]

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

\[ A_2 = A_{2, \infty} \left( \frac{k_1^*/k_1} \right) A_1 \]

Solving this equation with respect to \( A_1 \), one finds

\[ A_1 = \left( \frac{k_1}{k_1^*/k_1} \right) (A_{2, \infty} - A_2) \]  

The rate of accumulation in phase 2 is given by

\[ \frac{dA_2}{dt} = k_1 A_1 \]

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

\[ \frac{dA_2}{dt} = k_1 (A_{2, \infty} - A_2), \text{ or } -\frac{d(A_{2, \infty} - A_2)}{dt} = k_1 (A_{2, \infty} - A_2). \]

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

\[ A_{2, \infty} - A_2 = A_{2, \infty} e^{-k_1 t} \]  

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.

\[ k_1^* = \frac{k_1 A_{1, 0}}{A_{1, 0}} \]

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:
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.

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 $A^0$ mole stable isotope and $A^*$ mole radioisotope (see Fig. 20). In practice, $A^*$ will be a very small fraction of $A$; hence $A^0$ will remain practically constant.

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

$$-\frac{dA^*}{dt} = kA^*,$$

and by integration this gives

$$A^* = A^* e^{-kt}.$$  \hspace{1cm} (7)
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):

$$a^* = a^*_0 e^{-kt} \text{ (cpm per ml)}$$  \hspace{1cm} (7a)

$$s^* = s^*_0 e^{-kt} \text{ (cpm per mole)}$$  \hspace{1cm} (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.

$$A^*_{1} + A^*_{2} = A^*_{tot}.$$  \hspace{1cm} (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.
PHASE 1;

\[ \frac{A_1}{A_{1,0}} \]

PHASE 2:

\[ \frac{k_1 A_1}{k_2 A_2} \]

\[ A_2 (\text{ALL STABLE}) \]

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:

\[ k_2 = \frac{k_1 A_1}{A_2} \]  \hspace{1cm} (9)

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

\[ -\frac{dA_1^*}{dt} = k_1 A_1^* - k_2 A_2^* \]

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

\[ -\frac{dA_1^*}{dt} = \frac{k_1 A_1^*}{A_{1,0}} - k_1 \frac{A_1}{A_2} A_{1,0}^* \]

or

\[ -\frac{dA_1^*}{dt} = \frac{[(A_1 + A_2)/A_2] k_1 A_1^*}{A_{1,0}} - k_1 \frac{A_1}{A_2} A_{1,0}^* \]

or

\[ -\frac{dA_1^*}{dt} = \frac{[(A_1 + A_2)/A_2] k_1 (A_1^* - [A_1/(A_1 + A_2)] A_{1,0}^*)}{A_{1,0}} \]

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

\[ A_1^* - \frac{A}{A_1 + A_2} A_{1,0}^* = (A_{1,0}^* - \frac{A}{A_1 + A_2} A_{1,0}^*) e^{-(A_1 + A_2)/A_{1,0}^*} \]  \hspace{1cm} (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

\[ A_{1,\infty} / A_1 = A_{1,\infty} / (A_1 + A_2) \]

\[ A_1 / (A_1 + A_2) = A_{1,\infty} / A_{1,0}^* \]  \hspace{1cm} (11)
Introduction of equation (11) into (10) yields

\[ A_{1}^{*} - A_{1,\infty}^{*} = (A_{1,\infty}^{*} - A_{1,0}^{*})e^{-k_{1}^{*}t_1} \],

where \( k_{1}^{*} = [(A_{1} + A_{2})/A_{1}]k_{1} \). 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](image)

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:

\[ k_{1} = [A_{2}/(A_{1} + A_{2})]k_{1}^{*} = [1 - A_{2}/(A_{1} + A_{2})]k_{1}^{*} = (1 - A_{1,\infty}^{*}/A_{1,\infty}^{*})k_{1}^{*} \],

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

\[ k_{1} = [(A_{1,\infty}^{*} - A_{1,\infty}^{*})/A_{1,\infty}^{*}]k_{1}^{*} \]  \( \text{ (13)} \)

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:

\[ a_{1}^{*} - a_{1,\infty}^{*} = (a_{1,0}^{*} - a_{1,\infty}^{*})e^{-k_{1}^{*}t_1} \]  \( \text{(12a)} \)

\[ s_{1}^{*} - s_{1,\infty}^{*} = (s_{1,0}^{*} - s_{1,\infty}^{*})e^{-k_{1}^{*}t_1} \]  \( \text{(12s)} \)

\[ k_{1} = k_{1}^{*}(a_{1,0}^{*} - a_{1,\infty}^{*})/a_{1,0}^{*} = k_{1}^{*}(s_{1,0}^{*} - s_{1,\infty}^{*})/s_{1,0}^{*} \]. \( \text{(13a and 13s)} \)
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 $\text{O}^{17}$ by bombarding $\text{N}^{14}$ with $\alpha$-particles ($\gamma\text{N}^{14} + 2\text{He}^{4} \rightarrow 8\text{O}^{17} + \text{p}^{1}$) can, therefore, be written as $\text{N}^{14}(\alpha, p)\text{O}^{17}$.

The probability of a nuclear process is generally expressed in terms of a cross-section $\sigma$, 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 $\sigma$ is defined by the following equation:

$$-dI = \ln n \cdot d\tau,$$

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 $\sigma$. If we assume that $\sigma$ 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),

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

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

$$I_0 - I = I_0 (1 - e^{-n\sigma d}).$$

$\sigma$ is a characteristic of the reaction and the target nuclei.

Bombarding particles which have been used to effect nuclear reactions are neutrons, protons, deuterons, $\text{H}^3$, $\text{He}^3$, $\text{He}^4$, photons, electrons and others. Among the particles, the neutron is extensively used because of its ready availability from a reactor. The $(n,\gamma)$ reaction is the only type commonly occurring with slow neutrons. This reaction occurs with very nearly every target. After the emission of $\gamma$ 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:

\[ D = N \sigma (1 - e^{-\lambda t})E, \]

where
- \( D \) is the total number of disintegrations per second,
- \( N \) is the total number of target nuclei,
- \( \sigma \) is the cross-section of the element in cm\(^2\)/atom,
- \( \lambda \) 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.

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:

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

$$^2\text{He}^4 + ^4\text{Be}^9 \rightarrow ^6\text{C}^{12} + ^6\text{N}^1.$$ (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\textsuperscript{10} enriched BF\textsubscript{3} gas, in which the following nuclear reaction occurs:

\[ _{0}n^{1} + _{5}B^{10} \rightarrow _{3}Li^{7} + _{2}He^{4}. \]

(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\textsuperscript{3} of soil with normal moisture content, these elements, usually in concentrations on the order of a gram per m\textsuperscript{3}, have no influence as a rule. However, boron (19% B\textsuperscript{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\textsuperscript{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 (U₃O₈). This combines the required chemical stability and long half-life (4.5 x 10⁹ 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/cm²).

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" (Vₜ).
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 Vₚ 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), V₂ - V₁.
   The slope is
   
   \[
   \frac{(R₂ - R₁)/Rₚ}{(V₂ - V₁)/100} \times 100\%
   \]
   in which Rₚ = the count rate at the working voltage (see below).
As the counter ages, the threshold voltage \( V_T \) tends to increase and the racing voltage \( V_R \) to decrease. To allow for this, choose the working voltage at \( V_T + 75 \) volts or \( \frac{1}{2}(V_T + V_R) \) if the plateau 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 \( \tau \) 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:

\[
R^+ = \frac{R}{1 - R \tau}.
\]  

(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 \( \tau \) can then be estimated approximately with the aid of equation (1). (At very high count rates, it may turn out that \( \tau \) is no longer constant but equal to some function of \( R \).)

Another approximation of the dead time \( \tau \) 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)

$$\frac{R_1}{1 - R_1 \tau} + \frac{R_2}{1 - R_2 \tau} = \frac{R_{12}}{1 - R_{12} \tau} + \frac{r}{1 - r \tau}.$$ 

Since $R_1 \tau \ll 1$ and $r \tau \ll R_1 \tau$, the following approximations can be made:

$$\frac{R_1}{1 - R_1 \tau} \approx R_1 + R_1 \tau, \quad \frac{r}{1 - r \tau} \approx r.$$

Therefore, after substituting, we obtain

$$\tau \approx \frac{(R_1 + R_2 - R_{12} - r)}{(R_1 + R_1 \tau - R_2)} \quad (2)$$

or

$$\tau \approx \frac{(R_1 + R_2 - R_{12} - r)}{2R_1 R_2} \quad (3)$$

since

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

**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/cm$^2$, 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 $\beta$-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 $\tau$ 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 \( \sigma \) 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 \( \sigma_{\text{nat}} = S \), 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) \( \text{P}^{32} \) solution containing on the order of 0.05 \( \mu \text{c}/\text{ml} \).
(2) 1-ml pipette, and a pro-pipette (e.g. rubber bulb).
(3) 25 counting cups.

PROCEDURE

(1) Obtain a bottle of \( \text{P}^{32} \) 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 \( S_{\text{nat}} \) and the total standard deviation according to the following formula:

\[
\sigma_{\text{tot}} = \sqrt{\left\{ \sum (S - \bar{S})^2 / (n-1) \right\}}
\]

(5) Compare this \( \sigma_{\text{tot}} \) and \( \sigma_{\text{nat}} \), and if they are found to be significantly different, explain. Calculate \( \sigma_{\text{nat}} \) in per cent.

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(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 $\sigma_{tech}$ in per cent, using the equation

$$\sigma_{tot}^2 = \sigma_{nat}^2 - \sigma_{tech}^2.$$

(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 $\beta$-particles

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

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

**PROCEDURE**

(1) Pipette 100 $\mu$L of a solution containing approximately 0.1 $\mu$C $^{32}$P/ml into a counting cup, and dry under an infra-red lamp.

(2) Prepare a second sample by pipetting 100 $\mu$L of a solution containing approximately 50 $\mu$C $^{32}$P/ml into a counting cup and drying.

(3) Count the weak source until $10^4$ counts are registered. Place an aluminium filter of about 20 mg/cm$^2$ between the counter window and the source, and count again.

(4) Continue counting at increasing absorber thickness until a count rate of about 200 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 $\beta$-emitters.)
(8) Deduct the extrapolated bremsstrahlung from the net count rate, and plot the corrected curve.

(9) Judge by inspection the point at which the uncorrected transmission curve appears to run into the almost horizontal bremsstrahlung line. This point corresponds to the "range" and should be on the order of 800 mg/cm$^2$ for $^{32}$P.

(10) Repeat the exercise, using $^{14}$C instead of $^{32}$P. The "range" should then be about 30 mg/cm$^2$.

(11) The corrected curve should in both cases asymptotically approach a vertical line through the "range" point, as the beta particle transmission becomes zero (i.e. as the log becomes $-\infty$; see Lecture Matter, section 1.4.2).

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

1.5. Self-absorption and self-scattering of $\beta$-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 $\beta$-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 $\gamma$-emitting sources but is usually unimportant since this radiation has a greater power of penetration and the sources are relatively small.) A third source of error when voluminous samples of varying thickness are involved may be called "self-geometry", i.e. the top of the sample 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-$\beta$ samples of varying thickness may be circumvented, because only relative values are needed for the experiment. For instance, many experiments are based on formulae in which activity measurements are only entered in ratios such as $a_1^\gamma/a_2^\gamma$ (or $s_1^\gamma/s_2^\gamma$, but this again equals $a_1^\gamma/a_2^\gamma$ divided by $a_1/a_2$), where $a_1^\gamma$ and $a_2^\gamma$ are the activity concentrations of test substance in two samples. If in such a case the tracer is a low-energy $\beta$-emitter and the samples are unequal in thickness, their count rates cannot in general be entered directly; however, correction to "true" count rates need not be attempted, because correct relative 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 curve 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):

\[
a^*_{rel} = \frac{\text{net count rate of exp. sample of wt.} \ w}{\text{net count rate of ref. sample of wt.} \ w}
\]

When corrections for self-weakening to yield the "true" count rates of \(\beta\)-samples are necessary, two different methods are possible. On the one hand, using the material in question, one may prepare a set of increasingly thick samples of constant 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.

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 \( a^* = \text{number of } \beta-\text{particles per cm}^3 \text{ sample ejected in the direction of the G-M tube}, \)

\[ \mu = \text{linear absorption coefficient (characteristic of } E_{\beta_{\text{max}}} \text{ and density of sample material)}, \]

\[ x = \text{sample layer (cm)}; \]

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 \( \ell \) 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

\[
A^*/A_0^* = f = (1 - e^{-\mu X})/\mu X,
\]

where \( \mu \) may be taken as the mass absorption coefficient (characteristic of \( E_{\beta_{\text{max}}} \) 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 \( A_\infty^* \) as \( X \) approaches \( \infty \). In practice \( A_\infty^* \) becomes constant at a thickness equal to about half the "range" of the \( E_{\beta_{\text{max}}} \) of the \( \beta \)-particles. A sample of this, or greater, thickness is called "infinitely thick". It is well known that the net count rates of infinitely thick samples are proportional to the 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 infinitely thick samples).

In the first of two experiments, a series of sources will be prepared by precipitation of increasing weights of CaCO\(_3\) from a solution containing 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 \( \mu \)C 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

<table>
<thead>
<tr>
<th>Source No.</th>
<th>Sol. B (ml)</th>
<th>Sol. A (ml)</th>
<th>Water (ml)</th>
<th>5 N NH(_4)OH (ml)</th>
<th>1 M Na(_2)CO(_3) (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>-</td>
<td>7</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>-</td>
<td>6</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>-</td>
<td>4</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>6</td>
<td>-</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
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<td>10</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>6</td>
<td>-</td>
<td>1.5</td>
<td>10</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>7</td>
<td>-</td>
<td>2</td>
<td>10</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>8</td>
<td>-</td>
<td>3</td>
<td>25</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>9</td>
<td>-</td>
<td>4</td>
<td>25</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>10</td>
<td>-</td>
<td>5</td>
<td>25</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

(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\(_3\)OH). 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 CaCO₃ 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 on for a few minutes until the CaCO₃ precipitate on the filter paper is dry.

(9) Remove the filter paper at its CaCO₃-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 Ca⁴⁵ labelled samples of CaCO₃ 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 CaC¹⁴O₃

This procedure includes the conversion of BaC¹⁴O₃ to C¹⁴O₂, which is then precipitated as CaC¹⁴O₃. (There appear to be no obvious advantages to the precipitation of CO₂ as either the Ba or Ca salt for the purposes intended.) This complete system is normally not required; i.e. in practice the C¹⁴O₂ evolved from a system may simply be caught in the observing system and precipitated. On the other hand, on occasion contamination of the CaCO₃ or BaCO₃ may be suspected, e.g. with BaSO₄ (from SO₃) 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

\[
\text{BaC}^{14}\text{O}_3 + \text{H}^+ \rightarrow \text{C}^{14}\text{O}_2 + \text{Ca}^{2+} + \text{H}_2\text{O} \quad \text{CaC}^{14}\text{O}_3
\]

(1) Assemble an open system consisting of a reaction flask (main reaction flask plus funnel), connected to a gas-washing (CO₂-absorbing) bottle. The entire system is flushed with either N₂ or CO₂-free air. (See Fig. 27).

(2) Put N/10, CO₂-free NaOH in the gas-washing bottle and place in the main flask approximately 200 mg of BaC¹⁴O₃ containing about 0.2 μc C¹⁴. Into the funnel of the reaction vessel place 2-3 ml of 10% HClO₄ (perchloric acid).
(3) Sweep the system with CO$_2$-free gas at such a rate that discrete bubbles are produced in the gas-washing bottle.

(4) Add the acid to the reaction vessel portion-wise, so that CO$_2$ is not formed at an excessive rate. Continue gas-sweeping 10 min. beyond the final addition of acid.

(5) Remove the NaOH solution containing the C$^{14}$O$_2$ 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$_2$O and recentrifuge. Again pour off the supernatant, resuspend the precipitate in abs. CH$_3$OH and centrifuge. Pour off the supernatant; suspend in CH$_3$OH 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:

- \( \alpha \)-radiation: Zinc sulphide crystals spread thinly \((10 - 20 \text{ mg/cm}^2)\).
- \( \beta \)-radiation: Anthracene, or naphthalene containing 0.1% of anthracene, in the form of a large crystal. Recently, plastic scintillators have been developed.
- \( \gamma \)-radiation: Sodium iodide, activated by about 1% of thallous iodide, in the form of a transparent, single crystal, cut to the required size.

Scintillation detectors have three advantages over G-M tubes for counting \( \gamma \)-photons. These are

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

The \( \gamma \)-crystal is hygroscopic and therefore caséd 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 \( \gamma \)-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 \(R^+ - r^+\) 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 \(R^+ - r^+\) 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

\[ A^*_t = YD^*_t, \tag{1} \]

where \(Y\) is the counting yield.

\[ \dagger \] 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):

\[ D_f = D_0 e^{-\lambda t} \]

where \( D_0 \) is the disintegration rate at zero time, 
\( \lambda = \) disintegration constant \( = 0.693/t_i \), 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 \( t_i \), then the disintegration rate \( D_{f+T} \) at the end of the counting period will be significantly lower than at the beginning:

\[ D_{f+T} = D_0 e^{-\lambda (t+T)} \]

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 \( A^*_0 \) are related as follows:

\[ \bar{A}^* = \frac{Y D^*}{Y (N^*_0 - N^{*+T})/T} = Y (N^*_0 (1 - e^{-\lambda^* T})/T, \text{since (see equations (2) and (3))} \]

\[ N^{*+T} = N^*_0 e^{-\lambda^* T}, \]

hence

\[ \bar{A}^* = Y (D^*/\lambda^* T) (1 - e^{-\lambda^* T}), \]

which follows from \( D_0^* = \lambda^* N^*_0 \).

---

\[ \text{Fig. 28} \]

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

\[ \text{\dagger} \] If the counting time \( T \) is not over \( 1/4t_i \), the relation \( \bar{A}_T^* = A^*_{T+1/4} \) (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

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

where

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

With the aid of equation (4) the ratio of $A^*_i$ 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 $A^*_i : \bar{A}^*$ 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 $\gamma$-photons.

**REAGENTS AND MATERIALS**

1. SO$_3$-H type cation exchange resin (e.g. Amberlite IR-120 or Dowex 50).
2. About 200 $\mu$C Cs$^{137}$.
3. A conventional burette.
4. EDTA solution (about 0.3% adjusted to pH 11-12 with NaOH).
5. Plastic counting container.

**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 $\text{Ba}^{137m}$, against time on semi-log paper. Deduce the half-life of $\text{Ba}^{137m}$ 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 $\gamma$-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

$$I_d = \frac{k}{d^2},$$

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 $\gamma$-rays from a $5 \mu\text{c}$ $\text{Co}^{60}$ 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 $d = 20, 40, 60, \ldots 160$ 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 $\gamma$-photons is exponential. The attenuation of $\gamma$-rays from $\text{Co}^{60}$ which emits $\gamma$-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 thick-
ness on semi-log paper.

(6) Determine the half-thickness of lead for Co\(^{60}\) \(\gamma\)-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
\(\gamma\)-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 coinci-
dence 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 photomulti-
plier 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-
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

<table>
<thead>
<tr>
<th>Dioxane</th>
<th>5 parts 417 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellosolve†</td>
<td>1 part 83 ml</td>
</tr>
<tr>
<td>PPO (2-5 diphenyloxazole)</td>
<td>1% w/v ‡ 5 g 15 ml/vial</td>
</tr>
<tr>
<td>Naphthalene</td>
<td>5% w/v 25 g</td>
</tr>
<tr>
<td>POPOP (1-4-bis-2-5-phenyloxazolyl)</td>
<td>0.25 g</td>
</tr>
<tr>
<td>Benzene</td>
<td></td>
</tr>
</tbody>
</table>

Labelled compounds

Cl⁴ toluene, supplied as solution A
H³ toluene, supplied as solution B

TABLE VIII

SOLUTIONS FOR COUNTING VIALS

<table>
<thead>
<tr>
<th>Counting vial</th>
<th>Sol. A (ml)</th>
<th>Sol. B (ml)</th>
<th>Toluene (ml)</th>
<th>Solvent system (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.2</td>
<td>1.8</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0.4</td>
<td>1.6</td>
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<td>1.0</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td></td>
<td>2.0</td>
<td>15</td>
<td></td>
</tr>
</tbody>
</table>

† Ethyleneglycol-monoethylether.
‡ Weight per unit volume.
Solutions

A: 10 000 to 12 000 dis./min. or 0.005 µc/ml Cl\textsuperscript{14} toluene
B: 50 000 to 60 000 dis./min. or 0.025 µc/ml H\textsuperscript{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 Cl\textsuperscript{14} (using vial 1) and H\textsuperscript{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 Cl\textsuperscript{14} and H\textsuperscript{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\textsuperscript{32}, Ca\textsuperscript{45} and I\textsuperscript{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, BaCO₃.

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 CO₂ 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 (H₂SO₄, Cr₂O₇, KIO₃, H₃PO₄) 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 (K₂S₂O₈) at about
100°C. The CO₂ evolved is quantitative and is caught in a NaOH gas-washing bottle and converted to BaCO₃ for plating.

REAGENTS AND MATERIALS

(1) CH₃C¹⁴OONa.
(2) 200 mg K₂S₂O₈.
(3) 1 ml 5% AgNO₃.
(4) 0.1 N NaOH, CO₂-free.
(5) H₂O, CO₂-free.
(6) BaCl₂ (saturated aqueous solution).
(7) CH₃OH, abs.
(8) N₂ gas, or CO₂-free air.
(9) Flow apparatus, consisting of a two-necked reaction flask, an H₂O 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 C¹⁴ source of usual sample area and containing known number of dis./min.).

Fig. 29
Assembly for combustion of carbon compounds and conversion of CO₂ produced

PROCEDURE

(1) Arrange the flow apparatus as shown in Fig. 29.
(2) Calculate the amount of CH₃C*OONa needed to produce approximately 50 mg of BaCO₃, and place into the reaction chamber.
(3) Add about 20 ml H₂O, 200 mg K₂S₂O₈ and 1 ml 5% AgNO₃. (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 ClO₂.

(6) Wash the inlet of the gas-washing system with CO₂-free water, combining the washings with the N/10 NaOH.

(7) Add sufficient saturated BaCl₂ solution to precipitate all the ClO₂.

(8) Transfer quickly to a centrifuge tube, and centrifuge immediately (or stopper and centrifuge at will).

(9) Wash the precipitate once with CO₂-free water and then with abs. CH₃OH.

(10) Re-suspend in CH₃OH, 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 BaCO₃ 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 P³²/ml ("carrier-free" or of known P concentration).
(4) Fiske's reagent (13 g MgO, 175 g citric acid, 330 ml 25% NH₄OH in water to give 1-1 solution).
(5) 25% NH₄OH.

PROCEDURE

(1) Mark six 100-ml beakers as U₁, U₁', U₂, U₂', K and K', and pipette into them, respectively, the following aliquots:

- U₁ U₁' 5 ml unknown
- U₂ U₂' 20 ml unknown
- K K' 5 ml of 0.20 M H₃PO₄-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% NH₄OH 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 U₁ - and U₂- 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 U₁ and U₂ 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)

$$AX^* + BX^0 \rightarrow AX^0 + BX^*,$$

where $X^*$ represents a radioisotope of "X" and $X^0$ 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

$$\begin{align*}
(AX^*) + (AX^0) &= a \\
(BX^*) + (BX^*) &= b \\
(AX^*) &= x \\
(BX^*) &= y = c - x
\end{align*}$$

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

$$-\left(\frac{1}{a}\right) \frac{dx}{dt} = k_A \left(\frac{x}{a}\right) \left[\frac{b-y}{b}\right] - k_A \left[\frac{(a-x)}{a}\right] \left(\frac{y}{b}\right) = k_A \frac{x}{a} - k_A \frac{y}{b}$$

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

$$\frac{dx}{dt} = -\left[\frac{(a+b)}{b}\right] k_A x + \left(\frac{a}{a+b}\right) ck_A;$$

and on integrating, we obtain

$$x = C_1 e^{-\left[\frac{(a+b)}{b}\right] k_A t} + \left[\frac{a}{a+b}\right] c,$$

(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

$$x_\infty = \left[\frac{a}{a+b}\right] c; \text{ and } y_\infty = c - x_\infty = \left[\frac{b}{a+b}\right] c.$$

Hence

$$\frac{x_\infty}{y_\infty} = \frac{a}{b},$$

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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 $x_\infty$ into equation (1), the following final form is obtained:

$$x - x_\infty = (c - x_\infty) e^{-(a+b)/b}k_A t. \tag{2}$$

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

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

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:

$$k^*, x^*-x^* \text{ and } x^* \text{ (see Lecture Matter, section 4.3, example (3)).}$$

**REAGENTS AND MATERIALS**

1. Cation exchange resin, Ca$^{2+}$ saturated in solution (6).
2. Anion exchange resin, H$_2$PO$_4^-$ saturated in solution (5).
3. 5 μC P$^{32}$.
4. 10 μC Ca$^{45}$.
5. 10$^{-3}$ M KH$_2$PO$_4$.
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 $x^* - x_0^*$ on log scale against the time on linear scale.

(7) Obtain the slope from the graph, and calculate the rate constant $k_4$ 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 $\beta$- and $\gamma$-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**

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>24N HNO$_3$</td>
<td>15% w/v Na$_2$SO$_3$</td>
</tr>
<tr>
<td>16N HNO$_3$</td>
<td>20% w/v KSCN</td>
</tr>
<tr>
<td>N HNO$_3$</td>
<td>10% w/v Fe(NO$_3$)$_3$</td>
</tr>
</tbody>
</table>

† On the basis of activity concepts alone.
**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 24N HNO₃. 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 Na₂SO₃ and 1 ml of KSCN. Boil, and spin down CuSCN when it has settled, reject supernatant and wash precipitate with hot water saturated with SO₂.

2. Dissolve precipitate in 0.5 ml of hot 16N HNO₃; add 5 drops of Fe(NO₃)₃ and 1 drop of NH₄H₂PO₄, then 15N NH₃ till dark brown. Boil, spin down Fe(OH)₃ precipitate and wash it once with 2N NH₃.

3. Combine supernatant and washings in a fresh tube, and acidify with CH₃CO₂H till pale blue. Then add 0.5 ml of 16N HNO₃, 1 ml of Na₂SO₃ and 1 ml of KSCN; boil and spin down CuSCN. Pour away supernatant and wash precipitate with hot water saturated with SO₂.

4. Dissolve precipitate in 0.5 ml of 16N HNO₃; add 15N NH₃ until solution is deep blue and CH₃CO₂H 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**

\[
\text{μg Cu in sample} = \frac{\text{cpm sample}}{\text{cpm standard}} \times \frac{\text{wt standard Cu-salicylaldoxime}}{\text{wt sample Cu-salicylaldoxime}}.
\]

**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 Zn⁶⁴(n, p)Cu⁶⁴. 1 μg of zinc in the sample yields copper-64 equivalent to \(7 \times 10^{-4}\) 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**

- 36N H$_2$SO$_4$
- 16N HNO$_3$
- 15N NH$_3$
- 50% w/v MgCl$_2$
- 5% w/v H$_2$SO$_4$ 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$_4$H$_2$PO$_4$, 44.5652 g K$_2$SO$_4$ and 61.7689 g anhydrous Na$_2$SO$_4$ 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$_2$SO$_4$, 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$_2$SO$_4$ 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$_4$PO$_4$ three times with 5 ml of water.
5. Slurry MgNH$_4$PO$_4$ 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**

\[
\mu g \text{ P in sample} = \frac{10 \times cpm \text{ sample}}{cpm \text{ standard}} \times \frac{wt \text{ standard MgNH}_4\text{PO}_4}{wt \text{ sample MgNH}_4\text{PO}_4}
\]
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 ±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, α)P^{32} reaction.

The percentage contamination of the MgNH₄PO₄ 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)?
- neutron?

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

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

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

(5) If the activity fall-off factor F is defined as \( F = \frac{N_0}{N_*} \), show that \( F = 2^{\frac{t}{t_\frac{1}{2}}} \).

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

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

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

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

(10) A Na\textsuperscript{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\textsuperscript{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\textsuperscript{14} content. \( t_\frac{1}{2} = 5600 \) y.

(12) A 0.1-mg sample of pure Pu\textsuperscript{239} underwent \( 1.4 \times 10^7 \) 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 $(n, \gamma)$ 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?
### 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)

<table>
<thead>
<tr>
<th>Radionuclide</th>
<th>Critical organ**</th>
<th>Maximum permissible concentrations</th>
<th>Maximum permissible burden in total body</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>For 40-h week</td>
<td>For 168-h week</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(MPC)w (µc/cm³)</td>
<td>(MPC)a (µc/cm³)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(MPC)w (µc/cm³)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(MPC)a (µc/cm³)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(MC)</td>
</tr>
<tr>
<td>H³ (HTO or T³O)*** (sol.)</td>
<td>Body tissue Total body</td>
<td>0.1 5×10⁻⁶</td>
<td>0.03 2×10⁻⁶</td>
</tr>
<tr>
<td>(T³) (submersion)</td>
<td>Skin</td>
<td>2×10⁻³</td>
<td>4×10⁻⁴</td>
</tr>
<tr>
<td>Be⁷ (sol.)</td>
<td>GI (LLI) Total body</td>
<td>0.05 6×10⁻⁶</td>
<td>0.02 2×10⁻⁶</td>
</tr>
<tr>
<td>(insol.)</td>
<td>Lung GI (LLI)</td>
<td>0.05 10⁻⁶</td>
<td>0.02 4×10⁻⁷</td>
</tr>
<tr>
<td>C¹⁴(CO₂)*** (sol.)</td>
<td>Fat</td>
<td>0.02 4×10⁻⁶</td>
<td>8×10⁻³ 10⁻⁶</td>
</tr>
<tr>
<td>(submersion)</td>
<td>Total body</td>
<td>5×10⁻⁵</td>
<td>10⁻⁵</td>
</tr>
<tr>
<td>F¹⁸ (sol.)</td>
<td>GI (SI)</td>
<td>0.02 5×10⁻⁶</td>
<td>8×10⁻³ 2×10⁻⁶</td>
</tr>
<tr>
<td>(insol.)</td>
<td>GI (ULI)</td>
<td>0.01 3×10⁻⁶</td>
<td>5×10⁻³ 9×10⁻⁷</td>
</tr>
<tr>
<td>Na²² (sol.)</td>
<td>Total body</td>
<td>10⁻³ 2×10⁻²</td>
<td>4×10⁻⁴ 6×10⁻⁸</td>
</tr>
<tr>
<td>(insol.)</td>
<td>Lung GI (LLI)</td>
<td>9×10⁻⁴ 9×10⁻⁹</td>
<td>3×10⁻⁴ 3×10⁻⁸</td>
</tr>
<tr>
<td>Na²⁴ (sol.)</td>
<td>GI (SI)</td>
<td>6×10⁻³ 10⁻³</td>
<td>2×10⁻³ 4×10⁻⁷</td>
</tr>
<tr>
<td>(insol.)</td>
<td>GI (LLI)</td>
<td>8×10⁻⁴ 10⁻⁷</td>
<td>3×10⁻⁴ 5×10⁻⁸</td>
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</table>

* 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.
<table>
<thead>
<tr>
<th>Radionuclide</th>
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<th>Radionuclide</th>
<th>Critical organ(s)**</th>
<th>Maximum permissible concentrations For 40 h·week</th>
<th>For 168-h week</th>
<th>Maximum permissible burden in total body</th>
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** The daughter elements of Rn²₂⁰⁶ and Rn²₂₂⁰ 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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<td>9 x 10⁻⁴</td>
<td>3 x 10⁻⁴</td>
</tr>
<tr>
<td></td>
<td>(insol.) Lung</td>
<td>9 x 10⁻⁴</td>
<td>10⁻¹⁰</td>
</tr>
<tr>
<td>²³⁵ U</td>
<td>GI (LLI) Kidney</td>
<td>8 x 10⁻⁴</td>
<td>3 x 10⁻⁴</td>
</tr>
<tr>
<td></td>
<td>Bone</td>
<td>10⁻¹⁰</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GI (LLI) Lung</td>
<td>8 x 10⁻⁴</td>
<td>10⁻¹⁰</td>
</tr>
<tr>
<td>²³⁷ U⁻nat</td>
<td>GI (LLI) Kidney</td>
<td>5 x 10⁻⁴</td>
<td>2 x 10⁻¹⁰</td>
</tr>
<tr>
<td></td>
<td>Bone</td>
<td>10⁻¹⁰</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GI (LLI) Lung</td>
<td>5 x 10⁻⁴</td>
<td>2 x 10⁻¹⁰</td>
</tr>
<tr>
<td>²³⁹ Pu</td>
<td>Bone</td>
<td>10⁻⁴</td>
<td>2 x 10⁻¹²</td>
</tr>
<tr>
<td></td>
<td>(insol.) Lung</td>
<td>8 x 10⁻⁴</td>
<td>4 x 10⁻¹¹</td>
</tr>
<tr>
<td>²⁴¹ Am</td>
<td>Kidney</td>
<td>10⁻⁴</td>
<td>6 x 10⁻¹²</td>
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<tr>
<td></td>
<td>Bone</td>
<td>10⁻⁴</td>
<td>6 x 10⁻¹²</td>
</tr>
<tr>
<td></td>
<td>(insol.) Lung</td>
<td>8 x 10⁻⁴</td>
<td>10⁻¹⁰</td>
</tr>
<tr>
<td>²⁴² Cm</td>
<td>GI (LLI) Liver</td>
<td>7 x 10⁻⁴</td>
<td>2 x 10⁻⁴</td>
</tr>
<tr>
<td></td>
<td>Bone</td>
<td>7 x 10⁻⁴</td>
<td>2 x 10⁻¹⁰</td>
</tr>
</tbody>
</table>

* Provisional values for Th-nat.
** The abbreviations GI, S, SI, ULI and LLI refer to gastrointestinal tract, stomach, small intestine, upper large intestine and lower large intestine, respectively.
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".

---

** 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)

\[
\begin{align*}
\text{Counting time of sample + background} & \quad T \\
\text{Counting time of background (blank sample)} & \quad t \\
\text{Total counting time, } T + t & \quad \theta
\end{align*}
\]
Count rate of sample + background \( R \)

Count rate of background (blank sample) \( r \)

Fractional natural standard deviation of the net count rate of sample, \( \delta_{\text{nat}} \frac{R-r}{R-r} \)

Counting yield, over-all \( Y \)

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

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

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

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

\[
\frac{df}{dt} = \frac{R/(\theta - t) - r/t^2}{2(R/(\theta - t) + r/t)^{3/2}(R-r)} = 0 \text{ for } t^2 = (\theta - t)^3.
\]

The partition of \( \theta \) (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 \( t/T = (r/R)l \) are equivalent to

\[
t = \frac{ri}{(R + ri)} \text{ and } T = \frac{Rr}{(R + ri)}.
\]

which, by substitution into equation (1), gives

\[
f_{b.p.} = \frac{1}{\theta(Ri - ri)}.
\]

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", \( R^* - ri \) will attain its maximum value, and the natural uncertainty (for the best partition of the given \( \theta \)) 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 low activity samples (\( R \approx r \)) are to be measured. But when \( R \) is not much greater than \( r \), the difference \( R^* - ri \) may be approximated by \( \frac{(R-r)^2}{R} \), so that the optimal setting (corresponding to \( f_{b.p.\ min} \)) may be approximated by that for which \( \frac{(R-r)^2}{R} \) attains its maximum value. Since this approximate optimal criterion for low activities is equivalent to \( Y^2/r \) attaining its maximum value, it is independent 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.

\[\text{R}^* - ri = \frac{\left[ 1 + (R-r)/r \right]^{1/2}}{2} \]

\[= ri \left[ 1 + \left( R-r/r \right)^{1/2} - 1 \right] ; \text{ so, for } R \approx r, \]

\[R^* - ri \approx ri \left[ 1 + \left( R-r/r \right)^{1/2} \right]. \text{ or} \]

\[R^* - ri \approx ri \left( R-r/r \right)^{1/2}.
\]

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BIBLIOGRAPHY

Some basic books containing many further literature references


APPLIED PART

INTRODUCTION

SOILS

PLANTS

SOIL-PLANT

MENTAL EXERCISES
INTRODUCTION

The radioisotope has, in the past 15 y, become one of the important tools in advanced research within the area of soil-plant science. This is true because of the tracer possibilities that exist within the soil, from soil to plant and within the plant. Perhaps even more important is the fact that the kinetics involved in any system between soil and plant is measurable with the aid of the isotope, which measurement was physically impossible before the advent of the use of the isotope.

It is generally true that the more complex the problem under investigation, the more likely it is that an isotope is needed for elaboration of the results. It goes without saying that a well conceived research programme embodying conventional methods of measurement is necessary before introduction of isotopes into a research project will prove of much value; the use of isotopes in itself will solve few if any agronomic problems. On the other hand, properly used in conjunction with conventional methods, they will assist in the obtaining of more extensive results in less time.

Radioisotopes are being used in many countries today for evaluation of the fundamental chemical properties of soil, where, how and how much ion adsorption takes place on soil particles, the kinetics of ion exchange between soil surface and soil solution, the accumulation and degradation of the soil organic fraction etc. They are used to some extent in measurement of soil physical properties, in particular the storage and movement of soil water and the in situ bulk density of soil profiles. Understanding of the physiology of plant growth has been greatly extended by the use of isotopes, especially of carbon and hydrogen, from the early stages of photosynthesis through the nature and function of enzyme systems, the action of growth regulators etc. Complex relationships involved between the soil and the mineral nutrition of the plant growing on the soil are being widely studied with the ultimate goal of achieving more efficient fertilizer utilization.

There are limitations on the use of radioisotopes in soil-plant research, just as in any other scientific field: (1) There must be no chemical effect of the applied substance containing the activity on the system under investigation. For example, a toxic effect of the carrier chemical on a plant would probably invalidate results compared with what occurs in a normal system. (2) There must be no radiation effect. Again, in the case of a plant, there must be assurance that the radiation itself will not prove deleterious to plant cells or organs. Various studies have shown radiation damage to plants when exposed to P³² activities in excess of about 50 mc/g P₂O₅. This is a very much higher level of activity than is necessary for a good uptake experiment. The dangers in this respect will vary with the plant species and the element under investigation. They will also vary with the nature of radiation involved in the same approximate order of RBE as described under human health physics. Applied Part C, experiment 4.2 deals with this topic. (3) There must be no isotope effect. Any large variation in atomic weight among isotopes of the same element will show a corresponding difference in mobility of the constituent atoms, becoming a serious problem in diffusion-like systems where light elements are employed. Thus C¹⁴O₂ has been shown to be assimilated by barley seedlings about 17% slower than is
There is no severe problem of an isotope effect where neither bond making nor breaking is involved. As a result, C\textsuperscript{14} is a good label for tracing any organic molecule if the label is placed in a non-functional part of the molecule. (4) Radioisotopical purity is essential. An unknown mixture of radiocontaminants with the isotope intended for use in an experiment obviously would be quite intolerable. For example, interpretations of P\textsuperscript{32} uptake by a plant would be seriously in error had the original tag contained a slight percentage of P\textsuperscript{33} because of the longer half-life of the latter. (5) Radiochemical purity is also necessary. The radioisotope employed must be in a single chemical state. A mixture of phosphate and phosphite in a system will give decidedly different exchange or uptake results from one containing only phosphate. In this case, the phosphite-tagged molecules are more soluble and are not precipitated in the magnesium ammonium phosphate procedure. (6) In many cases it will be necessary to eliminate an exchange error in tracer studies. The radioisotope tag has been widely used to study exchange reactions as to reaction rates, bond strengths, molecular rearrangements and structures. In any plant-uptake and intermediary-metabolism investigation, however, one obviously must know that the phenomenon measured is neither a simple exchange reaction involving no new metabolic activity nor the uptake of a molecular species other than the original label. (7) The degree to which activity is distributed into different chemical forms must be known in many studies. This is especially true in pure chemical-tracer studies, likewise in metabolic studies with plants. It is not required in situations of gross uptake and utilization.

Planning the experiment which incorporates a radioisotope requires time and ingenuity on the part of the investigator. Radioisotopes are not inexpensive, and the use of larger amounts than required entails risk to human health and radiation damage to growing plants. For these reasons, no more tag is desirable than that required to assure an adequate count rate throughout the experimental period. Expected dilution effects, method of sample preparation, likely sample size, isotope decay rate, counting yield and counting statistics are all factors of significance in the planning of an isotope experiment. (See Mental Exercises.)

Many methods of sample preparation can be employed with the varied types of detection equipment now available on the market. Some of the more important methods employed in soil-plant studies are the following: (1) Counting a thin film of active material which was placed in a planchet as a solution form and then dried to a solid by evaporation. Pipetting accuracy, as has been detailed, is a primary factor. Otherwise, uniform spread of activity in the planchet and crystallization of solutes other than those containing the activity, with consequent variable thickness of the film, must be anticipated and corrected for in the case of beta emitters. (2) A given sample or portion thereof may be dissolved and the activity counted in liquid form by an end-window or dip counting tube. This technique will, however, not work for soft-beta- or alpha-emitting isotopes. In the case of the solution counting the tube wall thickness also limits the application to hard-beta and gamma-emitters (e.g. approximately 60 mg/cm\textsuperscript{2} for thin glass as compared with 1-2 mg/cm\textsuperscript{2} for thin-mica-window tubes). Nonetheless, liquid counting favours the geometry factor as well as the conditions for radiochemical assay. (3) Dried plant material may be finely
ground and compressed into briquets. In this case either a constant weight of sample or a sample weight providing a briquet in excess of infinite thickness should be employed. The method presents the advantage of simple and rapid preparation such that counting can be done with a minimum of decay loss. Shortcomings that diminish the value of this technique are the self-absorption factor which limits the count obtainable with soft-beta activity, difficulty in standard preparation and the problem of geometry when variable swelling of samples occurs. A modification is the cylindrical briquet of approximately 8-10 cm length with a hole in the middle for insertion of a thin-wall G-M tube. The enhanced geometry gives a several-fold increase in count rate, allowing a longer growth period of the plants for the same initial activity used. It may also be useful to weigh approximately 20 mg/cm² of dry, ground plant material into counting planchets, add few drops of an acetone-soluble gum and evaporate excess acetone. (4) A solid precipitate of the activity may be collected by filtration, centrifugation or sedimentation after proper treatment of an original solution state. By this means the activity is concentrated beyond the degree accomplished with any of the previous methods and, depending on geometry, an optimum count rate achieved. As in (1), there is the problem of achieving a constant weight of precipitate per unit area, in addition to which difficulties are frequently experienced with paper and precipitate distortion. The time and effort involved in effecting precipitation and collection also restrict the usefulness of the method. (5) Plant ashing by muffle furnace allows a maximum concentration from a relatively large bulk of plant material. The chief limitation is the difficulty in handling the ashed sample and the possibility of loss on ignition (volatile compounds, e.g. Zn-compounds). Fixation of an acetone-soluble gum may be recommended.

With these preliminary considerations for assuring validity of tracer investigations it is hoped the student will approach the relevant experiments with the realization that they have been conceived to broaden his perspectives on methodology in soil and plant studies. We hope this approach will teach him about some of the pitfalls encountered by earlier investigators.
SOILS

1. ADSORPTION OF CATIONS AND REPULSION OF SIMPLE ANIONS

1.1. Introduction

All cations and some anions are adsorbed by the soil. The adsorption process usually being reversible, the adsorption complex (clay minerals, organic matter, Fe- and Al-hydroxides) may serve as a storage reservoir for plant nutrition. For major cations the capacity of this reservoir may be 10 to 100 times the amount present in the soil solution.

The adsorption is caused in part by electrostatic interaction between ions and adsorption complex. The adsorption complex being negatively charged (as a rule), this implies that cations are accumulated (positively adsorbed), whereas at least the simple anions (e.g. Cl, SO₄) are repelled (so-called negative adsorption of anions).

Definitions:

(a) The "amount adsorbed" of (cat)ion X, \( \gamma_{ads}^+ (X) \) is defined as the total amount of ion X present in the system, per gram of soil, minus the amount (apparently) present in the soil solution, per gram of soil. Thus

\[
\gamma_{ads}^+ (X) = \Sigma(X) - Vc_0(X),
\]

in which \( \Sigma(X) \) indicates the total amount of ion X per gram of soil, \( V = \) true moisture content in ml per gram of soil, \( c_0(X) \) = normal concentration of ion X in the equilibrium solution (i.e. the electro-neutral soil solution at considerable distance from the soil particle surface). For anions the "amount adsorbed" is often negative, i.e. the total amount present in the system is less than the product \( Vc_0 \) (because the anions do not fill the whole volume \( V \)). This deficit of anions is named the "negative adsorption" of (an)ion Y. This "amount repelled" of (an)ion Y, \( \gamma_{rep}^- (Y) \) is defined by

\[
\gamma_{rep}^- (Y) = Vc_0(Y) - \Sigma(Y).
\]

(b) The cation exchange (adsorption) capacity \( \gamma^+ \) may be defined as the total amount of cations present in the system, per gram of soil, minus the total amount of anions present in the system, per gram of soil.

For a soil containing one species of cation X and one species of anion Y, one has \( c_0(X) = c_0(Y) \), and therefore

\[
\gamma^+ \equiv \Sigma(X) - \Sigma(Y)
\]

\[
= \Sigma(X) - Vc_0 + Vc_0 - \Sigma(Y)
\]

\[
= \gamma_{ads}^+ (X) + \gamma_{rep}^- (Y).
\]
In words: the cation exchange capacity in this case also equals the sum of the excess of cations (above the amount apparently present in solution) and the deficit of anions (below the amount apparently present in solution).

In systems containing several species of cation and anion one can define the "amount adsorbed" $\gamma_{ads}$ for each of the species concerned, in accordance with the above equation. The adsorption characteristic of a soil for a given pair of cations is then defined as the relationship between the amounts adsorbed $\gamma_{ads}^+ (X_1)$, $\gamma_{ads}^+ (X_2)$ and the corresponding composition of the electro-neutral part of the solution (i.e. the equilibrium solution). Such a characteristic may be determined experimentally and could then be presented in the form of a table listing the corresponding values of the amounts adsorbed and solution composition. It has been found, however, that the adsorption characteristic may be approximated by a rather simple mathematical equation of the form:

$$\frac{\gamma_{ads}^+ (X_1)}{\gamma_{ads}^+ (X_2)} = K_{1,2} \left[ \frac{c_0 (X_1)^1/z_1}{c_0 (X_2)^1/z_2} \right],$$

in which $z$ = valence of the cation involved (Gapon exchange equation). This indicates that the ratio of the amounts adsorbed is roughly proportional to the "reduced" ratio of the concentrations in electro-neutral solution. "Reduced" ratio then means that the concentration must be taken to a power equal to the inverse of the valence of the cation concerned. The proportionality factor $K_{1,2}$ is then called the exchange "constant"; it should be understood, however, that it is only approximately constant.

The experimental determination of the amounts adsorbed $\gamma_{ads}^+ (X_1)$, $\gamma_{ads}^+ (X_2)$, ... $\gamma_{ads}^+ (Y_1)$, ... etc. follows immediately from the definition. Thus one determines first the total amount present in the system and then subtracts the product $Vc_0$. The amount repelled is simply the "amount adsorbed with the opposite sign", e.g. $\gamma_{rep} (Y_1) = -\gamma_{ads} (Y_1)$.

The determination of the total amount present may be effected by observation of the isotopic dilution of a known amount of a labelled form of the ion "c" concerned, when added to, and equilibrated with, the system (assuming that all the "c" ions adsorbed are isotopically exchangeable). Following the outline given in the Basic Part, Lecture Matter, 4.2, one finds

$$V_{dis} = V_a \left( \frac{c_0^*}{c_e^*} \right) \left( \frac{\text{total cpm added to system}}{\text{cpm per ml}} \right),$$

in which ($V_{dis} =$"apparent volume of even distribution"), $V_a =$ volume of the label solution added, $c_0^* =$ activity concentration of the radioisotope of "c" in the label solution added, $c_e^* =$ activity concentration of the radioisotope in the electro-neutral part of the solution after equilibration with the added label solution. The total amount of test ion "c" in the system (after addition of the label solution) then equals

$$\Sigma_c = V_a \left( \frac{c_e^*}{c_e^*} \right) c_e,$$

in which $c_e =$ test ion concentration in the electro-neutral part of the solution after equilibration with the added label solution. Finally, the total amount
of test ion in the origin system is $\Sigma = \Sigma_c - \Sigma_a$, where $\Sigma_a$ is the total amount in the added solution (i.e. $V_aC_a$).

If the original moisture volume $V$ and the original equilibrium concentration $c_0$ are known (or measured), one may calculate the amount adsorbed, which should be expressed per gram of soil, as the total amount $\Sigma$ minus the product $Vc_0$.

As follows from the above, the determination of the amount adsorbed requires in general the knowledge of $c_0$, $c_a$, $c_e$, i.e. the concentration of the test ion (1) in the equilibrium solution before addition of the label; (2) in the label solution; (3) in the solution after equilibration with the added label solution. The procedure may be simplified by the use of a label solution which is identical in composition with the electro-neutral part of solution before addition of the label; then $c_0 = c_a = c_e$. Such a label solution often may be prepared by labelling of an amount of original equilibrium solution, separated from the system, with small amounts of label having a high specific activity. The two procedures, i.e. the use of a label solution with arbitrary composition and the use of a label solution identical in composition with original equilibrium solution, are demonstrated in experiments 1.2.1 and 1.2.2. In experiment 1.2.3, repulsion (negative adsorption) of chloride ions, the precise determination of the concentration of $Cl^-$ in the system is avoided altogether, as the purpose of this experiment is the determination of the apparent volume of exclusion $V_{eq}$ of $Cl^-$, i.e. the mean volume from which the $Cl^-$ ions are repelled because of the negative charge of the soil particles, expressed per gram of clay.

1.2. Experiments

1.2.1. Determination of the cation exchange capacity of a clay

REAGENTS AND MATERIALS

(1) Ca-Cl-clay suspension (preferably about 4% illite, or any other clay suspension containing approx. 1-2 meq. exchangeable cations per 100 ml of suspension).

(2) 0.0025 M CaCl$_2$ containing 0.1 µc Ca$^{45}$/ml.

(3) 0.0025 M CaCl$_2$ containing 0.05 µc Cl$^{36}$/ml.

(4) 1 N NaOH.

(5) Erichrome Black T, Calcon indicator powder.

(6) 0.01 N complexon (versenate) solution (ethylene diamine tetraacetic acid).

(7) 0.1% K$_2$CrO$_4$ solution.

(8) 0.01 N AgNO$_3$ solution.

PROCEDURE

(1) Pipette 5 ml of the clay suspension into a weighing flask, and determine the concentration of the clay after oven-drying at 105°C.

(2) Pipette 5 ml of clay suspension into a centrifuge tube, and add 5 ml of 0.0025 M Ca*Cl$_2$ solution.
(3) Into the other centrifuge tube, pipette 5 ml of clay suspension, and add 5 ml of 0.0025 M CaCl$_2$ solution. Both centrifuge tubes, after tight sealing with rubber stoppers, are placed on an end-over-end shaker.

(4) After 24 h the tubes are removed and subjected to centrifugation at about 2500 rev./min. for 20 min.

(5) Clear (i.e. electro-neutral) supernatant liquid is decanted into test tubes.

(6) 1 ml each of added solutions, 0.0025 M Ca$^{2+}$Cl$^-_2$ and 0.0025 M CaCl$^+$, and 1 ml of supernatant solutions are pipetted into counting pans (to yield the activity concentrations).

(7) After the solutions are dried under infra-red lamps, they are counted with a G-M counter. The count rates should be determined immediately after drying, both for the added and the supernatant solutions.

(8) Ordinary chemical procedures are used to determine Ca and Cl concentrations.

(9) Take 5 ml each of supernatant and added solutions into 50-ml beaker for Ca determination.
   (a) Add 5 ml of 1 N NaOH into each beaker.
   (b) A pinch of Calcon indicator is added into the beaker, and the samples are titrated with 0.01 N complexon to completion of colour change from purple to blue.

(10) Take 5 ml each of supernatant and added solutions into 50-ml beakers for Cl determination. Add 5 ml of 0.1% K$_2$CrO$_4$ solution into the beakers, and titrate with 0.01 N AgNO$_3$ until the first appearance of red colour of Ag-chromate.

(11) Calculate the cation exchange capacity $\gamma^+$ of the clay from the difference between "pool" of Ca and "pool" of Cl in the original system, employing the appropriate equations given in the introduction.

QUESTIONS

(1) Calculate the apparent volume of even distribution of the Ca$^+$ ions added to the system ($V_{\text{dis}}^+$).

(2) Is $V_{\text{dis}}^+$ smaller or larger than the true volume of the system ($V + V_a$)? What does this mean? Calculate the difference ($V_{\text{acc}}^+$: apparent volume of accumulation of cations).

(3) The same questions as above for Cl$^-$ ions. (The difference between $V + V_a$ and $V_{\text{dis}}^+$ is the apparent volume of exclusion of anions $V_{\text{ex}}^-$.)

(4) Calculate the total amounts of Ca and Cl present in the system after addition of the label solutions ($\Sigma_a$(Ca) and $\Sigma_a$(Cl)).

(5) Why is the (adsorption) exchange capacity $\gamma^+$ not equal to

$$\frac{\Sigma_a$(Ca) - $\Sigma_a$(Cl)}{\text{grams of clays}}$$

(6) Calculate the amounts of Ca and Cl present in the system before the addition of the label solutions ($\Sigma$(Ca)). Remember: $\Sigma_a = \Sigma^+$(amount added with label). How much was added with the label?
(7) Calculate the amount of Ca present in the system before addition of the label with the formula:

\[ \text{"Pool"} = V_a C_a (s*_a/s*_c - 1), \]

in which \( s* \) is the specific activity of Ca in cpm per meq. of Ca. Compare (7) with (6). Derive the equation used in (6) from the one given in (7). Remember: \( s* = c*/c \).

(8) Calculate the product of the apparent volume of accumulation of Ca (cf. (2)) and the equilibrium concentration of Ca. What does this quantity correspond to? Remember: The amount adsorbed equals the total amount minus the (apparent) amount in solution. Now:

This: \( V^+_{\text{diis}} \cdot c_e = \text{total amount (when?)} \)

Minus: \( (V + V_a) \cdot c_e = \text{amount in solution (when?)} \)

Gives: \( V^+_{\text{acc}} \cdot c_e = \text{amount adsorbed (when?)}. \) Express per gram of clay.

(9) Repeat (8) for the Cl ions.

Remember: The amount repelled = \( (V + V_a) c_e - V_{\text{diis}} \cdot c_e = V_{\text{ex}} \cdot c_e \).

Why is the sum of the amounts adsorbed and repelled calculated for Ca and Cl in (8) and (9) not equal to \( \gamma^+ \)? (Cf. (5)).

1.2.2. Determination of an exchange "constant"

REAGENTS AND MATERIALS

(1) 30 ml of Na-Ca-Cl-illite suspension (about 10%), previously shaken for at least 24 h.

(2) 70 ml of clear (electro-neutral) equilibrium solution of above, of known composition, containing approximately 0.002 N Ca and 0.01 N Na as chloride.

(3) Na*Cl solution (1 \( \mu\text{c} \)/ml in \( 10^{-5} \) N NaCl).

(4) Ca*Cl\(_2\) solution (5 \( \mu\text{c} \)/ml in \( 10^{-3} \) N CaCl\(_2\)).

PROCEDURE

(1) Determine the concentration of clay by pipetting out 5 ml into a weighing flask.

(2) Pipette 5 ml of the clay suspension into each of two centrifuge tubes.

(3) Label two portions of the supplied equilibrium solution (about 25 ml each) with 0.5 ml of the Na* and Ca* solutions, respectively.

(4) 5 ml each of the Na* and Ca* equilibrium solutions is added into respective clay suspensions in the two centrifuge tubes. The tubes are then shaken for 24 h.

(5) Centrifuge at about 2500 rev./min. for 20 min.
(6) After careful decantation of clear supernatants into beakers, 1 ml of each supernatant is placed into a separate planchet for counting with a scintillation crystal and a G-M tube, respectively. 1 ml each of the labelled equilibrium solutions is likewise taken for counting.

(7) Calculate the amounts of Na and Ca adsorbed, using the appropriate equations given in the introduction.

(8) Calculate the "Gapon exchange constant" from the amounts adsorbed and the given composition of the suspension solution, according to

\[ K_{Na,Ca} = \left( \frac{\gamma_{ads}^+(Na)}{\gamma_{ads}^+(Ca)} \right) \cdot \left\{ \frac{\left[ c_0(Ca) \right]^2}{c_0(Na)} \right\}, \]

in which \( c_0 \) is expressed in mole/l.

QUESTIONS

(1) Repeat questions (1) and (2) of experiment 1.2.1 for Ca and Na.

(2) Calculate \( \Sigma_e(Ca) \) and \( \Sigma_e(Na) \).

(3) Calculate the amounts of Ca and Na present in the system before addition of the label.
Remember: \( \Sigma_e = \Sigma + V_a C_a \). Express per gram of clay.

(4) Calculate the amounts adsorbed of Ca and Na before addition of the label.
Remember: Amount adsorbed = total amount - amount in solution (expressed per gram of clay or soil).

(5) Repeat question (8) of experiment 1.2.1 for Ca and Na. Compare with (4). Why are they the same this time?

(6) Derive the formula for the calculation of the amount of cation adsorbed in case the label solution is equal in composition to the original (electro-neutral) equilibrium solution, which should read

\[ \gamma_{ads}^+ = V_a \left( \frac{c_0^+}{c_0^-} - p \right) c_0, \]

in which \( p = \frac{\text{total volume of solution after addition of label}}{\text{volume of added label solution}}. \)

In the present experiment \( p = 2 \); therefore,

\[ \gamma_{ads}^+ = V_a \left( \frac{c_0^+}{c_0^-} - 2 \right) c_0. \]

(7) If the accuracy of the counting was assumed to be within about 1%, the ratio \( c_0^+/c_0^- \) should be accurate to within about 1.4%. Calculate the accuracy of \( (c_0^+/c_0^- - 2) \) in per cent for both Ca and Na. What would be the accuracy of the determination of \( \gamma_{ads}^+(Ca) \) and \( \gamma_{ads}^+(Na) \)? What is the accuracy of the value obtained for the exchange constant \( K \)? Compare with the reproducibility of \( K \) determinations by different members of the group.

In order to obtain sufficient accuracy, one should thus always strive towards a situation in which \( c_0^+/c_0^- \gg p \). Remember: In the above
case, $\gamma_{lds} = 0$ for $c_f^d/c_e^d = 2$ (simple dilution of the added label over twice the added volume).

1.2.3. **Determination of repulsion (negative adsorption) of chloride ion**

**REAGENTS AND MATERIALS**

1. 1.5 $\mu$C/ml Cl* in 0.1 N NaCl.
2. Cellophane (dialysis) bags.
3. Mohr titration reagents or conductivity bridge equipment for chloride determinations.
4. 2% Na-Cl-montmorillonite suspension in about 0.005 N NaCl. (If required, a range of electrolyte concentrations may be employed.)

**PROCEDURE**

1. 1 ml of the supplied Cl* solution is diluted to 100 ml (standard solution).
2. Bring exactly 100 ml of suspension and two dialysis bags containing 10 ml of water each into a 150-ml, wide-mouth Erlenmeyer flask.
3. 1 ml of the supplied Cl* is added to the suspension; the Erlenmeyer is stoppered and put on a shaker. Continue shaking for 48 h.
4. Remove the bags gently from the flask, and put them upright in 25-ml beakers.
5. Cut the bags with scissors close to the top.
6. Take out contents with a 10-ml pipette without contaminating the solution with any suspension adhering to the outside of the bag.
7. After the solutions are transferred into 25-ml beakers, one ml aliquots are dried on counting trays, as well as 1-ml portions from the standard solution prepared in (1).
8. The electrolyte concentration of the dialysate is determined.
9. The volume of exclusion of the Cl ions $V_{ex}^-$ is calculated from the observed count rates of standard and "bag" solutions and expressed in ml per gram of clay.

**QUESTIONS**

1. Calculate the apparent volume of even distribution of the Cl* ions.
2. Subtract the apparent volume of even distribution from the true liquid volume $V_t$ according to

$$V_{ex}^- = V_t - V_{ds}^- .$$

3. Express the apparent volume of exclusion $V_{ex}^-$ per gram of clay.
4. Show that $V_{ex}^-$/gram of clay = $\gamma_{cep}^-$ (Cl)/$a_e^-$ (Cl), where $a_e^-$ is the test anion (Cl) concentration in the bag. Remember the definition of the amount repelled, i.e. apparent amount in solution minus total amount.
In this case the anion repulsion is found for the system after addition of the label. Which data would be necessary to calculate the apparent volume of exclusion before addition of the label?

The volume of exclusion must be equal to the specific surface of the clay, multiplied by the apparent distance of exclusion of the CI ions, i.e. the average (or effective) distance over which the CI ions are repelled by the negatively charged surface of the soil particles. This distance is determined by the strength of the electric field emanating from the soil particles. Theoretical considerations indicate that the distance of exclusion for Na-CI-clays equals approximately

$$d_{ex}^+ = 2/(\beta \cdot \Sigma_{CI})^{1/4} - \sigma,$$

in which $\Sigma_{CI}$ = total electrolyte concentration in mole/l; $\sigma$ = about 4Å for montmorillonite and 2Å for illite; $\beta$ = a constant, equal to about $10^{15}$ cm/mole. In the present experiment $\Sigma_{CI}$ may be calculated from the conductivity of the dialysate. Calculate $d_{ex}$ in cm; remember 1Å = $10^{-8}$ cm.

Estimate the specific surface area of the clay from the measured value of $\gamma_{eq}/a_e$ in cm$^3$ per gram and $d_{ex}$ in cm. What are the units of $S$, the specific surface area?

2. ADSORPTION OF PHOSPHATE AND MOVEMENT OF PHOSPHATE, Ca AND CI IONS

2.1. Introduction

In contrast to the adsorption of the common cations and some simple anions, phosphate "adsorption" by soil is a complex phenomenon. Chemical bonding on mineral surfaces, precipitation and incorporation in organic compounds all play a role. As a result phosphate in soil is usually only slowly and incompletely exchangeable against other anions.

Several chemical extraction procedures have been employed to characterize the phosphate status of soil, but because of the specific character of phosphate these procedures allow at best a relative evaluation of different soils.

It may be expected that the determination of the amount of phosphate that is exchangeable against itself (by means of isotopic exchange) would be a less arbitrary estimate than the amount extractable. This amount of isotopically exchangeable phosphate has been defined as the $E$-value of soil (for phosphate)$^\dagger$. Again, because of the complexity of the phosphate-binding mechanism the $E$-value depends very much on experimental conditions. Aside from an influence of the duration of the equilibration, it has been found

$^\dagger$ This principle was first used by McAuliffe, C.D., Hall, N.S., Dean, L.A. and Hendricks, S.B., Exchange reactions between phosphates and soils: Hydrocyclic surfaces of soil minerals, Soil Sci. Soc. Amer. Proc. 12 (1948) 119-123.
that in certain soils also the carrier level of the added label influences the result. Although in principle one should perhaps attempt to add the label at a carrier level equal to the phosphate ion concentration existing in the soil system, practice requires the addition at a much higher carrier level. This is so because the determination of the specific activity implies a fairly accurate evaluation of the phosphate concentration, which in turn can only be done if a sufficiently high level is present. The addition at higher carrier level, however, implies changing the system, which gives a somewhat arbitrary character to the experimental procedure. It would thus seem advisable that the determination of the E-value be performed at two carrier levels as a check on whether the specific system investigated is sensitive to this level. If so, an attempt could be made to run a series of determinations at different carrier levels and extrapolate the results to zero carrier.

The E-value is found directly from the application of the isotopic dilution principle. Thus, if the amount of phosphate added per unit of soil equals b, then

\[ E = b \left( \frac{s^*}{s^*_c} - 1 \right). \]

In practice, the duration of the shaking period is taken as 24 h. In experiment 2.2.1 shaking times of 2 h, 24 h and 48 h are suggested. With regard to the carrier level, values of 0, 1 and 2 μg P per ml will be used. The 0.01 M CaCl₂ is introduced for imitation of the composition of the soil solution and facilitation of filtering.

In as much as the purpose of the determination of the E-value is often the assessment of the amount of phosphate available to the plant, use of the plant as a sampling device has been proposed. This would circumvent the changes introduced in the system by the dilution which is necessary for the determination of the E-value, although incurring other difficulties in interpretation if the "pool" involved in isotopic dilution differed in size from the "pool" available to the plant. The amount of "Larsen" phosphate present in soil as calculated by putting \( s^*_p \) = observed specific activity of P in the plant is named the "L" value.

The adsorption of various ions by soil as discussed above and determined in the preceding experiments is demonstrated readily by percolation of a soil column with a solution containing these ions. Obviously those ions adsorbed most strongly will be retained in the upper parts of the column. In contrast, ions which are repelled by the soil solid phase (negatively adsorbed) will not be retained at all but will follow the moving liquid. Radioisotopes provide a convenient tool for a demonstration of these phenomena. Isotopes emitting hard β⁻ or γ-ray may be spotted by simple monitoring of the soil column. For soft β-rays the column must be sectioned and samples prepared for counting, if necessary by an extraction.

In experiment 2.2.2 the mobility of PO₄, Ca and Cl ions in soil will be studied by leaching of soil columns which received a surface application of the respective ions some 24 h before the leaching.
2.2. Experiments

2.2.1. Adsorption of phosphate by soil

REAGENTS AND MATERIALS

(1) Molybdate solution
Dissolve 5 g of ammonium molybdate, (NH₄)₆Mo₇O₂₄ • 4H₂O, in 80 ml of warm water. Add 2.8 ml of concentrated H₂SO₄ to the cooled solution, and dilute to 100 ml with water.

(2) Hydroquinone solution
Dissolve 0.5 g of hydroquinone in 100 ml of water, and add 1 drop of concentrated H₂SO₄ to prevent rapid oxidation. The solution should be prepared freshly before use.

(3) Sulphite solution
Dissolve 110 g sodium sulphite, Na₂SO₃, in water and make up to a liter.

(4) Standard phosphate solution
Dissolve 0.1098 g of potassium dihydrogen phosphate in distilled water and dilute to 1 liter. Each milliliter contains 0.025 mg of phosphorus.

(5) Two soils, one acid and one neutral.

(6) P³² solution containing 10 μc/ml.

(7) Solutions containing 0, 1.0, 2.0 μg P/ml in 0.01 M CaCl₂.

PROCEDURE

(1) Weigh out 5 g of soil samples in three 100-ml stoppered Erlenmeyer flasks.

(2) Label 100-ml amounts of the stock solutions (7) above with 10 μc P³² (1.0 ml)

(3) Add 50-ml amounts of the solutions prepared under (2) to the soil samples, and shake for 24 h.

(4) After the shaking, let the flasks stand so that the majority of large particles can settle down and filter.

(5) Determine the radioactivity of 1-ml aliquots of the filtrates and standards (remainder of solution prepared under (2)) after drying of the samples under an infra-red lamp.

(6) For determination of the phosphate concentration, 10 to 30 ml of the filtrates, depending upon the phosphate concentration, are pipetted into 50-ml volumetric flasks. Add some water to make the volume close to 30 ml but not more.

(7) Prepare at the same time a series of standards by using 1, 2, 4, 6, 8 and 10 ml each of the standard phosphate solution (4) above instead of the filtrate of the soil suspension.

(8) Add 5 ml of molybdate solution to all systems, mix well, add 5 ml of hydroquinone solution, mix well, and then immediately add 5 ml of sulphite solution and mix well. Make up to the volume with water, and mix well.
(9) Allow the solution to stand for 30 min.; then measure the colour intensity with a colorimeter at a wavelength of 650 mum or using a red filter.

(10) The phosphate concentrations of the filtrates is determined by interpolations on the "standard" curve.

(11) From the activity measurement and phosphate concentration determination, the specific activity of phosphorus before and after the interaction of phosphorus with soil is calculated. The E-value of the soil is calculated from the isotope dilution equation.

There are other reducing agents used in the determination of phosphorus besides hydroquinone, such as SnCl₂, Metol (p-methylaminophenol sulphate), ascorbic acid or hydrazine sulphate. An alternate procedure with stannous chloride is given below:

REAGENTS AND MATERIALS

(1) Molybdate solution
Dissolve 15 g of ammonium molybdate (NH₄)₆Mo₇O₄•4H₂O in about 300 ml of 50°C distilled water. When the solution has cooled, slowly add 350 ml of 10 NHCl, stirring, to molybdate solution. Cool the solution to room temperature and dilute to 1 l with distilled water.

(2) Stock stannous chloride solution
Dissolve 10 g of stannous chloride SnCl₂•2H₂O in 25 ml of concentrated HCl. Place in stoppered glass bottle (coloured to prevent light transmission), and store in a dark cabinet.

(3) Diluted stannous chloride solution
Add 1.0 ml of the stock stannous chloride solution to 322 ml of distilled water. Shake well before use. The solution must be freshly prepared every day.

PROCEDURE

After pipetting the solution to be determined into a 50-ml volumetric flask, add 10 ml of the molybdate solution, and mix well. Immediately add 5 ml of the diluted stannous chloride solution, and shake well. Make up to the volume with water, and measure the light intensity after 10 min., using 650 mum wavelength or red filter with photometer.

2.2.2. Movement of phosphate, Ca and Cl ions in soil

REAGENTS AND MATERIALS

(1) Three soils: sandy loam, silt loam and clay.

(2) 5 ml portions of labelled solutions containing:
P³²: 0.2 μc P³²/ml in 0.01 M H₃PO₄ and 0.01 M CaCl₂
Ca⁴⁵: 0.2 μc Ca⁴⁵/ml in 0.01 M CaCl₂
Cl³⁶: 0.2 μc Cl³⁶/ml in 0.01 M CaCl₂.
PROCEDURE

(1) Place 40 g of soil in percolation tube, 2 cm in diameter, above a gravel base.
(2) Add water to wet the soil†, and add 5 ml of P\textsuperscript{32} solution to surface. Cover the surface of the soil with fine gravel.
(3) Similar procedures are carried out for Ca and Cl solutions.
(4) Let the soils stand for 24 h, and then leach the soil with 30 ml of water. Save the leachates for counting.
(5) Repeat leaching five times on subsequent days, each time using 30 ml of water.
(6) After complete drainage of the liquid, carefully remove the column of soil by blowing with a rubber ball, or pushing with a plunger.
(7) Cut the soil column into 1-cm segments, dry under infra-red lamp, grind, and place 1 g each onto counting discs for counting.
(8) Take 2 ml of an aliquot from each 30-ml leachate into counting pans, dry under infra-red lamp, and count.
(9) Compare the data.

3. DETERMINATION OF RADIOISOTOPES IN NATURAL SOIL

3.1. Introduction

In natural soil, usually very small amounts of radioactive isotopes of certain elements are present, notably K\textsuperscript{40}, Sr\textsuperscript{90} and Cs\textsuperscript{137}. Because of the extremely low activity of these elements in soil, their determination has two specific aspects. One aspect is the necessity of isolation and concentration of the element; the other is the determination of very low count rates with sufficient accuracy. The latter aspect has been dealt with in the Basic Part, Lecture Matter, section 2.4.2, and in particular the reader is referred to equation (2), which may be used for evaluating the counting time necessary in order to obtain a specified statistical accuracy. The use of anti-coincidence counting is described briefly in the Basic Part, Lecture Matter, section 2.5.1.

The isolation and concentration procedure, of course, depends on the particular element concerned. In experiment 3.2, a total count rate resulting from elements in soil extractable by concentrated hydrochloric acid and precipitated by oxalate at pH = 4 will be observed (the radioelement present most abundantly would presumably be Sr\textsuperscript{90}).

3.2. Experiment

REAGENTS AND MATERIALS

(1) 1 : 1 HCl and dilute HCl (1 : 20).
(2) Ammonium acetate 50%.
(3) Oxalic acid.

† Be careful to exclude all air from the soil prior to the addition of the labelled solutions.
(4) NH$_4$OH (10%).
(5) Methyl orange indicator.
(6) CH$_3$OH.
(7) "Formvar" or any other solidifying material.
(8) pH indicator paper.

PROCEDURE

(1) Weigh 25 g of soil into a 125-ml Erlenmeyer flask.
(2) Add 50 ml of 1:1 HCl, and shake for 5 min.
(3) Allow the solid parts to settle, and filter into a 400-ml beaker. Rinse the flask twice with water, and filter into the same beaker. Dilute the filtrate with 100 ml of distilled water.
(4) Add 1 ml of ammonium acetate solution and a few grams of oxalic acid. Heat the beaker on the hot plate.
(5) Add slowly 10% HN$_4$OH until the pH of the solution becomes 4. This can be checked by pH indicator paper.
(6) Digest on the boiling water bath for 5 min.
(7) Filter the contents, transferring all the precipitate onto the filter paper. Wash twice with distilled water.
(8) Pierce the filter paper with a sharp glass rod, and wash the precipitate down to a new 400 ml beaker with dilute warm HCl.
(9) Repeat the oxalate precipitation, now using a few drops of methyl orange as indicator.
(10) Filter the contents through a chimney and stick, allowing all the precipitate to be uniformly distributed on the filter paper. Wash the precipitate on the filter paper twice with a small amount of methyl alcohol.
(11) After careful removal of the filter chimney, the sample on the filter paper is transferred to a counting tray.
(12) After several minutes of air drying, the sample is fixed with a small amount of "Formvar".
(13) After complete drying of the sample, it is counted for 10 min. in an ordinary G-M counter and 5 min. in the anti-coincidence counter. The backgrounds are counted for the same lengths of time. Compare the results. How long should counting be done with each for 5% natural standard deviation?

4. DETERMINATION OF SOIL MOISTURE AND BULK DENSITY

4.1. Introduction

4.1.1. Soil moisture by neutron scattering

Because of the elastic nature of collisions between fast neutrons and hydrogen nuclei, and the resultant production of slow neutrons with associated back-scattering toward the source, it is possible to calibrate a given fast-neutron source to hydrogen content of any given medium. Thus moisture,
the primary carrier of hydrogen atoms in soil, can be measured in situ, provided an access tube is placed into the soil.

Instruments are usually provided with a factory calibration chart. The probe itself is commonly housed in a shield of lead and paraffin. The former reduces the gamma activity to a level safe for handling by the operator, and the latter effects shielding of the neutrons as a consequence of moderation by H in the paraffin. At the same time, the latter permits a check at any time on the proper functioning of the entire electronic system. If the shield reading varies from the value originally established for the instrument, erratic performance can be assumed until proper evaluation solves the problem.

Potential sources of error are hydrogen atoms in the soil organic fraction, water of crystallization of inorganic soil minerals, as well as other neutron-moderating elements, such as Cd, B, Li, Cl and Mn. These potential errors are usually small; however, in the case of extremes, calibration of the instrument can be readily accomplished in those soils of high "background". With such a calibration, it is even possible to measure moisture content of pure organic materials such as stored grains, coal piles or peat and muck soils.

Initial calibration in the case of a depth probe (which has greater application than has a surface probe for most agronomic considerations) has usually been done at the factory in a given medium and within a given casing. A difference in predominant soil minerals from this medium could, as noted above, necessitate a different calibration. A difference in the dimensions or composition of the casing would also make necessary a new calibration. Thus it must be considered a generally desirable practice always to calibrate the instrument in representative soil(s) of the area under investigation and with the proper type of tube and casing. This can be accomplished in various ways. One is to have a group of barrels filled with soil wetted to known moisture levels. Another is to extract several samples in the field immediately adjacent to holes where moisture readings have been made with the neutron gauge. A plot is then made of count rate against laboratory-determined soil moisture content by volume.

Thin-walled aluminium casing (approximately 0.025 in thickness) of specified diameter (access tube) has proved effective as the casing for the soil well into which the probe is inserted. Aluminium is superior to stainless steel as it is less subject to corrosive action of soil chemicals and in most places is substantially cheaper. It is particularly important that the hole prepared for the access tube is not larger than the tube so as to permit close contact between tube and soil. If this is not the case, excessive evaporation of water from the soil adjacent to the tube will facilitate development of large cracks and invalidate moisture readings at the particular site. Further, a gap between soil and tube would allow gravitational movement of water during rain or irrigation. Optimum access tube placement is accomplished with the aid of a hydraulic probe or coring machine which removes a constant diameter soil core to the desired depth. Satisfactory results are also obtained by hand with an auger.

The length of the access tube employed is determined by the problem under investigation. If the investigation involves measurement of water storage in the soil and its extraction by an annual crop, it is quite probable that a 6-ft (2 m) tube will be adequate. On the other hand, a 20-ft (6 m) tube
may be desirable (depth of soil permitting) in studies of tree crops or perennial tap-rooted legume crops.

4.1.2. Bulk density by gamma-ray attenuation

A 2 or 3 mc Cs137 source is commonly contained in a probe similar to the neutron probe for purposes of bulk density determination. Since the half-life of Cs137 is 33 y, the standard shield reading and ultimate calibration changes slightly with time, approximately a 2% count reduction/year. The detector consists of one or more G-M tubes thoroughly shielded from the source by lead. An additional lead shield houses the active portion of the probe when it is not in use. The probe can be obtained in two models, one for insertion into an access tube in the soil and another for placement on the soil surface. When made by the same manufacturer, density and neutron moisture probes are interchangeable for use with the same scaler and are accommodated by the same soil-access tubes.

Calibration beyond that provided by the manufacturer is desirable, especially because of possible differences in dimensions and composition of the access tubes. In this case, calibration is best carried out in a series of barrels, each of which contains a known volume and weight of soil (at least 30-cm radius). The plot of e.g. lb/ft$^3$ or g/cm$^3$ against cpm is essentially linear within the common range of soil bulk density values.

Shield readings are taken intermittently during calibration and actual soil measurement as a check. The bulk density of the soil is then calculated thus:

\[
\text{shield reading at calibration} \times \text{curve factor} = \text{bulk density}.
\]

Considerations with regard to kind and placement of access tubing in soil are the same as those discussed under calibration of the neutron moisture probe. Alterations in size and composition of tubing must be compensated for by recalibration.

No method has previously been conceived for accurate measurement of soil bulk density in situ. The accuracy of the gamma attenuation method equals or exceeds that obtainable by conventional, more laborious methods.

4.2. Experiments

4.2.1. Determination of soil moisture by neutron scatter

REAGENTS AND MATERIALS

(1) Neutron probe and scaler.
(2) Monitor.
(3) Tub of water (20-cm radius and 40 cm deep) fitted with an access tube of aluminium, which is sealed at the bottom.
(4) Moisture cans.
(5) Auger for boring holes to accommodate aluminium access tubes.
(6) Aluminium tube, 2 m long, with an internal diameter slightly larger than neutron probe diameter.

(7) Stop-watch.

PROCEDURE

A. Laboratory:

(1) Determine the proper operating voltage for the scaler. This can be done while the probe remains within its shield, the neutrons being moderated and back-scattered by the surrounding paraffin.

(2) Obtain the count rate in a substantial volume of water. The reading should check within about 2% of that prescribed for the instrument by the manufacturer.

(3) Determine $\gamma$-activity as a function of distance from the source during measurements in (2) above.

B. Field calibration:

Calibration is necessary not only as a check on the manufacturer but to allow for differences in dimension and composition of access tubes.

(1) Prepare a series of 200-cm deep holes. Each hole should be drilled in a different field so as to yield a wide range in soil moisture conditions (grass, alfalfa, winter-wheat, corn etc.). Save soil samples from the holes in moisture cans, corresponding to exactly 0-30 cm, 30-60 cm, 60-90 cm, 90-120 cm, 120-150 cm and 150-180 cm. The moisture content of these samples is determined by oven-drying.

(2) In each hole, insert an aluminium access tube, place the probe in the tube, and take two 1-min. readings at 20 cm, 45 cm, 75 cm, 105 cm, 135 cm and 165 cm. Take a shield reading before and after each set of readings.

(3) Calculate moisture in samples brought to laboratory as per cent by volume.

(4) Plot cpm against % water by volume. Prepare alternate curve on same chart of cpm against grams per dm$^3$ (or pounds of water per ft$^3$ of soil), and of cpm against cm or inches of water per dm or foot depth of soil. These calibration curves, of course, are based on shield readings obtained at the time of calibration. Any variation in shield readings must be compensated for in subsequent days as follows:

$$\text{Shield reading at calibr.} \cdot \text{soil reading} \cdot \text{curve factor} = \begin{cases} \% \text{ water by volume} \\
\text{g water/cm}^3 \text{ soil} \\
\text{cm water/dm depth} \end{cases}$$
4.2.2. Determination of soil bulk density by attenuation of gamma radiation

REAGENTS AND MATERIALS

(1) Gamma density probe and scaler.
(2) Monitor.
(3) Three barrels of at least 30-cm radius containing soil material of known total weight and volume: one of medium sand, one of poorly aggregated, medium-textured soil, and one of well aggregated clay or clay loam. An aluminium access tube is placed vertically in the centre of the barrel.
(4) Auger or probe for digging holes to accommodate aluminium access tubes, or use same access tubes as for neutron-scatter moisture experiment.
(5) Aluminium tubes, 180 cm long, with an internal diameter slightly larger than gamma-density-probe diameter.
(6) Stop-watch.

PROCEDURE

(1) Determine proper operating voltage for scaler as in neutron moderation experiment.
(2) Measure bulk densities of three prepared barrels of soil materials. Readings should be expressed as cpm per unit of bulk density. Compare results with standard curve provided by the manufacturer.
(3) In connection with (2) above, determine and record radiation exposure rate with monitor at varying distance from the probe.
(4) Prepare a series of 180-cm deep holes and insert aluminium tubes, as in the moisture determination experiment, or use same holes. Take readings at 20, 45, 75, 105, 135 and 165 cm. Record 1-min. readings by stop-watch, as well as shield readings before and after each series.
(5) Obtain moisture values for each position as well. Subtract moisture value in weight/unit volume from the density value in comparable units of weight/unit volume.
(6) Interpret results for each profile studied.
1. COUNTING YIELD OF VARIOUS SAMPLE PREPARATIONS

1.1. Introduction

The purpose of this exercise is the determination of counting yield Y as a function of sample preparation and beta energy. The shelf number and working voltage are to be kept constant throughout the exercise.

Each sample contains a known amount of absolute activity (e.g. in μc). Ca$^{45}$ is used as a soft-beta emitter and P$^{32}$ as a hard-beta emitter.

The counting yield in per cent is defined by the equation

$$ Y = \frac{A^*}{D^*} \times 100\% = \frac{R^+ - r}{\text{(number of } \mu\text{c}) \times 2.22 \times 10^6} \times 100\% $$

The sample variation comprises two parts:
A. Different sample types, and
B. Different thicknesses of one sample type.

A. Different sample types

The following four types of sample will be prepared:
(a) Dried and ground plant material
(b) Material (a) dry-ashed
(c) Material (a) wet-ashed
(d) Solution (c) dried.

B. Different thicknesses of one sample type

Six samples of increasing thickness (from 50 to 400 mg per counting cup) will be prepared from dried and ground plant material.

1.2. Experiment

REAGENTS AND MATERIALS

(1) Dried and ground plant material containing 0.09 μc Ca$^{45}$ per gram.
(2) Dried and ground plant material containing 0.09 μc P$^{32}$ per gram.
(3) 2:1 nitric-perchloric acid.
(4) Counting cups.

PROCEDURES

A. Different sample types

(1) Obtain a counting cup containing 500 mg of material (a) consisting of Ca$^{45}$-labelled, dried and ground plant material with an activity concentration of 0.09 μc/g. Determine the counting yield.
(2) Obtain a counting cup containing the dry ash from 500 mg of material (a), and determine the counting yield.

(3) Into a counting cup pipette 0.5 ml of solution containing the wet ash from 50 mg of material (a). Determine the count rate immediately. Calculate the counting yield.

(4) Dry sample (3) under infra-red lamp (avoid boiling!). Determine the counting yield.

(5) Summarize the results as follows:

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Counting yield</th>
</tr>
</thead>
</table>

### B. Different thicknesses of one sample type

(1) Obtain six counting cups containing 50, 100, 150, 200, 300, and 400 mg Ca$^{45}$-labelled, dried and ground plant material with an activity concentration of 0.09 $\mu$C/g.

(2) Determine the counting yields of the six samples, and plot Y as a function of sample-mass-thickness ($\times$ mg/cm$^2$).

(3) Plot net count rate of sample against mass-thickness, and determine approximately at what mass-thickness the sample becomes "infinitely" thick? How does this thickness compare with the maximum range of the Ca$^{45}$ beta particles?

Finally, repeat Part A and Part B, using P$^{32}$ instead of Ca$^{45}$.

### 2. THE FIXATION OF CO$_2$ AND SEPARATION OF PHOTOSYNTHATES BY PAPER CHROMATOGRAPHY

#### 2.1. Introduction

As a result of photosynthesis CO$_2$ is reduced by green plants to a variety of organic compounds, primarily carbohydrates. An overall equation describing the process for carbohydrate formation may be written as

$$\text{CO}_2 + 2\text{H}_2\text{O} \xrightarrow{\text{light}} [\text{CH}_2\text{O}] + \text{O}_2 + \text{H}_2\text{O}.$$  

However, many compounds are formed: sugars, amino acids and organic acids. (It should be noted that C$^{14}$O$_2$ may be exchanged in the dark appearing as carboxyl groups, viz.

$$\text{H}_3\text{C-CO-COOH} + \text{C}^{14}\text{O}_2 \xrightarrow{\text{light}} \text{HOOC}^{14}-\text{CH}_2-\text{CO-COOH}.$$  

However, there is no net gain in mass and the C$^{14}$O$_2$ thus incorporated is readily lost again by re-exchange with C$^{12}$O$_2$. In addition to these simple compounds formed more or less directly in photosynthesis, C$^{14}$ will be found,
in varying amounts of time, in all the compounds of the cells. In this experiment a relatively long time of photosynthesis is employed; consequently the Cl4-label is widespread, much of it being fixed into polymeric substances: polysaccharides as starch, cellulose etc.; polyamino acids, i.e. proteins; and polynucleic acids, i.e. ribonucleic and deoxyribonucleic acid (RNA and DNA). However, this experiment deals only with the isolation and separation of the main "soluble" categories of the cell: sugars, amino acids and organic acids; and with the identification of the major soluble sugars: glucose, fructose and sucrose. The major tools used in this isolation and identification are ion exchange resins and paper chromatography.

Paper chromatography is very widely applied in biochemical research. As generally used, it is a partition chromatography, based on differential separation of various substances according to the differences in the partition coefficients between two solvents of the substances being separated. One of the solvents, which is generally water, is firmly adsorbed on the inert support, filter paper, and is therefore called the "non-mobile" solvent. The other solvent, generally an organic solvent, moves over the support by capillarity and is called the "mobile" solvent. Depending upon the partition coefficient of a substance, the distribution of the substance in both phases may vary; and if the substance favours the organic phase, the distance of travel of the substance with the organic phase will be great. On the other hand, if a substance favours the aqueous phase, the distance of travel will be small.

In essence, the procedure is carried out by the application of a small drop of test solution a short distance from one end of a filter paper strip. After the drop has dried, this end of the strip is placed in a solvent so that the solvent moves past the spot by capillary movement. After completion of the separation, the individual zones or spots are identified by colour reaction, radioassay or other suitable method.

By running a square chromatogram in two steps at right angles to each other, using different solvent mixtures, one may separate even closely related compounds. See Fig. 30.

The identification of a substance can be accomplished by calculation of the Rf value of the individual substance in a particular solvent and checking of the value with known substances. The Rf value of a substance is the ratio of travel distance of the particular substance to that of the solvent. It should be pointed out here that the Rf value is a function of solvent composition, temperature, pH, presence of salts and nature of the paper as well as other factors.

The primary usefulness of paper chromatography may be listed as follows:

(1) Separation of mixtures into their constituents;
(2) Demonstration of homogeneity of chemical substances;
(3) Demonstration of identity of substances;
(4) Qualitative and quantitative estimation of substances in a mixture.

As is shown in experiment 3.2, a plant can fix atmospheric CO2 and transport the photosynthates from the spot of synthesis to other parts of the plant.

By varying the time of synthesis and identifying the substances by radioassay of paper-chromatograms (autoradiography), one is able to trace the
pathway of atmospheric carbon from its gaseous state to a major class of products, the sugars. Furthermore, by variation of the time of exposure of the plant to $^{14}$C, the kinetics of the individual products of photosynthesis may be ascertained.

2.2. Experiment

In this experiment, a technique of the initial identification of the photosynthates will be demonstrated with $^{14}$O$_2$ and a green plant.

2.2.1. MATERIALS

(1) Plastic chamber $18 \times 12 \times 5$ cm.
(2) Bean leaves or whole plants.
(3) $\text{BaC}^{14}$O$_3$.
(4) 10% $\text{HClO}_4$.
(5) Syringe.
(6) Rubber cap.
(7) Silicone grease.
(8) Plant-growth dessicator.
(9) Aspirator.
(10) Suction flasks.
(11) 80% ethanol.
(12) Beakers.
(13) Round-bottomed flask.
(14) Water bath.
(15) Dry ice or liquid N₂.
(16) Whatman No. 4 chromatographic paper (or equivalent).
(17) Schleicher & Schüll 2043 B chromatographic paper (or equivalent).
(18) Ion exchange columns with resins (cation & anion).
(19) 5% HCl.
(20) 2% NaOH.
(21) 2% diethylamine.
(22) Chromatography tank.
(23) Micro-pipettes.
(24) Hair dryer.
(25) Phenol.
(26) Butanol.
(27) Propionic acid.
(28) Glucose, fructose and sucrose.
(29) Ammonium molybdate solution. Add 20 ml of 10% ammonium molybd-
date to 3 ml conc. HCl, shaking constantly. Then add 5 g NH₄Cl.
(The solution is stable about 1 week in a refrigerator.)
(30) X-ray film.
(31) Developing and fixing solutions.
(32) Test tubes.

2.2.2. PROCEDURE

A leaf and a plant are labelled by C¹⁴O₂ as follows:

2.2.2.1. Leaf labelling

(1) Place a freshly detached leaf in a plastic box having a removable
lid and a small side hole through which a glass tube with a rubber
cap extends. (See Fig. 31.)

![Fig. 31](image)

*Fig. 31*

Plastic box for labelling of plant material with a removable lid and two holes
through which glass tubes extend, one fitted with a rubber cap (serum vial stopper)
and another with a stopcock.

(2) Place a small planchet containing solid BaC¹⁴O₃ (approximately
30 μc) just inside the side hole so that addition of HClO₄ onto the
planchet through the hole from the outside by means of syringe can conveniently be carried out.

3. The box is made air-tight by coating of the inside lip of the lid with silicon wax. Evacuate the chamber (containing the plant) to approximately 1/2 atmosphere.

4. Add 2 ml of 10% HClO₄ with syringe via the hypodermic needle through the rubber cap onto the BaCl₄O₃ particles on the planchet.

5. Let the plant leaf photosynthesize for about two hours.

6. Remove the cover under the fume hood so that any remaining Cl₄O₂ can be expelled into the atmosphere. (Never open the cover in the laboratory.)

7. Take out the plant leaf, and place it on top of the dry ice to stop further synthesis, or drop it into simmering 80% aqueous ethanol or into liquid N₂.

2.2.2.2. Growth-chamber labelling of plant

8. Place a plant which has been grown in a culture solution in a pot. The plant and pot are then put into a dessicator (see Fig. 32), the lid of which is provided with a burette.

9. Place a small beaker containing about 100 μc of BaCl₄O₃ just under the glass tip of the burette so that the HClO₄ added to it can combine with BaCO₃ to form Cl₄O₂.

10. Add about 5 ml of 10% HClO₄ on top of the burette, being certain that valve 1 is closed.

11. Open valve 2, evacuate the inside for about 30 sec. with an aspirator so that the final pressure is about 1/2 atmosphere. Close valve 2.

12. Open valve 1 so that 10% HClO₄ flows down into the small beaker, forming CO₂. Close valve 1 (and keep it closed for an hour).

13. Allow the plant to photosynthesize for 1 h. During this time, connect the outlet of valve 2 with two suction flasks containing 10% NaOH so that CO₂ gas can be caught. (Be sure that valve 2 remains closed until the experiment is to be concluded.)
(14) After an hour open valve 1 until the internal and external pressures are equal. Then open valve 2 and apply suction gradually so that Cl\textsubscript{4}O\textsubscript{2} in the dessicator can be replaced by Cl\textsubscript{12}O\textsubscript{2}. (Operations in this section should be carried out in the fume hood.)

(15) After applying suction for 10 min., open the cover under the hood, cut the upper part of the plant and extract immediately or kill in dry ice or liquid N\textsubscript{2}.

2.2.3. Separation, Identification and measurement of synthates

(16) Both samples (leaf and plant) are placed separately in beakers containing just enough 80% ethanol to cover the plant material. Extract for 10 min., and decant the solution into a round-bottomed flask; repeat twice or until the leaf is free of chlorophyll.

(17) Place the round-bottomed flask on a water bath having a temperature of 50 - 60°C; connect the tip of the flask to an aspirator, and apply suction so that rapid removal of ethanol can be accomplished. Removal of the residual water will require a flask evaporator. Concentrate down to about 2 ml.

(18) Take out an aliquot of 20 - 30 \(\mu\)l with micro-pipette, and place it at one corner of filter paper (e.g. Whatman No. 4) on a spot previously marked by pencil (approximately 3 cm from each edge of a corner). Dry with a hair dryer between each addition of the liquid so that the liquid does not spread to more than 5 mm in diameter. The remaining solution in the flask is used for ion-exchange fractionation.

(19) Count the radioactive spot on the chromatogram on both sides and average the value. Use this information to calculate the subsequent exposure time (see Introduction, section 4.1 below).

(20) Prepare the solvents for chromatographic separation. The first solvent is phenol-water (100 : 20 by volume) adjusted to pH 5 - 6. The second solution is
   (a) Butanol-water (1246 : 84 by volume), and
   (b) Propionic acid-water (335 : 88 by volume).
   Just before use, mix equal volumes of (a) and (b), and warm slightly to form a single-phase solvent.

(21) Place the phenol-water solution in a chromatographic tank, and saturate the air with its vapour. Then place the treated end of the filter paper, on which the sample is dried, in the solution. Let the solvent move by capillarity until the wetting front reaches 2 - 3 cm or less from the end of the paper. Mark the wetting front with pencil.

(22) Take out the paper and dry it. At this time the tank is emptied and cleaned and the second solution is added.

(23) After the paper has dried and the tank is saturated with the vapour of the second solution, hang the paper so that the origin is again on the bottom but rotated 90° from its initial position. See Fig. 30.

(24) Let the solvent move by capillarity until the wetting front reaches 2 - 3 cm or less from the end.

(25) Take out the paper; hang it and dry it, as above.
(26) Place the chromatogram in the darkroom on X-ray film, and leave it for the calculated exposure (see step (19)), after which the film is processed as usual.

(27) Prepare the resin columns: one H\(^+\) saturated (cation) exchanger, the other an OH\(^-\) saturated (anion) exchanger.

(28) The residual solution (of step (17)) is poured on top of the cation exchanger column, the outlet of which is connected to the top of the anion exchanger. Elute the unexchanged solutes from the resin with two bed volumes of H\(_2\)O. Any cationic groups will be retained by the cation exchange resin; and anionic groups, by the anion exchange resin.

(29) After elution of the anionic resin with two more bed volumes of water and combination with the previous eluent, all neutral groups such as sugars and neutral esters are eluted.

(30) Separate the cationic and the anionic columns, and elute the resins with 5\% HCl and 2\% dimethylamine, respectively. Collect the eluents.

(31) These three fractions, neutral, cationic and anionic groups, are concentrated in vacuum; and each fraction is separated independently by means of paper chromatography with butanol/acetic acid/water (4 : 1 : 5) in one-dimensional form, and the autoradiogram is made and developed as usual (see step (26)).

(32) To identify the unknown substances in the neutral fraction, place 20 \(\mu\)g each of glucose, fructose and sucrose in spots adjacent to the unknown spot of neutral fraction, and run the chromatography.

(33) After the separation and the drying of the paper, develop (spray lightly) with phosphomolybdate spray for colour reaction. Heat at about 70°C for 20 min. The sugars will appear as blue spots.

(34) For other unknown substances R\(_f\) values obtained from the standard book can be used for identification.

(35) To determine the activity quantitatively, an elution of spot should be carried out. The filter paper around the spot is cut in a wedge-shaped form and attached to a well-saturated chromatographic sheet (see Fig. 33).

![Fig. 33](image)

Assembly for elution of spots on paper chromatograms for quantitative activity determinations

(36) Using water as eluting solvent, let the liquid drop down to the receiving beaker. The activity of the liquid and its concentration are then measured by the usual procedures.
3. CO₂ FIXATION BY HIGHER PLANTS

3.1. Introduction

It is well known that green plants can fix gaseous carbon dioxide in the light and synthesize it into organic materials. Part of the synthesized products is usually transferred from the spot of synthesis to other parts of the plant. The synthesis and translocation of the product can conveniently be studied by autoradiography although a quantitative evaluation is difficult.

3.2. Experiment

REAGENTS AND MATERIALS

(1) BaC¹⁴O₃.
(2) J-shaped glass tubing; 1.5-cm diameter, having one end connected with a serum vial stopper and the other end with a flat surface (see Fig. 34).
(3) 10% HClO₄.
(4) Hypodermic syringe.
(5) Burette stand and holder.
(6) Bean plant, 20 - 30 cm high, with first two leaves fully expanded (approximately 15 d after germination).

PROCEDURE.

(1) Working under a fume hood, place a few small crystals of BaC¹⁴O₃ into the tube.
(2) Apply some vaseline on the flat end of the glass tube, which is held with a burette clamp, and place it under a leaf of the bean plant, applying slight pressure on the leaf so that complete adherence of the tube to the leaf occurs without any leak.
(3) By means of a hypodermic syringe, gently inject 2 ml of 10% HClO₄ into the tube through the serum vial stopper. CO₂ evolution will take place on contact of the HClO₄ with the BaCO₃.
(4) Allow the leaf to photosynthesize for 30 min.
(5) Disconnect the leaf from the glass tubing. The unused labelled C¹⁴O₂ should not be inhaled. The aerial portion of the plant is cut and an
(6) Place film and specimen into a deep freezer for the entire exposure time calculated according to Table IX below.

(7) Following autoradiography and processing of the autoradiogram, remove all leaves and petioles except that of the fed leaf.

(8) Remove the fed leaf at the base of the petiole and save for experiment 4.2.1, if desired.

(9) Cut the petiole and remaining stem below and above the fed leaf into 2-cm lengths.

(10) Extract each length separately with simmering 80% ethanol in a test tube. (The volume of the aqueous ethanol should be kept as small as possible, e.g. 1–2 ml.) Concentrate each extract and plate.

(11) Count the plates and plot the count rate against the distance along the petiole and stem from the fed-leaf base.

QUESTIONS

(1) How can the information from this experiment be used for calculation of the approximate rate of translocation of the photosynthate?

(2) When will it no longer be possible to utilize the radioisotopic information for calculation of the rate of translocation of the photosynthates in this experiment?

(3) State the disadvantage of using HCl and H2SO4, respectively, to liberate CO2 from the BaCO3.

4. AUTORADIOGRAPHY

4.1. Introduction

Ionizing radiations act upon photographic emulsion in the same manner as light does. If a film is exposed to an object containing radioactive material, upon development a photographic image which provides visualization of the location of the radioactivity in the sample is obtained. This image is variously known as autoradiogram, autoradiograph, autogram or radio-autogram. Depending upon whether the object is being exposed in a gross manner or at the cellular level, the processes may be further subdivided into macro- or micro-autoradiography, respectively.

In macro-autoradiography cellular detail is rarely observed, but localization is generally adequate to provide information concerning diverse identifiable tissues, such as veins and veinlets of the blade, or, in favourable circumstances, even the leaf islets themselves. In theory, the method could be made quantitative by counting of the grains produced. This provides a direct measure of the radiation flux. Alternatively, some degree of quantization, i.e. the relation between the degree of darkening and the activity in that area, can be obtained by insertion of a graded radioactive wedge onto the film adjacent to the leaf during its exposure. By comparison of equi-
valent levels of darkening of the known values of the wedge with unknown leaf values, a rough equivalence can be found. In this experiment, however, comparison will be made by direct counting of punched-out leaf discs.

The factors involved in macro-autoradiography are: (1) speed of film (including "graininess"), (2) thickness of tissue (with special reference to nature and energy of radiation), (3) distance between film and specimen, and (4) absence of artifacts.

Artifacts, i.e. film images resulting from processes other than specimen radiation, are generally the result of (a) chemical reactions from plant exudates or expressed sap etc., and (b) mechanical pressure, from excessive pressure of tissue onto the film, causing dislocations in the film silver grains. These are revealed as "pressure lines" mirroring elevated portions of the specimen.

The smaller the distance between the specimen and the film, the greater is the resolving power of the autoradiogram, as scattering is diminished. (The size of the film image of a point source of activity will be smaller, the closer the two.)

The thickness of the specimen is of importance primarily for micro-autoradiography, where the self-absorption of the radiation may be a critical factor. For very soft radiation, as that of H, thickness is obviously of little importance, since the only radiation leaving the specimen will come from the surface 1 μm (10⁻³ mm) of the section. For stronger radiation, e.g. from C¹⁴ or S³⁵, radiation may come from as much as 20 μm or more, i.e. more than a one-layer thickness of cells. Consequently, the film image obtained can depend on the specimen. This is even more true for tissue with still stronger radiation, as that from P³² or Cl³⁶, where many layers of cells are penetrated. Here there would be no chance for cellular delineation unless the specimens were thin. Similar considerations hold for the film: the greater the energy of the isotope radiation, the greater its penetrability into the film and, correspondingly, the more diffuse the image. Consequently, the softer the radiation, the better the resolution. The decreasing resolving powers of commonly used beta-emitting isotopes are H³ (0.005 MeV, average), C¹⁴ (0.05 MeV, average), S³⁵ (0.05 MeV, average), Ca⁴⁵ (0.075 MeV, average) and P³² (0.7 MeV, average).

The specific ionization of the isotopes must also be considered. Thus H³, with its soft radiation, will require, in theory, approximately ten times the surface specimen activity to produce the same film darkening as a similar specimen containing C¹⁴. In practice, because of the contribution from lower layers of the C¹⁴ specimen, approximately one hundred times the counted activity of H³ is required. On the other hand, the more energetic beta particles of P³² pass through the film without appreciable ionization (compared with C¹⁴). Therefore, more P³² than C¹⁴ is required for comparable darkening. Similarly, gamma rays, with their great range and low specific ionization, are relatively inefficient.

It is important to avoid both under- and over-exposure, the latter causing a loss of resolution. The simplest way to evaluate the amount of radioactivity in biological material necessary to produce an image within a reasonable exposure period is to make a rough determination, if possible, of the activity of the sample. It is generally stated that for C¹⁴ approximate-
TABLE IX

DATA OBTAINED WITH A HALOGEN-QUENCHED G-M TUBE (5 cm² ACTIVE AREA) AND ASSUMING 5% COUNTING YIELD

<table>
<thead>
<tr>
<th>cpm (leaf surface)</th>
<th>dis./min. per cm²</th>
<th>To give 5 x 10⁶ dis.</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>400</td>
<td>9-d exposure</td>
</tr>
<tr>
<td>500</td>
<td>2000</td>
<td>2-d exposure</td>
</tr>
<tr>
<td>1000</td>
<td>4000</td>
<td>1-d exposure</td>
</tr>
<tr>
<td>5000</td>
<td>20000</td>
<td>0.5-h exposure</td>
</tr>
</tbody>
</table>

ly 5 to 10 million beta particles per square centimeter must strike an X-ray film to produce adequate blackening.

Exposure times may be calculated with the aid of Table IX.

With a windowless G-M tube to count H³, the same cpm would require exposures approximately a hundred times longer, while the exposure times for P³² are approximately twenty-fold.

In experiments 4.2.1. and 4.2.2. bean plants fed through the roots are used for observation of the uptake and distribution of P³² in the upper parts.

Subsequently, following autoradiography, the plant is sampled by means of a cork-borer, and the activity per unit area of the leaf discs is determined by direct counting (neglecting self-absorption because of the high energy of P³² betas).

Macro-autoradiography provides information concerning the distribution of particular elements in a tissue or group of cells. Micro-autoradiography is concerned with the distribution of a substance at a cellular or subcellular level.

In experiment 4.2.3 use is made of a compound, thymidine, which is incorporated into a unique and discrete portion of a cell, the chromosomes, which lie solely within the nucleus. By the use of thymidine-H³ and the viewing of the autoradiograph microscopically, subcellular localization of the chromosome can be observed, thus indicating which cells are dividing and which are not. Furthermore, by careful control of feeding times, dose and microscopy, the rate of labelling of the different chromosomes and parts of a single chromosome may be noted under favourable circumstances.

4.2. Experiments

4.2.1. Macro-autoradiography of bean leaf by direct-contact method

REAGENTS AND MATERIALS

(1) P³² labelled ortho-phosphate solution.
(2) Hoagland solution.
(3) Developer.
(4) Stopping bath, 2% CH₃COOH.
PROCEDURE

(1) Place a trifoliate bean plant into a 100-ml Erlenmeyer flask containing 50 ml of Hoagland's solution with one-tenth of the normal phosphate level.

(2) Label the solution with 50 μc P³₂O⁴⁻³, and shake gently to mix well. Allow the plant to take up nutrients for 1 h.

(3) Cut off a leaf and place it on filter paper.

(4) Place a metal plate with 1-cm² hole onto the centre of the leaf and determine the activity through the hole with a count-rate meter.

(5) Estimate the exposure time required to obtain 100 million particles per cm² film. (If the labelled leaf from experiment 3.2 (step 8) is used, the appropriate C¹⁴ estimation must be made.)

(6) Take the leaf, mounted on cardboard and covered with Mylar or cellophane, to the darkroom; take the film out of the envelope; place the leaf onto the film and put film plus leaf back into the envelope, and place this in the cassette.

(7) Store in a deep freezer for the estimated exposure time.

(8) After exposure, develop the film according to the instructions at end of experiment 4.2.2.

4.2.2. Macro-autoradiography of bean plant by direct-contact method and evaluation of activity distribution by G-M counting and degree of film blackening.

REAGENTS AND MATERIALS

Same as for experiment 4.2.1.

PROCEDURE

(1) Place a trifoliate bean plant into a 100-ml Erlenmeyer flask containing 50 ml of Hoagland's solution with one-tenth of the normal phosphate level.

(2) Label the solution with 50 μc P³₂, and shake gently to mix well. Allow the plant to take up nutrients for 1 h.

(3) After the feeding is over, take out the plant; let the nutrient solution drain off the roots, and then dry these with blotting paper. Place plant into another Erlenmeyer flask containing non-labelled Hoagland's solution, and leave there for 20 min.
(4) Take out the plant; again let excess solution drain off the roots, and blot away the residual water. Mount the plant on blotting paper, dissecting the plant into sections, if necessary.

(5) Estimate required exposure time, and expose plant to film as in experiment 4.2.1 (steps 4-7).

(6) After processing of the film (see below), note the different degrees of blackening on the film. Choose three representative areas, one very dark, another very light and a third in between.

(7) Cut out the corresponding areas on the leaf with a cork borer.

(8) Count the leaf discs, and compare the degrees of blackening with the count rate of the leaf discs.

**PROCESSING THE FILM**

(1) Read instructions given by the manufacturer, and prepare processing solutions accordingly.

(2) Place the film into the developing solution for the appropriate time (depending very much on temperature; as a rule of thumb, 5 min. is a proper value). Make sure that the film is completely submerged in the solution. Agitate during developing.

(3) Take out the film, and dip it into a 2% acetic acid bath for 20 sec. to stop further development.

(4) Place into fixing solution for double the time it takes the film to "clear".

(5) Soak the film in water for 30 min., and hang up to dry.

**QUESTIONS**

(1) Why is the plant placed in non-radioactive Hoagland's solution following the exposure to radioactive solution?

(2) Discuss the relationships between grain size, resolution, speed of film and exposure time.

(3) Discuss the feasibility of quantification of this procedure by grain counting. How would you count these grains?

**4.2.3. Micro-autoradiography of tritiated thymidine in chromosomes**

**REAGENTS AND MATERIALS**

(1) Hoagland's nutrient solution.
(2) H3-thymidine.
(3) Maize seedlings.
(4) Tweezers.
(5) Scissors.
(6) Razor blade.
(7) 1 N HCl.
(8) 95% ethanol.
(9) 10% acetic acid; 40% acetic acid.
(10) KCr(SO₄)₂ · 12H₂O (chrome alum).
(11) Developer, such as D-19-b.
(12) Stripping film plate, such as AR-10.
(13) Gelatin.
(14) Acetic alcohol (3 parts C₂H₅OH + 1 part glacial acetic acid).
(15) Film fixing bath.
(16) Test tubes.
(17) Beakers.
(18) Stopped glass tubes.
(19) Prepared slides†.
(20) Petri dish.
(21) Eye dropper pipette.
(22) Needle.
(23) Cover glass.
(24) Glass rod.
(25) Light-tight box.
(26) Processing dish.
(27) Elder pith.
(28) 5% polyvinyl alcohol.

PROCEDURE

(1) Put 10 ml of Hoagland's solution into a test tube, and label the solution with 10 μc of tritiated thymidine.
(2) Two maize seedlings, grown for two or three days after germination, are placed on top of the test tube so that the roots can take up nutrient from the solution for 1 h.
(3) Take out the plants from the solution; wash the roots carefully with distilled water, and place the plants on another test tube containing non-labelled Hoagland's solution. Leave the plants for 2 d.
(4) Remove the seedlings, carefully blotting off the water on the plant roots with absorbent paper; cut off two root tips of about 2-cm length each, and put the roots into a beaker containing 50 ml of acetic alcohol to fix for 20 min.
(5) Take out one root tip from the fixing solution; put it into a test tube containing 5 ml of 1 N HCl, and place the tube in an oven at 60°C for 10 min. This root will be used for squashing, while the other root will be used for sectioning.

SECTIONING

(6) The root in the beaker is washed with distilled water for 5 to 10 min. and then placed between the two halves of elder pith to facilitate cutting.

† Prepare the slides as follows: 1.5 g of gelatin is dissolved in 7 ml of 15% acetic acid under moderate warming. To this solution a mixture of 30 ml of ethanol and 70 ml of water is added. Finally, 0.1 g of chrome alum dissolved in 2 ml of water is added to the solution. Mix well. The slides are dipped into the solution and dried.
(7) With a razor blade remove the tip 0.5 mm, consisting of the kalyptra (Fig. 35), and make the cutting of the next section as thin as possible. A microtome may be used for cutting, if available.

(8) Arrange the cuttings with a needle in two rows, near the centre of a prepared slide, and dry (Fig. 36).

(9) Wash the root in the test tube with water, and place on a prepared slide glass.

(10) Remove the kalyptra with a razor blade, and cut the root into a few slices of 0.25- to 0.5-mm length.

(11) Collect the slices in the centre of the slide; add a drop of 40% acetic acid, and cover with a cover glass.

(12) Apply light pressure from above with a match stick until the cells are spread to an almost monocellular layer. Avoid lateral movement of the cover glass.

(13) Put the slide upside down in a Petri dish, supporting both ends with glass rods.

(14) Add 10% acetic acid into the dish until the slide is just covered. A short time later, the cover glass will fall off, leaving most of the squash on the slide.

(15) Take out the slide; carefully dip into Petri dish containing water and dry.

(16) Cover the prepared slide with stripped film, as follows:
STRIPPING-FILM TECHNIQUE

(17) Kodak stripping-film plate AR-10†, 5-μm thick in emulsion with a 10-μm gelatin protective layer, is cut into sections in a dark room with a razor blade as shown in Fig. 37, leaving a margin of at least 5 cm on all sides.

(18) A corner of one film section is loosened by means of a razor blade. Remove it gently from the plate.

(19) Place the film upside down on the surface of distilled water at 21–24°C. The film should be floated on the surface for at least 3 min, or until it is fully expanded.

(20) Carefully lower the slide below the floating film and lift it so that the film adheres uniformly on the slide without wrinkling. Turn the slide so that the excess part of the film will adhere on the back of the slide.

(21) Place the slide in a light-proof box, and expose under cool conditions of 4°C for 1 week. Humidity control may be obtained by use of an adjacent vial of saturated aqueous CaCl₂.

FILM PROCESSING

(22) Prepare the developing and fixing solution, and adjust the temperature to 20°C.

(23) Transfer the slides into the slide dish containing the developing solution. Leave them without agitation for 5 min.

(24) Rinse the slides in another dish containing distilled water by leaving them there for 15 sec.

(25) Transfer the slides to a dish containing the fixing solution, and leave for 10 min.

(26) Turn on the light, having made sure that the films are clear; rinse in running tap water for 30 min. Dry the slides with warm forced air.

(27) Put a few drops of 5% polyvinylalcohol solution on the slides, cover the specimen with cover glass, remove the film adhering underneath the slide and observe through a microscope.

† This is an orthochromatic film, and suitable safe lights may be used so that the operations discussed below need not be carried out in complete darkness.
QUESTIONS

(1) Compare the use of H\(^3\) and C\(^{14}\) in exposure time and resolution.

(2) How would one alter the procedure to compare a water-soluble substrate?

(3) How would you change the procedure to detect RNA instead of the DNA of the chromosomes?
SOIL-PLANT

1. UPTAKE OF IONS BY PLANTS

1.1. Introduction

The amounts of ions taken up from solution by plants are measured as a function of concentration of the ions in the solution (experiment 1.2.1) and time (experiments 1.2.2 and 1.2.3). In these experiments, two methods of measuring amounts of nutrients taken up are demonstrated: measurement of the decrease in activity in the nutrient solution and measurement of the increase of activity in plant organs; in experiment 1.2.1 both methods are carried out simultaneously.

In experiment 1.2.2 the technique of double labelling is used. The difference of amount of sulphate and phosphate anions taken up by oats will be studied by use of S\textsuperscript{35} and P\textsuperscript{32}. As S\textsuperscript{35} emits much softer β-particles than does P\textsuperscript{32}, it is possible in the process of counting to distinguish between both sources by counting before and after absorption of the β from S\textsuperscript{35} by a suitable aluminium absorber.

In experiment 1.2.3 the excised-root technique is demonstrated in measurement of the uptake of rubidium in the presence and in the absence of potassium. This experiment shows that potassium decreases the uptake of rubidium. A possible approach to interpretation of this effect makes use of the carrier theory for ion uptake. According to this theory, ion absorption is a series of consecutive reactions starting with the combination of an ion with a specific "carrier" (some chemically related ions may combine with the same carrier) and culminating with the deposit of that ion in the cell, usually against a concentration gradient. These two events can be described by the following scheme:

\[
\begin{array}{c|c|c}
\text{outside} & \text{carrier zone of root} & \text{inside root cell} \\
(x^0) + & (c) \xrightarrow{k_1} (xc) \xrightarrow{k_2} (x^i) + (c^i) & k_1 \quad k_2 \\
\end{array}
\]

where (x\textsuperscript{0}) and (x\textsuperscript{i}) stand for concentrations of the test ion, (c) and (c\textsuperscript{i}) stand for concentrations of the carrier, (xc) stands for concentration of ion + carrier complex, and k stands for rate constant.

According to scheme (1), if the concentration of ion plus carrier complex remains constant and k\textsubscript{2} is negligible, the rate of ion uptake is constant, and it is proportional to the concentration of ion plus carrier complex given for a particular outside concentration of ions:

\[
v = \frac{d(x^i)}{dt} = k_2 \cdot (xc),
\]

in which v is the rate ("velocity") of ion uptake, which is controlled by k\textsubscript{2}.
If the test ion outside is labelled at time $t = 0$, assuming $k_{1}$ to be relatively great, all the ion plus carrier complex will be labelled very shortly after zero time; and in practice the ion uptake in moles as followed by the activity concentration in the roots should be a linear function of time, as follows:

$$x^{*} = (x^{*} c) + (x^{*} c)k_{2}t$$  \hspace{1cm} (2)

The rate of metabolic turnover of the complex can then be expressed as the difference of the two molar (or activity) concentrations $x_{f}^{*}$ and $x_{f}^{-}$ in the roots divided by the corresponding time interval:

$$v = \frac{d(x_{1})}{dt} = \frac{(x_{f}^{*} - x_{f}^{-})}{(t_{2} - t_{1})}.$$  \hspace{1cm} (3)

Plotting of $x^{*}$ against $t$ and extrapolation of the straight line to time $t = 0$ shows, in agreement with equation (2), that the formation and reverse splitting of the ion plus carrier complex (controlled by $k_{x}$ and $k_{-1}$) are rapid processes, whereas the metabolic intake of the complex occurs in measurable times and is the rate-limiting step in the overall series of consecutive uptake reactions.

Note: The experimental conditions must be such that the specific activity of the nutrient solution remains constant during the experiment.

1.2. Experiments

1.2.1. Uptake of phosphate ions as a function of phosphate concentration in the solution

REAGENTS AND MATERIALS

(1) Nutrient solution containing the following composition per liter:

- 1.2 mmole KNO$_{3}$
- 0.5 mmole Ca(NO$_{3}$)$_{2}$
- 0.2 mmole MgCl$_{2}$
- Trace amounts of Zn, Cu, Fe, Mo, B and Mn.

(2) Oat or barley plants, approximately 1 - 2 weeks old after germination.

(3) KH$_{2}$PO$_{4}$.

(4) Carrier-free P$^{32}$.

(5) Crucibles.

(6) Spatula.

(7) Glass rods.

(8) Aeration equipment.

(9) Holding plate with cardboard growing containers.

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PROCEDURE

(1) Take 5 containers (plastic or paraffinized cardboard) of 100-ml volume; cut out bottom, leaving a small rim, and replace it by a stainless-steel meshwork. Plant 10 pre-germinated seeds in each container; bring them in superficial contact with aerated nutrient solution, using holding plates, and cover them with wet cheese-cloth, the edges of which hang in the solution. The cheese-cloth should be removed when the green tops are approximately 3 cm long. For assurance of a steady state in the plant-solution system, the nutrient solution should be renewed daily, the last time 1 h before the experiment starts. After about two weeks, containers with five plants are transferred onto experimental solutions, as described in the following steps (2) - (5).

(2) Nutrient solutions containing phosphate in addition to the original composition are prepared so that the following phosphorus concentrations are obtained:
- 0.01 mmole P/l
- 0.025 mmole P/l
- 0.05 mmole P/l
- 0.10 mmole P/l
- 0.25 mmole P/l

(3) Approximately 350 ml each of these solutions in beakers is labelled with 1 ml containing $8 \mu c \mathrm{P}^{32}$.

(4) After thorough mixing of the solution, 1 ml of each solution is taken out for counting.

(5) The cardboard containers with the plants are placed on the beakers, with three pins as support, and left for 3 h with continuous aeration.

(6) Plants are taken out, rinsed rapidly, dried with cheese-cloth and cut between roots and shoots for separate measurements.

(7) 1-ml amounts of the remaining solutions are pipetted for counting so that the uptake of $\mathrm{P}^{32}$ can be determined from the difference in activity of the solution before and after the treatment. Calculate the percentage standard deviation of the difference in activity, assuming a standard deviation of 2% in individual counts, by using the formula:

$$\sigma_D = (\sigma_1^2 + \sigma_2^2)^{1/2},$$

where $\sigma_D$ is the standard deviation of difference of count rates and $\sigma_1$ and $\sigma_2$ are the standard deviations of individual count rates.

(8) Shoots and roots are separately cut into small pieces with scissors, placed in crucibles and ashed in the muffle at 550°C for 2 h. Take care to prevent losses resulting from air circulation.

(9) A known weight of ash is spread on the counting planchet. For spreading the ash it is necessary to use some water and detergent.

(10) The samples in the planchets are counted with an end-window G-M tube. Convert count rates to mmole P. Remember: $P = P^*/s^*$. 149
The amounts of phosphate absorbed by the plants are plotted as a function of the initial phosphate concentration.

Compare the results obtained by solution and plant counting.

1.2.2. **The rate of uptake of phosphate and sulphate ions by plants**

**REAGENTS AND MATERIALS**

1. Nutrient solution of similar composition as in experiment 1.2.1.
2. 50 oat plants, approximately 1-2 weeks old.
3. 1-1 Petri dishes.
4. Growing equipment as in preceding experiment (1.2.1).
5. \( \text{P}^{32} \)
6. \( \text{S}^{35} \).

**PROCEDURE**

1. Transfer 700 ml of nutrient solution to a 1-1 Petri dish, and label with 30 \( \mu \text{c} \) \( \text{P}^{32} \).
2. Take 1-ml aliquot for the determination of the activity after drying (standard).
3. Add 30 \( \mu \text{c} \) \( \text{S}^{35} \); then take 1-ml aliquot for the determination of the activity after drying (standard).
4. Grow the plants as described in experiment 1.2.1. Transfer a batch of 10 cardboard containers on a holding plate onto one Petri dish (= 50 plants). Aerate solution continuously.
5. Take 1-ml aliquots of the solution for counting at the following intervals after insertion of the plants: 2, 4, 8, 15, 30, 60, 120 and 240 min.
6. Harvest the shoots and determine the fresh weight.
7. The planchets, after drying under infra-red lamp, are counted once with a thin-mica-window G-M tube and then counted again with the same tube without change of the geometry but with the 40 mg/cm\(^2\) aluminium absorber placed between the sample and the tube.
8. The growth solution activities of \( \text{P}^{32} \) and \( \text{S}^{35} \) per unit weight of plant shoot are calculated by comparison of the count rates with those of standards. Plot these activities as a function of time.
9. Discuss the results obtained.

1.2.3. **Interaction of rubidium and potassium in ion uptake**

**REAGENTS AND MATERIALS**

1. \( 10^{-3} \) M RbCl containing 0.2 \( \mu \text{c} \) Rb\(^{86} \)/ml.
2. Same solution as (1) but containing \( 10^{-3} \) mole/1 KCl in addition.
3. Oat plants grown on 1:10 diluted Hoagland solution for 1 week after germination and transplanted into distilled water the day before the experiment, totalling about 15 g of excised roots.
(5) Counting cups.
(6) Scissors.
(7) Aeration equipment.

PROCEDURE

(1) Excise the roots of plants having been grown in water culture (but not on quartz sand); wash several times with distilled water, and place in a beaker containing distilled water. Continue aeration for 1 h.

(2) Take out and blot the roots between cheese-cloth. Roots should be handled with utmost care; beware of touching them unnecessarily.

(3) Weigh out 1 g each of excised roots into 10 wide-mouthed Erlenmeyer flasks containing approximately 100 ml of distilled water. Weighing should be done rapidly to avoid air-drying of the roots.

(4) Decant water and place flasks upside down to drain, leaving roots adhered on the flask walls. Do not lose any roots.

(5) Add 250 ml of $10^{-3}$ M RbCl containing Rb* into each of five beakers at 15-sec. intervals.

(6) Start to decant the solution into waste bottle shortly before the end of each experimental time period, so as to finish decanting 2, 4, 6, 8 and 10 min. respectively after immersion into the experimental solution. Immediately after decanting of the radioactive solution, the roots are washed four times with distilled water for 15 sec. each. The first wash-water should touch the roots at the end of the decanting period.

(7) Spread the roots on counting cups; dry under infra-red lamp and count. In counting the standard, it is necessary to spread 1 g of root on the counting cup before addition of 1 ml of the standard so that geometry of standard and sample is similar. To compensate for errors during weighing procedure and for possible losses of roots during rinsing, the dry weight of the samples can be taken and counting results can be expressed per unit of dry matter.

(8) Repeat these operations for the remaining five oat root systems with Rb*Cl solution containing KCl.

(9) Plot the uptake of Rb for both experimental results as a function of time on ordinary graph paper.

(10) Discuss the results.

2. DETERMINATION OF A-VALUE

2.1. Introduction

The nutrient status of the soil, the effectiveness and reaction of fertilizers in the soil etc. may be quantitatively measured with labelled fertilizer.
FRIED and DEAN† suggested a concept of measuring the amount of nutrient in a source in terms of a standard source of that nutrient. If a crop has access to two sources of phosphorus with no interaction between the sources, the plants will absorb phosphorus from the two sources in direct proportion to the respective amounts "available". Now, let these amounts of available phosphorus be A and B, and let the respective amounts of phosphorus per plant from the two sources be a and b; then

\[ a : b = A : B. \] (1)

In accordance with this concept, labelled fertilizers are particularly suitable for measurement of amounts of standardly available nutrient in soils. For practical purposes the following equation, which is another way of expressing (1), is most commonly used:

\[ A = B (a/b) = B[a/(a+b)]/[b/(a+b)] \text{ or } A = B \{ (1 - y)/y \}. \] (2)

where

- A (A-value) is the amount of soil phosphorus measured in units of the applied fertilizer standard;
- B is the amount of labelled standard P-fertilizer applied; and
- y is the fraction of the phosphorus in the plant derived from the labelled standard P-fertilizer (1 - y, thereby, the fraction from the soil).

By definition: \[ y = b/p, \] where \( p = a + b \) is the P-content per plant. Furthermore, the amount of fertilizer-P per plant is equal to the amount of activity per plant \( p^* \) divided by the specific activity of fertilizer-P (see Basic Part, Lecture Matter, section 4.1, equation (3)), i.e.

\[ b = p^*/s^*_{\text{fert.}}. \]

Substituting for b in the above y equation, we find

\[ y = (p^*/p)/s^*_{\text{fert.}} \text{ or } \]

\[ y = s^*_{\text{plant}}/s^*_{\text{fert.}}. \] (3)

It is worth noticing in equation (1) that an A-value calculation is based on a specific activity ratio (equation (3)); hence in determination of A-values it is only necessary to measure relative specific activities.

The determination of A-values by this procedure is subject to certain experimental limitations because the fertilizer standard interacts to a greater or lesser degree with the soil. In field experiments and with less water-soluble or granulated fertilizers, the experimental limitations are not as great. Experimental determinations of A-values should be so designed as to minimize the soil-fertilizer interaction.

If in an A-value experiment radioactive phosphorus is mixed throughout the soil as the standard and the reaction of the phosphorus with the soil is essentially completed, giving a constant specific activity of phosphorus throughout the system, then the experiment has accomplished the measurement of an L-value, as described in Applied Part, A. Soils, section 2.1.

In the past some confusion has arisen because the forms of the equations used for calculating A- and L-values are identical. However, it must be remembered that only the form and the units are the same; the ideal experimental designs are diametrically opposite limiting cases. Both are difficult to fulfill in practice.

Small grain crops will be grown on three different soils which have received radioactive phosphate fertilizer. After a certain period of growth they will be harvested and radioactivity measured; the phosphate content will be determined, and the A-value of the soils will be calculated from the equation.

2.2. Experiment

REAGENTS AND MATERIALS

(1) Vanadate solution (0.25%): 2.5 g of NH₄VO₃ is dissolved in 500 ml of boiling water. Cool the solution and add 20 ml of conc. HNO₃. Dilute to 1 l after complete cooling.
(2) Molybdate solution (5%): 50 g ammonium molybdate in 1 l of water.
(3) 1:2 HNO₃.
(4) Rye grass and/or millet seed (or other small grain crops, to minimize errors from seed phosphorus content).
(5) Soils differing in phosphorus status.
(6) Radioactive standard P-fertilizer, approximate specific activity of 0.25 mc/g P₂O₅ (granulated superphosphate approximately 1 mm φ).

PROCEDURE

(1) Weigh out samples of labelled standard fertilizer (radioactive superphosphate of approximately 20% P₂O₅, approximately 0.2 mc P³²/g P₂O₅) in amounts of 90 and/or 180 mg/pot.
(2) Mix the fertilizer portions with 1800 g each of soils in a porcelain bowl or plastic mixing device. Keep a sample of standard fertilizer for specific-activity determination.
(3) Transfer the soils into plastic, or plastic-bag-lined, pots of approximately 2-l capacity, and sow 20 seeds.
(4) Grow the plants for 3 weeks; harvest the upper parts of the plants, dry at 75°C and weigh.
(5) Weigh out 2 g of the finely ground plant material in a crucible, and ash at 550°C for 5-10 h.
(6) Moisten the ash with a few drops of water, add 10 ml of HNO₃ (dil. 1:2), evaporate half the volume, filter, rinse filter with distilled water, and make filtrate up to 50 ml.
(7) To obtain the standard fertilizer solution, weigh out 0.1 g into a 100-ml volumetric flask, add 10 ml of HNO₃ (dil. 1 : 2) into the flask, and dissolve the fertilizer by heating. Make up the volume to 100 ml with distilled water, and filter.

(8) To obtain the phosphate concentrations of the digests of plants and fertilizer, pipette 5 ml of each into 100-ml volumetric flasks, and dilute with water to about 30 ml.

(9) Add to the flasks 10 ml of 1 : 2 HNO₃, 10 ml of 0.25% vanadate and 10 ml of 5% molybdate solutions successively and in that order. Make up to volume with water and mix well.

(10) 45 min later, the per cent transmittance of the solutions is measured with a spectrophotometer of wave-length 470 μm. The measured phosphorus concentration is obtained from a calibration curve, which has been prepared by the running of these phosphorus determinations on known standard solutions.

(11) To obtain the activity concentrations of the digests (from steps 6 and 7) of plants and fertilizer, pipette 10-ml aliquots into a liquid containing tube. Count each activity up to a sum count of 10,000. If the count rate of the fertilizer exceeds 10,000 cpm, dilute digest obtained in (7) and recount.

(12) From the specific activities of the phosphorus in plant and fertilizer, calculate y (the fraction of the phosphorus in the plant derived from standard P-fertilizer) and the A-value of the soil in mg P/kg soil and in kg P/ha (assuming that 1 ha equals 3 X 10⁶ kg of soil). Units of A-values are units of the fertilizer applied (mg standard fertilizer phosphorus per kg soil or kg standard fertilizer phosphorus per ha).

3. THE EFFECT OF PLACEMENT OF FERTILIZERS AND OF INTERACTIONS BETWEEN NUTRIENTS ON THEIR UPTAKE BY PLANTS

3.1. Introduction

The efficiency of a fertilizer as a supplier of nutrient for plants may be influenced to a considerable extent by the method of placement of the fertilizer. When a fertilizer is placed, for example, in a band near the seed, roots of the crop may find it easier to reach and utilize the fertilizer than if it were broadcast throughout the soil. In addition, banding of the fertilizer reduces the area of contact and the interaction between soil and fertilizer. Although the effect of fertilizer placement on growth of crops can be studied in the normal way by field experiments, it is possible to obtain by the use of isotopes in pot experiments useful information of, for example, the effect of soil type on utilization of placed fertilizer and the influence of one nutrient in enhancing or depressing the uptake of another nutrient. This experiment is thus the reverse of experiment 2.2, because in this case the soil phosphorus supply is used as a standard with which different placements are compared.
3.2. Experiments

3.2.1. Phosphate uptake as influenced by phosphate and nitrogen placement

In this experiment the effect of soil type and nitrogen will be studied by measurement of the uptake by plants of phosphorus from $^{32}$P-tagged fertilizer placed by four different methods in the soils. Plant samples will be taken and assayed at an early stage in growth and also at harvest time (early dough stage).

REAGENTS AND MATERIALS

(1) Same chemical reagents for plant-P determinations, as for A-value experiment (2.2).

(2) At least three bulk soils:
   (a) Calcareous or high pH,
   (b) Near neutral,
   (c) Strongly acidic, and
   (d) Any other soil conditions desired.

   Each pair of students should use one soil.

(3) Seeds of oat, millet, barley or maize (refer to step (2) under procedure).

(4) Tagged superphosphate (approximately 20 or 45% $P_2O_5$) of uniform size particles and containing 0.2 mc $P^{32}$ per gram $P_2O_5$. (Alternatively, prepare a solution of KH$_2$PO$_4$ containing 51 mg of the salt and 10 $\mu$g $P^{32}$ per ml. Prepare sufficient solution to provide 1 ml per pot plus 4 - 5 ml extra to serve as standard.)

(5) Prepare a solution of ammonium nitrate containing 108 mg per ml. (1 ml in 1800 g soil corresponds to 40 kg N per ha.)

(6) Plant grinder.

(7) Equipment for liquid and/or solid sample G-M assay.

(8) Organic solvent containing glue (acetone-soluble gum is recommended).

(9) Two-pronged instrument with spacing of about 1.3 cm for making small furrows in soil.

PROCEDURE

(1) Use 1800 g dry soil in plastic-bag-lined containers (about 2000-ml capacity). Prepare the following fertilizer treatment:
   (a) 11.6 mg P/pot is mixed uniformly with the soil, which corresponds to about 30 kg $P_2O_5$ or 13 kg P per ha. Broadcast 1 ml of the N-solution after planting and another ml at thinning time. (If solution P is used, add 1 ml of the active solution to a small quantity of soil in an evaporating dish. Dry in warm oven for 1-2 h at 60-70°C, or allow to air-dry overnight. Grind thoroughly and then mix with main body of soil for the pot. Beware of dust contamination.)
   (b) 11.6 mg P/pot is banded 1.3 cm to side of seed row (make two small furrows through centre of pot, holding back 2.5 cm of
soil cover, placing fertilizer uniformly along one furrow and seed along the other). Broadcast N-solution in two increments as in (a).

(c) 11.6 mg P/pot plus 0.5 ml of N-solution is banded together 1.3 cm to side of seed row. Broadcast 0.5 ml N-solution after planting and a second increment of 1 ml at thinning time.

(d) 11.6 mg P/pot is broadcast on surface after planting, N as in (a).

(2) In all cases, the seeds should be placed at a uniform depth of 2.5 cm in a row through the middle of the pot. Uniform depth is assured by the holding back of a given volume or weight of soil to provide 2.5 cm cover as the pot is made ready for seeding. Plant 15 seeds per pot (more if larger container is used, less if smaller). Millet or barley or maize can be substituted for oats if desired, although grain will not result with maize; also plant only five seeds of maize and thin to two.

(3) At 25-cm growth stage thin to eight plants per pot. Cut thinnings at soil surface, and save for activity assay. Dry and weigh. Grind as finely as possible.

(4) Digest thinnings in nitric-perchloric acid mixture as detailed in step (6) of Procedure, experiment 3.2.2, Applied Part, C. Soil-Plant. Drying and weighing of thinnings are not required, the 10 ml of acid mixture being adequate for any amount of dry plant material likely to be produced during the early growth stage. Prepare a reference standard by adding 4 mg of fertilizer P to a 100-ml volumetric flask; dissolve (or dilute) in 10 ml of 1:2 HNO₃, and make up to 100-ml volume with distilled water, filtering if necessary. Determine P³² activity by solution counting, using an appropriate volume for the counting system to be employed. Determine total P in the solution in accordance with steps (8) to (10) of Procedure, experiment 2.2, Applied Part, C. Soil-Plant. Calculate per cent of P derived from fertilizer.

(5) Continue the "greenhouse" care of pots, watering up to field capacity whenever soil moisture is reduced to approximately one-half of available water capacity.

(6) Make final harvest when plants have reached early dough stage of seed development. Dry and weigh. Grind as finely as possible.

(7) Count activity in plant samples and in fertilizer reference, and determine total P as described above, using one or more grams plant material as needed for adequate count. If experience with alternative counting methods is desired, use one of the following.

(a) Plant briquet counting. Add sufficient air-dried, finely ground plant material to a compression cylinder to assure infinite thickness (about 3 g for 3.125 cm diameter briquet). Compress at 10,000 lb/in² for 1 min. Prepare two similar briquets for standard count by thoroughly mixing 1 mg fertilizer P/g inactive dry plant material, regrinding and compressing as above. Count briquets with end-window G-M tube. Alternatively it may be useful to weigh approximately 20 mg/cm² of dry, ground plant
material into counting planchets, add few drops of an acetone-
soluble gum for fixation of sample and evaporate excess acetone.

(b) Precipitate counting. Pipette 40 ml of digestion solution as in
(4) above (but using 2 g of dry, ground plant material) and
standard fertilizer solution into 150-ml beakers. Add 5 g of
ammonium citrate and 20 ml of magnesia reagent (latter pre-
pared by dissolving 400 g of MgCl$_2$·6H$_2$O and 300 g of NH$_4$Cl
in 1.5 l of warm water, adding NH$_4$OH until solution is alkaline
to litmus, filtering after 1 h and adding HCl to filtrate until
acid to litmus).
Neutralize with NH$_4$OH, using litmus as indicator; then add an
excess of 2 ml. Shake at intervals for 30 min. Filter through
demountable filter chimney, transferring all of precipitate in
the beaker onto the filter. Apply suction while filtering. Use
1:20 NH$_4$OH to transfer precipitate remaining in beaker to the
filter. When complete transfer is attained, wash precipitate
with methanol. Remove the filter from the suction flask, and
count the activity of the precipitate with end-window G-M tube.
This procedure is recommended especially for later samples
of low specific activity. If activity is very low, however, it
may be necessary to use more than 2 g of plant material for
assay.

(8) Compare and discuss relative P-uptake from the different types
of fertilizer placement, thus assessing the relative efficiency of
placement.

3.2.2. Influence of phosphate and nitrogen on zinc utilization

Maize will be grown in this experiment for studying the influence of
concurrent nitrogen and phosphate treatments on zinc utilization. The crop
will be sampled and assayed at an early growth stage and as it approaches
maturity. The influence of inherent soil properties on this interaction of
nutrients will be demonstrated by the use of two or more different soils as
the growth media.

REAGENTS AND MATERIALS

(1) Same chemical reagents for determination of plant P as used in
A-value, experiment 2.2.

(2) Fertilizer reagents:
(a) Phosphate. Use uniform grain, reagent grade Ca(H$_2$PO$_4$)·H$_2$O
or NaH$_2$PO$_4$·H$_2$O (grind to uniform size particles if lumpy).

(b) Nitrogen. Use solution of ammonium nitrate containing 216 mg
of the salt/ml.

(c) Zinc. Use standard solution containing 36 mg ZnCl$_2$ and 15 μc
Zn$^{65}$/ml. 1 ml corresponds to approximately 20 kg Zn/ha.

(3) Two or more soils:
(a) Moderately acidic, pH 5.6 or less,
(b) Alkaline, preferably calcareous, and
(c) Any other desired soil condition.

(4) Plant grinder.

(5) Well-type scintillation counter for measuring gamma activity.

(6) Chemical reagents plus equipment as required for colorimetric,
polarographic, spectrographic or X-ray fluorescence quantitative
determination of zinc. One satisfactory colorimetric procedure
is based on the complexing of heavy metals by cyanide and releasing
of Zn with chloral hydrate. "Zincon" produces an unstable blue
colour in the presence of Zn, the intensity of colour being approxi-
ately proportional to the Zn concentration. The procedure involves
the following analytic grade reagents:
(a) Concentrated HNO₃ and HClO₄ (60-70%).
(b) Congo-red indicator, 0.025% solution in distilled water.
(c) NH₄OH and HCl.
(d) Buffer solution of 38 g of NaOH + 124 g H₂BO₃ + 149.2 g KCl,
dissolved in distilled water and diluted to 2 l. Adjust to pH 9
with NH₄OH or HCl.
(e) Vitamin C (sodium ascorbate), 12.5% solution in distilled water.
(f) KCN, 1% solution in distilled water.
(g) Zincon, 0.39% solution. Dissolve 0.39 g of Zincon in 4.5 ml
of 1 N NaOH, add 60 ml buffer solution and dilute to 100 ml
with distilled water.
(h) Chloral hydrate, CCl₃CH(OH)₂, 30% in distilled water.
(i ) Zn solution of 100 ppm, prepared by dissolving 0.1 g of elemen-
tal zinc in 50 ml of 0.02 N HCl and diluting to 1 l with distilled
water (ppm = parts per million, e.g. mg per kg).

(7) Maize seeds, single cross variety.

PROCEDURE

(1) Use plastic-lined containers of approximately 2-1 capacity and 1800 g
of soil per pot. (If larger containers and quantities of soil are
employed, adjust fertilizer application rates accordingly.) Tag
the soil for each pot with Zn⁶⁵ in the following manner: Add 1 ml of
Zn⁶⁵ solution to about 15 g of dry soil in an evaporating dish. Allow
to dry overnight in a warm room, or dry in 1-2 h by heating in a
warm oven (60-70°C). Grind with mortar, and mix this sample
thoroughly with the entire bulk of soil (1800 g) that goes into one
pot. These operations are carried out in a hood for the avoidance of
dust contamination.

(2) Make the following fertilizer treatments in duplicate on each soil:
(a) Mix 144 mg of Ca(H₂PO₄)₂ · H₂O thoroughly with the soil. After
planting, add 1 ml of fertilizer N solution to soil surface before
watering. This results in treatments of about 17 kg P and
80 kg N/ha.
(b) Place 144 mg of Ca(H₂PO₄)₂ · H₂O in a band 1.3 cm to side of
seed row. Make two small furrows through the centre of the
pot by means of bi-pronged implement having prongs spaced 1.3 cm apart.

(c) Same as (b) but also add 0.5 ml of N solution to the P band and 0.5 ml to surface after planting, as in (a).

(d) Add N solution to surface, as in (a).

(3) In all cases, the seeds should be placed at a uniform depth of 2.5 cm in a row through the middle of the pot. Uniform depth is assured by holding back a given volume or weight of soil to provide 2.5 cm cover as the pot is made ready for seeding. Plant five seeds of maize per pot (preferably a single cross variety with minimum genetic variability). Grow plants by effective "greenhouse" procedure, watering to field capacity whenever soil is reduced to about one-half of available water capacity.

(4) At 25-cm growth stage, thin to 2 plants per pot, cutting thinnings and saving for chemical and radioactivity assay. Dry and grind finely. Beware of dust contamination.

(5) Count 1-g samples of ground plant material in a well-type scintillation counter, effecting a uniform compaction (equal volume) of samples in the sample holder. By comparison with a standard sample, express as pc Zn$^{65}$/g plant material (1 picocurie = 10^{-12} c).

(6) Digest a 1.5-g sample of plant material in a 125-ml Erlenmeyer flask, using 2.5 ml of HClO$_4$ and 7.5 ml concentrated HNO$_3$. Place reflux funnel on flask, and leave on steam bath overnight. Then place on sand bath, and heat until white fumes of HClO$_4$ have been given off for about $\frac{1}{2}$ h, such that about $\frac{1}{2}$ ml of liquid remains. Note: Never leave digestings unattended, and be careful to avoid explosive destruction by heating too fast.

Cool, wash reflux funnel with distilled water, swirl flask and filter through Whatman No. 31 (or similar) filter paper into 100-ml volumetric flask. Rinse flask several times with warm distilled water, pouring rinses through filter set-up. Make up to 100-ml volume with distilled water. Carry through a blank digestion as above for each group of samples.

(7) Determine total P of this solution as in steps (8) to (10) of Procedure, experiment 2.2, Applied Part, C. Soil-Plant.

(8) Determine total Zn of the plant material by any convenient procedure. If colorimetric method is used on above digestion, proceed as follows:

(a) Dilute 10 ml of Zn stock solution to 500 ml, which solution then contains 2 ppm Zn. Pipette standard series of 0, 1.5, 3, 5 and 10 ml of 2-ppm Zn solution into 25-ml volumetric flasks, and to each add 5 ml of the blank solution. Bring each flask to about 15 ml with distilled water.

(b) Pipette 5 ml of each unknown solution into 25-ml volumetric flasks. Add 10 ml of distilled water. From this point, treat unknowns and standards in identical fashion.

(c) Add 0.5 ml of 0.025% Congo-red indicator.

(d) Neutralize with NH$_4$OH or HCl; pH should be between 7 and 9.

(e) Add 2 ml of buffer solution. Shake,
Add 1 ml of KCN. Shake. Allow to complex for 12-20 h.
Add 1 ml of vitamin C. Shake.
Add 1 ml of Zincon. Shake.
Add 1 ml of chloral hydrate. Make up to volume. Shake. Read at 530 nm within 15 min.

Precautions

(i) Glassware must be Pyrex (heat-resistant glass), or equivalent, and immaculate (accomplished by soaking overnight in 1-2 N HCl followed by rinsing with distilled water).
(ii) Distilled water will require redistillation or passage through an exchange column if stored or conveyed through conventional metal containers or pipes.
(iii) KCN is poisonous on contact; thus rubber gloves must be employed in all stages where it is involved.
(iv) Vitamin C, chloral hydrate and Zincon must be made up fresh daily.
(v) Caps and stoppers must be of polyethylene.
(vi) NaOH may be used in place of NH₄OH for neutralization.

(9) Continue greenhouse care of pots until about the tasselling stage. Then harvest, dry, weigh and grind the total yield.
(10) Determine total P and Zn, and count Zn⁶⁵ as above. Calculate P and Zn yields.
(11) Evaluate effects of N and P treatments on Zn utilization (both concentration and yield) and of N and Zn on P utilization.
(12) Compare total Zn⁶⁵ uptake with Zn yield. Calculate specific activity of Zn, and interpret.

4. RADIATION DAMAGE

4.1. Introduction

Radiation, as pointed out in the section on the interaction of radiation with matter (Basic Part: Lecture Matter, section 1.4), causes ionization when it interacts with matter. Ionization is a function of both the flux (number of particles or photons per cm² per sec.) and the energy of radiation.

Radioisotopes are used in the study of a number of different reactions in plants. When larger than tracer amounts of activity are used, the possibility of radiation damage increases. The degree to which a plant suffers from radiation greatly depends upon environmental conditions, the exact effects of which are still not fully understood. It should be remembered that radiation damage may not necessarily result in visual symptoms.

In this experiment, oats will be grown at various phosphorus levels coupled with different P⁳² levels for the purpose of observation of radiation damage.
4.2. Experiment

REAGENTS AND MATERIALS

(1) Polyethylene or glass pots.
(2) Porcelain dish and spoon.
(3) Metal spoon.
(4) Polyethylene flask.
(5) Volumetric flasks (50 ml).
(6) Measuring cylinder (100 ml).
(7) Nutrient solution containing \(4 \times 10^{-3}\) mole/l \(\text{Ca(NO}_3\text{)}_2\), \(2 \times 10^{-3}\) mole/l \(\text{KNO}_3\), \(1 \times 10^{-3}\) mole/l \(\text{MgSO}_4\) and trace amounts of \(\text{Fe, Mn, Cn, Zn, and Mo}\).
(8) \(\text{KH}_2\text{PO}_4\) and \(\text{P}^{32}\).
(9) Oat seeds.
(10) Sand.

PROCEDURE

(1) Weigh out 500 g of dry sand into each of 9 polyethylene pots.
(2) Prepare 50 ml each of solutions of the composition shown in Table X.

<table>
<thead>
<tr>
<th>No.</th>
<th>(\text{M P}^{31}) (mmole/ml)</th>
<th>(\text{P}^{32}) (µc/50 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>(5 \times 10^{-6})</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>(5 \times 10^{-6})</td>
<td>100</td>
</tr>
<tr>
<td>3</td>
<td>(5 \times 10^{-6})</td>
<td>300</td>
</tr>
<tr>
<td>4</td>
<td>(5 \times 10^{-6})</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>(5 \times 10^{-6})</td>
<td>100</td>
</tr>
<tr>
<td>6</td>
<td>(5 \times 10^{-6})</td>
<td>300</td>
</tr>
<tr>
<td>7</td>
<td>(5 \times 10^{-5})</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>(5 \times 10^{-5})</td>
<td>100</td>
</tr>
<tr>
<td>9</td>
<td>(5 \times 10^{-4})</td>
<td>300</td>
</tr>
</tbody>
</table>

(3) Into porcelain dish add 500 g of sand, 30 ml of \(\text{H}_2\text{O}\) and 50 ml of the radioactive solution. Mix well, and transfer into polyethylene pots.
(4) After filling 9 pots with the different radioactive solutions, 20 oat seeds are sown in each pot at a depth of 1 cm.
(5) Grow the plants in the greenhouse with occasional watering to keep the moisture content reasonably constant by adding nutrient solution up to the original pot weight.
(6) Observe leaf appearance and length of leaves daily. After four weeks of growth, the upper parts of the plants are cut and final measurements of the number of leaves, the length of each leaf and total dry weights are made. Compare and discuss the results.
MENTAL EXERCISES

(1) Potassium salt containing Na as an impurity of 1% of potassium present is irradiated under the neutron flux of $10^{12}$ n cm$^{-2}$ sec$^{-1}$ for 2 h. Assume that the $(n, \gamma)$ reaction is the most likely reaction and that the shielding effect is negligible. What is the fraction of radioactivity attributable to Na?

(2) How many $\mu$g of $^{32}$P should be present in the centre of a counting tray at a distance of 5 cm from the window of a G-M tube of 2 cm diameter to give a count rate of 5000 cpm (window thickness 3 mg/cm$^2$, dead-time 300 usec), assuming no backscattering or self-absorption?

(3) The background of a G-M set has been determined during 2 h and appeared to be 12 cpm. How long would one have to count a sample to obtain its count rate with a natural standard deviation of 1% if the sum of the count rate plus background is (a) 500 cpm? (b) 15 cpm?

(4) 50 g of a Ca-saturated clay with an adsorption capacity of 40 meq./100 g is in equilibrium with 1 l of 0.01 M CaCl$_2$. The suspension has to be labelled with Ca$^{45}$ in such a way that 1 ml of the supernatant liquid will give 3000 cpm. How much Ca$^{45}$ will have to be added to the suspension? Assume that the counting yield is 3%.

(5) Wheat is grown on a soil in pots, to which radioactive phosphorus has been added and thoroughly mixed at a rate corresponding to 40 kg P$_2$O$_5$ per ha. The plants are harvested after 20 d and the following measurements made.

<table>
<thead>
<tr>
<th>Weight of plant</th>
<th>$P$ (%)</th>
<th>Specific activities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard</td>
<td>-</td>
<td>$5.00 \times 10^5$ cpm/g P</td>
</tr>
<tr>
<td>Wheat (20 d)</td>
<td>5.75 g</td>
<td>0.21</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$2.11 \times 10^5$ cpm/g P</td>
</tr>
</tbody>
</table>

Calculate the A-value of the soil.

(6) A plant is known to absorb phosphorus in proportion to the amount of available phosphorus present. Assume that a soil has 20 kg/ha of available phosphorus (P$_2$O$_5$) and one wishes to determine the A-value by applying 20 kg of radioactive phosphorus (P$_2$O$_5$) per ha.

If one wishes to obtain a count rate of 200 cpm per ml of the solution which is prepared by digesting 2 g of dry plant material grown for 50 d, containing 0.2% P, and making up to 100-ml volume by dilution, what should be the specific activity of the radioactive phosphorus? Assume that the counting yield of the G-M counter is 5%.

(7) In a pot experiment with alfalfa a yield of 10 g of dry matter per pot is expected. Calculate the necessary amount ($\mu$g) of Ca$^{45}$ which must be added to each pot in order that 1-g compressed briquet
samples, corresponding to maximum count resulting from self-absorption, would give a count rate of 500 cpm per sample with a counting yield of 20%. It is assumed that 25% of the applied Ca is taken up in the plants and that the concentration in the plants, which have grown 100 d from seeding, is 0.4%.

(8) You plan a greenhouse tracer experiment with P\textsuperscript{32} and, of course, are interested in level of activity to assure adequate count rate with the end product and at the same time minimum radiation exposure to plant and researcher. You know that an 8-g dry-weight yield of crop can be expected in the 4000 g of soil to be used per pot, with P percentage of crop approximately 0.3. How much P-fertilizer containing 21% P should be added to each pot to correspond to an application of 120 kg P/ha (1 ha = 2 \times 10^6 kg of soil)? If a total count rate of about 1000 cpm of one-tenth of the total sample is desired, if counting yield has been determined as 20%, if 80 d will elapse between planting and radioactivity assay of harvest, and if an estimated 25% of the plant P is derived from tagged fertilizer (note: this does not say 25% of the applied fertilizer is utilized), what amount of P\textsuperscript{32} activity should be incorporated with the added fertilizer per pot? What is the resulting fertilizer specific activity?

**Experiment**

**OBJECT OF THE EXPERIMENT**

The experiment is for the purpose of the determination of the amount of isotopically exchangeable K in an illitic clay under conditions comparable to field conditions.

**PROCEDURE**

A suspension of the clay is prepared, and the composition of the equilibrium solution is determined. Next a label solution is made up containing a suitable amount of K\textsuperscript{42} in the presence of K\textsuperscript{39} carrier and other cations, such that its composition is equal to that of the equilibrium solution.

At the beginning of the experiment ("time zero") suitable amounts of suspension and label solution are combined and put on the shaker. At different time intervals the systems are removed from the shaker and centrifuged. A sample of the clear supernatant is then radio-assayed to determine the activity concentration $a_\text{s}$ in the supernatant. The ratio of $a_\text{s}$ and $a_\text{L}$ (the activity concentration of the label solution) then yields the total amount of K, exchangeable against K\textsuperscript{42}, present in the system. Subtracting the amount of K in solution then gives the amount of isotopically exchangeable K present in and on the solid phase.

**CALCULATIONS**

For the above experiment the actual procedure (i.e. the amount of tracer necessary, dilutions, safety precautions, counting procedure etc.)
should be outlined in a flow sheet, together with predicted count rates and accuracy of the determination and specification of counter assemblies to be used.

NECESSARY DATA

The suspension contains 10% clay; the cation exchange capacity of clay is 50 meq./g; the percentage K as found from extraction with N NH₄NO₃ is 2%. The equilibrium solution contains \(0.10 \times 10^{-3}\) N K, \(6 \times 10^{-3}\) N Ca with Cl as anion. The experiment is run in 20-ml centrifuge tubes, which limits the total volume to about 10 ml. The isotope is available as K⁴² in solid K₂CO₃; the specific activity is 80 mc/g K at time zero. The half-life of K⁴² is about 12 h; emission consists of \(\gamma\) at 1.5 MeV and \(\beta^-\) at 3 MeV.

Long-lived impurities in the tracer are present to an amount of 0.5 mc/g K. Background count rates are about 300 cpm in the scintillation counter, 12 cpm in an ordinary G-M counter, 1 cpm in an anti-coincidence counter. The counting yield was determined to be about 2.5% in the scintillation counter, 6% in the G-M counter and 30% in anti-coincidence counter.
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