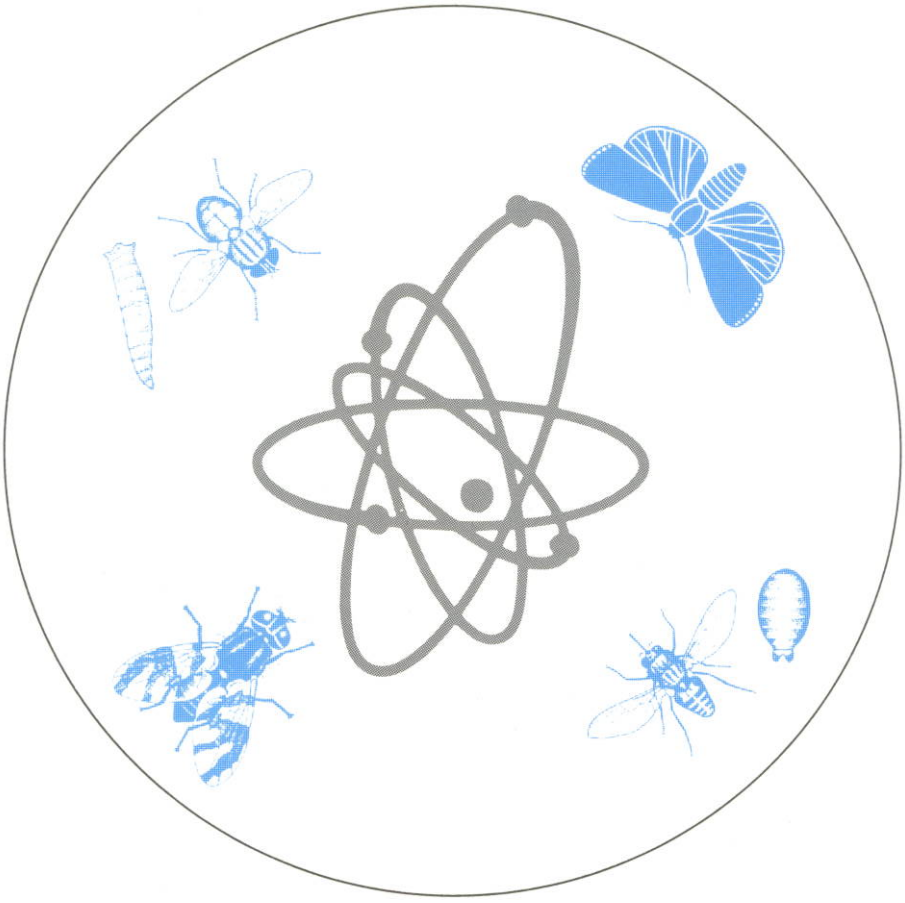

TECHNICAL REPORTS SERIES No. 336

Laboratory Training Manual on the Use of Nuclear Techniques in Insect Research and Control

Third Edition

A JOINT UNDERTAKING BY FAO AND IAEA

INTERNATIONAL ATOMIC ENERGY AGENCY, VIENNA, 1992

LABORATORY TRAINING MANUAL ON THE USE OF NUCLEAR TECHNIQUES IN INSECT RESEARCH AND CONTROL

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VIC Library Cataloguing in Publication Data

Laboratory training manual on the use of nuclear techniques in insect research and control : a joint undertaking by the Food and Agriculture Organization of the United Nations and the International Atomic Energy Agency. — 3rd ed. — Vienna : The Agency, 1992.
p. ; 24 cm. — (Technical reports series, ISSN 0074-1914 ; 336)
STI/DOC/10/336
ISBN 92-0-101792-8
Includes bibliographical references.

1. Entomology—Laboratory manuals. 2. Radioactive tracers in entomology. 3. Insect pests—Control. I. Food and Agriculture Organization. II. International Atomic Energy Agency. III. Series: Technical reports series (International Atomic Energy Agency) ; 336.

VICL 92-00018

Printed by the IAEA in Austria
May 1992

FOREWORD

The Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture was established in 1964 by the Food and Agriculture Organization of the United Nations and the International Atomic Energy Agency. It is responsible to these two organizations for research and development involving nuclear techniques in food and agriculture. This Manual replaces the Laboratory Training Manual on the Use of Isotopes and Radiation in Entomology (Technical Reports Series No. 61) published in 1977. Extensive revision of the isotopes part of the Manual was undertaken by D. Anthony, University of Florida, Gainesville, Florida, United States of America. That part of the Manual dealing with the sterile insect technique (SIT) is, for the most part, new and was prepared by D.E. Weidhaas of the Insects Affecting Man and Animals Research Laboratory, Agricultural Research Service, United States Department of Agriculture, Gainesville, Florida, USA. Co-ordination of this revision was undertaken by D.A. Lindquist of the IAEA.

Isotopes are so commonly used in agricultural research in developed countries that it may seem superfluous to produce a laboratory training manual for the use of isotopes and radiation in entomology. However, in developing countries these techniques are not commonly used because of a lack of both training and equipment.

The purpose of this Manual is to help entomologists and others responsible for the entomological research and control of insects in developing countries become familiar with the potential use of isotopes and radiation in solving some of their research and insect control problems.

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PART IV. APPLICATION TO ENTOMOLOGICAL PROBLEMS

PART V. STERILE INSECT TECHNIQUE

Part I

RADIATION SAFETY[1]

I-1. INTRODUCTION

Those who have worked with very toxic pesticides such as parathion or temik and/or with powerful mutagens already have experience with many of the precautions recommended for the safe use of radioactive isotopes. A major difference is the need to shield against radiation 'shining' on a person at a distance. With these similarities and differences in mind, a careful study of the following safety rules for a radioisotope laboratory is necessary. Copies of these safety rules should be given to everyone working with radioisotopes and should be displayed prominently in every laboratory in which work with radioisotopes is performed.

I-2. SAFETY RULES FOR A RADIOISOTOPE LABORATORY

(1) No smoking, eating, drinking, application of cosmetics or storage of food is permitted in the area in which radioactive materials are used or stored.
(2) Personal belongings (books, bags and clothing) must not be placed on laboratory work benches.
(3) Good housekeeping must be maintained at all times. All containers (beakers, test tubes, etc.) must be labelled with the type of radioisotope, the activity and the date.
(4) Trays, benches and hoods must always be lined with absorbent paper before using radioactive material. This helps to confine spills and to facilitate easy decontamination. Further steps to prevent contamination, especially the principle of *multiple containment,* should be practised.
(5) *No* pipetting of radioactive materials is to be done by mouth; a syringe, propipette or other remote control device should be used.
(6) Plastic or rubber gloves are to be worn whenever radioactive material is handled; the gloves are to be removed before leaving the area in which radioactive material is being used in order to prevent the spread of contamination. Other protective clothing (laboratory coats, goggles, etc.) should be worn when necessary.

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[1] Much of the material in Part I is adapted from the Radiation Control Guide, University of Florida, Gainesville, Florida, United States of America, and is augmented by material from the New England Nuclear Products Division of E.I., Du Pont de Nemours and Co., Boston, Massachusetts, USA.

(7) Before leaving the laboratory hands should *always* be washed and then monitored with the appropriate survey meter.
(8) All hard (high energy) beta and gamma emitting isotopes should be shielded to an exposure dose rate below 0.02 mGy/h (2 mR/h) at 30 cm. Long handled equipment should be used when working with highly radioactive material. No work should be done over open containers of hard beta or gamma emitting isotopes.
(9) A film badge and/or a pocket dosimeter should be worn at all times while working in the laboratory; the devices should be worn on the chest.
(10) All radioactive waste should be collected in suitable containers. These shall be labelled 'Caution — Radioactive Material' and must show the isotope, the amount, the date and the name of the principal investigator.
(11) All injuries occurring while handling radioactive material should be monitored for contamination.
(12) Radioactive material should be used and stored in such a manner as to prevent unauthorized persons from using or removing such material
(13) All spills and other incidents should be reported to the responsible authorities.

I–3. PERSONNEL AND LABORATORY RADIATION MONITORING

I–3.1. Units

Safety rules (7), (8) and (9) of Section I–2 refer to the monitoring of exposure or the exposure potential. Subsequent sections mention the amount of activity. Unfortunately, there are two sets of units for such quantities: the old, or traditional, units (which are those that may appear on many of the radiation monitoring devices still in use), and the new, or International System (SI), units. Table I-I gives the conversion factors from one set of units to the other.

I–3.2. Instructions for use

Regardless of which units are employed in the calibration or reading of a personnel monitoring device, the commonly available ones are: a film badge, a thermoluminescent dosimeter (TLD) or a pocket dosimeter. All these devices provide integrated dose measurement over the period in which the dosimeter is used.

If the body is exposed fairly uniformly, the dosimeter should be worn on the chest. This allows the dose to critical organs (gonads, red bone marrow) to be accurately estimated by the dosimeter. When wearing a protective lead apron, the film badge should be worn on the collar, outside of the apron. Ring badges or wrist badges should be worn when large radioactive sources are handled, since the dose to the hands may be high.

TABLE I–I. CONVERSION OF OLD UNITS TO NEW SI UNITS

Quantity	Old unit	New SI unit
Activity	Curie (Ci)	Becquerel (Bq)
Exposure	Roentgen (R)	Coulomb/kilogram (C/kg)
Dose	Rad	Gray (Gy)
Dose equivalent	Rem	Sievert (Sv)

Conversion factors from the old units to the new units

1 curie = 3.7×10^{10} disintegrations/second (dis/s)
1 dis/s = 1 becquerel (Bq)
1 curie = 3.7×10^{10} Bq
1 millicurie = 37 MBq
1 nanocurie = 37 Bq
1 roentgen = 2.85×10^{-4} coulomb/kilogram (C/kg)
100 rad = 1 gray (Gy)
100 rem = 1 sievert (Sv)

I–3.3. Types of personnel monitor

I–3.3.1. Film badge

A film badge is the most commonly used personnel monitoring device for X ray and gamma radiation and charged particles. A film badge is composed of a piece of photographic film and a special film holder. The film emulsion is made up of a gelatin base embedded with silver bromide grains. The grain size and concentration of the silver bromide is important in determining the sensitivity and resolution of the film. The constituents or type of emulsion are varied to best respond to the type and intensity of the radiation involved. Radiation loses energy by exciting or ionizing the atoms of silver bromide. The freed electrons migrate about the crystal until trapped by impurities or deformities in the crystal lattice. Silver ions accumulate about the trapping centres, because of their attraction to the negative electrons, and form silver atoms. These atoms constitute the latent image of the emulsion and act as a catalyst during the development process. The effect of radiation exposure is a darkening of the film. The amount of darkening of the film is proportional to the absorbed dose. The film is placed inside a light tight packet, which is placed in

the film holder. The film holder contains various filters, i.e. copper, lead, cadmium and plastic. Radiation passing through the filters produces a density distribution on the film from which the energy range of the radiation can be determined.

As a personnel monitoring device, photographic film has several disadvantages:

(1) Fogging may result from mechanical pressure, high temperatures or exposure to light before development
(2) Fading of the latent image, which is dependent on the time interval between exposure and development
(3) Development of the image is a critical process; ordinarily, it is necessary to send the exposed film badge to a commercial laboratory for carefully controlled development and dosage estimation.

I-3.3.2. Thermoluminescent dosimeter

A TLD is also used for monitoring beta, X ray and gamma radiation. Energy absorbed from incident radiation excites and ionizes the molecules of LiF or CaF_2, the thermoluminescent material. The free electrons and holes migrate in the crystal and are trapped by impurities or deformations. They remain in the traps until the crystal is heated to a high temperature, which enables the electrons and holes to return to their ground state, with the emission of light. The amount of light emitted is proportional to the energy absorbed within the thermoluminescent material, i.e. proportional to the radiation dose to the detector. The emitted light is measured with a photomultiplier tube, the output of which is applied to a readout instrument; quantitative readouts can be made locally by laboratory personnel.

A TLD is a better indicator of radiation exposure than film because it is composed of elements of low atomic number (human tissue also contains elements of low atomic number). TLDs are less affected by environmental conditions (heat, light and humidity) than film, but they do not provide as much information on the energy of the incident radiation. TLD materials are available in many sizes and shapes, e.g. rods, ribbons, pellets and single crystals.

I-3.3.3. Pocket dosimeter

A pocket ion chamber dosimeter can be of the direct or indirect reading type. A direct reading dosimeter consists of a small capacitor in pen type housing. Before use, the dosimeter is charged using a dosimeter charger. Radiation exposure results in a loss of charge and a corresponding deflection of the fibre. This dosimeter also contains a lens and scale by which the amount of fibre deflection (dose) can easily be determined.

The dosimeter is held up to a light and the dose read from the scale. Using this dosimeter, a personal dose can be determined immediately, without interrupting the monitoring ability of the device. Direct reading dosimeters are usually calibrated to read exposure in milliroentgen, but some read in roentgen.

Indirect reading dosimeters are also shaped like a pen, but must be read using a charge reader. The latter is a voltmeter calibrated in roentgen.

A pocket ion chamber dosimeter is sensitive to moisture and rough handling, causing the charge to leak from the capacitor and to give erroneous readings. If the dosimeter is accidentally dropped, it should be assumed that the instrument has been rendered invalid, at least temporarily, and it should be replaced or recharged.

I–3.4. Types of laboratory monitor

I–3.4.1. Survey meter

A survey meter, which is an instrument designed to monitor radiation exposure in and around the workplace, measures the amount of ionization produced per unit of time. Many, or most, of these meters are calibrated to give readings in milliroentgen per hour, although new instruments may be calibrated in SI units. Other survey meters, especially those using a Geiger–Müller (GM) tube as the detector, produce audible signals whose pitch or frequency is a function of the count rate being detected.

Before, during and after carrying out any operations with radioisotopes it is important to monitor laboratory surfaces for contamination as well as various laboratory operations for their personnel exposure potential. As an example of the latter type of monitoring, it is instructive to survey the exposure rate around and over open containers of a hard beta emitter such as ^{32}P (thus verifying the caution given in safety rule (8) of Section I–2).

A survey meter is also essential in monitoring radioisotope shipments upon receipt (see Section I–4), and in dealing with spills of radioactive material, should they occur (see Section I–4.3).

I–4. RECOMMENDED PROCEDURES TO BE FOLLOWED WHEN RECEIVING RADIOACTIVE SHIPMENTS

If an institution does not have a system for processing radioactive shipments, it is recommended that this Manual be distributed to all radioisotope users.

I–4.1. Introduction

A package should always be opened and inspected immediately upon receipt. A radioactive solution inadvertently stored upside down may gradually leak and cause contamination. Furthermore, vendors often do not accept claims for shipments not inspected within 15 days of delivery.

I–4.2. Processing of soft beta, hard beta and gamma emitters

(1) Wipe-test the package to check for removable contamination.
(2) Note the radiation units stated on the package, and verify and record these in the receipt log *(hard beta and gamma only).*
(3) Place the package in the vented hood.
(4) Open the outer package and remove the packing slip; open the inner package and verify that the contents agree in name and quantity with the packing slip.
(5) Measure the radiation field of the unshielded container; if necessary, place the container behind the shielding to reduce the field to the allowable limits and proceed with remote handling devices *(hard beta and gamma only).*
(6) Check for possible breakage of the seals of the container, loss of liquid or change in the colour of the absorbing material.
(7) Wipe-test the inner contents and document any pertinent findings on the packing slip. *Note:* The liner, shield and isotope container may show surface contamination; they should be discarded in 'hot' waste disposal containers.
(8) Record the type of activity, the quantity present and the location of the delivery in the receiving log.
(9) Deliver the processed package to the correct laboratory; if delivery is delayed, notify the recipient of its arrival and clearance.
(10) If the material has been packed in dry ice, refrigerate or deliver immediately to the ultimate user.
(11) If contamination, leakage or shortages are observed, notify the vendor's customer service department immediately, by telephone if possible; request instructions.

I–4.3. Radioactive material spill emergency procedures

I–4.3.1. Minor spills (less than 100 mCi (3.7×10^9 Bq) of activity)

(1) *Notify:* Inform persons in the area that a spill has occurred.
(2) *Prevent the spread:* Cover the spill with absorbent paper.
(3) *Call for help:* Report incident to the appropriate authorities.
(4) *Clean up:* Use disposable gloves and remote handling tongs; fold the absorbent paper and carefully wipe up the spill; place used papers in a plastic bag and

dispose in the radioactive waste container; include all other contaminated material such as disposable gloves.

(5) *Survey:* Use a survey meter to check the area around the spill, as well as hands and clothes for contamination.

I–4.3.2. Major spills (more than 100 mCi (3.7×10^9 Bq) of activity)

(1) *Clear the area:* Notify all persons not involved in the spill to vacate the room.

(2) *Prevent the spread:* Cover the spill with absorbent paper, but do not attempt to clean it up unless one is the trained, responsible authority; confine the movement of all personnel potentially contaminated in order to prevent the spread.

(3) *Shield the source:* If possible, the spill should be shielded, but only if it can be done without further contamination or without significantly increasing one's own radiation exposure.

(4) *Close the room:* Leave the room and lock the door(s) to prevent entry.

(5) *Call for help:* Notify the appropriate authorities.

(6) *Personnel decontamination:* Contaminated clothing should be removed and stored for further evaluation; if the spill is on the skin, flush thoroughly and then wash with mild soap and lukewarm water.

(7) *Facility decontamination:* (a) Proceed to clean up the spill, as described in items (4) and (5) of Section I–4.3.1; (b) shoe covers can be worn to prevent personal contamination; and (c) both body and extremity film (or TLD) badges may be required.

Part II

RADIATION AND ISOTOPES

II-1. TYPES OF RADIATION AND BASIC DEFINITIONS

II-1.1. Atomic model: radioactivity

Although the modern view of subatomic structure includes a veritable 'nuclear zoo' of particles and forces, it is sufficient for our purposes to view atoms simply as being composed of a positively charged nucleus surrounded by shells of negatively charged (orbital) electrons.[1] The nucleus contains protons and neutrons as its major components of mass. A proton carries a positive (elementary) charge and a neutron has no charge. The nucleus has a diameter of the order of 10^{-12} cm and contains almost the entire mass of the atom. The atom, including the orbital electrons, has a diameter of the order of 10^{-8} cm.

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

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

$$Z + N = A \triangleq A_r \text{ in unified atomic mass units}^2$$

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

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

where El represents the chemical symbol for the element.

The nuclei of some nuclides are not stable. One by one they disintegrate spontaneously, each nuclide at a characteristic rate, and are called 'radioactive'. In nature, a number of unstable nuclides are known; nowadays, radioactive isotopes of

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

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

nearly every element are produced artificially (e.g. in atomic reactors and by particle accelerators). Disintegration of radioactive nuclei is accompanied by the emission of various types of ionizing radiation. Radioactive nuclides are termed radionuclides; other similar abbreviations are radioactive isotopes, radioisotopes, etc.

Radioactive nuclei, upon disintegration, may emit alpha (α) or beta (β) particles as well as gamma (γ) rays. Alpha particles are fast moving helium nuclei (^4_2He), i.e. a combination of two protons and two neutrons.

Alpha particles from a given disintegration are all emitted with the same energy, i.e. they have a discrete or line energy spectrum. In contrast to alpha particles, beta particles, fast moving electrons (β^-) or their anti-particle positrons (β^+), are emitted with energies varying from zero to a maximum energy (E_{max}) characteristic of the particular nuclear disintegration occurring, i.e. beta particles have a continuous spectrum. Conservation of mass/energy in beta emissions is maintained by the simultaneous emission of a very small, unchanged (and therefore usually undetectable) particle called the neutrino, which carries energy equivalent to the difference between the energy of the beta particle and E_{max}.

Gamma rays are electromagnetic energy packets (photons) of very short wavelength compared with that of visible light, but travelling at the characteristic speed of light.

Natural isotopes of elements with low Z numbers (except ordinary hydrogen) have approximately the same number of neutrons as protons in their nuclei ($N \triangleq Z$), and are usually stable. As the atomic number of the element increases, the number of neutrons increasingly exceeds the number of protons with stability being maintained, but finally only unstable nuclei occur (above $Z = 83$, bismuth). Thus, the majority of radioisotopes in nature are found for elements of high Z number with a neutron to proton ratio of the order of 1½:1. Emission of alpha particles is characterstic of these heavy, unstable elements. The alpha particle is a very stable nuclear form which is ejected as a single particle from the nucleus of the heavy atom when it disintegrates.

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

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

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

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

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

Here, 1.02 MeV (1.63×10^{-13} J) is the minimum energy required for a β^+ emission and is equivalent to the rest mass of a positron plus an electron. An excess of protons in the nucleus may alternatively be reduced by the nucleus capturing one of its own orbital electrons, a process known as electron capture (EC) or K capture

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

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

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

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

In some instances, two alternative modes of decay of the nucleus may occur. An example is seen for ^{40}K decay

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

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

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

Another example is

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

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

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

TABLE II–I. EMISSIONS DETECTABLE BY INSTRUMENTS USED IN ISOTOPIC TRACER TECHNIQUES

Emission	Source	Type of spectrum
Alpha particles	Nuclear disintegrations, especially from heavy elements	Discrete (line)
Electrons/positrons		
β^- particles	Nuclear disintegrations from nuclei with excess neutrons	Continuous ($0 \rightarrow E_{max}$)
β^+ particles	Nuclear disintegrations from nuclei with excess protons	Continuous ($0 \rightarrow E_{max}$)
Conversion electron	Internal conversion, gamma to e^-	Discrete
Auger electron	Internal conversion, X ray to e^-	Discrete
Photons		
Gamma rays	Nuclear disintegrations	Discrete
X rays (1)	Extra nuclear origin, electron capture, *or*	Discrete (often called characteristic X ray)
X rays (2)	Bremsstrahlung, extra nuclear 'braking' radiation from the charged particle being deflected or decelerated	Continuous

When a large nucleus such as ^{235}U captures a neutron, the nucleus divides into two parts of approximately equal mass; this process is called fission. Primary fission products are unstable (excessive N), and each forms a series of radioactive daughter nuclides terminating with a naturally occurring stable nuclide.

The nuclear and extra nuclear emissions which are detectable by the instruments used in isotopic tracer techniques are summarized in Table II–I.

II–1.2. Einstein's equation

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

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

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

$$E = mc^2$$

where E is the energy release (in the above case Q), m is the mass decrease and c is the speed of light; since c is a very large number, E is also large.

Alternatively, Einstein's equation may be expressed either as

$$E = 931\ m$$

where E is the energy release in megaelectronvolt (MeV) and m is the mass decrease in unified atomic mass units,

or as

$$E = 5810\ m$$

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

II–1.3. Radioactive decay law: specific activity

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

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

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

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

Rearranging Eq. (II–1) to solve for λ

$$\lambda = \frac{1}{N}\frac{dN}{dt} \tag{II–2}$$

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

Equation (II-1) may be integrated to give

$$N = N_0 e^{-\lambda t} \tag{II–3}$$

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

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

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

$$1/2\ N_0 = N_0 e^{-\lambda T_{1/2}} \tag{II–4}$$

Hence

$$1/2 = e^{-\lambda T_{1/2}} \quad or \quad e^{\lambda T_{1/2}} = 2 \tag{II–5}$$

Thus, taking the natural logarithm of both sides

$$\lambda T_{1/2} = \ln 2 = 0.693 \tag{II–6}$$

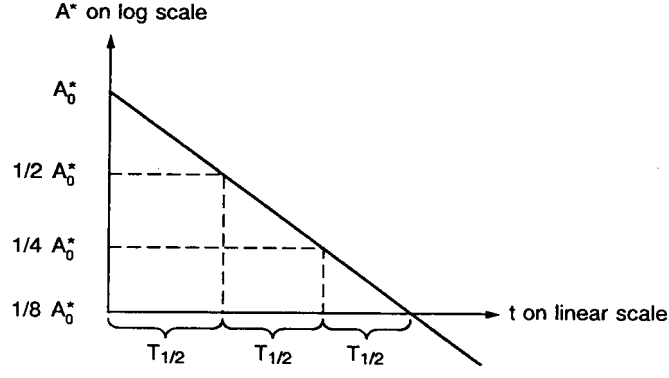

FIG. II–1. Decay curve of a single radionuclide (log–linear plot).

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

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

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

From Eq. (II–3), one therefore obtains

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

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

$$A^* = A_0^* (0.5)^{t/T_{1/2}} \tag{II–9}$$

The half-life of a radionuclide may be determined graphically by plotting the disintegration rate (or a constant fraction thereof, as determined by a suitable counting instrument) versus time on log–linear graph paper. Referring to Eq. (II–9), if the common logarithm (logarithm to the base 10) is taken on both sides, the result is

$$\log A^* = \log A_0^* - \frac{\log 2}{T_{1/2}} \tag{II–10}$$

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

The special unit of activity (radioactivity) has, for many years, been the curie. This was originally defined as the radioactivity associated with the quantity of radon in equilibrium with 1 g of radium (1910). The formal definition agreed to in 1964 by the Conférence générale des Poids et Mesures, when the curie was accepted for use with the SI, was

$$\begin{aligned} 1 \text{ Ci} &= 3.7 \times 10^{10} \text{ dis/s} \\ &= 3.7 \times 10^{10} \text{ s}^{-1} \text{ (exactly)} \end{aligned}$$

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

$$\begin{aligned} 1 \text{ Bq} &= 1 \text{ dis/s} \\ &= 1 \text{ s}^{-1} \end{aligned}$$

Hence

$$1 \text{ Ci} = 3.7 \times 10^{10} \text{ Bq} = 37 \text{ GBq (exactly)}$$
$$1 \text{ Bq} = 27.0\dot{2}\dot{7} \text{ pCi} \simeq 27.03 \text{ pCi}$$

The old unit, the curie, is to be phased out.

Before research results are sent for publication, it is suggested that a check be made with journals as to the units they require. Indeed, disintegrations per unit time (such as disintegrations per minute (dis/min), or dis/s) should be used at all times, i.e. the observed counts per unit time should be corrected to disintegrations per time. Conversion of dis/min or dis/s to other units is then simple.

The following list will assist in obtaining a feel for the interrelationship:

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

In practice, a radionuclide will often be accompanied by variable quantities of one or more stable isotopes of that element. The stable form is called the carrier. Specific activity is the term used to describe the ratio of radioactive atoms to carrier atoms. The specific activity is defined, in general, as the activity of a particular radionuclide per unit mass of its element or compound. The common units of specific activity are microcuries per gram of substance (i.e. kilobecquerels per gram of substance).[4]

The absolute (carrier free) specific activity of a (carrier free) radionuclide, i.e. the special case in which all atoms of the element present are of the same radioisotope, can be obtained from Eq. II-7 by substituting N_A, Avogadro's constant[5], for N, the number of atoms

$$A_0 = \lambda N_A \qquad \text{(II-11)}$$

---

[4] Disintegrations per minute per milligram ($\text{dis} \cdot \text{min}^{-1} \cdot \text{mg}^{-1}$).

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

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

$$A_0(\text{Bq/mol}) = \lambda N_A \quad or \quad A_0(\text{Ci/mol}) = \frac{\lambda N_A}{3.7 \times 10^{10}} \tag{II-12}$$

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

$$A_0(\text{Bq/g}) = \frac{\lambda N_A}{M} \quad or \quad A_0(\text{Ci/g}) = \frac{\lambda N_A}{(3.7 \times 10^{10})M} \tag{II—13}$$

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

$$\lambda(s^{-1}) = \frac{\ln 2}{T_{1/2}} = \frac{0.693}{T_{1/2}} \tag{II–14}$$

An example of the production of radioisotopes that are carrier free is given in Section II–1.6.4.

II–1.4. Principles of radiocarbon dating

The method of radiocarbon dating is based on two assumptions. First, that when living matter dies the carbon atoms contained in the dead material do not exchange with the carbon atoms (e.g. in CO_2) outside the material. Second, that the specific activity of ^{14}C in nature has been in a steady state and has thus remained at an essentially constant value for many thousands of years (up to the detonation of nuclear weapons).

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

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

$$A = A_0 (1/2)^{t/T_{1/2}} \tag{II–15}$$

For instance, if the value of a is found to be 0.063 Bq/g, i.e. $dis \cdot s^{-1} \cdot g^{-1}$, then the specimen is dated as being about 11 000 years old (i.e. $2T_{1/2}$).

II–1.5. Energy of radiation

The energy unit most commonly used with regard to radiation is the electron-volt (eV). This is equivalent to the kinetic energy acquired by an electron (or any other singly charged particle) accelerated through a potential difference of 1 V in a vacuum. This unit is used by SI and has been determined experimentally to be

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

In nuclear science, a commonly used multiple is the megaelectronvolt (1 MeV $= 10^6$ eV)

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

The kinetic energies of the particles and photons emitted by radionuclides have characteristic values. The energies of alpha particles, characteristic gamma and X ray photons are constant or discrete. The energies of beta particles ejected by a given radionuclide vary, however, from zero up to a specific maximum energy (E_{max}) that is available to the beta particle.

The beta energies given in a table or chart of nuclides are the E_{max} values. The average beta particle energy is usually about one-third that of E_{max}. IC electrons, on the other hand, are monoenergetic.

The characteristic radiation and energy for a given radionuclide are often shown in the form of a decay scheme. The decay schemes of six radionuclides are shown in Fig. II–2.

II–1.6. Interaction of radiation with matter

II–1.6.1. Alpha particles

The alpha particles ejected from any particular radionuclide are monoenergetic. Their initial kinetic energies are of the order of several megaelectronvolts and, since ionization potentials and bond energies are in the range of 112 eV, the alpha particles are capable of causing many ionizations and electronic excitations of the atoms or molecules along their path. Ionization is the complete removal of the valence electron, and excitation is the raising of electrons to higher energy levels in their orbits. Since the valence electron participates in any chemical bond of the atom, ionization destroys the integrity of that bond. Alpha particles are doubly charged and of comparatively heavy mass and, therefore, form a dense track of ion pairs (i.e.

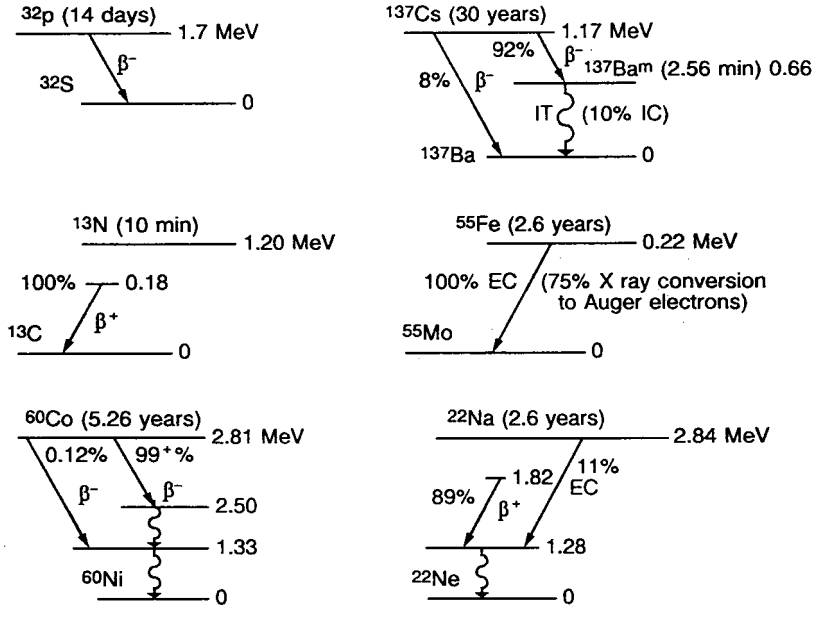

FIG. II–2. Decay schemes showing the characteristic radiation and energy of six radionuclides (IT = isomeric transition; different types of the same nucleus are called isomers; IC = internal conversion of a gamma photon; EC = electron capture (K capture)).

ejected electrons and positively charged ions) along their path. Hence, alpha particles lose energy in matter relatively rapidly by these processes. As the alpha particle dissipates energy along its path, its velocity decreases, and at zero kinetic energy the particle acquires two electrons from its surroundings and becomes a helium atom. The range, i.e. the distance that an alpha particle can penetrate into any matter (absorber), depends on the initial energy of the particle and on the density of the absorber. The range of an alpha particle is relatively small and amounts to several centimetres in air and several micrometres (1 μm = 10^{-3} mm) in tissue for energies of the order of 1–10 MeV.

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

Because of this high LET, and also because alpha particles are characteristically emitted with several megaelectronvolts of energy, alpha emitters are of maximum hazard if they find their way into living tissue. External to an organism, alpha emitters are generally considered to be of minimum hazard. Even here, mention should be made of certain caveats concerning the biological hazards from externally deposited alpha emitters. Unfortunately, one of the most widely distributed alpha

emitters occurring naturally is ^{226}Ra, which decays to ^{222}Rn (radon). Radon is a gas, and radon decay products are a series of non-gaseous alpha emitters with a total energy of tens of megaelectronvolts. Radium, and therefore its gaseous daughter radon as well as its series of daughters, are associated with phosphate deposits and are now recognized as a substantial human health problem in those regions of the world that have phosphate deposits. Even non-gaseous, supposedly non-volatile, alpha emitters may show an apparent volatility, since emission of a high energy alpha particle sometimes imparts sufficient recoil energy to the emitting atom to cause it to be detached from the sample into the air, i.e. to be sublimed. It is easy to understand why knowledgeable users of radioisotopes avoid having anything to do with alpha emitting isotopes if it is at all possible.

II-1.6.2. Beta particles

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

The biological hazard from beta emitting nuclides deposited in living tissue is intermediate between the very severe hazard of alpha emitters and the moderate hazard of the gamma emitters so deposited. The hazard from beta emitters external to an organism is also intermediate between the severe hazard of gamma emitters and the low hazard of externally positioned alpha emitters. It is necessary to insert here a precautionary note on the hazard of beta emitters connected with bremsstrahlung and shielding. One may be tempted to determine the range of the particular beta emitter being used and then provide lead shielding for a stock of this isotope of a thickness greater than the range of the beta, assuming that all the damaging radiation would be stopped in so doing. This is definitely not the case, since such a procedure ignores the substantial contributions of bremsstrahlung. Particular concern should be shown for bremsstrahlung if the isotope used is ^{32}P (as it is for many of the experiments described in this Manual) and if one erroneously contemplates using lead shielding. The importance of bremsstrahlung when one is attempting to absorb beta radiation can be seen from the following equation

$$\frac{\text{Energy loss by bremsstrahlung}}{\text{Energy loss by ionization}} = \frac{E_e Z}{800} \qquad \text{(II-16)}$$

where E_e is the electron energy in megaelectronvolt and Z is the atomic number of the stopping material. If one substitutes the values for ^{32}P E_{max} (1.71) and the Z for lead (82), one finds that about 17% of the beta energy is converted to bremsstrahlung. Significant exposure could result from handling a large amount of ^{32}P 'shielded' by a small amount of lead. It is far better to use plastic ($Z < 5$) as the initial shielding around large samples of beta emitters.

Partly owing to the fact that beta particles have a continuous spectrum of energies up to E_{max}, their absorption in matter is, by chance, approximately exponential with absorber thickness. Thus, when the beta radiation transmitted by an absorber is plotted on log–linear graph paper as a function of the mass per unit area[6] of the absorbing material, a fairly straight line is obtained over a portion of the curve (Fig. II–3).

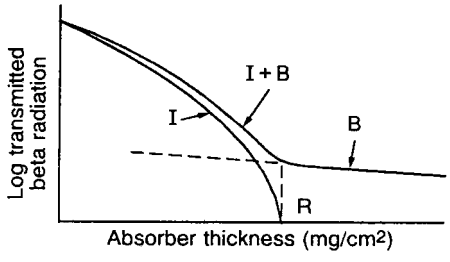

FIG. II–3. Curve demonstrating beta radiation as a function of the absorber thickness (I = intensity of the transmitted beta radiation; B = bremsstrahlung (and gamma ray) component; R = appropriate maximum range of beta particles in the absorber material).

The total transmission curve becomes almost horizontal at R, which is the range of straight pathed beta particles with an initial energy close to E_{max}. Although all the beta particles are stopped by this thickness of absorber, there is still some transmission of radiation because the beta particles interact with the atoms of the absorber, giving rise to non-characteristic X rays, the bremsstrahlung. It should be pointed out that bremsstrahlung radiation has a continuous energy spectrum from essentially zero energy to nearly the E_{max} of the beta emitter causing the bremsstrahlung. This is in contrast to the discrete or line spectrum of the other photon emissions, characteristic X rays and gamma rays. In addition, any gamma rays will contribute to this component. By subtracting this component (B) from the composite curve (I + B), the pure beta transmission curve (I) is obtained.

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[6] Mass per unit area is a product of the density of the absorber multiplied by its 'thickness' parallel to the incident radiation. Its units are: g/cm^2; mg/cm^2; kg/m^2 (SI). Other terms used in the literature for the quantity mass per unit area are: thickness, area density, surface density, density thickness, etc.

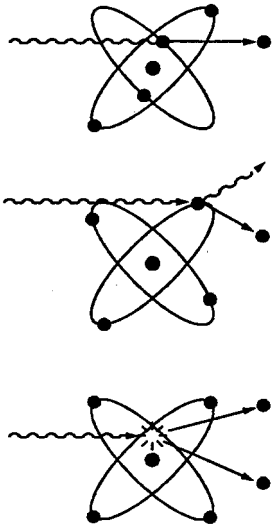

FIG. II–4. Gamma ray interactions.

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

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

II–1.6.3. Gamma and X ray photons

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

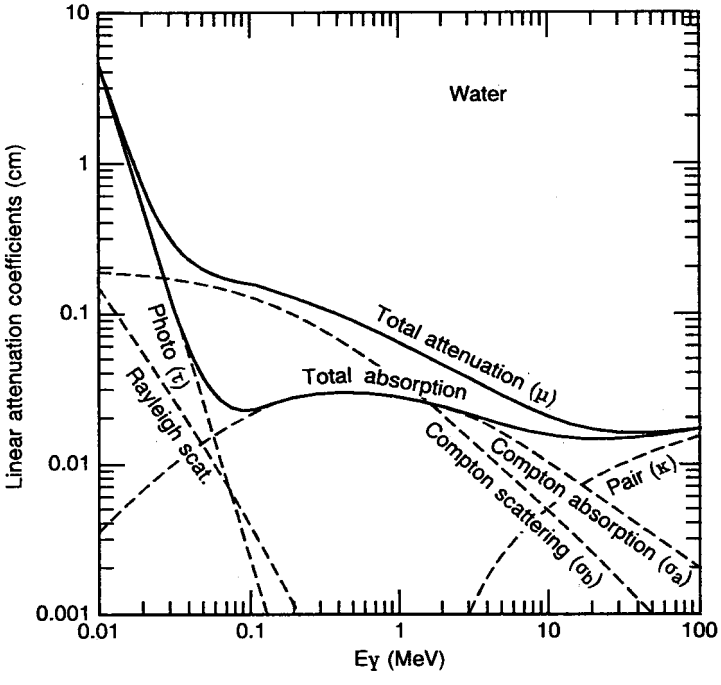

FIG. II–5. Linear attenuation coefficients for gamma rays in water (the total Compton attenuation is $\sigma_c = \sigma_a + \sigma_b$).

their origin; they interact identically in matter). The following are the three types of attenuating event to which various detectors may respond (Fig. II–4).

(1) *Photoelectric absorption* is predominant for relatively low energy gamma photons and for absorbing material of high Z. The gamma ray interacts with a K or L electron of the absorber atom and expels the electron from the atom with a kinetic energy equal to the initial gamma photon energy minus the binding energy of that K or L electron. Thus, an electron is ejected with kinetic energy, enabling it to produce ionization and excitation along its path comparable to those of a beta particle. In Fig. II–5, the coefficient for photoelectric absorption (τ) is given for water as a function of gamma photon energy, E_γ. The absorption coefficient is a measure of the probability of absorption (average number of events per centimetre).

(2) *A Compton effect* is the interaction between a gamma photon and an outer electron of the absorber atom or a free electron. Part of the initial kinetic energy is absorbed (transferred to the electron) and the photon is scattered in a new direction at a lower energy. The photon will, eventually, after multiple scattering, be absorbed through the photoelectric effect. As can be seen in Fig. II–5, the coefficient for

Compton absorption (σ_a) is at a maximum in water for gamma rays of about 0.5 MeV. The effect rises only slightly with increasing Z. The fast electron arising from a Compton event produces ionization and excitation in the same manner as a beta particle.

(3) *Pair production absorption* may occur when the gamma photon has an initial energy of at least 1.02 MeV (1.6×10^{-13} J). The gamma ray photon interacts with the positive field of the nucleus of the absorber atom and is completely used up in producing a positron–electron pair.[7] For this process, a minimum energy of 1.02 MeV is required. Any gamma photon energy above this required level is imparted as kinetic energy to the positron and the electron, both of which cause ionization and excitation along their respective paths. Upon annihilation of the positron, two 0.51 MeV photons are subsequently absorbed by a photoelectric event or a combination of the Compton and photoelectric effects. In Fig. II–5, the absorption coefficient for pair production is labelled κ.

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

$$\frac{dI}{dx} = -\mu L \tag{II-17}$$

Equation (II–17) is identical to the well known Lambert–Beer law for attenuation of monochromatic light. The proportionality constant (μ) is termed the **total attenuation coefficient** (Fig. II–5). Exactly analogous to the radioactive decay constant, λ, μ is the fraction of the original intensity removed from the beam per unit linear thickness of absorber. Equation (II–17) is mathematically identical to the radioactive decay law (Eq. II–1) and may be integrated to give

$$I = I_0 e^{-\mu x} \tag{II-18}$$

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

$$\mu = \tau + \sigma_c + \kappa \tag{II-19}$$

where τ is the photoelectric attenuation coefficient, σ_c is the total Compton attenuation coefficient ($= \sigma_a + \sigma_b$) (see Fig. II–5) and κ is the pair production attenuation coefficient.

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

The numerical value of μ is dependent on the gamma photon energy (E_γ) and the type of absorber material. Figure II–5 illustrates the attenuation probabilities for water as a function of E_γ.

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

$$X_{1/2} = \frac{\ln 2}{\mu} = \frac{0.693}{\mu} \qquad \text{(II–20)}$$

an equation of a form similar to Eq. (II–14), i.e. Eq. (II–6).

Equation (II–18) may alternatively be expressed as

$$I = I_0(1/2)^{x/X}\ 1/2 \qquad \text{(II–21)}$$

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

II-1.6.4. Neutron production and interaction processes

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

Neutrons are produced by fission processes; the most common sources of neutrons are nuclear reactors that control fission chain reactions.

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

(1) *Elastic collisions:* Neutrons of high initial energy, fast neutrons, interact with other nuclei in billiard ball like collisions, losing a fraction of their kinetic energy with each collision. By this moderation process, they eventually reach an energy that is the same as that of molecules in thermal equilibrium with their particular environment. They are then termed thermal neutrons. Light elements, especially hydrogen, are the most efficient for this moderating process. A fast neutron undergoing an elastic collision with another atom or molecule will generally produce a fast recoil ionized atom (e.g. a proton from hydrogen). This recoil ion will then cause ionization and excitation along its path.

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

(a) (n,γ) reaction

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

This type of reaction is used to produce many artificial radionuclides.

Note: The n,γ reaction produces a radioactive isotope of the element being neutron irradiated.

In the example, the ^{60}Co produced cannot be chemically separated from the stable ^{59}Co in the sample; this is an example of the production of a non-carrier free radionuclide.

(b) (n,p) reaction

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

(c) (n, fission) reaction

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

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

(d) (n,α) reaction

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

This is a reaction used to detect neutrons.

II-1.6.5. Neutron activation analysis

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

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

often abbreviated

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

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

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

$$\left(\frac{dN^*}{dt}\right)_{prod.} = \sigma\, n_T\, \phi \qquad \text{(II-22)}$$

where σ is the cross-section (square centimetre per nucleus)[8], n_T is the total number of specified stable nuclei exposed, and ϕ is the thermal neutron flux density ($cm^{-2} \cdot s^{-1}$).

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

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

$$\frac{dn^*}{dt} = \sigma\, n_T\, \phi - \lambda N^* \qquad \text{(II-23)}$$

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

$$A^* = \sigma\, n_T\, \phi\, (1 - e^{-\lambda t_{irr}}) \qquad \text{(II-24)}$$

where σ, n_T and ϕ have the same meaning as given above, and where A* is the activity produced at the end of the irradiation period (Bq, i.e. dis/s), λ is the decay constant of the radioisotope produced (s) (see Eq. (II-14)), and t_{irr} is the duration of the irradiation period (s).

Usually, the technique is used to determine the number of atoms, n_T, of a certain stable nuclide in a sample. If the flux, ϕ, irradiation time, t_{irr}, and cross-section, σ, are known, and the activity produced, A*, is determined, this theoreti-

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

cally allows calculation of n_T. Otherwise, a comparator technique can be used (i.e. simultaneous irradiation of a standard with a known amount of the sample element), as is frequently done in practice.

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

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

II–1.6.6. Induced X ray emission

The neutron activation analysis discussed above involves the induction of activity with appreciable lifetimes. In contrast, another system of analysis has been developed that uses the non-nuclear activation of atoms by irradiation with electrons, protons, X rays or gamma rays, followed by immediate release of the absorbed radiation in the form of characteristic X rays and bremsstrahlung. Characteristic X rays are used to make quantitative determination of the elements. The method is limited to elements heavier than phosphorus, but does allow simultaneous determination of 10–20 elements in the 10 ppm range. The technique has been very useful in studies of air pollution and in analysis of archaeological samples. Of all the possible inducers of X rays in this method, protons have proved to be the most useful; the system of X ray analysis based on irradiation of the sample with protons is known as proton induced X ray emission (PIXE) analysis.

Although analysis by induced X rays, in particular PIXE, is a powerful, sensitive method of elemental analysis, there are some major drawbacks to the method. First, it requires a rather elaborate and expensive accelerator to produce, accelerate and focus a beam of protons on the target; second, preparation of the target may be difficult; and, finally, the method requires an expensive, high resolution solid scintillation detector coupled with a multichannel analyser.

II–2. GENERAL ISOTOPES USED BY ENTOMOLOGISTS

II–2.1. Non-radioactive tracing

As thought is being given to the use of radioisotopes for solving entomological problems, it may be surprising to find in a manual on the subject that the first suggestion is *not* to use a radioisotope unless it is really necessary. It is much easier, safer

and has far fewer constraining regulations if problems can be solved without radioisotopes. For example, using a dye to trace an insect may have the same effect as tagging it with radioactive tracers, and less sophisticated equipment is required. If, after careful consideration of other alternatives, it appears that use of a radioactive tracer provides the best solution, then the question arises as to which isotope should be used. The advantages and disadvantages of the isotopes most commonly used by entomologists are listed below in their approximate order of overall usefulness.

II–2.2. Phosphorus-32

This isotope is probably the most widely used among entomologists. It is a pure beta emitter (no primary gamma emission, only bremsstrahlung). Its strong beta emission (E_{max} 1.71 MeV) makes it easy to measure with a GM counter or liquid scintillation equipment. In solid samples, it shows relatively little self-weakening. It is a good isotope for Cerenkov detection, allowing scintillation measurement in aqueous solution without contamination with fluors, etc.; therefore, further chemical or biochemical processes can be carried out after measuring the radioactivity. It is the safest isotope to use because of its lack of gamma emission and because of its short half-life (14.3 days). This short half-life makes decontamination very easy. For example, even a pot of soil treated with ^{32}P for growing labelled plants or for labelling soil inhabiting insects, ordinarily an almost impossible task to decontaminate, can be decontaminated simply by storing the pot for about ten half-lives (about 5 months) while radioactive decay converts the ^{32}P to natural, stable ^{32}S. This short ^{32}P half-life has sometimes been cited as the major disadvantage of this isotope for research. It is true that only about 6% of an administered label remains after 2 months. However, particularly if a 'yes' or 'no' answer is required (e.g. was this a tagged insect or was it not?), ^{32}P could be a useful label for 2 months, or even slightly longer. The lack of gamma emission makes it unsuitable for tagging insects for later detection in deeply buried locations, such as in soil or under the bark of trees. One should always bear in mind that the energy of the beta emission from ^{32}P is high and, accordingly, a strong beam of radiation is emitted from open containers containing solutions of this isotope.

II–2.3. Iron-59

This isotope, which emits both beta and gamma radiation and has a half-life of about 45 days, is a good all purpose isotope for biological studies. Its longer half-life makes it useful for experiments lasting 6 months or more, and its strong gamma emission allows detection even when buried deeply. Multiple emissions permit a wide range of choice of instrument for detection and measurement, i.e. beta and/or gamma detecting equipment. Counting solid samples of ^{59}Fe with a GM counter is more difficult than with ^{32}P, since the preponderance of its beta emission is of much

lower energy. This means that, although such counting is possible, one would have to correct for self-weakening, even in small samples. Although its toxicity (hazard) to personnel is in the same general class (lower medium) as ^{32}P, its longer half-life and strong gamma emission present problems of decontamination and shielding.

II–2.4. Carbon-14

This isotope is particularly useful in labelling organic compounds, or parts of the same, for biochemical metabolic studies confined to equipment used in laboratories. Its major disadvantages are its long half-life (>5700 years) and the low energy of its beta emission (E_{max} 0.155 MeV). These factors make the isotope generally unsuitable for uses involving release into the environment. It is elegant for labelling plants, since $^{14}CO_2$ is taken up readily and, via photosynthesis, is incorporated into all parts of the plant. Similarly, it is very useful in measuring respiration in plants or animals, since this process is the reverse of photosynthesis and therefore evolves CO_2. The low energy of its beta emission can be a virtue or a problem. The low energy allows relatively high resolution in autoradiography, but it is a problem in measurement, especially of solid samples. The very long half-life makes decontamination by decay totally impossible. Therefore, when using ^{14}C the following precautions should be taken: small amounts should be used; volumes should be kept small and it should be confined. An exception to the latter is that in some countries venting of *small* amounts of $^{14}CO_2$ to the environment is allowed because, when the entire atmosphere of the world is taken into consideration, huge amounts of $^{12}CO_2$ as a diluent are present. Therefore, the regulations in one's own country should be checked.

II–2.5. Sulphur-35

Many of the uses mentioned for ^{14}C also apply to ^{35}S, since their low energy beta emissions have the same advantages and disadvantages. The 87 day half-life of ^{35}S opens the possibility of releasing, for example, labelled insects to the environment. In general, the precautions for its use are similar to, although slightly less rigorous than, those for ^{14}C.

II–2.6. Hydrogen-3

This is the isotope of choice for autoradiography at the subcellular level. Its low toxicity permits some release to the environment; however, for almost any uses other than autoradiography it presents problems to the investigator. It is almost impossible to measure quantitatively, except by the liquid scintillation technique, and, with difficulty, using quantitative autoradiography. Unfortunately, hydrogens in organic compounds are very labile (they will exchange with hydrogens almost

anywhere else), making interpretation of ^{3}H labelled experiments extremely difficult. Indeed, one of the ways of producing ^{3}H labelled compounds is simply to seal them in a glass bulb for 1 month or more with $^{3}H_2$ gas of the order of a few curies (10^{10} Bq). Labelling occurs via exchange, but it should be remembered that such exchange works in both directions.

II–2.7. Carbon-60 and ^{137}Cs

These isotopes are considered together, since they have virtually the same advantages and disadvantages. They are used generally when a long lived gamma emitter is desired. However, their long life and gamma emission place them in a higher toxicity/hazard class than any of the isotopes previously mentioned and, correctly, this has led to regulatory restrictions on their use where release to the environment is contemplated. It is recommended that ^{59}Fe be used if a relatively long lived gamma emitter is needed.

II–2.8. Sodium-24

This isotope has much the same usefulness and drawbacks as the cobalt isotopes discussed above. However, the greater solubility of sodium compounds under physiological conditions may be a basis for choosing sodium over cobalt, or vice versa.

II–2.9. Iodine-131 and ^{125}I

Iodine-131 is used when a short lived gamma emitter is desired. It is in the same (higher, medium) toxicity class as ^{60}Co and ^{137}Cs, but it is too short lived for many uses (half-life 8 days). To a considerable degree, ^{125}I has supplanted ^{131}I as the radioactive isotope of iodine used as a tracer. Its longer half-life (60 days) makes it attractive. The absence of beta emission is an advantage for its use as a gamma source, but this is a disadvantage for tracer laboratories that are not equipped with instruments for measuring gamma emission. Of greatest importance, although not stressed often enough, is the fact that certain of the valence states of iodine are volatile. This presents major problems of contamination for personnel, laboratories and equipment. Use of acidic solutions to minimize volatilization should be avoided and solutions should be stored above the freezing temperature, because freezing results in subsequent volatilization of the iodine from the sodium iodide solutions. Radiation safety officers in some institutions insist that all personnel using any radioisotope undergo regular thyroid monitoring.

Part III

RADIATION DETECTION AND ASSAY OF RADIOACTIVITY

III-1. METHODS AND EQUIPMENT

The radiation which comes from radioisotopes interacts with matter (gaseous, liquid or solid), causing chemical changes, ionization and excitation. These effects are utilized in the various methods of detection and measurement of radioactivity. In radiography, for example, ionizing radiation is detected by its effect on photographic, X ray or nuclear emulsion. In the ionization chamber, the gas flow detector, the GM tube and the neutron detector, ions produced directly or indirectly by radiation are collected on charged electrodes. In solid and liquid scintillation counting, emission photons (in the blue to ultraviolet region) form the basis of detection. Solid state detectors, more properly called semi-conductor radiation detectors, are crystals whose electrical conduction is altered by the absorbed radiation. Their operation depends on their semi-conductor properties; this class of detector is of great importance in dosimetry.

Some of the radiation detection and measurement methods are described below. Additional details are provided when a particular method or instrument is called for in various laboratory exercises in subsequent sections of this Manual.

III-1.1. Autoradiography

This method is a photochemical process, the one used by Becquerel in 1896 in the discovery of radioactivity. In autoradiography, ionizing radiation interacts with the silver halide in photographic emulsions. When radioactive material is placed near a photographic plate or film, blackening is produced on development of the emulsion. The blackened areas constitute a self-portrait of the activity in the material. The intensity of the blackening (as determined by the eye or with a densitometer) at a given place is a function of the exposure time and the amount of activity in the sample at that place. It is also a function of the LET of the particular radiation. Gamma rays and X rays have a low LET and are of little use in autoradiography, because the photons from a given place in the sample material penetrate throughout a large area of emulsion, producing an almost uniform fogging on development. Conversely, alpha particles and low energy beta particles (^{3}H, ^{14}C, ^{35}S and ^{45}Ca), which have a high LET, are very effective. High energy beta particles produce diffuse radiograms owing to the relatively long path lengths of these particles in the emulsion. The emulsion properties should compromise between fine grain to increase the resolution and high sensitivity to reduce the exposure time. Usually,

exposure times are long because, to obtain a good image, absorption of about 10^7 soft beta particles is needed per square centimetre of emulsion. Thus, a thin histological section containing 1–10 Bq/cm^2 (1 to 10 dis·s^{-1}·cm^{-2} = 27–270 pCi/cm^2) requires several weeks of exposure to show optimal blackening.

The autoradiography method is particularly suitable when distribution of a radioactive compound in biological material is to be studied. However, precautions should be taken to avoid any chemical or pressure effect of the material on the emulsion, since 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 arise; film development conditions will also affect the image. Hence, interpretation of the autoradiograms of biological material is not straightforward. Autoradiography is frequently applied to determine the components of a paper or thin layer chromatogram. If the laboratory exercise described in Section IV–3.4.2 is carried out, one will become very familiar with this type of autoradiography application.

Micro-autoradiography is useful when the distribution of radioactive compounds in a section is to be studied. Either the sections on the slides can be coated with melted emulsion or, if the resolution required is not quite as high, a stripping film can be used to cover the sections on the slide. Micro-autoradiography, at the electron microscopic level, is useful when the distribution of radioactive compounds in a section is studied. Usually, the slide is dipped in a liquid (melted) emulsion to produce a very thin layer; this is to allow precise orientation of the darkened emulsion with the closely underlying tissue structure. Special emulsions with a very high silver content have been produced, so that even very thin films have enough silver to produce an autoradiographic image within a reasonable exposure time. The isotope of choice for such ultramicro work is ^{3}H, since it is the only isotope of an element naturally occurring in biological materials with a low enough range (i.e. low enough E_{max}) to provide the kind of precision necessary to locate, with subcellular accuracy, the origin of the radioactivity producing the darkening of the emulsion.

III–1.2. Ionization detectors

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

Alpha and beta particles and IC electrons have a high specific ionization, i.e. they 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 is

released by each photoelectric effect or Compton scattering (or pair production if the energy is very high); these fast electrons ionize in the same manner as beta particles. Neutrons may also produce ions, directly (by collision) or indirectly (following nuclear absorption). Detection by ionization of these types of radiation is based on the fact that the atoms of a gas (in the detector) become ionized when they are hit by the radiation particles or photons. The number of ionizations in the gas is a direct measure of the quantity of ionizing particles or photons (α, β, e, γ, X or n) that reach the detector. When an electric field is created in the detector, the negative ions and/or electrons start moving and, by hitting the positive electrode (anode), discharge. Likewise, positive ions move towards the cathode.

In the following subsections, five different types of ionization instrument are described.

III–1.2.1. Electroscope

In an electroscope or simple electrometer (see Fig. III–1), the positive electrode is a rod with a wing or metal string and the negative electrode is the wall of the detector.

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

The type of detector most commonly used as a pocket dosimeter is an electroscope, usually using a quartz fibre as the charged object. The charge leaks off and the fibre 'relaxes' at a rate proportional to the amount of ionization occurring in the

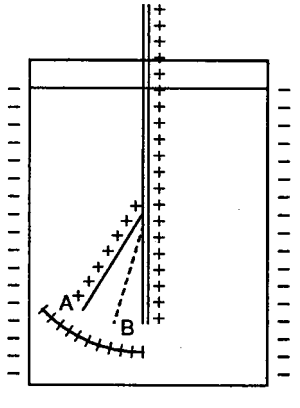

FIG. III–1. Electroscope.

dosimeter which, of course, is a function of the amount of ionizing radiation that has passed through the air in the meter. What one actually sees in these meters is the shadow of the quartz fibre thrown on to an illuminated scale etched on a ground glass screen.

III–1.2.2. Gas filled detectors with a collector/cathode voltage bias

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

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

Figure III–2 shows a plot of pulse size against bias voltage. Region I is the ionization chamber region. The curves are drawn for an alpha and a beta particle traversing the sensitive volume of the detector.

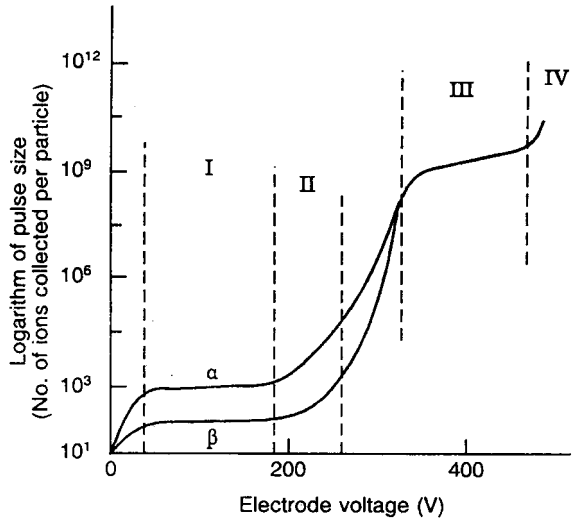

FIG. III–2. Plot of the logarithm of pulse size versus electrode voltage: I = ionization chamber region; II = proportional region; III = GM region; IV = continuous discharge region (the voltage corresponding to a given region varies greatly from one make of instrument to another).

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

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

In Region III, the bias voltage is so great that the charge collected on the anode attains a maximum size, independent of the number of primary ions formed. In this region, at a given voltage, all the pulses are of the same size, irrespective of the number of primary ions; this is termed the GM region.

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

III-1.2.3. Ionization chamber

When a detector is operating in Region I (Fig. III-2), each ionizing particle or photon gives rise to an electric pulse on the electrodes. A constant stream of particles or photons gives rise to a continuous series of pulses, forming a weak electric current, which may be amplified and registered by an electronic circuit. Such detectors are termed ionization chambers and are often filled with air. 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 type of detection instrument is thus a dose rate meter. (A well known version of this type of meter is the Cutie Pie.) When the walls of the ionization chamber are constructed of air equivalent material, the instrument can be used to measure the absorbed dose of gamma or X rays in air. Some chambers also have biological tissue equivalence.

A small, electrically charged ionization chamber, held in place, for instance, by a finger ring, can 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.

Ionization chambers have proved to be useful when built into a laboratory reaction train, including, for example, reaction vessels, condensers, scrubbers and absorbers, in order to follow the path of radioactive gases. These chambers have

been of particular use to organic chemists in monitoring gaseous substances containing ^{14}C or ^{3}H.

III–1.2.4. Proportional counter

Proportional counters operate in Region II (Fig. III–2) when secondary ionization has become important. Electrons that have arisen from primary ionization 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 increased further. The final pulse size is 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 measurement. Usually, the radioactive sample is placed inside the detector, which is transfused by a gas at atmospheric pressure (gas flow counters). In this way, particles of low energy, such as the β^- from ^{14}C, can be counted effectively ('windowless' counting), provided suitable amplification precedes the register.

III–1.2.5. GM counter

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

Energetic beta particles, electrons and gamma or X photons emitted by radioactive liquids can be counted with a thin glass wall 'dip counter' GM tube that is immersed in the liquid, or with a specially designed liquid detector consisting of a cylindrical glass container around the GM tube. Thus, in both cases radioactive

liquid surrounds the GM tube. Particles of low energy can obviously not be counted in this way because of absorption in the wall of the GM tube.

Detectors operated in the GM region are very sensitive to beta particles, and very little additional amplification of the pulse is necessary to drive a counting circuit. In addition, they are almost insensitive to normal voltage fluctuations. Furthermore, they are relatively inexpensive.

GM counters can be used for a great many applications of radioisotopes to entomology. They are relatively free of operational problems, and are found in a large number of laboratories throughout the world. Accordingly, substantial coverage of this device is given below.

When the term GM counter is used, it includes, besides the GM tube, a high voltage supply, an amplifier (possibly with a discriminator), a recorder and a timer.

GM counters are used most widely for the detection and measurement of beta particles. Monitors are used to detect beta particles on glassware, benches or trays. A monitor consists of a GM tube connected to a power unit and a count rate meter. Often, a small loudspeaker is connected to the rate meter, so that a noise warns the operator when the tube is in the vicinity of a contaminated spot.

It should be pointed out that GM counters are *not* very effective (1–3% efficiency) for counting pure gamma emitters, because most of the photons pass through the gas in the tube without any interaction. Fortunately, there are relatively few pure gamma emitters among the radioisotopes. Most, but not all, gamma emitters also emit one or more beta particles, which can be measured effectively by a GM counter.

III–1.2.5.1. Plateau of a GM tube

GM counter assemblies in normal operation often show an appreciable variation in performance from one measurement to another. Thus, it is useful to have a reference source by which day to day counting can 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 can be made from black uranium oxide (U_3O_8). This combines the required chemical stability and a long half-life (4.5×10^9 years). The oxide should not have been treated chemically for at least 1 year, during which time any significant daughter products removed by previous treatments will again have 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 can be done by covering the source with aluminium foil (35 mg/cm^2 (0.35 kg/m^2)). The foil provides a certain amount of mechanical protection to the reference source, and also prevents contamination from a damaged source.

With this or a similar standard source, the following properties of a GM tube can be determined: the threshold or starting potential and the length and shape of the

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

PROCEDURE

(1) Obtain a source counting of about 130 counts/s ($\simeq$ 8000 counts/min)
(2) Place the source in the holder (within the lead castle), and increase the electrode bias voltage (high voltage) slowly until the first counts are obtained; this voltage level is called threshold voltage (V_T)
(3) Determine the count rates with increasing voltage; a total of 10 000 counts registered for each voltage step is adequate (voltage increments may be 20–50 V)
(4) When the count rate does not change appreciably as the high voltage is increased, the GM tube is operating in the plateau region; *no further* high voltage steps should be attempted once it is noticed that the count rate is beginning to increase again, since at higher voltages (V_R in Fig. III–3) the counter will start to race, and damage to the GM tube is likely to occur
(5) Calculate the slope as the percentage increase in count rate per 100 V and the plateau length (see Fig. III–3, $V_2 - V_1$)

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

where R_w is the count rate at the working voltage

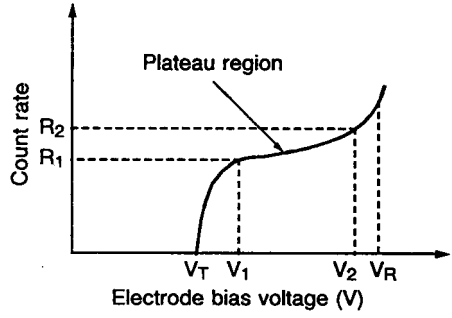

FIG. III–3. Characteristics of a GM tube showing the relationship between the count rate and the voltage.

(6) As the counter ages, the threshold voltage (V_T) tends to increase and the racing voltage (V_R) to decrease; to allow for this, choose the working voltage at $V_T + 75$ V, or ½ ($V_T + V_R$) if the plateau length ($V_2 - V_1$) is less than 150 V. Occasional checks on the plateau characteristics of a tube are necessary with age (number of counts).

III-1.2.5.2. Resolving time of a GM counter

The fact that some time is required to collect the flood of positive ions from each discharge of a GM tube as well as for the quenching process to take place implies that during this period the detector tube is insensitive to other ionizing particles entering its sensitive volume. This period is 100–300 μs for GM tubes. At high count rates, the counts will be lost because of the inoperative period of the counting system; hence, a resolving time correction must be made to obtain the true counts from the figure registered.

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

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

$$R_{true} = \frac{R}{1 - R\tau} \qquad \text{(III-1)}$$

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

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

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

Assuming that the background rate is negligible when compared with the high counting rates of samples needing substantial correction for resolving the time error, a simplified expression for closely approximating τ is

$$\tau \simeq \frac{R_1 + R_2 - R_{1+2}}{2R_1R_2} \qquad \text{(III–2)}$$

In this experiment, τ is determined by using split or paired sources, as described in the following procedure.

PROCEDURE

(1) Two counting cards are provided or prepared (source 1 and source 2), as illustrated in Fig. III–4. Each should contain an amount of radioactive material that gives a counting rate of approximately 10 000 counts/min. A convenient method of preparation is to deliver a small volume of high specific activity radioisotope solution to a circle of blotting paper (conveniently cut out with a cork borer). After drying, cut the circle in half and mount one half on each card. Rubber gloves should be used and the contaminated blotting paper should be handled with tweezers. To mount the blotting paper on the card, double stick tape should be used on the card. A strip of tape as covering is desirable.

(2) Mount source 1 on the left half of a sample holder, with a blank card mounted on the right half; count for 1 minute or longer to establish the count rate; this is R_1.

(3) Remove the blank card *without touching or otherwise disturbing source 1;* mount source 2 in the position formerly occupied by the blank; count and establish the count rate; this is $R_1 + R_2$.

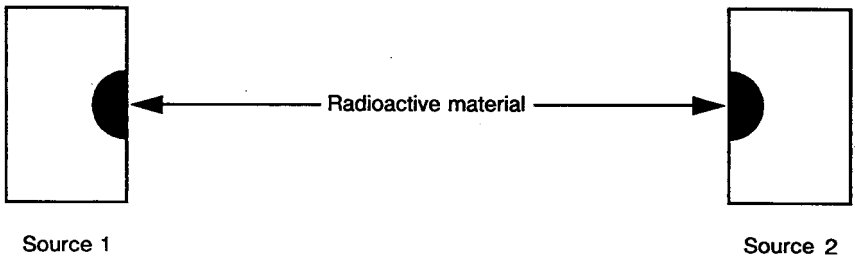

FIG. III–4. Paired or split sources.

(4) Now remove source 1 from the holder and replace it with the blank card, again without disturbing source 2; count and establish the count rate; this is R_2.
(5) Calculate τ using Eq. (III–2).

III–1.2.5.3. Natural and technical sources of uncertainty (statistics)

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

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

When a series of identical counts is made on a sample that is not moved between individual counts, assuming the counter functions correctly, the standard deviation of the sum count is found to be $\sigma_{nat} = C^{1/2}$, where C is the sum count. This is a measure of the natural uncertainty inherent in radioactive decay. It should be noted 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) is called technical uncertainty. Experimental evaluation of these two types of uncertainty is made below.

MATERIALS

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

PROCEDURE

(1) Obtain a bottle of ^{32}P solution that gives a count rate of 170 counts/s ($\simeq$ 10 000 counts/min) per millilitre.
(2) Using a rubber bulb, pipette 25 samples containing 1 mL each; as soon as each sample has been pipetted, dry it under the infrared 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 minutes each, without moving the sample; record the result of each counting.
(4) Calculate the natural standard deviation according to $C^{1/2}$ and the total standard deviation according to the following formula: let C_1, C_2,..., C_n be the sum counts registered in n countings; calculate

$$\bar{C} - \left(\sum_n C_i\right) \Big/ n$$

which is the mean sum count; calculate

$$C_1 - \bar{C},\ C_2 - \bar{C},\ \ldots,\ C_n - \bar{C}$$

The total standard deviation of the sum count is then

$$\sigma_{tot} = \left(\frac{\sum_n (C_i - \bar{C})^2}{n - 1} \right)^{1/2} \qquad \text{(III-3)}$$

(5) Compare the values for σ_{tot} and σ_{nat}, and if they are found to be significantly different, explain; calculate σ_{nat} in per cent.
(6) Count each of the 25 samples separately for 2 minutes, and record the results.
(7) Repeat the calculation according to Eq. (III-3), and compare with $C^{1/2}$; calculate σ_{tech} in per cent, using the equation

$$\sigma_{tot}^2 = \sigma_{nat}^2 - \sigma_{tech}^2 \qquad \text{(III-4)}$$

(8) Calculate the count rate (R) and its total standard deviation in per cent and in counts/s or counts/m.

III-1.2.5.4. Self-absorption/self-scattering of beta particles

It is often necessary to measure the radioactivity of sources that contain appreciable amounts of solid material. When a thick source is counted, errors from self-absorption and source scattering are introduced. Absorption tends to decrease the count rate below the expected value; it is most important with soft beta emitters whose maximum energy is less than about 0.5 MeV. Scattering tends to increase the count rate and is most noticeable with high energy beta emitters. (The effect of self-absorption and self-scattering also exists with gamma emitting sources; however, it is usually unimportant, since such 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 is called self-geometry, i.e. the top of the sample is relatively closer to the counter as the thickness increases. The combined effect of self-absorption, self-scattering and self-geometry, which normally results in a diminution of the count rate, is termed self-weakening.

Very often, corrections for self-weakening in soft beta samples of varying thickness can be circumvented, because only relative values are needed for the experiment. For instance, many experiments are based on formulas in which activity measurements are only entered in ratios such as A_1/A_2, where A_1 and A_2 are the specific activities of the test substance in two samples. If, in such a case, the tracer is a low energy beta emitter and the samples are unequal in thickness, their count rates cannot, in general, be entered directly; however, correction to true count rates need not be attempted, because correct relative specific activities also suffice, since they are to be used as a ratio only. Correct specific activities relative to any arbitrary laboratory reference can 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 reference material containing any unknown, but uniformly distributed, specific activity of the radioisotope used in the experiment, a series of samples covering the thickness range of the experimental samples is prepared. From these reference samples a reference curve is constructed that has the count rate plotted against the mass of sample. This is now (whatever shape the curve may have) a reference of the constant specific activity throughout the length of the curve, so the relative specific activity, SA_{rel}, of any experimental sample is obtained by weighing and counting the sample (under the same conditions as those used for counting the reference samples) and simple division of the net count rate of the experimental sample by the net count rate of the same mass (thickness) of the reference material (see Fig. III-5)

$$SA_{rel} = \frac{\text{Net count rate of the experimental sample of mass m}}{\text{Net count rate of the reference sample of mass m}} \qquad \text{(III-5)}$$

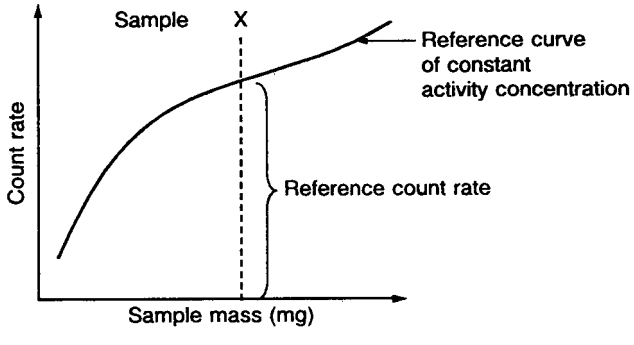

FIG. III-5. Reference curve showing the relationship between the count rate and the sample mass of constant activity concentration.

When corrections for self-weakening are necessary in order to yield the true count rates of beta samples, one should prepare a set of increasingly thick samples of constant specific activity (or constant total activity) and from these construct a self-transmission curve (the count rate plotted against the mass per unit area (mg/cm^2)).

In this graphic method, one extends the self-transmission curve to samples that are as thin as possible; this part of the curve is then extrapolated to yield the zero self-weakening value, or the 'true value', of the activity concentration in count rate per milligram of sample (or the total activity in counts/s or counts/min). Care should be taken in this extrapolation, because very thin samples often exhibit negative self-weakening, i.e. self-scattering into the detector slightly exceeds self-absorption.

In practice, one often chooses simply to correct all the counts to some reference sample mass. One is now counting with equal efficiency and counts can be compared on a relative basis. Of course, this does *not* give the absolute disintegration rate (dis/s or dis/min). For this, one needs to know the counting efficiency, i.e. ((counts/min)/(dis/min)) × 100, of a known activity sample counted under reference standard conditions.

In the experiment described below, a series of sources is prepared by precipitation of the increasing weights of $CaCO_3$ from a solution containing ^{45}Ca. Self-weakening is determined by counting the series of sources. A plot of the counting yield versus weight yields a curve suitable for correcting the counts on the varying weight samples to some standard counting condition.

It should be pointed out here, that if one contemplates using ^{14}C or ^{35}S and plans to count solid samples, one can and should plan an exactly analogous experiment to develop the self-weakening correction curves for these isotopes. Even if a higher energy beta emitter is being used, such as ^{32}P or ^{59}Fe with solid samples, it is a good idea to run such curves to learn how heavy a sample must be before correction is required. Samples containing ^{35}S are converted to the sulphate and are precipitated as $BaSO_4$. Samples containing ^{14}C are converted to the carbonate and are precipitated, washed and mounted exactly as described below for the ^{45}Ca samples. One can precipitate the ^{14}C carbonate samples as either the Ba or Ca salt. There seems to be no obvious advantage of one salt over the other.

In the case of ^{14}C carbonate samples, if one suspects the possibility of contamination, for example, with sulphate from SO_3 in the Van Slyke combustion procedure, one can purify the carbonate simply by reconversion to gaseous CO_2 and reprecipitation. This procedure is given as an addendum to the ^{45}Ca procedure below.

When the self-geometry effect is negligible, a self-transmission curve based on increasingly thick samples of constant specific activity approaches asymptotically a constant value A^*_∞ as X approaches infinity. In practice, A^*_{obs} becomes constant at a thickness equal to about half the range of the E_{max} of the beta particles. A sample of this, or greater thickness, is called infinitely thick. It is well known that the net

count rates of infinitely thick samples are proportional to the specific activities of the samples.

Experiment: Self-weakening using $^{45}CaCO_3$

PROCEDURE

(1) Prepare 50 mL of a solution (solution A), 0.25M $CaCl_2$ and 0.1N HCl, containing 1 μCi ^{45}Ca (37 kBq ^{45}Ca).
(2) To prepare solution B, pipette 5 mL of solution A into a 50 mL volumetric flask, and make up with 0.1N HCl.
(3) Prepare about 50 mL of 5N ammonium hydroxide and 50 mL of 1M Na_2CO_3 solution.
(4) Pipette the solutions A, B, water, ammonia and sodium carbonate by source number order according to Table III–I.
(5) After precipitation, heat the precipitates over a hot water bath or in a beaker with water on a hot plate; do not boil.
(6) Decant the supernatant, and wash twice with methanol (CH_3OH); leave a $CaCO_3$/methanol slurry.
(7) Assemble the filtering apparatus (see Fig. III–6) after the filter paper has been weighed carefully, and pour the $CaCO_3$ slurry into the glass cylinder. After it has settled for 2–3 minutes, gently initiate suction action until the first 2–3 mL of methanol have come through; then gradually apply full suction.
(8) When filtration is complete, turn on the infrared lamp, situated 10–15 cm above the glass cylinder, and keep it and the suction on for a few minutes until the $CaCO_3$ precipitate on the filter paper is dry.
(9) Remove the filter paper at its $CaCO_3$ free edge with tweezers and transfer to a counting plate; weigh and count. After counting is completed, it is a good idea to reweigh the samples to make sure they have been dried to constant weight.
(10) Plot the sample count rate as a function of the sample mass (mg).
(11) From this plot, determine the correct specific activity (counts$\cdot s^{-1}\cdot mg^{-1}$), taken as the slope of the initial straight part of the curve where the effect of self-absorption is negligible. For each sample, determine the correct count rate, which is equal to the correct specific activity (counts$\cdot s^{-1}\cdot mg^{-1}$) multiplied by the sample mass (mg).
(12) Finally, determine the self-weakening factor (SWF) of each sample. The SWF is defined as the ratio of the correct count rate to the observed count rate. Plot the SWF as a function of the sample mass.

TABLE III-I. SOLUTIONS IN THE $^{45}CaCO_3$ PROCEDURE

Source No.	Solution A (mL)	Solution B (mL)	Water (mL)	5N NH_4OH (mL)	1M Na_2CO_3 (mL)	
1	—	1	7	1	1	In 10 mL centrifuge tubes
2	—	2	6	1	1	
3	—	4	4	1	1	
4	—	6	2	1	1	In 40 mL centrifuge tubes
5	1	—	10	1	1	
6	1.5	—	10	2	2	
7	2	—	10	2	2	
8	3	—	25	2	2	In 50 mL beakers
9	4	—	25	2	2	
10	5	—	25	2	2	

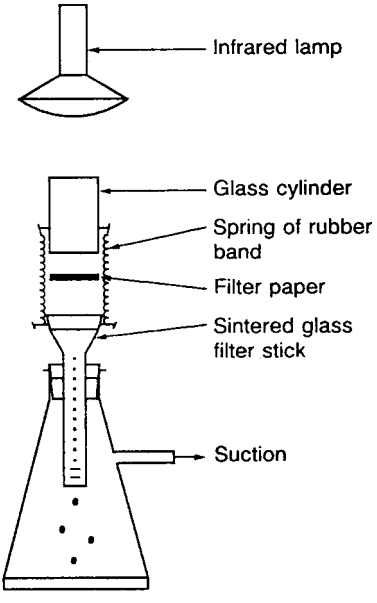

FIG. III–6. Exploded view of the filtering assembly.

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

$$\text{Correct counts/s} = \text{observed counts/s} \times (\text{SWF}) \qquad \text{(III–6)}$$

ADDENDUM PROCEDURE IF FURTHER PURIFICATION IS NEEDED

A Ba (or Ca) $^{14}CO_3$ sample may be purified by reconversion to the gas, followed by trapping and reprecipitation. The reaction employed is

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

(1) Assemble an open system consisting of a reaction flask (main reaction flask plus funnel) connected to a gas washing (CO_2 absorbing) bottle; the entire system is flushed with either N_S or CO_2 free air (see Fig. III–7).

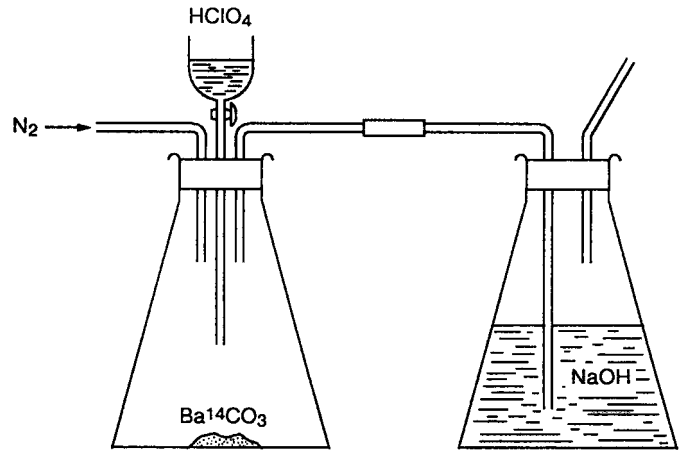

FIG. III-7. Open assembly for the conversion of $^{14}CO_2$.

(2) Put 0.1N CO_2 free NaOH in the gas washing bottle; then place approximately 200 mg of $Ba^{14}CO_3$, containing about 0.2 μCi (7.4 kBq) of ^{14}C, in the main flask; finally, place 2–3 mL of 10% $HClO_4$ (perchloric acid) in the funnel of the reaction vessel.

(3) Sweep the system with CO_2 free gas at a rate such that discrete bubbles are produced in the gas washing bottle.

(4) Add the acid to the reaction vessel portion wise so that CO_2 is not formed at an excessive rate; continue gas sweeping for 10 minutes beyond the final addition of acid.

(5) Move the NaOH solution containing the $^{14}CO_3^{2-}$ to a 50 mL centrifuge tube by repeated washing of the CO_2 absorbing vessel with small volumes of CO_2 free water. Add enough CO_2 free water to make a total volume of about 40 mL and precipitate the $Ca^{14}CO_3$ by adding several drops of saturated aqueous $CaCl_2$ solution.

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

III–1.2.6. Solid crystal scintillation counting

When ionizing radiation enters a scintillator, a number of light photons (in the visible and ultraviolet wavelengths) is liberated. This number is proportional to the energy dissipated by the incident radiation. Many of these light photons hit the photocathode of the PMT, which is optically linked to the scintillator, and a proportional number of electrons is liberated. Within the PMT, a multiplication of electrons by a constant factor takes place. The whole sequence, from ionization onwards, gives rise to an output pulse which is proportional to the energy dissipated in the crystal by the primary ionizing event (Section III–1.2.3).

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

Alpha radiation: Zinc sulphide crystals spread thinly (10–20 mg/cm^2).

Beta radiation: Anthracene, or naphthalene containing 0.1% anthracene, in the form of a large crystal; plastic scintillators are also used; for low energy beta emitters, liquid scintillators are used.

Gamma radiation: Sodium iodide, activated by about 1% thallous iodide, in the form of a transparent, single crystal, cut to the required size.

Scintillation detectors have three advantages over GM tubes for counting gamma photons: (1) higher counting efficiencies (20–40 times); (2) no significant resolving time corrections up to approximately 1700 counts/s (10^5 counts/min); and (3) the output pulse height is proportional to the input photon energy.

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

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

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

To establish optimum high voltage/input bias voltage settings, one tries to maximize $(R^{1/2}-R^{1/2}b)$ or, as an alternative test, to maximize $(R-R_b)^2/R_b$, where R is net count rate of the sample and R_b is the background count rate at the same bias/high voltage settings. In the experiment below, samples are used whose activity differs by a factor of 10^2 in order to provide experience with moderate and low sample activity levels such as may be experienced in field work.

For this experiment, it is assumed that a simple gamma scintillation counter is used, not one equipped with pulse height discrimination capability, because only a single isotope and equipment that could conceivably be taken into the field are of interest. If the gamma counting equipment in the laboratory is capable of pulse height discrimination and has a multichannel analyser, this experiment can be carried out by switching to the 'integral mode'. The multichannel analyser is invaluable if more than one isotope is to be used, such as in dual labelling experiments and for activation analysis, or if environmental contamination, such as that resulting from bomb testing or reactor accidents, is to be determined.

PROCEDURE

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

(1) Obtain a source containing about 0.2 μCi (7.4 kBq) of a gamma emitter such as ^{137}Cs, ^{60}Co or ^{59}Fe

(2) Obtain a second source containing about 0.002 μCi (74 Bq) of the same isotope

(3) Set the bias voltage at 5 V, and determine the count rate of each sample at 50 V intervals of the high voltage setting (HV)

(4) Determine the background at 5 V bias and at 50 V intervals of the HV setting

(5) Repeat the sample and background counts for 20 V bias

(6) Plot $R^{1/2}-R_b^{1/2}$ as a function of the HV, and determine the optimum working voltage for the two samples at biases of 5 and 20 V

(7) Determine the maximum value of $(R-R_b)^2/R_b$ for the two samples at biases of 5 and 20 V

(8) Calculate the percentage natural standard deviation at which the samples have been counted, both at maximum $R^{1/2}-R_b^{1/2}$ and maximum $(R-R_b)^2/R_b$.

III-1.2.7. Liquid scintillation counting

III-1.2.7.1. Introduction

The method of choice for counting radioisotopes that emit soft (low energy) beta particles is the liquid scintillation system. Since several of the radioisotopes of greatest interest to biologists are soft beta emitters (e.g. ^{3}H: 0.019 MeV E_{max}, ^{14}C: 0.156 MeV E_{max}, ^{35}S: 0.167 E_{max} and ^{45}Ca: 0.256 E_{max}), it is important to study this method.

In essence, the method involves the interaction of ionizing radiation with a substance called a fluor, which emits a flash of light when the interaction occurs. The flash of light is 'observed' by a PMT (or, usually, by two tubes), which emits a few electrons when the photon strikes it. The PMT is also an amplifier, building up the few original emitted electrons through as many as ten stages of amplification. Total amplification in the PMT may be of the order of 10^{6}. The signal is then led directly to a pre-amplifier to convert it to the proper form (low impedance) for transmission to the rest of the counting assembly, where it undergoes additional amplification in a linear amplifier.

Normally, two PMTs are used to collect the light emitted from the scintillation vial. This is done to increase the sample to the background counting ratio as follows. After each single ionizing event, light photons are normally registered simultaneously at both photocathodes. The coincidence circuit is designed to produce one output pulse if it receives simultaneously an input pulse from each of the two photomultipliers (within about 1 μs), i.e. in coincidence. Background or electronic noise pulses from either of the PMTs are seldom in coincidence with those from the other, and are therefore rejected. Thus, the ratio of true count rate to background, and thereby the sensitivity, is increased. Since the size of the output pulse is proportional to the energy lost in the liquid scintillator, limited pulse height analysis is possible. It is limited because of the shape of the beta spectra and because of the poor pulse height resolution. However, it is generally possible to count, for instance, ^{3}H and ^{14}C simultaneously.

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

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

Additional parts of the counting assembly usually consist of the following:

(1) *Co-incidence circuitry,* which allows only pulses arriving simultaneously from both photomultipliers to be counted; that is, it rejects random thermal noise pulses originating in a single tube.

(2) *One or more pulse height selectors (discriminators),* where the externally visible controls for the discriminators are either on-board computer controls, preset plug-in cassettes, or two 10 turn potentiometers, often labelled 'lower' and 'upper', which can be set to admit pulses of an energy corresponding to that emanating from the particular emitter being measured. The discriminators can be set to reject pulses of higher or lower energy, thus reducing background. The actual pulse height is a function of the energy of the radiation absorbed in the fluor, and of the carefully controlled, stabilized voltage applied to the PMT, and/or the linear amplifier.

(3) *One or more scalers,* to 'remember' the number of pulses passed to it by the appropriate discriminators.

(4) In the most modern (and most costly) assemblies, *on-board computers* are present which can be programmed to set up the machine for optimum counting, to correct for a variety of errors and, therefore, to express the observed counts as the true number of disintegrations. The experiment to follow and this Manual are written assuming that *the less costly, but often more reliable, manually operated system is used.*

III–1.2.7.2. Controls

Study and learn to operate the controls in groups, for example:

(1) Individual channel control
(2) Sample counting time, group counting, number of event controls, etc.
(3) External standardization (if any), sums of and/or differences in channels, channel ratio correction, automatic background subtraction (if available).

III–1.2.7.3. Bookkeeping

Most important: Control of sample numbering and machine position number. Errors negate all the care taken in setting the controls, preparing the sample and carrying out the biological or chemical experiment.

III-1.2.7.4. Laboratory experiments

(1) *How to set up the spectrometer for maximum sensitivity with any given isotope*

Following the instructions given by the instructor and after examining the operating manual for the instrument, set one channel with an appropriate window width for ^{14}C or ^{3}H. Insert into the counting position an 'unquenched standard' of the isotope chosen. (It is marked with the number of dis/min as assayed on some indicated date.) Consult the operating manual for a reasonable gain setting. Raise or lower the gain until satisfied that the maximum count has been obtained. Record the settings and the counts/min. Repeat the above procedure with the 'unquenched standard' of the other low energy isotope in another channel.

(2) *Calculation of the counting efficiency of 'unquenched' samples*

Calculate the percentage counting efficiency for the standards from the data given in item (1) above, as follows

$$\% \text{ counting efficiency} = \frac{\text{net counts/min observed in standard}}{\text{dis/min (today) in standard}} \times 100$$

(3) *Preparation and determination of the counting efficiency of the ^{14}C and ^{3}H samples of known dis/min*

(a) Non-aqueous samples

(i) *Containing ^{14}C:* The instructor provides a sample of a ^{14}C labelled compound that is soluble in the organic phase of the liquid scintillation cocktail, with a known activity of _____ . Carefully pipette the recommended volume of this sample into a liquid scintillation counting vial in the manner described by the instructor. Add 10 mL of scintillation cocktail, swirl and count under maximum counting yield conditions. Calculate the percentage counting efficiency.

(ii) *Containing ^{3}H (sample No. C-1-b):* Repeat the procedure outlined in item (i) above with a sample of a ^{3}H labelled compound of similar solubility to that described in item (i) above. The compound has a known activity of _____ . Calculate the percentage counting efficiency.

(b) Aqueous samples

(i) *Containing ^{14}C:* An aqueous sample of a ^{14}C labelled compound whose activity is known to be _____ is provided. Deliver _____ mL of this sample to _____ mL of water in a counting vial. Add 10 mL of an appropriate liquid scintillation cocktail (i.e. one designed for counting aqueous samples), swirl thoroughly and count in a channel of the spectrometer whose gain has been set to maximize the counting efficiency with that obtained with the other ^{14}C samples already measured.

(ii) *Containing ^{3}H (sample No. C-2-b):* A sample of a ^{3}H labelled water soluble compound whose activity is known to be _____ is provided. Deliver _____ μL of this sample to _____ mL of water in a counting vial. Proceed in a manner exactly analogous to item (i) above.

(4) *Observation of the effect of quenching and correction for it by the sample channels ratio (SCR) method*

(a) Introduction

The substances present in samples, in the liquid scintillation cocktail, etc., may either absorb energy from beta emission in processes other than those that produce light (chemical quenching), or be coloured and thus absorb some of the emitted light before it reaches the PMTs (optical quenching). Either kind of quenching reduces the light energy output from the liquid scintillation vial, i.e. the output will appear to be that of a lower energy isotope and one would therefore need a channel set with a *higher gain to maximize its count in that channel,* or alternatively one can adapt the channel width while keeping the gain constant. The latter strategy is examined. If, by discriminator settings, the ratio of a lower energy part (channel) to a higher energy part (channel) of the spectrum can be obtained, then it is possible to observe the relation between the counting efficiency, ϵ, and the channel ratio. This is done using a set of standards with a known constant amount of activity and measuring with increasing amounts of chemical quencher (i.e. increasing the channel ratio). A standard curve of ϵ versus the channel ratio can then be prepared and subsequently used to correct the sample measurements for any decrease in count rate due to quenching.

The liquid scintillation spectrometer should be set up as follows:

(i) At a given appropriate gain setting, set the upper and lower discriminators to give the maximum net count on the least quenched sample.

(ii) At the same gain setting as in item (i) above, with the same sample, set the discriminators in another channel with the upper limit in the same way as in item (i) above, but roll the lower discriminator upwards until the count rate is cut approximately in half, i.e. one is looking at the upper half of the energy spectrum. Predict what might happen to the ratio of counts in the two channels (called SCR) as one counts a series of samples of increasing degree of quenching. Check the predictions by obtaining data on a series of chemically quenched samples.

(b) Preparation and observation of a series of variously quenched samples under conditions of constant gain and varying channel width

Set the channels so that, initially, about 50% of the counts (the higher energy pulses) are in the upper channel and about 50% in the lower channel. Observe what happens to the distribution of counts between the two channels with increasing amounts of quenching agent.

(i) *Chemical quenching — ^{14}C:* The chemical quenching agent used is acetone. Other materials that quench strongly include chloroform, aldehydes, nitro compounds and colours; they should be treated in the same way as acetone. It is better to attempt decolourization or oxidation of highly coloured samples. Prepare a series of samples, each containing the same amount of ^{14}C (0.1 mL of _____) (see Table II–II for the acetone volumes).

(ii) *Chemical quenching — ^{3}H:* Repeat the protocol of item (i) above with a ^{3}H sample (0.1 mL of _____) and the same schedule of acetone volumes.

(iii) *Correction for quenching using the SCR method:* Using the data from items (i) and (ii) above and (if available) similar counting data on commercially supplied, sealed, variously quenched series of ^{14}C or ^{3}H samples, prepare quench correction curves, i.e. plot the percentage counting efficiency ((counts/min)/(dis/min)) versus the ratio of counts in the two channels. One can use such curves to convert the counts/min observed to dis/min in subsequent experiments with insects, etc., provided the other conditions (liquid scintillation cocktail, total volumes, counting vial type, spectrometer settings, etc.) are kept constant. If conditions dictate that these must change, check their effect on the counting efficiency.

TABLE III-II. VOLUMES OF WATER AND ACETONE FOR DEVELOPING QUENCH CORRECTION

Sample No.	Volume of acetone (mL)	H_2O
1-a-1	0	0.9
1-a-2	0.1	0.8
1-a-3	0.2	0.7
1-a-4	0.3	0.6
1-a-5	0.4	0.5
1-a-6	0.5	0.4

(5) *Various cocktails for liquid scintillation counting*

There are scores of different cocktails, adopted for various special uses. If cost and availability permit, it is recommended that the product literature is obtained from one of the major commercial suppliers and that a cocktail with qualities that meet the requirements is selected and purchased. Solubilizing agents and cocktails compatible with such agents may be required. A CO_2 trapping agent and a cocktail compatible with the trapping agent[1] may also be needed. Some samples may contain finely divided solids, so a gelling agent must be added. Very commonly, biological samples are aqueous, so the cocktail must be invisible with water. Triton X-100 (octylphenoxy propylethoxy ethanol) produced by Rohm and Haas is often used as the detergent for miscibility. The standard cocktail for non-aqueous samples uses tolnene as the solvent, although recently the less hazardous solvent pseudocumene (1,2,3 trimethyl benzene) is becoming more widely used. Nearly all the cocktails use paraphenyloxazole (PPO) as the primary fluor and 2,2'paraphenylene bis-5-phenyloxazole (POPOP) or dimethyl POPOP as the secondary fluor (the 'wave length shifter'). For a critical discussion of the various cocktails and several of their 'recipes', see L'Annunziata, 1987 [1].

[1] An excellent CO_2 trapping cocktail has been developed by Perschke (personal communication) in the IAEA Laboratory at Seibersdorf, Austria. He traps CO_2 from a Harvey oxidizer (Harvey Instrument Corporation, Hillsdale, New Jersey, USA), in 30 mL of 12% ethanolamine in methanol with 5 mL of a standard tolnene/PPO/POPOP cocktail. Later, he adds another 5 mL of the same tolnene/PPO/POPOP cocktail and counts.

[1] L'Annunziata, M.F., Radionuclide Tracers: Their Detection and Measurement, Academic Press, New York (1987).

III-2. BASIC INSTRUMENT CALCULATIONS

III-2.1. Counting efficiency (counting yield)

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

$$\epsilon = \frac{\text{Count rate of sample}}{\text{Disintegration rate in sample}} = \frac{R - R_b}{A^*} \qquad \text{(III-7)}$$

in counts/s per becquerel or curie.

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

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

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

(3) *Self-absorption in the sample:* Alpha and beta particles, and to a much lesser extent gamma photons, may be absorbed by the sample material in which the radionuclide is contained; therefore, a significant fraction of the activity radiation is not counted. This is a very important consideration for low energy beta particles. Consequently, the count rate from a given sample does not increase in proportion to its thickness. For a sample of a given area, as the sample thickness of constant activity concentration material increases, the count rate tends towards a maximum (Fig. III-8). At thickness X (measured in units of mass

per unit area), the sample is considered to be of infinite thickness. A common method for assaying low energy beta emitters using GM counting is to count all the samples at infinite thickness. The count rate, R_x, is then proportional to the activity concentration in the sample. The value for the infinite thickness of beta emitters is approximately equal to the range of beta particles in units of mass per unit area.

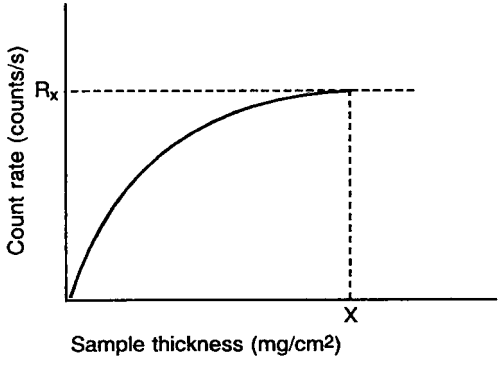

FIG. III–8. Count rate as a function of the sample thickness of constant activity concentration.

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

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

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

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

III–2.2. Counting statistics (natural uncertainty)

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

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

$$\sigma_{nat,\,C} = \sqrt{C} \qquad \text{(III–8)}$$

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

TABLE III–III. NATURAL STANDARD DEVIATION OF ACCUMULATED COUNTS

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

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

Referring to Eq. (III–8), if both sides are divided by the counting time, T, the result is the natural standard deviation of the count rate, R, since R = C/T and T in this respect is constant. Thus

$$\sigma_{\text{nat, R}} = \frac{\sigma_{\text{nat, C}}}{T} = \frac{\sqrt{C}}{T} \qquad \text{(III–9)}$$

However, since C = RT

$$\sigma_{\text{nat, R}} = \sqrt{\frac{R}{T}} \qquad \text{(III–10)}$$

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

A useful rule for counting is to try to accumulate so many counts that the percentage natural standard deviation is two to three times less than the percentage technical standard deviation. If, for instance, 10 000 counts are accumulated, then from this single assay it can be stated that there is a 68% probability that the true mean C value is within 10 000 ± 100, or in the range of 9000–10 000.

Two standard deviations (2σ) account for approximately 95% of the area under a normal distribution curve; in this case it can be stated that there is a 95% probability that the true mean C value is within 10 000 ± 200. An experiment testing some of these statistical considerations is included with the material on the GM counter.

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

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

One important reason for running background counts at least daily, and certainly before counting any series of samples anticipated to be low in activity, has

nothing to do with the sources of background described above. Contamination of the counter and/or its immediate surroundings may be occurring. A sudden increase in apparent background should alert the investigator to this possibility. If one has more than one counter, and they all show a sudden increase in background count, one should look for problems external to the counters, or even external to the counter room. The cause could be a Chernobyl type event, or something much less dramatic such as someone in the next room intermittently ashing rat carcasses containing substantial amounts of ^{95}Zr or ^{95}Nb.

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

$$\sigma^2_{C_s} = \sigma^2_C + \sigma^2_{C_b} \tag{III-11}$$

where $\sigma^2_{C_s}$ is the variance of the net sample count, σ^2_C is the variance of the sample plus background count and $\sigma^2_{C_b}$ is the variance of the background count.

The natural uncertainty of the net sample count then follows from

$$\sigma_{\text{nat, } C_s} = \sqrt{C + C_b} \tag{III-12}$$

where $\sigma_{\text{nat, } C_s}$ is the natural standard deviation of the accumulated net sample counts, C is the total of the accumulated counts due to sample plus background and C_b is the part of the accumulated counts due to background.

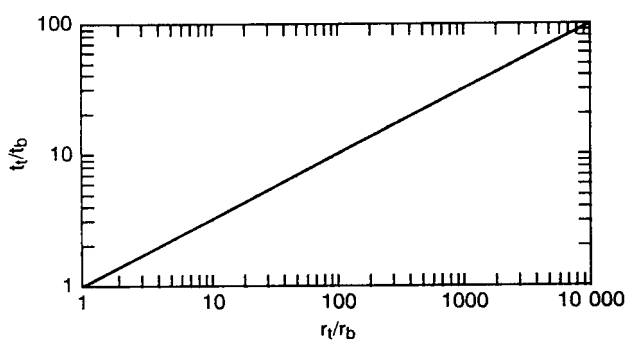

FIG. III–9. Most efficient distribution of counting time: t_t = total (sample + background) counting time; t_b = background counting time; r_t = count rate, total (sample + background); r_b = count rate, background.

Similarly, it follows from Eq. (III–12) that the natural standard deviation ($\sigma_{nat,\ R_S}$) of the net count rate ($R_s = R - R_b$) of the sample is given by

$$\sigma_{nat,\ R_S} = \sqrt{\frac{R}{T} + \frac{R_b}{T_b}} \qquad \text{(III–13)}$$

where R is the count rate of the sample plus background, R_b is the count rate of the background, T is the time used for counting the sample plus background and T_b is the time used for counting the background.

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

$$\frac{T_b}{T} = \sqrt{\frac{R_b}{R}} \qquad \text{(III–14)}$$

where the symbols have the meanings given in Eq. (III–13). However, strict adherence to this criterion for optimum statistical partition of the counting time is not critical in practice. Fig. III–9 is of help in choosing the most efficient counting time for both background and sample counting.

Part IV

APPLICATION TO ENTOMOLOGICAL PROBLEMS

IV-1. INTRODUCTION

The material in this section of the Manual is intended to serve two purposes:

(1) As a manual for specialized courses for entomologists, such as those given at intervals and sponsored jointly by the Food and Agriculture Organization of the United Nations (FAO) and the International Atomic Energy Agency (IAEA)

(2) As a guide to field investigators who wish to use radioisotopes to help solve problems. It is assumed that these investigators have obtained a body of basic knowledge, either from this Manual or elsewhere, which includes:

(a) The nature of radioisotopes and their emissions (radiation)
(b) The interaction of radiation with matter
(c) Radiation detection and assay
(d) Radiation hazards and protection therefrom (radiation safety)
(e) The basic principles of tracer methodology.

In organizing the material in this section, it was felt that some additional basic, but rather specialized, information and experience were needed. Hence, the section on basic techniques (IV-2) contains a subsection on laboratory and field safety (IV-2.1) so that investigators have rather specific directions on how to avoid contamination of personnel and equipment. This subsection also contains an experiment on decontamination, to familiarize investigators with the proper techniques.

Once investigators have their laboratories (and themselves) set up to use radioisotopes safely, a logical next basic step is to answer the question, How does one label insects with radioisotopes? Since there are a variety of ways of doing this, the section has a number of subsections, in most of which details of specific illustrative experiments are given. These provide investigators with detailed instructions on how they can gain experience in using a particular labelling technique.

Once investigators have accumulated experience with basic, but specific, techniques in the application of radioisotopes to entomological problems, logically, they wish to apply this knowledge of insect labelling to the solution of frequently recurring problems related to insect populations or to the behaviour of individual insects. This information, with illustrative experiments, is given in Section IV-3.

Alternatively, investigators may need to study metabolic or environmental problems related to insects or insecticides. In such cases, one needs to label some substance, rather than a whole insect or a population of insects. Coverage of such

applications of radioisitopes, with an illustrative experiment, is provided in Section IV–3.4.

IV–2. BASIC TECHNIQUES

IV–2.1. Laboratory and field safety

Before starting to apply radioisotopes in the laboratory or the field, one must be thoroughly familiar with the essentials of radiation protection given in Part I. One should also consider whether the desired objective can be achieved *without* using radioisotopes (see below). Assuming that one has fully considered all these items and is about to proceed with the use of radioisotopes in the laboratory for the first time, what has to be done physically to prepare the laboratory and oneself?

The highest priority in a radioisotope laboratory is the *prevention* of contamination. First, this requires being constantly on the alert to the potential for contamination, even in what appear to be simple, routine operations. For example, in pipetting a radioactive sample: think ahead about what to do with the used pipette whose externally wet tip, following use with a radioactive solution, is a likely source for the spread of contamination. It is also very important to use the principle of *multiple containment* wherever possible, so that even if one has used an ill advised technique or been clumsy any resulting contamination is strictly limited in its spread and/or is on an easily decontaminated surface. To illustrate: assume, for example, that an experiment calls for the injection of a high specific activity solution of a radioisotope (e.g. ^{32}P) into an insect, followed shortly by surgical and biochemical fractionation of the insect. One starts by laying out a rectangular piece (perhaps 0.5 m in its shortest dimension) of a special bi-layer sheet (often called 'diaper paper' in the USA) consisting of an impervious layer on the one side and an absorbent layer on the other. A porcelain, glass or plastic tray should be placed on top. The stock bottle of ^{32}P in its plastic and metal shield should be on this tray, as well as the injection syringe, surgical tools, etc. Any test tubes, volumetric flasks, centrifuge tubes, etc. to be used should also be on the tray, but should be placed in an upright position in appropriately sized breakers for stability and further containment. A small box of absorbent paper tissue wipes and a container in which to discard the used, contaminated wipes should also be immediately available. Additional, necessary precautions should be taken, e.g. wearing surgical rubber gloves during any operations with a high potential for contamination, lining the tray with diaper paper, reserving part of the tray for operations having the highest contamination potential, providing an additional level of containment in this area, and having an additional person on standby to assist when needed. Above all, there is no substitute for careful, advance planning and continued thoughtfulness. Going through a 'dry run', i.e. a run through of everything one plans to do in the real

experiment, except substituting a highly coloured non-radioactive dye for the radiotracer, is a good way of checking the adequacy of planning. Investigators are cautioned: in the laboratory, the more experienced one is with non-radioactive techniques, the more likely one is to use inappropriate procedures by rote. However, this does not apply to those persons who have been working with highly toxic materials, or with human pathogens, because such techniques are very familiar and easy to learn.

If, despite all precautions, some surfaces have become contaminated, it may be possible to decontaminate them. Often a fresh spill on a clean and polished surface can be washed without detectable contamination resulting. However, if such a spill is allowed to react with the surface, drastic action is required to remove residual radioactivity.

Decontamination can be divided into physical and chemical methods. The former include washing, vacuum cleaning and polishing, and steam or sand blasting; the latter include use of acids and alkali with or without a carrier, detergents, complexing agents and ion exchange material.

An important experiment consists of contaminating several different materials with various isotopes, followed by decontamination of these materials using chemical agents. It should be mentioned that ordinary, untreated concrete was not included in the samples for decontamination, since its microporous nature makes it almost impossible to decontaminate, except by vigorous mechanical abrasion. Does the laboratory have bare, untreated concrete floors?

REAGENTS AND MATERIALS

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

PROCEDURE

(1) Take a background reading of each material to be tested
(2) Dry 10 mL of each radioactive solution on separate pieces of the material being tested

(3) Make a reading on each spot, and record as initial activity
(4) Wipe the surface with damp tissue paper, dry the sample and measure the activity
(5) For a second series, use a washing solution as the decontaminating agent; dry the sample and measure the activity
(6) Try all the relevant decontaminating agents in a similar manner; if necessary, as a final step with linoleum, try removing the wax with acetone
(7) Record all the measured activities, and compare the effect of the different decontaminating agents on the various substances and radioactive isotopes
(8) Discuss the results.

IV-2.2. How to label insects

IV-2.2.1. Non-radioactive methods for marking insects

In a manual dealing with radiotracer methods, it may be surprising to find a short introduction on non-radioactive methods for marking insects. There are a number of reasons for including this material here. In many cases, non-radioactive methods are easier to use and interpret than radioactive methods. They generally require much less equipment; the procedures are usually simple and allow marking of individuals as well as of populations. Non-radioactive procedures involve no health problems and (in field work) no problems of environmental pollution by radiotracers. Therefore, one should always consider whether it is preferable to use a non-radioactive or a radioactive method. The choice depends not only on the actual problem and efficiency of the method but also on the equipment, costs, experience and licence to do field work with radiotracers, etc.

Moreover, non-radioactive methods can be of great help when combined with radiotracer techniques in double labelling, thus permitting differentiation between the individual specimens and groups of insects or populations.

Methods of marking insects have been reviewed briefly by Gangwere et al. [1]. Generally known techniques include: notching parts of the cuticle; clipping off single tarsae; marking with paint spots; and applying numbered or coloured labels, bright aluminium foil or light reflecting glass dust on the thorax. Recently, several methods have been developed extensively because of the increased interest in ecological field studies. Thus, fluorescent dyes have been used by several authors for marking and recovering released insects or ticks (see Medley and Ahrens [2] and the literature quoted by them). Dust applied in adequate amounts to insects with abundant hair or setae may adhere long enough for later recognition. However, dust

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[1] GANGWERE, S.K., CHAVIN, W., EVANS, F.C., Ann. Entomol. Soc. Am. **57** (1964) 662.

[2] MEDLEY, J.G., AHRENS, E.H., J. Econ. Entomol. **61** (1968) 81–84.

tends to get lost and contaminate the environment; this is of no importance in field experiments, but it is disadvantageous in experiments involving small areas or traps. Thus, it is preferable to mark the insects or ticks individually on the dorsum with dyes dissolved in alcohol or acetone.

Soluble fluorescent dyes can be detected on whole insects under black light, provided sufficient dye adheres externally and the self-fluorescence of the insect does not lead to errors. Portable ultraviolet (UV) lamps allow detection of the labelled specimens under field conditions. When the amount of fluorescent dye is so small that it can no longer be detected in this manner, it is recommended that the dead insects be placed on white filter paper and acetone dripped over the bodies. Thus, even small amounts of any dye (whether fluorescent or not) can be seen on the filter paper, either directly as a coloured spot or, under black light, as a fluorescent spot. As a last check, the insects should be squashed on the filter paper. These techniques have been used very successfully in the Netherlands for labelling radiosterilized onion flies (*Hylemia antiqua* Meigen) [3]. The flies were sterilized as pupae and transferred as such into the soil of an experimental field for 'self-release' after emergence. Distribution of these specimens after emergence was monitored using a 'self-marking' process: the pupae were buried under a layer of sand over which a thin coating of dye was applied, followed by a second layer of sand and soil. Emerging flies had to bore their way up to the surface and thus some dye was deposited on the ptilinum. Retraction of the ptilinum into the head precluded the loss of the dye, which was later detected by squashing the head on filter paper and dissolving the dye (fluorescent or not) with a few drops of acetone.

With a zinc or cadmium sulphide fluorescent dust, which is neither water nor alcohol soluble, millions of insects can be marked simultaneously for field observation. In most cases, these compounds are superior to metallic powders and soluble dyes. There being no human safety problems with fluorescent dusts, and because equipment for their detection is inexpensive, these materials can be very useful in entomological research. A major advantage of these dusts is that they do not wash off when the specimens are immersed in water or preserved in alcohol. The insects remain marked even after digging in sandy soil for months. Information regarding manufacturers, detection, equipment and application methods has been provided by Stern and Mueller [4].

Liposoluble dyes such as Calco® oil N-1700 have been used successfully for internally mass marking field populations of insects attracted by oil baits (e.g. boll-weevils attracted by cottonseed oil and fire ants by soybean oil) (see Ref. [5]).

Genetic markers can be used in field studies of insect distribution and flight range. For example, Cordovan bees are used frequently because their distinctive

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[3] NOORDINK, J.P.W., Personal communication.

[4] STERN, V.M., MUELLER, A., J. Econ. Entomol. **61** (1968) 1440–1444.

[5] LOYD, E.P., et al., J. Econ. Entomol. **61** (1968) 1435–1439.

body pigmentation permits easy identification in the field [6]. A prerequisite is, of course, that the vitality of genetically labelled strains is not less than that of normal wild insects. Kloft (unpublished data) succeeded several times in estimating unknown population densities of *Drosophila* by means of a genetic marker. Also, the same results have been obtained in the laboratory with radioactively labelled specimens.

With honey bees, a very interesting capture–mark–recapture system has been developed by Gary [7]. Ferrous labels are glued lightly to the abdomen of bees foraging in the field (these can be given individual numbers). When the bees thus marked return to the hive, their labels are taken off by magnetic traps as they pass through the hive entrance, thus recording to which hive each individual bee has returned. This method could possibly be adapted to other insects; however, its primary limitation is the improbability of the insect passing precisely within the recovery range of the magnets. It may be helpful to use a combination of attractive baits and magnetic traps for solitary insects.

IV–2.2.2. Topical application of isotopes (external application)

A precise volume of radioactive solution can be placed on a selected spot of the cuticle. Usually, the centre of the pronotum is preferred, a point which most insects cannot reach with their antennae or legs; thus, contamination of the insects while they clean themselves is prevented. It is advantageous to restrain the insects. Micropipettes, Hamilton syringes and micrometer syringes are used for topical application. The injection needle points should be sharpened and siliconized. Bending the needle allows the syringe to be held in a horizontal position so that any outflow of the liquid is prevented. For example, $^{60}Co(NO_3)^2$ diluted in cellulose acetate with acetone can be applied in dots on the cuticular surface of insects. Since the acetone evaporates immediately, the dots dry within a short time. For adult beetles, the solution can be applied on the lower surface of the elytrae (during narcosis beetles sometimes spread out the elytrae), thus totally preventing any mechanical rub-off.

Experiments described later in this Manual (Section IV–3.4.2) use topical application of radioisotopes as a means of approaching problems such as the fate of an applied insecticide.

An experiment carried out particularly with a view to studying the fate of a topically applied radioisotope, ^{32}P, in cockroaches is described.

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[6] LEVIN, M.D., J. Econ. Entomol. **52** (1959) 969–971; **54** (1961) 431–434.

[7] GARY, N.E., J. Econ. Entomol. **64** (1971) 961–965.

IV-2.2.2.1. Topical application of aqueous ^{32}P phosphate or radioiodine for testing penetration and excretion through the cuticle of insects

The cuticle of insects appears to permit the passage of substances from the inside to the outside (cuticular excretion) as well as from the outside to the inside. Advantage is taken of the latter property in the application of organosynthetic insecticides; these are soluble in the lipoids of the outer layers of the cuticle, which facilitates their penetration. From tracer experiments with aquatic or semi-liquid media (soils) it is known that the cuticle of living insects is also permeable for inorganic substances. This is important for the osmoregulation of aquatic insects.

PURPOSE

To study the permeability of the cuticle of non-aquatic insects to externally administered radioactive ^{32}P (as phosphate).

MATERIALS

(1) Cockroaches at different stages
(2) Cardboard, scissors and Scotch tape
(3) A calibrated syringe with a hypodermic needle for topical application
(4) Phosphorus-32 as phosphate in aqueous solution with a specific activity of 0.1–0.5 mCi/mL (3.7–18.5 MBq/mL)
(5) Glass jars
(6) Microcapillaries.

PROCEDURE

(a) Mounting of the cockroaches: The legs and antennae of the cockroaches are fixed on cardboard strips with Scotch tape. The insects remain in this position until the end of the experiment to prevent contamination of the cuticle.

(b) Topical application of the tracer: Avoid any other contamination of the cockroaches. In the middle of the pronotum apply a 3 μL drop (corresponding to ___ mCi or ___ MBq ^{32}P) of the radioactive solution. Note the exact time and temperature. After tracer application, the mounted cockroaches are placed in containers with a high relative humidity (provided by wet cellulose layers on the inside of the cover of the container).

(c) Checking of cuticle penetration of the tracer: After various time intervals (24, 48, 72 hours, etc.), the specimens of each series are checked for cuticle penetration of the tracer. If penetration occurs, one can expect the tracer to circulate with the haemolymph. Therefore, the cuticle is pierced between the coxae of the hind legs with a calibrated capillary tube and 2 μL of haemolymph is soaked up. Before this operation, the background activities of the capillary tube and the planchet have to be counted for 10 minutes. After soaking up the haemolymph, measurement is repeated (for 10 minutes) to detect the radioactivity in the blood.

The experiment can also be modified to detect cuticular excretion, since radioactive substances that have been absorbed are excreted after some time. To prove this, the abdomen is wiped gently with soft cellulose, which is then checked for radioactivity. The best way of checking cuticular excretion is to inject some tracer solution into the haemolymph.

IV-2.2.3. Isotopes in labelled food

Incorporation of a radioisotope in food is a common method of labelling insects for various important studies. Two studies, one on the social behaviour of ants and the other involving determination of the biological half-life, are described below.

IV-2.2.3.1. Demonstration of the rate of food uptake and exchange among social insects

Social insects such as ants, bees and termites have a highly developed social food exchange between the different individuals of the community. With the help of radioisotopes, it is possible to demonstrate this behaviour (in laboratory experiments) with ants over a short period. Using ants of different subfamilies, certain differences between them can be demonstrated; these have to be taken into consideration in tracer field experiments. The time required for this experiment is 4–8 hours, after completion of the preparatory work with the equipment and insects (see Refs [8–10]).

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[8] KLOFT, W.J., DRESCHER, W., DJALAL, A.S., Apidologie **7** (1976) 49–60.

[9] KLOFT, W.J., ROBINSON, F.A., Am. Bee J. **116** (1976) 106–107, 118.

[10] GÖSSWALD, K., KLOFT, W., "Tracer experiments on food exchange in ants and termites", Radiation and Radioisotopes Applied to Insects of Agricultural Importance (Proc. Symp. Athens, 1963), IAEA, Vienna (1963) 25–42.

PURPOSE

To demonstrate the uptake and distribution of food from ant to ant or caste to caste.

MATERIALS

(1) Glass containers, 10 cm × 10 cm × 5 cm, with covers
(2) Insect tweezers
(3) Glass slides and cover slips
(4) Chloroform or ether
(5) Honey or sucrose solution (20%)
(6) Phosphorus-32 (0.5–1.0 mCi (18.5–37 MBq)) in water, with a specific activity of about 0.5–1.0 mCi/mL
(7) Hollow plastic stoppers for holding the ants during measurement (containers), with a diameter corresponding to the size of the ants; talcum powder
(8) Non-radioactive phosphorus of the same composition as item (6)
(9) Vials, bottles, ordinary laboratory glassware and plastic squeeze bottles
(10) Radiation measuring equipment
(11) Ants from the subfamily Camponotinae and from the subfamily Myrmicinae (500 each)
(12) Decontamination solution: 1% sodium phosphate, plus a small amount of detergent such as Triton X-100 or Tween 20.

PROCEDURE

Experiment A: Rate of direct food uptake by ants. Prepare liquid radioactive food for the ants by mixing two parts of a 30% solution of honey in water with one part of a ^{32}P solution. The specific activity of the food should be 0.5–1.0 mCi/mL (18.5–37 MBq/mL). Place 10–15 specimens of each species in separate jars. Feed the ants by putting a drop of the prepared radioactive food on cover slips and placing these in the jars. Record the starting time, duration of feeding and air temperature. After careful decontamination, the ants should be measured individually. For records, use the following form:

Ant species:
Caste: Date of collection:
Composition of food: Specific activity:
Mode of radioisotope application: Time at beginning of count:
Air temperature: Time at end of count:

Measurement: GM tube No.: Working voltage:

Ant No.	Counting time	Counts	Counts/min	Background (counts/min)	Counts/min minus background (counts/min)

Experiment B: Social food distribution among ants. Individual ants with the highest radioactivity are now used as donors. Mark the donors carefully with a colour spot and place each donor in a jar containing 30 hungry worker ants (unfed for 2–3 days) of the same species and from the same colony. Prepare three jars of each species. Using chloroform or ether, kill the ants in one jar after 4 hours, those in the second after 20 hours and those in the third after 40 hours. Measure and record the radioactivity of each ant. Group the number of specimens in each jar in a category of radioactivity, i.e. 0–50, 50–200, 200–500, 500–2000 and over 2000 counts/min. Compare the food distribution of the two species on a time basis.

Experiment C: Social food exchange among honey bees (Fig. IV–1) [8, 9]. Place about 250 honey bees in two small wire screen cages; keep them in the dark and without food for about 2 hours (at 30°C). Take out one worker bee from each cage and feed it with a labelled honey solution (about 0.5 mCi/mL (18.5 MBq/mL)) through a pipette. Check the radioactivity (counts/min) of the labelled individuals; put them back into the cages. Give the insects of cage (a) water but no additional food. Give the insects of cage (b) additional food and water. Keep both cages dark (at 30°C) for 4 hours; finish the experiment by very slow and careful anaesthetization with CO_2, followed by killing with chloroform. Measure each individual for radioactivity (counts/min).

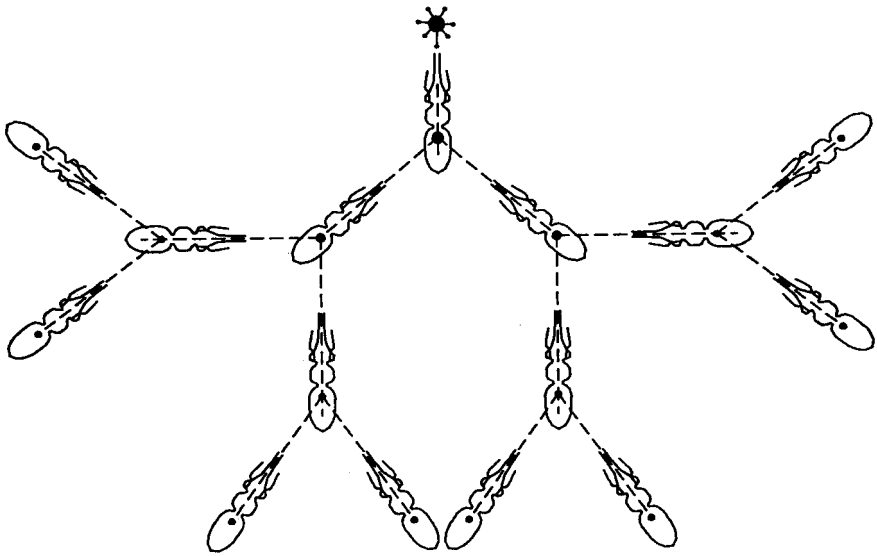

FIG. IV–1. Social food distribution in honey bees. The donor bee (top) imbibes a radioactive sugar solution, which is distributed by multiple regurgitation to other members of the hive. With a single donor bee fed a 50 μL radioactive sucrose solution and kept at 31 °C, a maximum crop content distribution was recorded: 349 individuals. (These results vary among races of the western honey bee Apis mellifica *L., see Refs [8, 9].)*

QUESTIONS

(1) What was the time required for the maximum distribution of food?
(2) Which ant subfamily accomplished the distribution first?
(3) Are there any differences in the honey bee experiments with and without a food supply? What are the sources of error?

IV–2.2.3.2. Measurement of the biological half-life of radiotracers in insects

The biological half-life is the time required for the loss of half the amount of an ingested substance. It is primarily dependent on the rate of excretion. The biological half-life (T_{biol}) cannot be measured directly. For a radioactive substance, it can be computed by measuring the effective decrease in the count rate of a radioactive

substance (in a time unit) as a result of both the biological and physical decay of radioactivity (Fig. IV-2) [11]. This can be expressed as

$$\frac{1}{T_{eff}} = \frac{1}{T_{phys}} + \frac{1}{T_{biol}}$$

The effective decrease in activity of a radioactive substance follows an exponential law, as does the physical and biological decrease in the count rate. Since the physical half-life of a radioisotope is known, the biological half-life can be calculated after having graphically obtained the effective half-life (T_{eff}) from the following formula

$$T_{biol} = \frac{1}{\frac{1}{T_{eff}} - \frac{1}{T_{phys}}} \quad \text{or} \quad T_{biol} = \frac{T_{eff} \times T_{phys}}{T_{phys} - T_{eff}}$$

Excretion being a physiological process, the biological half-life of radionuclides depends on the temperature and follows the Q_{10} law (except for some substances which are stored, e.g. radioiodine, some of which remains in the cuticle, especially of newly moulted individuals). Effective and biological half-lives can have different phases (Fig. IV-3) [12] (see also Ref. [13]).

PURPOSE

To determine the biological half-life of ^{32}P and ^{131}I in cockroaches with emphasis on temperature dependence.

[11] KLOFT, W., EHRHARDT, P., "Studies on the assimilation and excretion of labelled phosphate in aphids", Radioisotopes and Radiation in Entomology (Proc. Symp. Bombay, 1960), IAEA, Vienna (1962) 181–190.

[12] WHITEHOUSE, W.J., PUTMAN, J.L., Radioactive Isotopes, Clarendon Press, Oxford (1953).

[13] SCHLAGBAUER, A., Int. J. Appl. Radiat. Isot. **19** (1968) 757–763, 765–769.

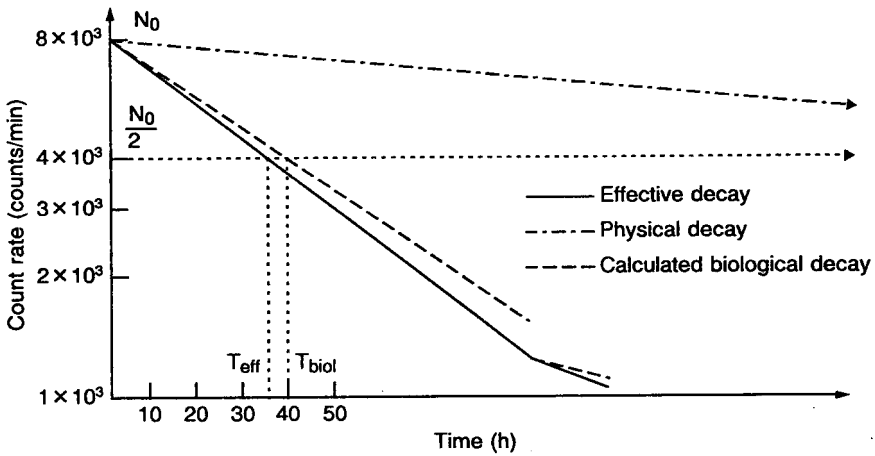

FIG. IV-2. Relationship between the effective, physical and biological decay of radioactivity in an imago of Megoura viciae *Buckt. [11]. The half-lives, T_{eff} and T_{biol}, were found graphically. N_0 is the initial activity of the aphid.*

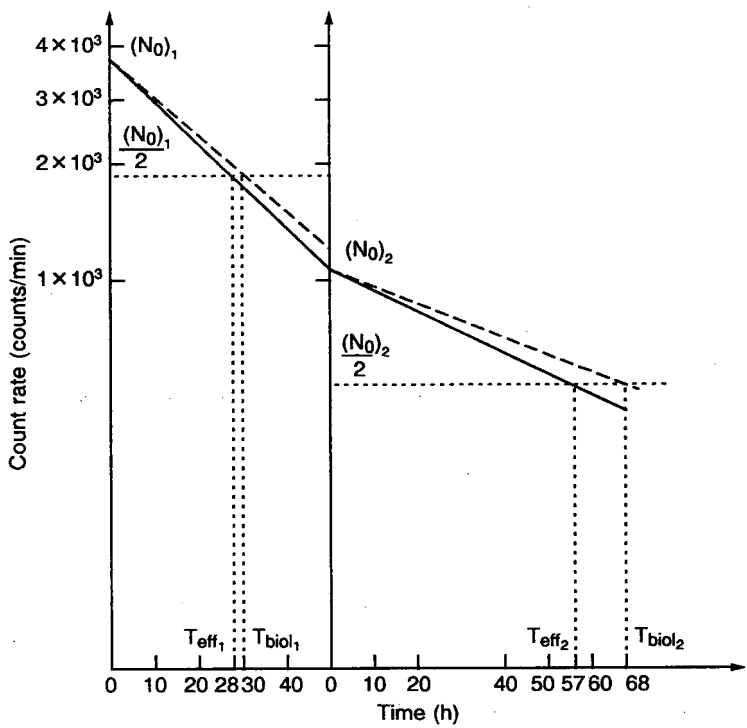

FIG. IV-3. Change in the biological half-life of ^{32}P in a larva of Megoura viciae *Buckt. The effective and biological half-lives change, owing to the rapid elimination of radioactivity through the gut in the first phase [12]. $(N_0)_1$ is the initial activity for the first phase; $(N_0)_2$ is the initial activity for the second phase.*

MATERIALS

(1) Medicine droppers
(2) Pipettes
(3) Syringe (1/4 mL)
(4) Phosphorus-32
(5) Iodine-131
(6) Petri dishes
(7) Gelatin capsules to hold the cockroaches firmly
(8) Sucrose solution
(9) Counting equipment
(10) Glass slides
(11) Ordinary laboratory glassware
(12) Carbon dioxide for anaesthetizing the insects
(13) Sodium phosphate
(14) Incubators set at two different temperatures
(15) Log–linear graph paper.

PROCEDURE

Anaesthetize the cockroaches with CO_2 and fix them on glass slides with the dorsum next to the glass. Scotch tape is adequate for this purpose. As the cockroaches recover from the CO_2, feed them ^{32}P or ^{131}I in a sucrose solution using a medicine dropper with a fine tip. Insects readily imbibe sufficient amounts of the solution. The specific activity of both preparations should be about 0.5 mCi/mL (18.5 MBq/mL). The cockroaches may become contaminated externally during the feeding process and must be decontaminated. Decontaminate by washing freely, first with a 1% solution of sodium phosphate in water containing a detergent, and then with pure water. Allow the insects to dry.

Radiation measurements should be taken soon after feeding and repeated 2, 24, 48, 72 and 96 hours later. Measure ^{32}P with a GM tube and ^{131}I with scintillation counters. Each measurement must be taken under the same geometric and physical conditions. Confining the cockroaches in a small gelatine capsule is a good way of maintaining the same geometry for different measurements.

Keep the cockroaches isolated in Petri dishes within incubators set at different temperatures (if possible, with a difference of 10°C). Renew the filter paper in the dishes daily and provide food and water. Excrements also have to be measured for radioactivity to obtain the values for passage through the alimentary canal. Wipe the pronota with filter paper after 2–3 days and measure for cuticular excretion.

Plot the results on log–linear graph paper (log of count rate (counts/min) against time (days)) and determine T_{eff} graphically. Calculate T_{biol} for the different time units.

QUESTIONS

(1) Are there any differences in the biological half-lives of the ^{32}P and ^{131}I solutions?
(2) If there are differences, to what can they be attributed?
(3) If the biological half-life is significantly temperature dependent, what can be concluded?

IV–2.2.4. Use of isotopes in an artificial medium (semi-synthetic medium)

IV–2.2.4.1. Speed of absorption and elimination of labelled (^{32}P) food in the larvae of *Trichoplusia ni*

The speed of food passage through the insect's alimentary tract can be measured very well with radiolabelled food. In this regard, there are great differences between the various nutritional types of insect. Continuous feeders, e.g. caterpillars of the cabbage looper *Trichoplusia ni* (Hbn.) (Lepidoptera, Noctuidae), have a high speed of food passage. These larvae are becoming increasingly preferred objects of research in insect physiology. One of the main reasons for this is that they can be bred on semi-artificial diets.

PURPOSE

To demonstrate the speed of normal food passage through the gut (see Fig. IV–4, λ_7) and especially to check the transfer of food through quick absorption into the haemolymph to the Malpighian tubules, and the excretion through these (compare with Fig. IV–4, λ_6).

MATERIALS

(1) Cabbage looper larvae
(2) Cabbage looper diet such as dry powder (for composition, see Ref. [14])

[14] RU, N., KLOFT, W.J., Entomol. Germanica **2** (1976) 242–248.

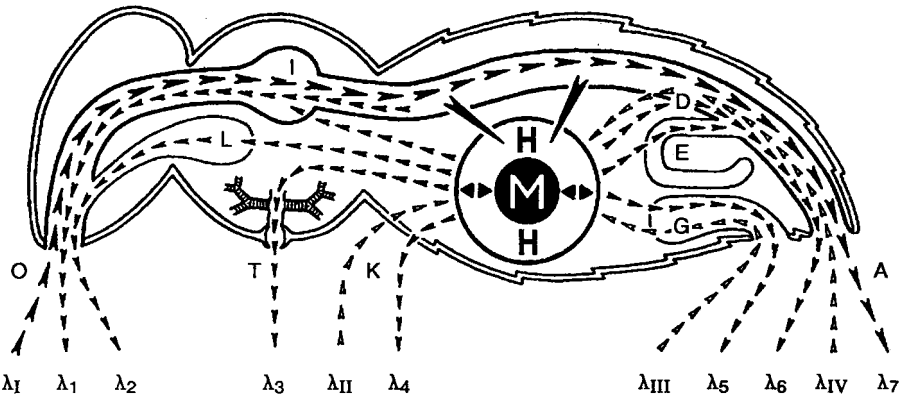

FIG. IV–4. Pathways of radioisotope uptake (Roman numerals) and excretion (Arabic numerals) through the insect organism (unpublished original drawing by W.J. Kloft and E. Wolfram). O = os (mouth); L = labial (salivary) glands; I = ingluvies (crop); T = tracheal system; K = cuticle; H = haemolymph; M = metabolism; D = gut; E = excretion through Malpighian tubules; G = genital organs; A = anus. Further explanations are given in the text.

(3) Small plastic beakers; stand to hang these over a rotating disc
(4) Synchronous clock
(5) Cardboard disc
(6) Tangle-foot® glue
(7) Radiophosphate
(8) Decontamination fluids
(9) Several jeweller's forceps for preparation
(10) Capillary tubes for haemolymph samples
(11) Laboratory glassware
(12) Graph paper
(13) Stopwatch.

PROCEDURE

The cabbage looper diet contains crushed pinto beans, dried Torula yeast, wheat germ, ascorbic acid, methyl-p-hydroxy-benzoate, sorbic acid, HWG[1], formaldehyde 40%, vitamin mixture, tetracycline and water. The cabbage looper diet must be mixed and 'cooked'. While placing some of the hot prepared diet into a

[1] HWG is a protein formula commonly used in laboratories in the USA.

beaker, a small amount of radiophosphate is added and thoroughly mixed with the diet. The beaker is fixed, hanging (upside down) over a prepared disc, divided into time sectors and smeared with 'tangle-foot' glue. The caterpillar is fixed, with the prolegs on a horizontal insect pin or piece of wire. The caterpillar feeds on the radioactive diet for *only* 1 minute (record the time carefully, using a stopwatch) and is then transferred to non-radioactive food. Note the temperature. To demonstrate the fast absorption of food (70 seconds were recorded by Ru and Kloft [14]), haemolymph samples are taken and checked for radioactivity. Plot the count rate (counts/min) of the faecal pellets against time in minutes.

QUESTIONS

(1) How many minutes after feeding started were the first radioactive pellets dropped?
(2) How can one distinguish between the first elimination of ^{32}P via excretion through the Malpighian tubules and the radioactivity eliminated after normal passage through the gut?
(3) After what time is the first radioactivity found circulating in the haemolymph?
(4) How much time is required to eliminate most of the ingested radioactivity?

(1) Uptake

λ_I Via mouth parts and the gut system
λ_{II} Through the cuticle; sorption of tritiated water is also cuticular uptake
λ_{III} Transfer via sperm or auxiliary secretion during copulation with radioactively tagged males
λ_{IV} Via the anus during rectal respiration and/or rectal osmoregulation.

(2) Elimination

λ_1 By regurgitation from the alimentary tract; in social insects, especially the crop
λ_2 The tracer carried by the haemolymph enters the salivary (labial) glands (L) through their epithelium and is secreted with the saliva
$\lambda_{3/4}$ The tracer is excreted during respiration ($^{14}CO_2$ given off) through the tracheal system (T) or during transpiration (tritiated water) through the cuticle (K), together with cuticular secretion or pheromones
λ_5 The tracer is eliminated by females with the eggs, viviparous larvae or auxiliary secretion, likewise by males with sperm and secretion; measurement of the activity in sperm and secretion is, in many cases, the only definitive method for determining the competitiveness of sterile males in the sterile insect techniques (SIT), particularly with species in which no spermatophores are deposited in the females

λ_6 The tracer is excreted via the Malpighian tubules (E) and the hind gut

λ_7 The tracer is excreted with the faeces after normal passage through the alimentary tract.

IV-2.2.5. Tagging insects with radioisotopes by rearing in an isotope labelled environment

PURPOSE

Tagging of insects with radioactive markers has been used in studying dispersion, the speed of flight, the total number of a natural insect population in an area and in other biological investigations. Phosphorus-32 is a useful tagging agent, but the short half-life (14.3 days) precludes its use for studies extending for more than a few weeks. However, ^{32}P has the distinct advantage that mere storage of any contaminated materials for 5–6 months ($> 10 \times T_{1/2}$) fully removes the contamination. For experiments that continue for a few months, one might consider using ^{59}Fe ($T_{1/2}$ of about 45 days) (see Refs [15, 16] for some of the ^{59}Fe labelling methods).

It is necessary to be familiar with handling mosquitoes and tagging them in the laboratory before field dispersal studies are undertaken. The best way of obtaining tagged adult mosquitoes is to apply the tracer to the larvae via water. Suitable radioactive substances such as ^{32}P as a phosphate or phosphoric acid are absorbed through the thin larval cuticle, taken up with food and imbibed with water. These processes ensure good internal labelling of the adults emerging from the pupae.

MATERIALS

(1) Pans or glassware, holding 0.5–1 L of water, suitable for rearing mosquito larvae
(2) Phosphorus-32 as phosphoric acid or a phosphate
(3) Insect emergence cages
(4) Pipettes and other laboratory glassware
(5) Counting equipment
(6) Mosquito larvae (In Gainesville, Florida, USA, for example, one used *Culex pipiens quinquefasciatus* Florida, USDA laboratory strain, in their late second or early third instar, 200 individuals in a normal rearing pan of 100 mL H_2O).

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[15] KABAYO, J.P., RUHM, M.E., BARNOR, H., ZEILLER, E., Appl. Radiat. Isot. **39** 3 (1988) 207–211.

[16] SMITTLE, B.J., LABREQUE, G.C.L., J. Econ. Entomol. **62** (1969) 1242–1244.

PROCEDURE

To reduce the amount of radioactive solution, only 0.1 L of salt water (sea salt solution) is used, to which 0.05 mCi (1.85 MBq) ^{32}P is added and thoroughly mixed. The larvae are fed the normal artificial diet. The pan must be covered with glass to avoid water loss (which would change the specific activity) in a laboratory without controlled air humidity.

After 24 hours, remove about half of the larvae from the radioactive medium; after washing in salt water, transfer them to another pan containing inactive salt water and normal larval food (at a ratio of 1/2:1). For the remaining larvae, follow the same procedure after 48 hours.

After pupation, remove the pupae, wash them briefly and transfer to waxed paper cups containing inactive salt water. Place the cups in a hatching cage.

Measurement: The adults must be measured for radioactivity for a period of 2 days. Record the males and females separately in both time groups (24 and 48 hours). Check the larval radioactivity at intervals (from the second day).

QUESTIONS

(1) Which sex has the greatest radioactivity?
(2) How does the uptake of radioactivity compare in the 24 and 48 hour treatments?

IV-2.2.6. Tagging insects through implants or by injection

Sometimes it is necessary to detect and measure the movement of insects that spend relatively long periods hidden in some environment, such as underground or under the bark of trees, where they cannot readily be observed. Radioisotopes, especially those emitting gamma rays, provide the easiest and most accurate method for studying such insects. Two methods of labelling these insects are:

(1) If the insect is large enough and durable enough, an isotope labelled wire can be implanted
(2) Some radioisotope labelled solution can be injected.

Examples of the two methods of labelling are given below. Although the experiment illustrating the injection method is actually used to measure the biological half-life, the principle could apply to a variety of experimental situations.

IV-2.2.6.1. Determination of the movement of insects not readily observed

PURPOSE

To determine the movement of mole crickets (or other species such as wireworms or corn rootworms) and to plot such movement on graph paper.

This experiment involves the use of ^{60}Co, but in a controlled laboratory situation. Before contemplating use of such a long lived isotope ($T_{1/2}$ = 5.3 years) involving release to the environment, one should study the regulations of the country in which the release is to be made in order to determine whether this is permitted. Also, consider substituting a less hazardous isotope (see Ref. [17] for a list of the relative hazards of radioisotopes).

MATERIALS

(1) One large shallow tray, about 45 cm × 75 cm × 7.5 cm, of wood or metal
(2) Good quality topsoil with a high moisture content
(3) Fine ^{60}Co wire or a water soluble ^{60}Co salt; the activity of the ^{60}Co should be sufficient to penetrate 7.6 cm of soil, i.e. about 0.05 mCi (1.85 MBq) per millimetre of wire
(4) Cellulose tape
(5) Ball of string or fine wire
(6) Portable counter
(7) Graph paper
(8) Small beakers and pipettes
(9) Forceps, scissors, etc.

PROCEDURE

Fill the tray with soil and make a grid over the tray with either string or wire. Place ^{60}CO wire into the abdominal cavity of the test insect (see Fig. IV-5(b)) or put a drop of the ^{60}CO salt solution on the adhesive side of a small piece of cellulose tape and allow the solvent to evaporate. Place the tape under the wing of the mole cricket. As the method of application, one may choose to inject the gamma emitting tracer into the insect being studied.

The tagged insect should then be placed on the surface of the soil at any particular location relative to the grid. The location should be marked on graph paper with the same number of grid spaces as the tray.

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[17] L'ANNUNZIATA, M.F., Radionuclide Tracers: Their Detection and Measurement, Academic Press, New York (1987) 474.

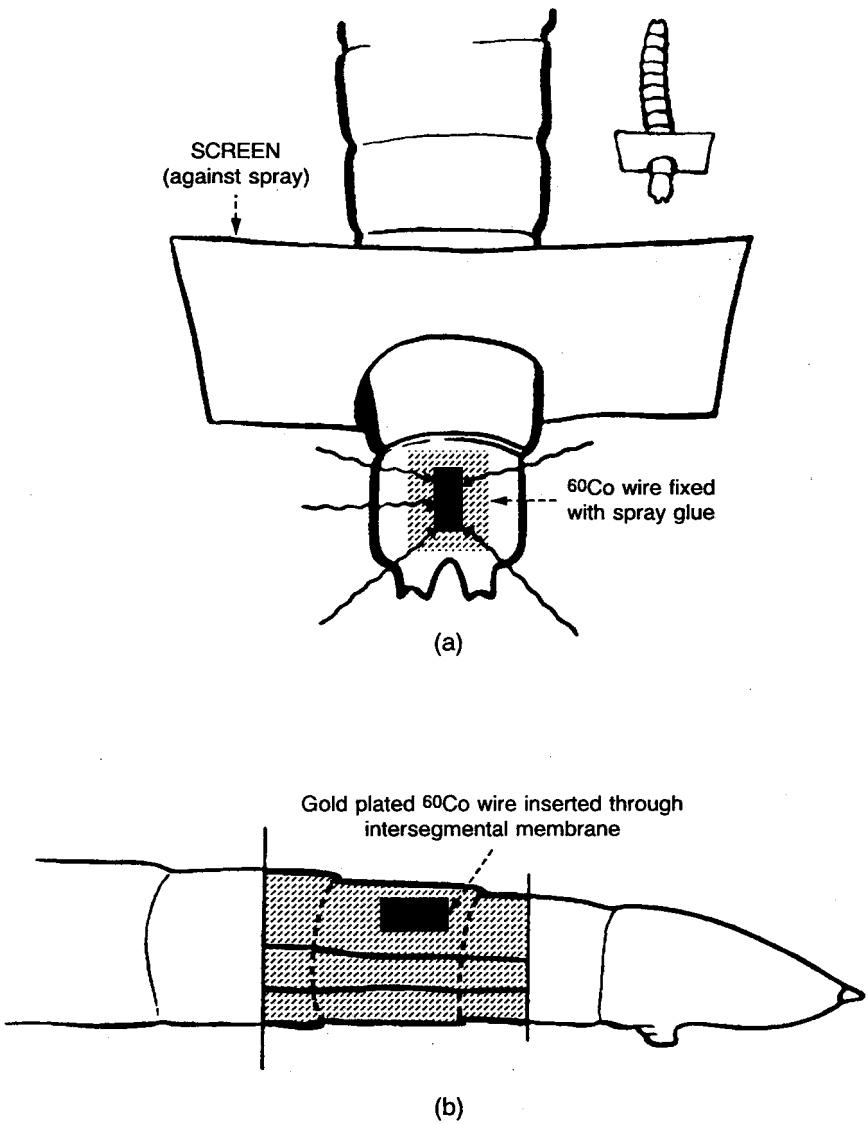

FIG. IV–5. (a) External fixation of a ^{60}Co wire on the pygidium of a click beetle (Elateridae) larva with spray glue; (b) internal label with gold plated ^{60}Co wire, inserted through an intersegmental membrane of a click beetle (Elateridae) larva.

The position of the insect should be located periodically with the counter. Active insects should be located at 5 minute intervals. The highest activity is at a point immediately above the insect. Record the new position of the insect on the graph paper and connect the various points with lines.

IV-2.2.6.2. Injection of ^{32}P to determine the biological half-life

This is a repetition of the experiment given earlier for measuring the T_{biol} of ^{32}P in cockroaches. In the earlier experiment, the ^{32}P was administered with food; here, it is administered by injection into the underside of the abdomen. This obviously requires a calibrated microsyringe, such as the Hamilton, and a suitably fine glass or metal needle. Otherwise, the materials, procedures and discussion are identical to those given in the earlier experiment (see Section 2.2.3). Compare and contrast the two methods of tracer administration.

IV-2.2.7. Tagging insects by feeding on an isotope tagged host

Predacious arthropods can be labelled by feeding them with radioactive prey [18]. Spiders can be fed with flies which have imbibed a ^{32}P sucrose solution (Fig. IV-6) [18]; larvae of Myrmeleontidae (ant lions) can be labelled in the same way by feeding them with radioactive ants [19]. Using a natural foodchain, ladybird beetles can be labelled by feeding them with radioactive aphids. Ants can be labelled by feeding them with the aphid's honeydew; this can be done in the laboratory as well as in the field.

A very interesting method of labelling parasitic Hymenoptera via radioactive insect hosts has been worked out by Steffensen and La Chance [20]. Since this method permits separate marking of the sexes and, subsequently, of the sperm or eggs, it is also of interest for experiments in genetics and radiobiology. *Habrobracon juglandis* can be bred in Petri dishes, and a stock colony of *Ephestia kuehniella* is used to supply the Hymenoptera with insect hosts. The caterpillars are supplied with the tracer by an oesophageal probe (Fig. IV-7) [20].

Plant sucking insects such as phloem feeding aphids and other Homoptera can be labelled via the host plant or artificial diets. Other nutritional types such as 'local imbibers' [21] can be fed on leaf discs floating in radioactive solutions. For mass rearing labelled crawlers (of the Coccid family Diaspididae), green melons can be tagged with $^{14}CO_2$ through photosynthesis or by injection with ^{14}C glucose. The melons are more evenly labelled when $^{14}CO_2$ is used.

[18] KULLMANN, E., KLOFT, W., Verh. Dtsch. Zool. Ges. (1968) 487-497.
[19] BUSCHINGER, A., BONGERS, J., Z. Vgl. Physiol. **62** (1969) 205-213.
[20] STEFFENSEN, D., La CHANCE, L.E., Radioisotopes in the Biosphere (Proc. Symp. Minneapolis, 1960) (1960) 132-145.
[21] KLOFT, W., KUNKEL, H., Z. Pflanzenkr. Pflanzensch. **76** (1969) 1-8.

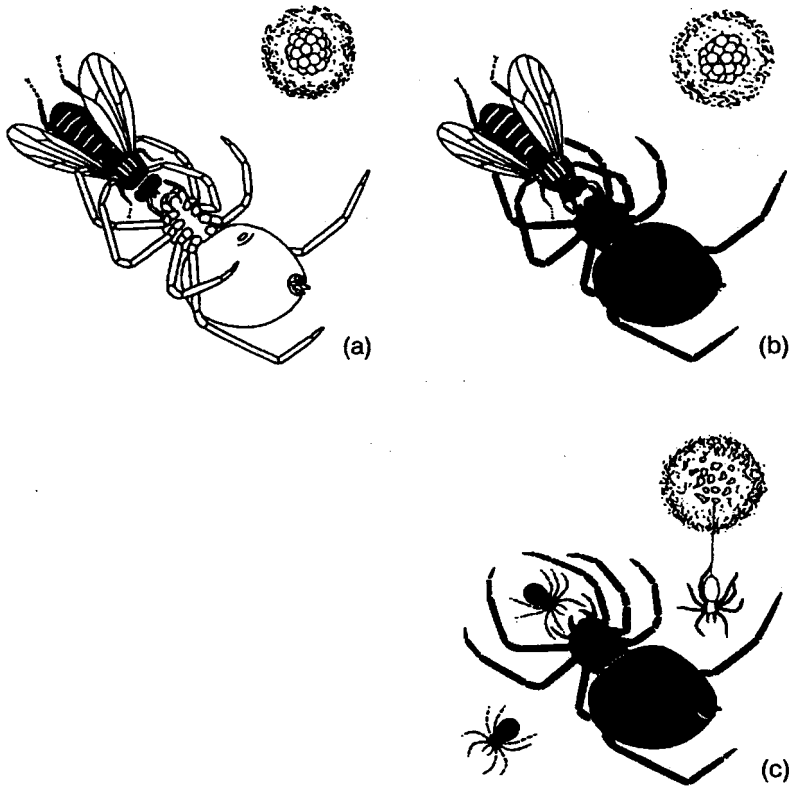

FIG. IV–6. Labelling of a female spider and her newly hatched larvae by feeding the female spider with radioactive flies. This experiment demonstrates the social behaviour between the mother and the newly hatched larvae in spiders [18]. (a) A female spider (Araneae, Theridiidae) is fed with a radioactive (black) fly after production of its egg cocoon; (b) by feeding on the radioactive fly, the spider becomes radioactive (black) itself; (c) the newly hatched larvae are fed by maternal regurgitation, becoming, in turn, radioactive (black).

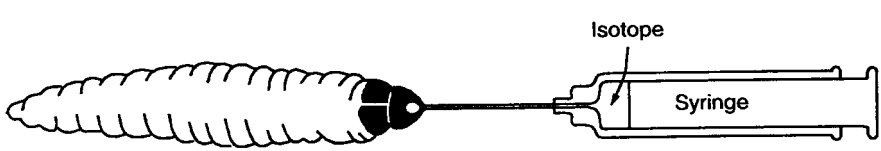

FIG. IV–7. Injection of a radioisotope into an Ephestia *spp. caterpillar, which will then serve as the radioactive host for the parasitic Hymenoptera (*Habrobracon *spp.) [20].*

Woodboring beetles in living stems can be marked systemically via plant injection. Leaf eating insects such as sawfly larvae and caterpillars can be labelled very easily on plant twigs dipped in radioactive solutions. For exact application (as regards time or amount), the sandwich method is convenient using sandwiches made of leaf pieces with the inner surface 'smeared' with the radiotracer; also of use are radioactive meridic diets [14].

While the experiments given below demonstrate how aphids can be labelled by feeding on a labelled host plant, or by a simulated 'host' plant, they also show how such labelling can yield much useful information.

IV–2.2.7.1. Tracer experiments with aphids

Plant sucking insects, especially aphids feeding on phloem sap, are of great importance in plant pathology. Basic knowledge on the nutritional physiology of aphids is necessary to learn more about host plant/insect relationships, the mode of virus vectoring and control with systemic insecticides. Considering the small size of most of the species and the small amounts of food they take up and saliva they inject, use of radiotracers can be of great advantage.

PURPOSE

(1) Demonstration of the time required by aphids to reach the phloem and start feeding
(2) Differentiation between the host and the non-host plants
(3) Deposition of saliva in the host plant during piercing and sucking
(4) Demonstration of food uptake through artificial membranes and of the feeding preferences for different diets
(5) Determination of the beginning of food uptake of a phloem feeding aphid using a radioactive host plant
(6) Use of double membranes to check the repellent effect of an insecticide and the attractant or repellent effects of some plant surface substances
(7) Indirect transfer of substances from aphid to aphid via the host plant
(8) Transfer of ingested radioactively labelled substances from virgin females to their larvae.

MATERIALS

(1) Two species of aphid (Aphididae): good mass rearing with predominantly apterous virgins (keep the host plant under long day conditions, i.e. 13 hours of light or more); *Myzus persicae* Sulz. (green peach aphid) and *Aphis craccivora* Koch (black cowpea aphid) are suitable

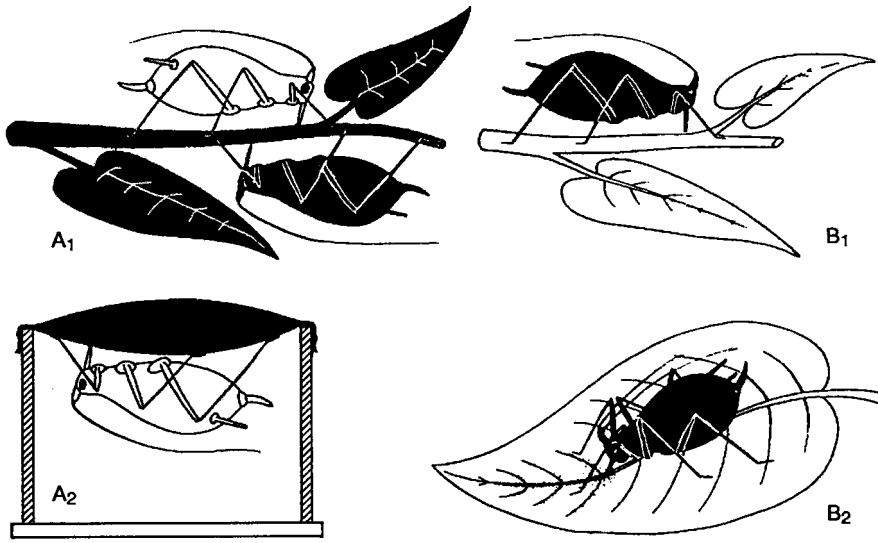

FIG. IV–8. Schematic summary of the radioisotope methods used [22]. A_1: non-labelled aphids (white) are transferred to a radioactive plant (black); A_2: aphids feeding on an artificial diet; the radioactively labelled diet (black) is enclosed between two membranes of Parafilm stretched over a glass ring to form an aphid cage; B_1: labelled aphid (black) is transferred to a non-radioactive plant (white); and B_2: radioactive aphid injects labelled saliva into a non-radioactive leaf; the radioactivity disperses in the leaf (dotted area), primarily along the veins.

(2) Small cages (glass rings) for artificial feeding
(3) Parafilm (product of Marathon, Inc., Menasha, Wisconsin, USA, or Lindsay and Williams, Ltd, London, UK) for making artificial membranes
(4) Phosphorus-32 as phosphate
(5) Chemicals for the artificial diet (see *Experiment D*)
(6) Fine paint brushes
(7) Thin cellophane wrap
(8) Scissors, tweezers and glassware.

PROCEDURE

The various methods used in radioisotope research with aphids are shown in Fig. IV–8 [22].

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[22] KLOFT, W., EHRHARDT, P., KUNKEL, H., "Radioisotopes in the investigation of interrelationships between aphids and host plants", Isotopes and Radiation in Entomology (Proc. Symp. Vienna, 1967), IAEA, Vienna (1968) 23–30.

Experiment A: Time required for food uptake. About 10–20 aphids are transferred with a fine camelhair brush to a young sprout or seedling of tobacco (for *M. persicae*), or cowpea (for *A. craccivora*); the plants should be radioactive for 24 hours. Check the aphids individually for radioactivity: after 30 minutes (first group), after 1 hour (second group), after 3 hours (third group) and after 6 hours (fourth group). Plot the number of radioactive individuals against time.

To save radioactivity, the same radioactive plant is used by the different groups.

Experiment B: Differentiation between the host and the non-host plants. Use the same radioactive plants as in Experiment A. At the same time and on the same leaf of tobacco or cowpea plants, place 5–10 individuals of both aphid species. Observe the behaviour of the aphids. After 3 hours, check, individually, the radioactivity of the specimens and compare the two groups. According to data from several species, aphids have first to obtain a good 'gulp' of the sieve-tube sap before they can differentiate between the host and the non-host plants. At first, the radioactivity reaches about the same level in both groups, but later it increases in the species feeding on its host plant (see also Ref. [23]).

Experiment C: Deposition of saliva. Aphids fed for about 20–24 hours on radioactive plants are transferred to the non-radioactive host plants. For external decontamination ('self-cleaning') of the mouth parts, induce the aphids to pierce the non-radioactive plants 1–2 times before the salivation experiments are started. Place the radioactive aphids on new leaves. Observe exactly the piercing behaviour, note the time and course of piercing and mark the piercing point by a colour spot on the opposite side of the leaf. Avoid contamination by honeydew. After 1 hour, or more, remove the aphids.

There are two ways of determining the deposition of radioactive saliva. (1) Cut out that part of the leaf on which the aphid has settled (use a cork borer). Place the pieces in water in liquid scintillation counting vials. Measure the Cerenkov effect (to save chemicals) using the ^{3}H channel of Tricarb. (2) Cut off the whole leaves immediately (or after some time, thus making it possible to observe saliva translocation); freeze dry (or use the quicker and cheaper, but rough, method of heat fixation at 60°C for 1 hour) and expose for 14 days on X ray film for autoradiography. In each case, use only highly radioactive aphids. For checking, they can be fixed for a short time in a small droplet of water (see also Refs [24, 25]).

[23] EHRHARDT, P., Experientia **17** (1961) 461.
[24] KLOFT, W., Z. Angew. Entomol. **45** (1960) 337–381.
[25] DANNEEL, J., Z. Angew. Zool. **54** (1967) 181–182.

Experiment D: Food uptake through artificial membranes

(1) *Preparation of an artificial diet:*

(a) **Amino acids**

Alanine	100 mg
Asparagine	300 mg
Glutamine	600 mg
Leucine	200 mg
Methionine	100 mg
Valine	200 mg
(b) **Sucrose**	20 g
(c) **Water**	100 mL.

(2) *Preparation of feeding cages:* Parafilm is stretched and fixed over a cover ring. In the middle of this membrane drop a little radioactive 'diet' (about 0.15 mL/ring). Cover the membrane carefully with a second membrane of stretched Parafilm, thus spreading out the fluid. Make the fluid container 'pillow' tight by pressing the membranes along the periphery of the ring with warm fingers. An outflow of liquid and contamination must be avoided (try the technique first with non-radioactive fluids).

(3) *Transfer of aphids and measurement:* Line the bottom of each of the three feeding cages with a disc of cellophane to collect the radioactive honeydew. Place 20–30 aphids inside the cages with: (a) a complete diet, (b) 20% aqueous sucrose, and (c) plain water. Note the time and temperature.

Measure the radioactivity of the living aphids individually, and compare the results for the three different 'diets'. The aphids can be fixed during the measuring time in a droplet of water in the centre of the planchet (transfer gently with the wet tip of a camelhair brush, laying the insects on their dorsum). Good results can be obtained by lightly squeezing the aphids in water and measuring with a liquid scintillation counter (Cerenkov effect).

The rates of radioactivity measured in parallel for the various diets at suitable time intervals provide information on the feeding preference. As can be seen, in short time experiments there is no difference between the complete diet and the aqueous sucrose [25].

The first detection of radioactivity from honeydew on the cellophane discs determines the passage time through the alimentary tract as well as the food uptake.

Experiment E: Determination of the beginning of food uptake of phloem feeding aphids using a radioactive host plant. Because of the function of the stylets and the anatomy of the host plant, some time is needed before the aphids reach the phloem and can begin food uptake. The tracer method is excellent for monitoring this phenomenon. Experience has shown (Ehrhardt [23, 26], Kloft and Ehrhardt [11],

[26] EHRHARDT, P., Z. Morphol. Oekol. Tiere **52** (1963) 597–677.

and Kunkel [27]) that the minimum time required is 10 minutes. However, about 6 hours are needed for 100% of the aphids to reach the phloem and become radioactive.

Place a large group of aphids on a radioactive host plant and check at different time intervals (e.g. 5, 10, 15, 20, 40, 60, 80 minutes, etc.) whether or not they have become radioactive. Plot the number (or percentage) of radioactive aphids against the time (in minutes) elapsed since externally visible feeding started. Note the temperature and air humidity.

Experiment F: Use of double membranes to check the repellent effect of an insecticide and the attractant or repellent effects of some plant surface substances. Artificial diets are advantageous for checking the aphicidal effect of insecticides, since no plant metabolic effects are involved. Addition of a tracer or use of a radioactively labelled insecticide permits determination of the relations between the dose (concentration), the amount of ingested fluid and the insecticidal effect. A double labelling technique (^{14}C labelled insecticide, tritiated water) has proved to be excellent for estimating the absolute uptake of insecticides and liquids by aphids [28].

Two compartment experiments with double stretched Parafilm membranes are particularly valuable. First, prepare a normal sachet of two membranes filled with diet. Then place on the upper (outer) membrane another drop of liquid diet and stretch over this a third membrane. Thus, two sachets are obtained that may contain different diets. However, the diets should have the same pH value to avoid the pH effects. One diet may contain an attractant or a repellent. The sequence (upper or lower sachet) of the different diets may be changed from one experiment to another, but the upper sachet should always be the one labelled with ^{32}P. Thus, the aphids feeding underneath can become radioactive only if they pierce through the lower sachet and reach the upper one containing the radiotracer.

Experiment G: Indirect transfer of substances from aphid to aphid via the host plant. Aphids can inject radioactive saliva into the plants (see Experiment C). It is possible that, initially, non-radioactive aphids ingest from the host plant radiotracers deposited by radioactive aphids [29].

Ten highly radioactive (^{32}P) aphids (check individually) are enclosed in a leaf cage; another cage contains the non-radioactive aphids. The two cages are placed either on opposite sides of a plant or at some distance from one another. After a certain time (2–4 days), check the unlabelled aphids. Use of a liquid scintillation spectrometer provides higher efficiency.

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[27] KUNKEL, H., PhD Thesis, Bonn University, Bonn, Germany (1966).

[28] HOLTGRÄWE, D., KLOFT, W., Oecologia **14** (1974) 229–236.

[29] FORREST, J.M.S., NOORDINK, J.P., Entomol. Exp. Appl. **14** (1971) 133–134.

Experiment H: Transfer of ingested radioactively labelled substances from virgin females to their larvae. Radioactive substances ingested by virginoparous females are transferred to their larvae, which are thus radioactive when born (see Fig. IV–4, λ_5). It is even possible to demonstrate the very high speed of radioactivity transfer within the ovary to unborn embryos (only 1 hour after the virginopara start feeding on the radioactive ^{32}P diet).

Place adult virgins on the radioactive diet and record their whole body activity after 1 hour of feeding (in other cases, after 2, 4 hours, etc.). Immediately thereafter, extract (with sharp pointed jeweller's forceps) the first unborn larva from its mother's abdomen. Decontaminate the extracted larva carefully from outside and measure its activity. After 2, 4 hours, etc., proceed likewise with the remaining larvae. By this technique, the radioactivity of the unborn larvae can be related to the original total radioactivity of their mother (for the literature, see Refs [30, 31]).

IV–3. PRACTICAL APPLICATIONS

IV–3.1. Population sizes

One of the most frequently employed and successful uses of radioisotopes in entomology has been to measure population sizes using the isotope dilution technique.

IV–3.1.1. Laboratory simulated radioecological experiments (tagging adult house flies to estimate the population density by the isotope dilution method (see also Section IV–2.2.2 for the method of labelling house flies))

Estimates of insect populations in certain areas are important, since they permit the exact planning of activities for insect control, ecological studies, etc. Many problems can be solved by tagging large numbers of individual insects with a radioactive tracer and employing a mark–recapture method for estimating the density.

PURPOSE

To provide training in tagging techniques and in the handling of large numbers of insects and special equipment by simulated field experiments under laboratory conditions. Adult house flies are convenient to use because, through their activity, they ensure a good mixture of tagged and untagged insects.

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[30] DANNEEL, J., Z. Angew. Zool. **54** (1967) 433–434.

[31] KLOFT, W.J., "Radioisotope methodology in aphid research", Aphids as Virus Vectors (HARRIS, K.F., MARAMOROSCH, K., Eds), Academic Press, New York (1976).

MATERIALS

(1) Cage for holding 500 house flies
(2) Large cubical fly cage (about 1 m × 1 m × 1 m)
(3) About 500 newly hatched house flies of mixed sexes, unfed, with a water supply; count the flies to determine the exact number
(4) Two days later, about 5000 newly hatched house flies of mixed sexes (they need not be counted exactly)
(5) Milk and sucrose solution
(6) About 1 mCi (37 MBq) of ^{32}P as sodium phosphate
(7) Tweezers
(8) Waxed cardboard cups
(9) Filter paper
(10) Small fly traps.

PROCEDURE

(1) *Tagging of house flies:* A known number of house flies (500) are placed in a small cage (with filter paper on the bottom) and provided with radioactive food. Milk, a sugar solution and ^{32}P (total amount, about 25 mL) are well mixed in a cardboard cup, and small pieces of styrofoam are placed in the feeding solution to prevent the insects from drowning. After consumption of the feeding solution, add normal milk or a sugar solution to the remainder of the radioactive solution. Thus, more ^{32}P is available to the flies. After 24 hours, check several captured individuals under the scaler for radioactivity (counts/min). Remove the contaminated feeding cup and also the filter paper that may have been contaminated by excrement, etc. This is necessary to prevent later contamination of a population of unknown density.

(2) *Release of labelled individuals in a population of unknown density:* Remove the dead flies from the small cage and subtract their number as well as that of the flies taken out for informal measurement from the total number of tagged flies. The small cage is then introduced into the big cage, which contains about 5000 newly hatched flies. Cut several openings in the cloth of the small cage. Make sure that there is a thorough mixing of tagged and untagged flies.

(3) *Recapture and measurement:* The first samples of flies (about 200 specimens for each sample) should be recaptured with traps, mechanical aspirators or by hand after 4–5 hours. Check the flies individually for radioactivity. The last samples of flies (2 × 200 specimens) should be recaptured after 72 hours and checked individually for radioactivity.

(4) *Calculations of the population density:* The population density is calculated using the formula of Bailey [32]

$$N = \frac{T(n + 1)}{t + 1} \qquad \text{(IV-1)}$$

where N is the total number of the whole population, T is the number of tagged individuals introduced, n is the number of specimens recaptured and t is the number of individuals marked in the recaptured sample. (In the calculation of final recapture, remember to subtract from T the number of recaptured, marked individuals from the first recapture.)

Variance is calculated as follows

$$V = N^2 \frac{n - t}{(n + t)(t + 1)} \qquad \text{(IV-2)}$$

The calculation according to Jenkins [33] is simpler

$$\text{Total population} = \frac{\text{No. of insects marked and released} \times \text{total No. recaptured}}{\text{No. of insects marked annd recaptured}}$$

(5) *Final examination of the method:* To determine the efficacy of the method, count (by hand) the population remaining in the cage (after killing with chloroform). To this number add the number of the flies recaptured.

In using this technique for measuring insect populations, the question often arises as to whether laboratory reared and/or laboratory labelled insects behave in exactly the same manner as the wild population into which they are released. Thus, a more passive insect labelling procedure (in situ) in the field is called for. The various methods of doing this are discussed in Section IV–2.2.5. Application of passive field labelling (with ^{32}P) of the corn earworm (*Heliothis zea*) to estimate its popula-

[32] BAILEY, N.T.J., On estimating the size of mobile populations from recapture data, Biometrika **38** (1951) 293.

[33] JENKINS, D.W., "Radioisotopes in entomological studies of endemic and tropical diseases", Radioisotopes in Tropical Medicine (Proc. Symp. Bangkok, 1960), IAEA, Vienna (1962) 235–266.

tions on the Island of St. Croix using the isotope dilution technique has been described well by Snow et al. [34]; this study serves well as an illustrative model.

IV–3.2. Insect movements (including the behaviour of social insects)

In Section IV–2.2.3 of this Manual, which describes the labelling of insects via incorporation in food, an experiment was designed to show, among other things, the movement of labelled ants. Also, in Section IV–2.2.6 the movement of male crickets is shown by following ^{60}Co labelled individuals. Kloft [35] provides references to many other examples of insect movement by tagging individuals with radioisotopes.

If one wished to obtain some of the information developed by labelling a host plant, but at the microscopic or electron microscopic level, one could label the host with ^{14}C or ^{3}H. Figure IV–9 shows a simple device that can be made to confine and control $^{14}CO_2$ or $^{3}H_2O$ during the labelling of plant material. In operation, one assembles the apparatus with the two terminal stopcocks open, pulls a slight vacuum (equivalent to a few centimetres of mercury below atmospheric pressure) on the whole assembly and then closes both the terminal stopcocks. This seals the desiccator lid and stopper, making the whole a closed system, despite the release of CO_2 (in ^{14}C experiments) and irrespective of the temperature or pressure changes during the course of labelling. If any leaks should occur, they would leak ambient air inwards, rather than contaminated gas/vapour outwards. The entire assembly is exposed to the sun or, better, to the light in a controlled temperature plant growth chamber. At the close of the labelling period, i.e. after insolation, the stopcock atop the desiccator is opened slightly, but with care, to allow slow equilibration of the pressure and to begin sweeping the gas or vapour in the desiccator towards the traps. After equilibration is complete, turn on the source of the vacuum, then slowly and carefully open the right terminal stopcock until the flow rate seen in the traps is one to two bubbles per second. Allow sweeping to continue overnight, or for at least 10 hours. The entire system is now ready for disassembly. Before using the ^{3}H labelled leaf, it must be well rinsed to remove external contamination and its petiole end should be placed in unlabelled H_2O. Surgical rubber gloves should be worn during this procedure, because the leaf transpires water, including $^{3}H_2O$.

Mention should be made of what should be in the traps during these alternative labelling procedures. For $^{14}CO_2$ trapping, both traps should contain 5–10 mL of 1N (or greater) NaOH in water. (Assay of the two traps separately for ^{14}C provides

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[34] SNOW, J.W., CANTELO, W.W., BOWMAN, M.C., Distribution of the corn earworm on St. Croix, US Virgin Islands, and its relation to suppression programs, J. Econ. Entomol. **62** (1969) 606–611.

[35] KLOFT, W.J., "Isotopes and radiation in agricultural sciences", Animals, Plants, Food and the Environment, Vol. 2, Academic Press, New York (1984).

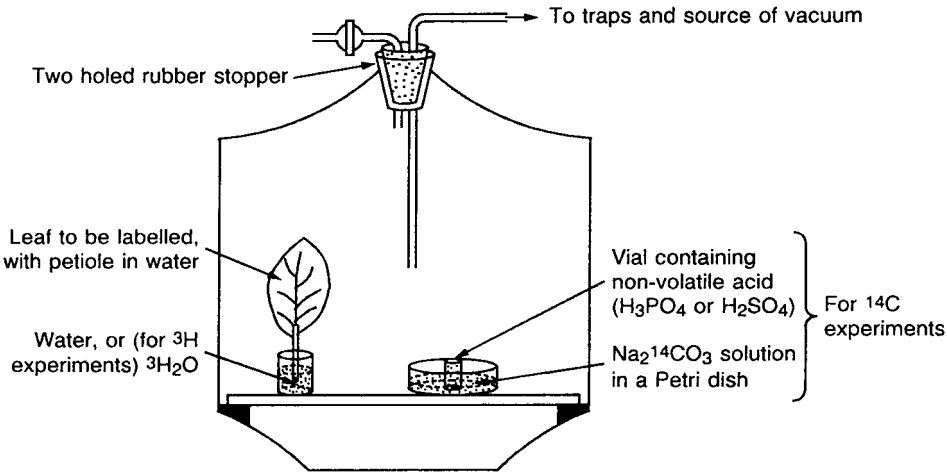

FIG. IV–9. Vacuum desiccator modified for labelling leaves with ^{14}C or ^{3}H.

information on the efficiency of trapping.) For ^{3}H trapping as $^{3}H_2O$, both the traps should contain 5–10 mL of water and be deeply immersed, i.e. to their full length in an ice water mixture. More efficient trapping of $^{3}H_2O$ is possible using concentrated H_2SO_4, but an acid mixture remains that is of no further use for biological studies, should one wish to reuse the $^{3}H_2O$.

Labelling a host plant with ^{32}P is easily accomplished in the laboratory, without any of the elaborate precautions cited above to contain radioactive gases or vapours when using $^{14}CO_2$, ^{3}H (either as a gas, or as $^{3}H_2O$) or ^{131}I. In contrast, an arrangement as simple as immersing the end of the petiole of a detached leaf in an aqueous solution of ^{32}P labelled phosphate will label the leaf. Of course, the roots of an intact plant can be immersed similarly in ^{32}P solutions for labelling the entire plant.

In the field, it is often desirable to be able to label an undisturbed plant growing naturally, either as a cultivated crop or as a wild plant. This enables one to label, in a passive manner, a wild population of insects for which the plant is host. This procedure avoids all the uncertainties and traumas of labelling insects in the laboratory for subsequent release in the field. Safety regulations in some countries (check the regulations of one's own country) and/or practical considerations, such as problems of uptake or measurement, eliminate a number of isotopes as candidates for labelling hosts in the field, and therefore for labelling wild, undisturbed insect populations. This leaves ^{32}P as the most usable isotope in such situations. It has been criticized as having too short a half-life (14.7 days) for biological studies;

however, even after 2 months about 6% of an original label remains. This is more than sufficient for many studies, especially those in which one only needs to know if the insect is labelled.

An excellent method of labelling a field crop (corn, *Zea mais*) by injection of microlitre quantities of high specific activity ^{32}P (for passive labelling of a wild population of the corn earworm (*H. zea*)) has been described in detail by Snow et al. [34]. A good review of various other plant labelling methods has been given by L'Annunziata [36].

IV–3.3. Host plant resistance

In IV–2.2.7 of this Manual, tracer experiments with aphids was cited as being a particular method of tagging insects by having them feed on labelled plants. However, part of this experiment illustrated that differentiation is not absolute. Aphids have first to obtain a good 'gulp' of the sieve–tube sap before they can differentiate between the host and the non-host plants if those with a feeding preference for a particular host and others not having such a preference are placed on a radioisotope tagged leaf of that host. At first, the radioactivity reaches about the same level in both groups, but later it increases in the species feeding on its host plant. This means that one needs to make quantitative and qualitative measurements over time.

One of the best ways of checking plant/insect relationships with insects that inject saliva as they feed is by using radioactively tagged insects feeding on non-labelled plants. The saliva injection transfers the radioactivity to the plant. This can be observed via autoradiography of the non-labelled leaf, looking for 'hot spots' where the insect has fed. Before subjecting a leaf to autoradiography, it should be dried, preferably freeze dried. Interposing a very thin plastic film between the dried leaf and the X ray film reduces possible chemically induced darkening of the X ray film, a process called chemography. Such effects can be reduced further by developing the film exposures in a refrigerator. If one is looking for low amounts of radioactivity in the specimen, long exposure times are needed — perhaps days or even weeks. If very long exposure times are contemplated, one should run a time series of exposures with non-labelled plant material prepared in the same manner to determine the rate of development of fogging or other non-radiation artifacts. A very detailed and extensive (more than 70 pages) review of film autoradiography is given in Ref. [17].

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[36] L'ANNUNZIATA, M.F., Radiotracers in Agricultural Chemistry, Academic Press, New York (1979).

IV–3.4. Labelling of defined substances

IV–3.4.1. Insect nutrition

In mass rearing projects, it is of great help if the insects are grown successfully on a defined media. This is especially true with insects that feed on blood, since blood is hard to store and transport, may be variable in its composition, may contain potent pharmacological agents and pesticides, and may be a disease carrier.

The tsetse fly (*Glossina* sp.) is an example of an insect that is being mass reared for use in SIT control projects in Africa. The medium now used contains aged, freeze dried pig's blood, a rather awkward material for projected multinational mass rearing SIT operations. The blood requirement raises questions about the role of haemoglobin in the iron metabolism of *Glossina*. Such questions must be answered in order to develop synthetic diets for blood feeding arthropods.

The haemoglobin/iron dietary role in *Glossina* has proved to be an excellent candidate for isotope assisted research. Kabayo et al. [15], from the IAEA Laboratory at Seibersdorf, injected guinea pigs with ^{59}Fe and found that more than 95% of the iron in their blood was in the haeme of haemoglobin. Tsetse flies, *Glossina palpalis palpalis*, were fed on the ^{59}Fe tagged guinea pigs and the results suggest that some haemoglobin–iron is absorbed by the flies. The authors of this study quite properly close their paper with this thoughtful paragraph:

> "Studies aimed at testing the nutritional requirement of such essential elements as iron can be complicated and hampered by impurities, so that even with a holidic diet trace amounts can negate the results. However, the present study does not demonstrate the essentiality of iron, merely its absorption as haeme or haemoglobin–iron."

IV–3.4.2. Fate of applied insecticides

Once a compound has been shown to have desirable insecticidal properties, but before it is approved for field use, the authorities need to know not only its metabolism in target and non-target organisms but also the answer to a number of other questions concerning its fate in the environment, especially if area wide aerial spraying is contemplated. Unfortunately, most of the field data available on such matters are from the temperate regions of the world and may or may not be applicable to the particular region (such as the humid tropics) in which use is intended. Some of the more important questions for which answers are needed include:

(1) How rapid is the decomposition of the insecticide compound: (a) by photolysis; (b) by hydrolysis; or (c) by metabolism?
(2) What are the decomposition products?

The following experiment, using ^{14}C labelled malathion, illustrates how one can obtain some of the required data. If possible, the entire experiment should be maintained at an elevated temperature, say 35°C. Further, the experiment should be carried out in a fume hood with the exhaust fan turned on and the front open at least 5–10 cm in order to maintain a constant flow of air inwards. This is to make sure that any volatile ^{14}C containing malathion degradation products do not result in exposure to personnel.

MATERIALS

(1) Malathion ^{14}C labelled: (a) a solution of 1 μCi(3.7×10^4 Bq)/μL in acetone, and (b) a solution of 1 μCi/10 μL in acetone
(2) Cage for holding flies
(3) Five houseflies
(4) CO_2
(5) Acetone
(6) Sand (clean and washed)
(7) Mortar and pestle
(8) Erlenmeyer flasks, 50 mL (several)
(9) Micropipettes (1 μL, 10 μL)
(10) Five thin layer chromatography (TLC) plates
(11) Malathion degradation products
(12) TLC developing solvents: (a) toluene, (b) methanol, (c) acetic acid, and (d) ammonium hydroxide
(13) Radioactive ink (ink and any conveniently available radioactive solution)
(14) X ray film and film developer
(15) Thin plastic film (the thinnest obtainable)
(16) Vials for liquid scintillation counting
(17) Liquid scintillation cocktail(s), including one with a gelling agent (or obtain a gelling agent to add to the cocktail)
(18) Shaker suitable for shaking 50 mL flasks
(19) Broad leafed plant, e.g. *Phaseolus* sp.
(20) Light source, e.g. 'Gro-Lux'
(21) Soil sample, preferably one not highly coloured with organic matter
(22) Sample oxidizer of choice to oxidize the residues
(23) Three glass microscope slides
(24) Thin plastic sleeve to cover the slides
(25) UV lamp (short wavelength, if there is a choice)
(26) Vacuum source (water aspirator)
(27) Glass traps and tubing as per Fig. IV–10
(28) Ethylene glycol
(29) 1N NaOH.

TABLE IV-I. FLOW SHEET (at about 35°C) (1 μCi = 3.7×10^4 Bq)

	Insects	Leaf	Soil	Glass	Water
Dose	1 μCi	1 μCi	1 μCi	1 μCi	1 μCi
Exposure t (and special conditions)	4 hours (three flies)	4 hours	1 day	4 hours UV, plastic bag, air flow, trap	1 day
Extraction	Grind with sand + acetone, then shake for 2 hours[a]	As with insects[a]	Shake for 2 hours with acetone[a]	Rinse with acetone	
	↓ 4 hour flies	↓	↓	↓	↓
TLC	Spots	Spots	Spots	Spots	Spots
Liquid scintillation	↓ ↓	↓ ↓	↓ ↓	↓ ↓	↓ ↓

[a] Aliquots of residues must be counted with a GM counter and corrected for self-absorption and backscatter to dis/min. Alternatively, aliquots of the residue can be oxidized to CO_2, the CO_2 trapped and then measured by the liquid scintillation technique.

Safety Note: If one chooses to oxidize aliquots of solvent extracted residues, one *must* first exhaustively remove all traces of the solvent (e.g. by air flow under reduced pressure), otherwise, during oxidation, a violent explosion may result.

PROCEDURE (Table IV-I)

(1) *House flies:* Apply topically 1 μL of the 1 μCi/μL ^{14}C malathion solution to each of five houseflies. After evaporation of the solvent under a gentle, warm air stream, the flies are placed in a cage for 4 hours. At the end of the exposure period the flies are killed by the administration of CO_2. All five flies are ground with 5 mL of acetone and a few grams of sand in a mortar and pestle. The sand and acetone are rinsed into a 50 mL Erlenmeyer flask with two successive rinses of acetone of 5 mL each. The flask is stoppered and shaken for 2 hours on a shaking machine. Three successive aliquots of 10 μL each are placed and dried at the origin of a TLC plate. Make every effort to keep the diameter of the spot as small as possible. Also deliver to the same spot 10 μL of a solution of a mixture of several known (non-radioactive) degradation/metabolic products of malathion for co-chromatography

with the fly extract. One should have established the Rf values of these products earlier, individually and collectively, under exactly the same chromatographic conditions. The solvents used should be tolnene:methanol:acetic acid, 50:50:1 (vol./vol./vol.) in one direction and tolnene:methanol:ammonium hydroxide, again, 50:50:1 in the other direction. After development and drying, the plates are marked in two places with radioactive ink and checked for radioactivity (in the dark room) by placing them face down on top of a sheet of X ray film previously covered with a thin plastic film to prevent chemical attack on the film and/or to protect against the contamination spreading from the TLC plate to the film. Allow exposure of the film to continue overnight in the dark (12–20 hours). Develop the film photographically according to the supplier's recommendations. Any spots on the chromatogram that correspond to the black spots on the developed X ray are scraped into a liquid scintillation vial containing a suitable cocktail, i.e. one with a gelling agent. The cap is placed on the vial, then the vial is shaken and put in the counter for about 30 minutes before counting. Make any corrections necessary to convert counts/min to dis/min.

(2) *Leaf:* In typical aerial applications of insecticide, much of the active material is deposited on vegetation. Take the following approach to obtain some idea of the rate of destruction/chemical alteration of such deposition by a combination of environmental and biochemical attack. On the upper surface of a leaf of some broad leafed plant, such as some *Phaseolus* (bean), deposit 10 μL (1 μCi) of the 1 μCi/10 μL acetone solution of ^{14}C malathion, either in a series of tiny spots or by streaking in irregular lines. The petiole of the leaf should be kept in water and the leaf illuminated during the entire 4 hour exposure period. At the end of exposure, the leaf is treated in the same manner as the flies, i.e. ground with sand and acetone, shaken for 2 hours, with an aliquot of the extract spotted on TLC plates for chromatography; another aliquot is taken for liquid scintillation measurements; and an aliquot of the acetone extracted leaf residue is taken for ^{14}C measurement with a GM counter. The TLC plates are developed in the same solvents, in the same manner (with the same non-radioactive standards added and co-chromatographed); the TLC plate is exposed to X ray film overnight (12–20 hours) and the spots thereby identified as radioactive are scraped into liquid scintillation vials for counting.

(3) *Soil:* To a 10–30 gram sample of soil from the proposed treatment area in a 50 mL Erlenmeyer flask add 10 μL (1 μCi) of the 1 μCi/10 μL solution and mix very thoroughly. Stopper the flask and let it stand for 1 day. At the end of the exposure period, add a known volume (20–30 mL) of acetone, shake for 2 hours, and then proceed with the TLC technique and the direct scintillation counting of aliquots, exactly as done with the fly and leaf samples. Acetone extracts of highly organic soils may be so strongly coloured as to be unsuitable for direct assay via liquid scintillation counting. If such is the case, an aliquot of the acetone extract may be subjected to wet oxidation; a sample of the CO_2 so produced and trapped can be

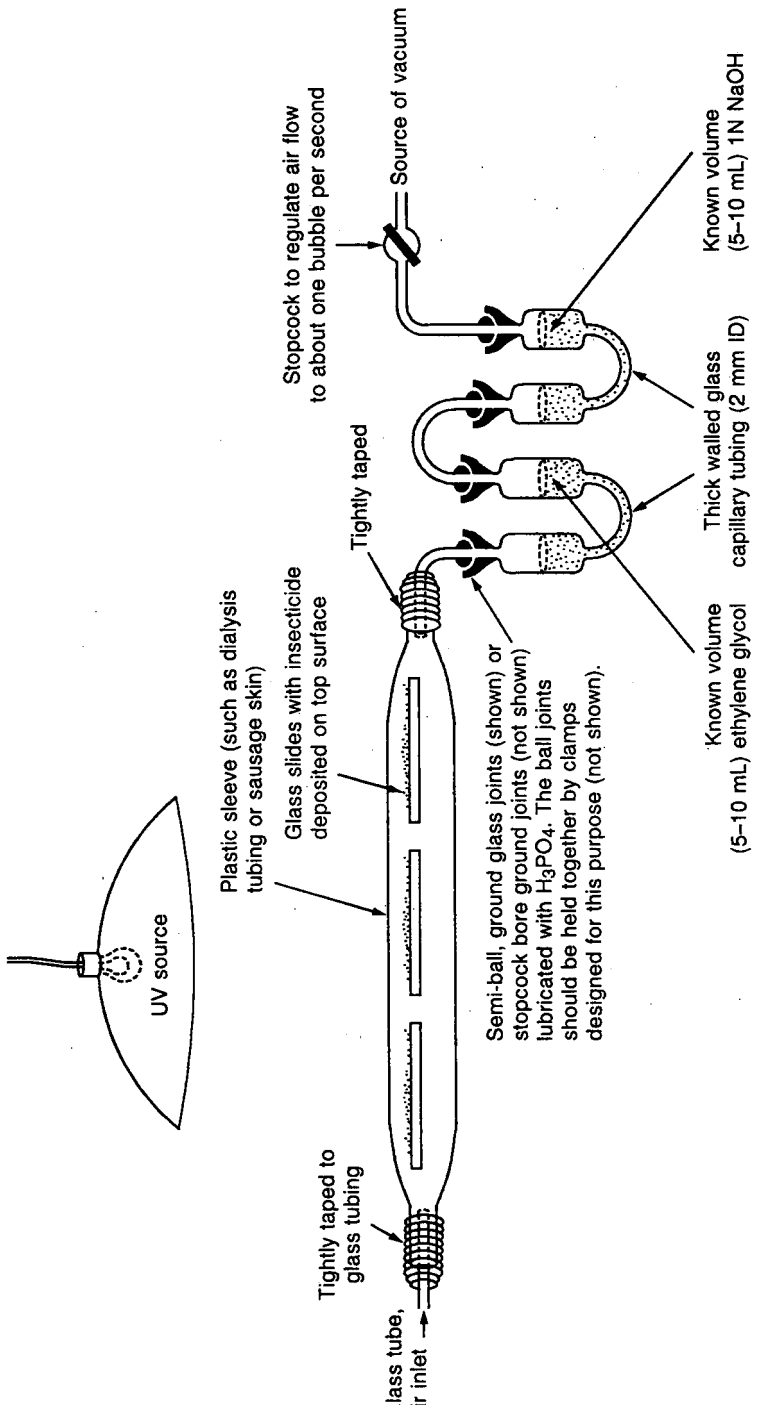

FIG. IV–10. Apparatus for the exposure of ^{14}C labelled insecticide to the effects of UV radiation.

assayed by liquid scintillation. A sample of the extracted soil residue is assayed for ^{14}C by counting with a GM counter or by oxidation to CO_2, followed by counting with the liquid scintillation technique.

(4) *Glass:* To simulate the fate of the insecticides falling on impervious, non-biological surfaces and to observe the destructive effects of UV light on the insecticides deposited on such surfaces, one should proceed as follows. Deposit 10 μL (1 μCi) of the 1 μCi/10 μL ^{14}C labelled malathion on each of three glass microscope slides and allow the acetone to evaporate. Enclose the three slides together, treated side up, in a plastic sleeve through which air is pulled (see Fig. IV–10 for the design of this apparatus). For 4 hours, expose to radiation from a UV source. Then, wearing rubber gloves (because the plastic may have become contaminated by the absorbing ^{14}C labelled degradation products), cut open the plastic sleeve and remove the glass slides. Rinse the slides into a container with a known volume of acetone. In the usual manner, take an aliquot of the acetone for direct measurement via liquid scintillation, and a second aliquot for TLC/X ray film/assay of spots as previously described. Also, take an aliquot of the ethylene glycol trap and the NaOH containing trap (Fig. IV–10) for direct liquid scintillation measurement of the ^{14}C. Recovery in all the fractions tested may be substantially less than 100% of that originally delivered to the slides. If this is the case, one can at least qualitatively look for the sources of loss by wiping all the joints with small pieces of filter paper or paper tissue, which are then counted in a liquid scintillation vial, and also by cutting out a piece of the plastic sleeve and counting it using the liquid scintillation technique. Since the counting of filter paper and plastic is of unknown (but $< 100\%$) counting efficiency, one obtains only a crude, minimum value for these losses in dis/min.

(5) *Water:* To simulate and observe the fate of an insecticide that may reach the surface water, one should proceed as follows. Deposit 10 μL (1 μCi) of the 1 μCi/10 μL ^{14}C labelled solution in 10 mL of water in a flask, swirl to mix, stopper the flask and let it stand for 1 day. At the end of the exposure period, proceed as usual by taking one aliquot for direct counting by liquid scintillation, and one aliquot assay using the complete TLC process described previously.

PART V

STERILE INSECT TECHNIQUE

V-1. INTRODUCTION

V-1.1. Early history and development

The concept that economically important insect populations may be controlled, managed or eradicated from defined geographical areas by affecting their reproductive capability was conceived by E.F. Knipling in the late 1930s. At that time, he was working with the screwworm fly problem in cattle in Texas, USA. The methods of control of insects depended, for the most part, on pesticides. He perceived that the screwworm problem persisted because of the high reproductive capacity of this fly and was looking for a method or approach that would affect its reproduction, and therefore its survival.

World War II delayed the development of this concept. In the late 1940s, interest was increased by a radiation biology publication showing that sterility in insects was caused by exposure to ionizing radiation. This led to initial laboratory experiments in the early 1950s on the sterilization of the screwworm by X rays, to the demonstration that both males and females could be rendered sexually sterile by exposure to either X rays or gamma radiation without excessive damage to their survival or mating ability, and to initial field trials of the new concept in insect control. The concept was that the release of a large number of mass produced insects (with both the males and females rendered completely sexually sterile) into natural populations would limit the reproductive ability of the natural population by the overwhelming number of sterile insects present. The reproductive capacity would be so reduced that the density of populations would be reduced. As the density of the natural population decreased, the influence of continual releases of sterile insects would increase, causing a continuing reduction in the natural population and possible elimination from the area in which the releases were being made. The validity of the concept was demonstrated by field trials with natural populations on the Island of Curacao off the coast of Venezuela in the mid-1950s and the elimination of screwworms from the southeastern USA in the late 1950s. Development of this new approach, along with its demonstrated usefulness, led to related research on other insects. A better understanding of the technique, including its potential and limitations, and more data on insect population dynamics, movement, migration and management problems evolved from these studies.

V–1.2. Potential uses of the method

The SIT approach has been developed successfully for several insects and some illustrations of this successful approach have been demonstrated. The screwworm fly was eliminated from the Island of Curacao originally in 1954 and again in 1977, following reintroduction in 1975. This fly has also been eliminated from Mexico, Puerto Rico and the USA. Various species of fruit flies have been eliminated from some islands in the Pacific Ocean and Mexico. Also, several introductions of fruit flies into the USA have been eradicated. The method has also been successful in quarantine operations, i.e. prevention of the successful movement of pest insect populations into uninfested areas. For example, quarantine operations using pesticides against the Mexican fruit fly along the USA–Mexican border have been replaced by the release of sterile males to prevent movement of this fly into California and Texas. The operation is highly successful. A similar programme is operational in the San Joaquin Valley, where the release of sterile pink bollworm insects protects cotton against the immigration of fertile natural individuals and therefore the establishment of a sustaining population in this area. In western Canada, use of the release of sterile codling moths in fruit orchards is planned. In the southern USA, the method has been demonstrated against the bollweevil in an integrated control programme. Research with other insects has shown the validity of the approach, but practical use of this sophisticated methodology awaits further technical, economic and cultural development.

Development of a new approach of control such as the sterility principle is complex. Original screwworm eradication programmes did not rely solely on the release of sterile insects. Quarantine controls had to be carried out, and animals were checked for infection and then treated with insecticide. Fruit fly programmes include use of insecticidal bait sprays to reduce the number of insects that have to be released. The original programmes were, in a very real sense, 'integrated programmes' in that they used all the available technology to accomplish their objectives.

One of the most interesting aspects of the development of the sterility principle is that its successful application requires more intuitive or actual data and information on the biology of the species and the dynamics of the density of its populations than other approaches. Integration of the sterility principle into a comprehensive control or eradication programme is an extremely difficult undertaking. It cannot be done on small populations within a generally infested area. The technology is sophisticated, requiring a thorough understanding of how each control component affects the reproductive capacity of the population as well as the interaction of control methods on the dynamics of the population.

It should be emphasized that there is a large difference between control of insects on a field by field basis within a generally infested area and population control over the whole area. Insecticides and other methods which kill any or all of the stages

of a pest species have value in that they temporarily eliminate the problems caused by the species. However, insecticides may not protect against future immigrants or individuals that survive the treatment. In general, field by field treatments protect the treated area only and affect such a small proportion of the total population that the treatments have to be made repeatedly to maintain protection. The sterile insect release technique requires dealing with the total population of insects. Density reduction occurs over time (i.e. generations). SIT, then, can only be used for total population control over a large area. Use of this method is completely different in approach and execution than use of protective methods on a field, farm or problem area basis.

V-2. THEORY AND MATHEMATICAL CONCEPTS

V-2.1. Release of sterile insects

The theory of the release of sterile insects is based on the concept that an insect species can be mass reared, sterilized and transported for release to the problem area. When released into the natural environment, the artificially produced insects must be sufficiently similar in quality, vigour, mating ability and movement to compete in the mating process of the natural population in the natural environment. The insects released must be in numbers sufficient to outnumber the natural population and effectively overcome the reproductive potential, causing a decline in density. It is obvious that when adults do damage, introduction of an additional number of insects can lead to an increased problem before density reduction can occur in subsequent generations. Therefore, it is important to remember that the sterility approach does not provide immediate relief but is rather a management tool that provides effective control at some later date. For this reason, the sterility approach should be considered a management tool within a large and ongoing programme.

The theory of the release of sterile insects can best be seen and understood by following the original theoretical example given for the method illustrated in Table V–I, which is a theoretical example. It is assumed that no practical problems such as immigration into or emigration out of this hypothetical population exist and that both the males and females are completely sterile and equally competitive with the naturally occurring insects. These practical problems are discussed after consideration of the theory in its simplest form.

The unit of time in this example is a generation. The simplest definition of a generation is the development time from one group of eggs to the time that the adults from that group again produce eggs, i.e. the life-cycle. By using generation as the unit of time it is possible to generalize for any type of insect. All insect populations have some total number of individuals in the population.

Knipling, in developing the sterility principle, dealt with the concept that there is a definite, although indeterminate, number of insects in a population with variable,

TABLE V–I. HYPOTHETICAL EXAMPLE OF THE STERILE INSECT RELEASE TECHNIQUE (AFTER E.F. KNIPLING)

Generation	No. of insects	No. of sterile insects released	Ratio of sterile to fertile insects	% sterility	No. of insects remaining fertile	Growth rate
P	1 000 000	9 000 000	9:1	90	100 000	Fivefold
F_1	500 000	9 000 000	18:1	94.7	26 316	Fivefold
F_2	131 579	9 000 000	68:1	98.6	1 896	Fivefold
F_3	9 480	9 000 000	949:1	99.9	10	Fivefold
F_4	50		180 000:1	99.99945	<1	Fivefold

but definite, growth potential in each generation. His model describes the density trends when one makes assumptions on the averages of these two primary characteristics of populations, i.e. the density and growth rate from generation to generation.

It should be noted that Table V–I follows a hypothetical insect population for five generations (P–F_4). The assumption is made that in the parent (P) generation there are one million insects in the population; these can be considered as individuals without regard to sex, or they can be considered as being composed of both males and females. If the sexes are considered, then the sex ratio of the insects must be known. In the simplest case (1 male:1 female), there would be 500 000 males and 500 000 females. Here, sex is ignored for simplicity. The theoretical example is the same in both cases. In the last column of the table, it should be noted that the population is characterized by an assumed population growth rate from generation to generation. Although not shown in the table, this insect population would increase to large numbers if no control or environmentally constraining factors were operative. One million insects in a parent generation would increase to 625 million in the F_4 generation by increasing at a fivefold rate. In the example, the trend is reversed by the release of nine million completely sterile insects. Assuming that the released insects compete fully and normally in the mating process of the wild insects, this number would establish a ratio of nine sterile insects to every one wild insect. If one were keeping track of both males and females, this would also mean that the ratio of males was nine steriles to one fertile. It should be remembered that the females released have been sterilized completely before release and are therefore incapable of producing progeny. The sterile females could absorb matings from the wild males. If there

is no difference in the matings between the released and the wild insects, the presence of the released females can be ignored for theoretical examples. The wild females would be subjected to matings, which would result in 90% sterility (or 90% reduction in fertility). Only the equivalent of 10% of the wild population would be capable of producing progeny. In other words, where one million individuals would be reproducing without releases, with releases only the equivalent of 100 000 would be reproducing. If these individuals can increase their numbers fivefold, then the next generation (F_1) would be represented by only 500 000 individuals. The positive side of this outcome is that the population is reduced by 50% in the first generation — a result quite different from the fivefold increase to the five million individuals that could occur with no releases. The next assumption is simply that if it is possible to produce and release nine million insects during the parent generation, it should be possible to do the same during the F_1 generation. The interesting aspect is that the wild population will now be overflooded with a ratio of sterile to fertile (18:1) that is twice as high as it was in the parent generation (9:1), since the density is reduced and, consequently, the reduction in fertility is increased. The theoretical outcome is the development of ratios of steriles to fertiles that are so high that the population becomes extinct.

The basic mathematical concept of the sterile insect release technique can be illustrated with simple formulas, if the following symbols are used:

P is the number of individuals in the parent generation
N is the number of sterile insects to be released in each generation
S is the fractional degree of sterility in the population caused by the release of sterile insects
R is the growth rate of the population each generation
F_1 is the number of individuals expected in the next generation

$$F_1 = P \times R \qquad S = \frac{N}{(N + P)}$$

$$5\,000\,000 = 1\,000\,000 \times 5 \qquad 0.9 = \frac{9\,000\,000}{(9\,000\,000 + 1\,000\,000)}$$

$$F_1 = P \times (1 - S) \times R$$

$$500\,000 = 1\,000\,000 \times 0.1 \times 5$$

Theoretically, release of sterile males only works when the sterility resulting from the ratio of released to sterile insects is sufficiently high to overcome the

TABLE V-II. GROWTH RATE DATA

Potential growth rate	Degree of sterility (%) required for decreasing density	Ratio of sterile to fertile insects required
1	Any amount	Any ratio
2	>50	>1:1
4	>80	>4:1
10	>90	>9:1
20	>95	>19:1

reproductive ability of the wild population. It is interesting to note that little is known about the growth rate of populations under practical field conditions where these insects are a serious problem. One should think of the insect(s) with which one is concerned. What were the average, maximum and minimum growth rates that occurred in the area of one's programme over the last 5 years? How do they change seasonally? If one were attempting to write a model of the potential of SIT, what growth rates would give the model some degree of validity? One could ask the same question about the total number of insects in the population. An illustration of the importance of the data on the growth rates can be seen in Table V-II.

Anyone can take the example or the equations given above and calculate models related to the feasibility of the sterile insect approach to their problem area. This is good practice and a valuable exercise. However, it should be remembered that there are many practical problems (discussed in later sections) which can affect the outcome of SIT.

V-2.2. Sterilizing natural populations

Primary use of the sterility principle has been through the release of sterile insects. However, Knipling proposed further theoretical use of the sterility principle that involved treating natural populations with sterilizing agents rather than mass producing and releasing colonized insects. This approach has some interesting possibilities for increasing the effectiveness of control methods. Materials have been found that cause sterility in males and females, or just females. The primary interest has been in finding materials that sterilize males as well as females, for the theoretical reasons illustrated shortly. Although materials have been found, and even shown in field tests with house flies, that confirm the expected theoretical advantage postu-

lated by Knipling, they have not been developed for use. The reason is primarily that the effective materials (those that sterilize males as well as females) are too toxic and hazardous to be developed for use. Female sterilants have been developed that result in a reduction in or elimination of progeny. However, in terms of affecting the reproductive capacity of a population, sterilizing a female does not have advantages over killing the female; in both cases, no eggs or progeny are produced by that female. Furthermore, a sterilized female is still alive and remains so until her natural death, but she may still cause problems. Female sterilants will have to offer some special advantage over insecticides if they are to replace them or be used in conjunction with them. For example, a sterilant that affects a different part of a population or affects the population at a different time after application could be very useful. Such usefulness has been shown with cockroaches, where growth inhibiting substances have resulted in inhibition of the reproduction of survivors after initial killing with toxicants.

Knipling has called the interesting theoretical advantage of chemosterilants applied to natural populations a 'bonus effect'. He starts from the premise that it is now possible, with insecticides, to treat some proportion of the natural population by a variety of methods. To illustrate this point, he assumes that it is possible to control 90% of the natural population. If 90% of the existing individuals are killed, 10% are left to reproduce. If the growth rate of the population is fivefold, the density decreases from generation to generation. (Ten individuals remaining from every 100 after treatment would only increase to 50 individuals in the next generation, a net 50% reduction in density.)

If the treatment is by sterilization (rather than by killing), if it affects both the females and the males, and if it can reach 90% of the population, a much better result could be achieved. Ninety per cent of the females would be sterilized and rendered incapable of producing progeny. In terms of the effect on the density of the next generation of insects, this is equivalent to an insecticide treatment. In addition to this effect on the females, 90% of the males would also be sterilized. The net result is that there would be a ratio of nine sterile males to one fertile male in the mating of the wild population. Ninety per cent of the females would be sterilized completely by the treatment. The remaining fertile females (10%) would be 90% sterile from the matings of the sterile males. This would result in 99% of the females becoming sterile and unable to reproduce. A 99% control effect is much greater than a 90% effect. Theoretically, this effect can be illustrated in several ways. Of the 10 female individuals from every 100 remaining fertile from sterilizing exposure, only one would remain fertile after the effect of the sterile matings. Where 10 individuals would produce 50 progeny at a fivefold growth rate (50% reduction from the original 100), one individual would produce only five at the same growth rate (95% reduction from the original 100). Considering the difficulty of getting treatments to the last 1, 5, 10 or 20% of a population, this 'bonus effect' from the matings of sterile males is a promising theory to pursue. The effect can be visualized in another way. To

cause 90% sterility requires a ratio of 9:1, or the release of nine times as many insects as there are in the wild population. To cause 99% sterility in a population would require a ratio of 99:1 or 99 times as many insects as the wild population. To increase the sterility effect in this case from 90 to 99% would require the release of 11 times as many insects. Hopefully, a useful *male* sterilant will one day be found and developed. Until that time, the concept is only a theoretical one.

V–2.3. Competitiveness of released insects

There is no reason to assume or believe that insects reared and sterilized in a laboratory or factory and then released back into nature will be fully competitive in the mating process. First, problems in behaviour and vigour arise that may result from the artificial colonization of a wild species for mass production. Second, stresses of artificial rearing, handling, sterilization and release occur. Third, factors associated with the quality and distribution of the insects once they are released into natural environments exist.

Colonization of a species in itself may cause changes in behaviour. Two cases have been demonstrated in mosquito research. In the late 1950s, releases of the mosquito, *Anopheles quadrimaculatus*, were attempted in Florida, USA, to determine whether sterility could be induced into a natural population and the density reduced. After several months of sterilized male releases there was no evidence that they had mated any wild, natural females, i.e. no sterility much above the level occurring naturally was found in the wild females. In a series of experiments carried out over two summers it was shown that the released virgin females were mated readily in nature — over 90% of the recaptured females had been mated. However, the experiments also showed that females from the laboratory colony were mated primarily (over 90%) by laboratory colony males and that the wild females were mated primarily by wild fertile males. Obviously, the differences in the mating behaviour of the two types had been caused by colonization of the species.

In Africa, some *A. gambiae* sterile hybrids were released in an attempt to control a native population of this mosquito. The lack of success was attributed to the behavioural differences between the hybrid forms.

Another example of this type of problem was that of laboratory colonized Lepidopteran species. The females 'called' the males at different times during the night than would females of the wild population. Such behavioural differences negate the effectiveness of sterile releases.

Normally, the term 'competitiveness', when used in developing SIT, refers to the mating ability of sterilized insects, i.e. males, compared with the mating ability of unsterilized or wild insects. It is assumed that the sterilization and handling procedures may lessen mating competitiveness. Tests carried out in cages in the laboratory or outdoors have been used to study this problem. For example, one can make the following types of cross in cages with insects from laboratory colonies or outdoor

TABLE V-III. CROSSES OF INSECTS FROM LABORATORY COLONIES OR OUTDOOR COLLECTIONS

Crosses	Sterile males	Normal males	Sterile females	Normal females
1	0	1	0	1
2	1	0	0	1
3	0	1	1	0
4	1	0	1	0
5	1	1	0	1
6	1	1	1	1
7	2	1	0	1
8	2	1	1	1
9	5	1	0	1
10	5	1	1	1
11	9	1	0	1
12	9	1	1	1

collections in the proportions indicated (Table V-III). It is essential that the adults be separated before they become sexually mature so that they are virgin before the crosses are made.

Other crosses could be used, but these provide the general approach. Cross 1 provides a control, showing that the insects can reproduce normally under the test conditions. Cross 2 shows the degree of sterility induced by sterile males. Cross 3 shows the same information for treated females. Cross 4 may seem useless if one thinks only of the mating of completely sterile males and females. However, if the sexes are less than completely sterilized, it might be interesting to see the effect when they are mated. Crosses 5, 7, 9 and 11 provide a means of looking at the competitiveness of the males when they are at different ratios. The expected per cent sterility caused by these ratios would be 50, 67, 80 and 90 for ratios of 1:1, 2:1, 5:1 and 9:1, respectively. Crosses 6, 8, 10 and 12 are included only to make one see what might happen when sterile females are included in releases. Both arguments — that the presence of released females will have an effect and that the presence of released females will not have an effect on the outcome of SIT — have been put forth. If the behaviour of the released females is different to that of the wild females, this may have an effect on the outcome; otherwise, they should not influence the effect from the ratio of released to wild males.

Although laboratory and outdoor cage studies are an essential step in the development of SIT, it should be remembered that the final answer is in the natural environment of the field problem area. Competitiveness testing should be done in the field if possible. When it is done in cages or in the field, the expected degree of sterility (calculated from the ratio) is compared with measured or observed sterility.

V–2.4. Release of genetically altered insects

Development of SIT, along with development of theories on the use of genetic mechanisms, led to the consideration of many mechanisms that might be used for insect control or elimination. These mechanisms include many complex factors such as cytoplasmic incompatibility, various types of homozygous and heterozygous translocations, hybrid sterility, compound chromosomes, inversions, F_1 sterility and sex linked lethals or growth factors.

Three mechanisms have been field tested and show considerable promise for the future. They are F_1 sterility, hybrid sterility and sex linked translocations and deleterious genes. Early work with the sterile hybrids of mosquitoes was not successful in field studies. The different hybrid types behaved differently in the field, so competitive matings did not occur. However, more recently, studies with sterile hybrid Heliothis moths have shown predicted results in field studies. The discovery of sterile hybrids in *Boophilus* ticks has led to the consideration of using this approach against these ticks.

The intriguing theoretical possibility of sterile hybrids is that sterility is passed from one generation to the next without further release. Matings between the hybrids result in males that are completely sterile and females that are fertile but pass male sterility on to the next generation. In theory, a single release (releases during a complete generation time) produces the same theoretical effect as continuous releases of sterile males until elimination occurs. For example, when releases of sterile hybrids are made at a ratio of 9:1, there would be a mixed population of 1000 male and 1000 female wild types with 9000 male and 9000 female hybrids. The hybrid males are sterile, so only 100 wild type females and 900 hybrid females would be capable of reproduction. If these females reproduce at a fivefold rate in the next generation, there would be 250 male and 250 female wild types and 2250 male and 2250 female hybrids. The ratio of sterile to fertile males would be still 9:1 and the number of both types would be reduced by 50%. Without further releases, the ratio would be maintained each generation and the population would decrease to elimination by 50% each generation, as long as there is no deterioration in sterility through the generations.

Apparently, in Heliothis hybrids the sterility is transmitted from generation to generation without deterioration and, therefore, without recovery of fertility. In *Boophilus* ticks, fertility is recovered after four to six or seven generations. Nevertheless, it is still possible to achieve theoretical elimination prior to recovery

of fertility in this tick complex. This mechanism is perhaps the most efficient one found to date; unfortunately, it has not as yet been found in other insects.

V-3. GENERAL REQUIREMENTS FOR THE DEVELOPMENT OF SIT

V-3.1. Area wide (total population) versus conventional field by field control

Insect control throughout the world has generally been carried out using the field by field approach. For example, an individual farmer, with the help of extension or commercial specialists, decides where and when to treat and what methods or chemicals to apply. He may either do this himself with his own equipment or he may hire someone to do it for him. This field by field approach has proved effective and provides protection from the damage caused by insect problems.

An area wide insect control approach involves management of the total insect population of one or more species. Several examples of this approach are given below. Cattle fever ticks (two species of *Boophilus*) were eliminated from the southern USA in the early to mid-1990s by a programme utilizing animal dipping in arsenic and pasture rotation. The success of this programme is maintained by quarantines that protect the tick free areas from reinvasion from infested areas such as those in Mexico. Locust outbreaks in the world have been managed by applying insecticides over extensive areas of land. The cassava mealy bug is being attacked on an area wide basis in Africa by releasing parasites. Also in Africa, tsetse flies have been controlled, and in some cases eliminated, in large scale programmes using insecticidal and other treatments. Currently, a large scale programme in West Africa is preventing the transmission of onchocerciasis (river blindness) by applying pesticides to rivers in which the larvae of vector black flies develop. This programme covers an area of over 750 000 km^2. Pest and disease carrying mosquitoes are generally managed and controlled by governmental agencies created to deal with the problem over large areas.

There are many important differences between the field by field and the area wide approaches. Area wide insect control has not been utilized other than in situations where governments or agricultural production co-operatives, working with many producers in a defined area, assume responsibility. Such control has the disadvantage that producers or individuals may not have direct input into the decision making process. Agricultural producers, being somewhat more individualistic than other groups in society, tend to dislike this approach. However, effective insect control and management strategies depend more and more on the area wide approach. Development of new control strategies, including SIT, pheromones, biological control agents, growth regulating compounds, attractants and genetic methods, all require managing insect problems in terms of the total insect population in an area rather than on a field by field basis.

Development and implementation of SIT depends on an area wide approach with additional information on populations, e.g. numbers, density patterns and growth rates. The total population of insects in a geographically defined area must be considered when planning the control or elimination programme. In the past, when insecticides were used, it was possible to think in terms of individual fields or farm plots. If insects from a surrounding area reinvaded, or if the survivors from the treatment produced new pest individuals, the treatment had to be repeated. It was not necessary to survey the total infested area and deal with the total population. Some programmes took into account the biology and life-cycle of the insect and planned the timing of treatments to provide maximum protection from damage. This approach led, in some cases, to a preventative type of treatment schedule, i.e. insecticides were applied on some schedule — say weekly — to ensure that little or no insect damage occurred. This resulted in excessive treatments in some cases and therefore led to concepts associated with integrated pest management (IPM). In IPM, if the insect pest population and its natural enemies are determined, a decrease in the number of treatments would follow, with resulting reductions in cost and environmental contamination. This approach has been very successful, but it is still not the approach required for the successful development of SIT.

In a SIT programme there is no immediate reduction in the number of pest insects. The mode of action of SIT, i.e. reducing the ability to reproduce rather than destroying or killing, can take a few generations to reduce the population below the damaging level; it often requires that the population be reduced below the damaging levels by insecticides or other means prior to the releases. In any case, the technology is not effective unless the total population of insects in an area is defined in terms of the numbers and growth patterns. (For more detail, see the review given in Section V–2.) However, once the concepts of the method are understood and the approach and requirements developed, the technology can be the most cost effective method of control.

Since the SIT approach generally involves large areas, it can involve several regions, states within a country, or countries. In such cases, it is essential that an organized and co-operative approach be developed among all the groups involved.

V–3.2. Insect movement and migration (isolation)

Any development of a SIT programme must be applied to the whole population of the species or that part of the population which can be isolated from the rest by natural or artificial barriers. As mentioned elsewhere in this Manual, as a method of control or eradication SIT differs from the protection of limited areas or individual fields against given pests. The total area must be defined and managed. If this area is less than the total range of the insect, some means of isolating the treatment area from the remaining area must be developed.

In most cases, not enough is known about the movement, flight range, migration and dispersion of populations to deal with total population management by area wide control methods.

The movement or range of any insect population has tended to be underestimated. As a consequence, some insect release programmes may have failed because they were conducted in too small an area, where the population was not isolated. This is a common error when planning experimental programmes. For example, a 9 km^2 area may seem an imposing, large area when determining the requirements for conducting a programme. On the other hand, a 9 km^2 area is a square with 3 km on each side, the distance from the centre of this square to its perimeter being only 1.5 km. For most insects, such a distance is covered easily in a very short time. One must know the movement and range of insects well enough to determine the area required. In most cases, initial work has been carried out on islands or other naturally isolated populations. The literature provides many descriptions of experiments in which the results of releases have been clouded by the presence of immigrants from outside the test area. The present recommendation would be that, whenever possible, researchers should accumulate as much information as possible on insect movement, migration and dispersion. With current emphasis being placed on IPM and population control below the threshold levels of injury or damage, much more information will become available on this subject.

V-3.3. Integration with other control methods

The mode of action of SIT, i.e. reducing the reproductive ability of an insect population, is an ideal mechanism to be combined with other conventional or new methods of control. For example, in screwworm programmes the method has been integrated effectively into use of wound treatments, i.e. insecticidal formulations applied directly to the infested wounds on animals containing screwworm larvae. Such treatment kills the larvae, reducing the population numbers and, thereby, the number of sterile insects that have to be released. Since the treatments do not kill the released adult sterile males, there is no concern about reducing the effectiveness of the release technology. During the southwestern USA and Mexican screwworm programmes, a new attractant–toxicant bait was developed that could be dispersed by aircraft into the problem areas. The bait attracted screwworm adults and killed those feeding on it — another effective way of reducing the numbers in the natural population and making the release of sterile insects more effective. In this case, however, the attractant–toxicant bait had to be used before the releases so that the released sterile insects would not be affected or killed by the additional treatment. The same approach, use of attractants combined with insecticides as bait sprays, is an essential part of fruit fly sterile insect programmes.

Biological control programmes, where predators or parasites are released to affect or kill the immature stages, eggs, larvae or pupae, are compatible with SIT.

Reductions in the number of pests would reduce the number of insects required for release.

Movement of the released sterile males in seeking out the wild females is an advantage to any control programme where it is difficult to get treatments to the remaining few individuals.

Since area wide or total population control is being considered in these new approaches, it will be necessary to depend on a more complete knowledge of the biology, ecology and population dynamics of the insect population, as well as on the way in which each control method affects the density and dynamics of the population.

V–3.4. Pre-release activities

Detailed planning and preparation of a release programme are essential. The entire programme area has to be mapped and divided into regions. Regional headquarters, facilities, personnel and equipment have to be provided. Surveys before release are needed to determine the areas with and without infestations, the density levels and the number of insects required for release. Data managing systems useful to both the regions and the headquarters of the programme should be provided. It is anticipated that computer systems will be used increasingly in this type of operation. Since density levels can vary within each area or region, such information must be accumulated and included in maps for decisions on release patterns. The type of releases for each area (ground or aerial) as well as the distances between releases and the timing and scheduling of releases must be determined. Assuming that all the production, sterilization and storage activities have been planned, the method of transport to the distribution and release sites must be outlined. Such transport should include ways of ensuring that the insects arrive at the release sites as healthy and vigorous individuals. Release of insects, even on a small scale, is a difficult operation; on a large scale, it is an operation that requires planning, implementation and continuing supervision and maintenance.

V–3.5. Economic considerations and evaluations

From the above descriptions of the theory and development of SIT, it is obvious that this methodology is sophisticated, difficult and not easily accomplished. For the most part, SIT is restricted to insect pests of major economic concern, where the current costs of damage and control justify large expenditures for research and development and, eventually, programmes. An assessment of costs versus benefits should be made in developing SIT programmes. The goals of any research work or programmes should be well developed.

Programmes must be justified on economically sound judgements and evaluations. Development of the economics of loss, damage and control costs is different. It is difficult to assign monetary values to injury or damage resulting from insect pests. If 50% of a crop is lost to insect damage, while at the same time no one is hungry or starving because of this particular damage, it may be difficult to assess a value of the loss. Many people around the world suffer from malaria. How does one ascribe a monetary value to the sickness, death or working hours lost that may result? One way would be to justify the development costs for SIT or general insect control or eradication programmes on the basis of costs that are less than those being spent at this time. Since a considerable amount of money is being spent on insect programmes, this can be a good approach. For example, if so much money is spent per hectare or per person for a control programme, it would be of interest to determine if a programme of total population management or elimination, by any method including SIT, is possible within the currently available resources. Such an assessment would have to take into consideration that the costs of eliminating the problem in a few to several years have to be weighed against the total continuing costs for many years of temporary control.

V-3.6. Planning

From the information given above it is obvious that a great deal of research, development and planning is required to bring about an effective SIT or integrated control programme. Most of the comments have been generalized and a detailed plan has not been presented. Each programme differs somewhat and cannot be outlined in detail. A few other general considerations should be mentioned. Before considering use of SIT, the economic advantages of such an approach should be well defined and accepted, i.e. the total costs of the programme must be justified in terms of the savings that can be brought about in relation to the current costs devoted to the problem. For example, SIT was developed and demonstrated in southwest Canada for use against the codling moth, but its successful application had to await the decision that it was more economical to use this technology than current methods.

Large programmes require the co-operation of many different agencies and groups. Each group must understand these requirements and be prepared to make the commitments necessary to carry out such long range programmes. The method is effective only when the technology is understood and commitment is made to the total programme. Each group will have a role to play in effecting a successful outcome. No group can be the sole executor of the total programme. The operational parts of the programme should be organized into a working, responsible unit. Periodic and annual reviews of the programme and its results are essential. All the problems likely to be encountered in such a programme cannot be fully anticipated and it is helpful to provide support by development and research methods.

V–4. SPECIFIC REQUIREMENTS FOR THE APPLICATION OF SIT

V–4.1. Colonization and rearing

The basic requirements of colonization and rearing should not be underestimated. There are many species of insects that cannot as yet be colonized in the laboratory. The reasons vary and, in some cases, are unknown. Some species may not mate in cages because spatial or other requirements are not satisfied. Others may not develop or lay eggs. Some species may be maintained in laboratory colonies only with great difficulty, requiring artificial copulation or special handling techniques. Sometimes, an insect may be selected for special behavioural traits or mating behaviour during the colonization process. Obviously, if a species cannot be colonized, or can be reared only with great difficulty or by changing its behaviour, it is a poor candidate for SIT until further research and development are completed. Even when it appears that a healthy vigorous colony is available, it should be measured for its vigour, mating and reproductive abilities. Any, or all, of the methods mentioned in Section V–2.3 can be used to determine the quality of an existing colony. The reproductive capacity of the colony should be estimated, i.e. for every 100 individuals in one generation, how many can be produced in the next generation? For most insects, this reproductive capacity is of the order of 50 or more. There are always exceptions to every generalization; for example, the reproductive capacity of the tsetse fly is less than 10.

Basic and developmental research on SIT has done more to increase the knowledge of insect rearing and its requirements than any other development in the field of entomology.

V–4.2. Mass rearing

Following colonization and small scale rearing of an insect species, there is generally an increase in size of the rearing operation in the laboratory for experimental field studies. Although this type of rearing may be on a large scale from the technical, laboratory point of view, it is not the same as mass rearing for a SIT programme. In fact, it is difficult to envision the requirements for mass producing insects for release in SIT. In many SIT programmes, millions or hundreds of millions of insects are required per week. Production cannot be conceived without a visit to the facilities that produce such numbers.

The operation can best be described as an insect factory and involves all the components and operations of a large factory — planning, location, facilities, budgets, accounting, personnel and shifts, equipment, supplies, purchasing, waste management, data management, maintenance, security, training, safety and public information systems, to mention only a few of the operations of a large commercial factory type operation.

One of the new concepts in facilities that mass produce insects for SIT is replacement of a single rearing plant or building with separate modules, each of which is capable of producing insects for sterilization and release independently of the others. This approach has many advantages. If there is a production problem in one unit — disease, contamination or mechanical control of the environment — the other units can continue and increase production to maintain the programme. New strains can be produced first in one unit and, when successfully mass produced, moved to the other units without reducing overall production. Variations in production technology can be tried in one unit to improve efficiency or quality. For example, in fruit fly programmes use of two modules has led to the comparison of 'tumbled' larval collection techniques (larvae are forced to leave the media by rotating large cylinders containing media) with that of a tray system in which the larvae crawl naturally from the media and drop into collection devices.

Facilities must have the capacity to produce the millions of insects required and include provisions for: adult colonies, egging and egg collection, immature larval rearing, larval collection and pupation, used diet disposal, irradiation, marking and packaging, storage and loading, quality control with development methods, and security from insect escape.

Planning for and purchase of equipment and supplies are of major concern. In some cases, these can be accomplished on the basis of concepts and plans developed in other similar factories. In any case, planning requires determination of the type and location of equipment and may involve the development of new equipment for special or improved operations. Media must be purchased in large quantities, be stored and be available for several months in the event of supply failure. Methods of preparing the components of the media in the proper proportions and consistencies for use in rearing must be provided, as well as methods of transporting these media to their place of use.

In any facility, the flow of operations from stock colonies to egging, rearing, collection, pupation, irradiation, packaging, storage and transport must be engineered for top efficiency and at minimum cost. Operations can require a few to several hundred personnel and, in most cases, around the clock operations of three separate shifts. Training programmes and safety for employees play an essential role in operations. Data management as well as facility maintenance and security must be provided.

Insect rearing requires special environmental conditions. Even different stages of any one insect may have various temperature, humidity and light requirements. Environmental specifications have to be developed, maintained and monitored. Occurrence of a disease in an insect or any contaminant — be it in another insect species competing for the media or in just being annoying — is always a possibility for which special procedures, and possibly additional staff, are required.

It is highly recommended that visits to existing insect rearing factories be made to become more familiar with this new development in entomology and insect con-

trol. Development of rearing factories is a difficult process, requiring planning and development by co-operative efforts. When SIT was first implemented, there was a trend towards a large, single rearing facility for a single programme. As SIT and related integrated area wide programmes continue to increase, further consideration will have to be given to the number of rearing factories needed and their location. In a programme involving several countries, it appears economically infeasible for each country to have its own rearing facility. Consideration will also have to be given to the location of these facilities, their proximity to airports or other forms of transportation and the distance that the insects can be transported. These are difficult processes but they have to be developed for each programme and probably involve further research of a technical, economic, logistical and political nature.

V–4.3. Radiation sources

There are a number of radiation source product but the manufacturers change continually. For a list of the firms which manufacture the radiation sources that can be used in SIT programmes, contact the Insect and Pest Control Section, Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, IAEA, Vienna.

V–4.4. Sexual separation prior to release

In the early development of SIT, separation of millions of individuals by sex was not possible and both sexes were reared, sterilized and released. It was important that all the females released were rendered completely sterile. The major problem with the release of females was that, in some cases, they damaged agricultural products, annoyed people or animals, or transmitted diseases through bites. Many attempts were made to separate the sexes. The methods first involved mechanical means such as looking for differences in the size or weight of the pupae, or even the adults, and using sieves or air streams for the separations. Such methods were generally not successful on a large scale. With one species of mosquito it was possible to separate the sexes (with an accuracy of about 99%) by determining the differences in the size of the pupae by forcing them through sieves. With house flies, it was possible to separate pupae in an air stream because of their differences in weight and shape. However, these methods were not useful or practical for those insects for which SIT was first developed.

Even if ways of separating the sexes in the later stages of the insect's life are developed, females would still have to be reared. In large scale programmes, the important advantage of sexual separation would be in decreasing the manpower and costs of rearing. In most insect species there is a 1:1 sex ratio, which means that the rearing requirements can be reduced by almost one-half if females are not reared. Sexual separation is, then, best accomplished in the egg or early larval instar stages;

this implies that some genetic method probably has to be developed for maximum efficiency.

Research is under way using a colour mutant in the pupae of the Mediterranean fruit fly. A type of seed separating machine separates the pupae rapidly on the basis of colour. Unwanted females would still have to be reared, but they could be removed before releases. More recently, thermolethal strains have been developed in which the females of the Medfly can be killed in the egg stage by increased temperature.

A genetic means of separating the sexes prior to rearing was first developed in mosquitoes for use in practical field release trials. Many strains of mosquito are known, and have been described, in which resistance to insecticides has occurred. The Genetic Unit at the Insects Affecting Man and Animals Research Laboratory in Gainesville, Florida, developed such a strain for field trials against the malaria vector, *A. albimanus*, in El Salvador. A strain of *A. albimanus* from the UK resistant to the insecticide propoxur was exposed to irradiation with a view to inducing chromosome breaks. The progeny were followed until a strain was found in which resistance to this insecticide was linked to the male determining chromosome and susceptibility to this insecticide to the female. Once this strain was developed, it was reared and tested for vigour, mating ability and reproduction, and then developed for mass rearing. It was possible to put this strain into larger scale rearing for field trials. The eggs produced for rearing the released males were exposed to propoxur in the water in which the eggs were hatched. Hatching female larvae were killed, while the male larvae were kept alive for rearing. Special field tests of males released in this manner (the rearing capacity was about one million per week) showed them to be vigorous and capable of competitive mating in the field. The results of the field releases also showed induction of sterility into the wild population with density reduction resulting from the releases. Such methodology is possible and under development with other insects. Further genetic research is needed to develop this complex and sophisticated approach.

Some advances in this type of technology can be expected with the current capabilities available from genetic engineering. In some cases, successful development of SIT will depend on this development; in other cases, the efficiency of SIT use will be improved. One field study with fruit flies has shown that the effectiveness of males in mating wild females was increased by 50–100% through releasing males only, rather than both males and females. In addition, fruit was not stung by the sterile females.

V–4.5. Insect population dynamics related to SIT

V–4.5.1. Introduction

Insect population dynamics is a complex field and over many years has been developed into a science of its own. It is not intended to develop a detailed study of

this area, but the reader interested in such research is referred to texts on the subject. The main interest here is to look at population dynamics as it can be applied directly to the development and application of SIT and other integrated control measures. The principles of SIT lead to a basic understanding of the interaction of populations and the effectiveness of control methods. This presentation is designed to summarize the theory behind SIT, as outlined by Knipling, and then to develop the original model into more complex life history models and finally computer simulation models.

V-4.5.1.1. Simplified generation modelling

Perhaps the most fundamental modelling technique applicable to insect population dynamics and control was proposed by Knipling to illustrate SIT. It utilizes a growth potential or reproduction rate per generation (R) and a factor representing the effect of a control technique (S) to relate the density in one generation (P = the parent generation) to the next generation (F_1)

$$F_1 = P\ R\ (1 - S) \qquad \text{(V-1)}$$

If no control is visualized (S = 0), then Eq. (V-1) reduces to

$$F_1 = P\ R_a\ (a = \text{actual}) \qquad \text{(V-2)}$$

In this case, the actual change in density (F_1/P) is equal to R_a. When a control technique is applied, a distinction must be made between R, the potential growth rate, and the actual growth rate or change in density. From Eqs (V-1) and (V-2), the actual change in density is a function of the growth potential and the effect of the control method on reproduction

$$R_a = R\ (1 - S) \qquad \text{(V-3)}$$

The requirement is to understand the potential growth rate of natural populations in the field where they are a problem and the effect of a single or multiple control strategy on this growth potential. In a population with a growth potential (R) of fivefold and a control action that is 90% effective in reducing reproduction over one generation (S = 0.9), the actual growth rate (R_a) would be 0.5 (R_a = 5 (1–0.9) = 0.5) and F_1 is only 50% of P. The model can be used repeatedly for successive generations to establish the trend of a population over time for a given control measure. Knipling used this type of model very effectively to illustrate SIT theoretically and practically in the field against the screwworm. He also used the model to illustrate the difference between the sterility and insecticidal approaches to insect control.

Effective use of this type of model depends on a knowledge of the key parameters R and S. The accuracy required for the parameter estimates depends on

the purpose of the modelling effort and the uses to be made of the final result. Intuitive estimates based on experience or limited data can be very useful in models to demonstrate the theoretical potential of a particular control procedure. However, for more critical applications or predictions of actual population changes, more precise parameter estimates are required; the results are only as good as the data used to develop the estimates. Estimates of R_a for natural populations may be obtained when population density data are available over a period of time and the generation time is known. In this procedure, the density ratios at generation intervals provide the values of R_a. Currently, many survey methods are used for sampling the various stages of the insect and a large amount of data is available. Not all data are adequate for use in analytical models. To be useful, the survey method must be applied consistently over a sufficient period of time and the sampling efficiency of the method must be reasonably constant over time and for different locations. Also, the population being surveyed must be uncontrolled or the effect of any control actions known. A basic set of data that will allow the modelling process to become more and more practical must continue to be built.

The equations represent a very useful approach to modelling insect population dynamics and control, particularly for SIT. More complex models are needed for many systems and control measures relative to integrated control strategies. The above models should be considered as a first step in a modelling process that can be made as complex as the system under consideration requires.

At this time, it is still astounding that so little is known about the potential growth rates of populations as they occur in problem areas. Operational programmes now represent the best source of this type of data and information.

V–4.5.1.2. Mathematical life history models

An extension of the above modelling approach can be made by including additional parameters related to the life-cycle or life history of the insect under study. In a classical life history analysis, the reproductive rate (R) is related to survival and progeny production by the following equation

$$R = e\, l_x\, m_x \qquad \text{(V–4)}$$

where e is the proportionality constant, l_x is the probability of survival to age x from birth or oviposition and m_x is the female progeny produced per live female at age x.

This relationship can be used to calculate the values of R from normal life table data. Also, another useful equation for insect populations can be derived from this relationship by applying certain generalizations common to many life histories. First, since oviposition occurs only in the adult stage, immature survival can be included as a separate parameter. Then, by assuming that the rates of adult survival and ovipo-

sition are constant with age and that oviposition occurs at discrete intervals after a preoviposition period, the following equation results

$$R = \frac{SI\ m\ (Sa)^D}{(1 - (Sa)^C)} \qquad (V\text{–}5)$$

where SI is the probability of survival from egg to emerging adult, Sa is the average daily survival rate of adult females, m is the average number of female eggs per live female per oviposition, D is the preoviposition time in days and C is the egg laying cycle in days. In this equation, SI can be subdivided into age classes, if desired. For example, if the development time (in days) for eggs, larvae and pupae (E, L and P, respectively) is known and a daily survival rate for each stage (Se, Sl and Sp, respectively) is also known, then

$$SI = Se^E\ Sl^L\ Sp^P \qquad (V\text{–}6)$$

Also, if a daily survival rate, Si, is given for the complete immature development period, I, then $SI = Si^I$. As a numerical example for Eq. (V–5), consider an insect population where SI = 0.0853, Sa = 0.85, m = 60, D = 6 and C = 3; then

$$\frac{0.0853 \times 60 \times 0.85^6}{1 - 0.85^3} = 5$$

Again, a model such as this is no better than the parameter estimates that are used; in most cases, field data on these parameters are not available. However, the method is available for theoretical calculations and will be more applicable for practical field problems as the database on field populations is accumulated. Currently, good estimates can be made for values in this equation for many insect species. It should be noted that the values for R in Eq. (V–5) can be used in Eqs (V–1) and (V–3) to calculate density trends for the control measures that limit the production rate, as discussed earlier.

However, since Eq. (V–5) includes survival parameters, one can now make theoretical calculations relative to other control techniques that can be envisioned as having an effect on the survival rate of specific stages. To do this, the effect of a control technique must be expressed as constant daily mortality, M, on the basis of specific daily age classes in the insect life-cycle. This is possible for many control techniques such as continuous treatment with biological agents, insecticides or traps that kill a constant proportion at given stages. For a given M, a survival rate due to control, Sc, can be calculated (Sc = 1 − M) and used in Eq. (V–5) to reflect the actual growth rate, R_a, after the control technique is applied. For control techniques

that affect the adult population, where Sa is already a daily survival rate, Sa would be replaced by Sa Sc and the following equation would be obtained

$$R_a = \frac{SI\ m\ (Sa\ Sc)^D}{1 - (Sa\ Sc)^C} \qquad (V\text{-}7)$$

For example, assume that one has an attractant or trapping device which traps an average of 16% of the female adults each day. Then, survival after trapping, Sc, equals 0.84. Using the numerical example for R = 5 above and calculating R_a gives an answer of 1.06. In other words, trapping out 16% of the adult females stabilizes a population at no increase rather than at fivefold growth. In the case of control techniques that cause mortality in the immature stages, SI would be multiplied by SC^N, where N represents the number of daily age classes that are affected by the control. The resultant growth can than be represented by

$$R_a = R\ Sc^N \qquad (V\text{-}8)$$

For example, consider an insect population where the development times are 2 days for eggs, 6 days for larvae and 2 days for pupae. If the control affects all the larvae equally, then N = 6 and $R_a = RSc^6$. Likewise, if the control affects all the eggs or pupae, then $R_a = RSc^2$. If S affects only one age class (1 day), such as the last day of larvae, then $R = R_aSc$. These calculations are theoretical; however, they illustrate several interesting practical considerations. For example, one can ask what average daily mortality would have to be applied to all stages of the immatures (egg, larvae and pupae) to reduce the growth rate from 5 to 1

$$Sc = 1 - M\left(\frac{R_a^{(1/6)}}{R}\right) \text{ or } M = 1 - \left(\frac{R_a^{(1/6)}}{R}\right)$$

In this case, M equals 0.24, or 24% of each stage would have to be killed each day by insecticides, biological agents or other means. If only the pupae were considered

$$M = 1 - \frac{R_a^{(1/2)}}{R}$$

Then the average daily mortality of pupae would have to be 55% to reduce the growth rate from 5 to 1.

These modelling techniques allow theoretical calculations to be made that interrelate dynamics and control from one generation to the next. However, the mathematics for these equations require that daily average mortality or survival be used. Unfortunately, many control agents are not applied every day or do not act in

a manner which permits the use of the average daily survival or mortality. Thus, more complex modelling techniques are required to give added realism to theoretical calculations and to simulate more accurately the actual effects of complex IPM control strategies.

V–4.5.1.3. Computer simulation models

Computer technology can be used to extend the complexity and versatility of the life history approach to the modelling of insect populations. The computer is used essentially as a 'bookkeeping' or storage unit for all the parameters and life stages of the insect. Interaction of all the controlling parameters is made during some defined unit of time, e.g. a day, and then the population is converted to the expected population during the next unit of time, e.g. the next day. Details of this simulation do not need to be explained here; instead, the reader is referred to computer simulation papers for the various approaches, some of which are quite varied. However, such simulations permit mimicking of the observed density trends of field populations. Simulations can include control treatments. The 'power' of computers in terms of storage, mathematical manipulations and output offers much potential in developing simulations for specific uses.

Such approaches to modelling and simulation may seem highly theoretical. The complexity of the problems of dealing with dynamics and control, along with the difficulty of estimating the parameters used to control the simulation, may seem overwhelming. However, if simulations can be verified by the results obtained in field studies, this should help in designing improved control strategies. Such simulations are currently useful in attempting to correlate the available knowledge on biology and control and will permit the development of hypotheses on management strategies. Such hypotheses should be of guidance in developing plans for the use of new control agents or the combination of control agents into strategies.

V–4.5.2. Total population numbers and estimates

After reviewing the previous section on insect population dynamics, one reaches two conclusions: application of this basic type of ecological study to field populations is very difficult, and the amount of actual field data that quantitates the various parameters controlling the density of populations is limited. Of particular interest is the fact that experiments on the development of sterility approaches to control can themselves be used to obtain estimates of parameters that regulate the dynamics of populations. By parameters one means, in particular, the absolute density and the population growth rates from generation to generation, but also such aspects as the survival rates of various life stages and their development times.

Before one considers actual examples of field research in which the sterility principle and approach were used to study absolute densities and population growth

rates, an outline should be given of a possible approach with an insect such as the Mediterranean fruit fly. In ongoing programmes, the following procedures are possible and, to a large degree, routine. First, the density levels of the adult flies (males) are monitored by use of traps containing trimedlure. When sterile insects are released, they are generally marked with a fluorescent dye so that they can be identified in the field upon capture. They can also be captured in the trimedlure baited traps, as are the native males. Although not a routine procedure, it is possible to recover eggs from infested fruit and check the eggs for hatching, thereby determining the degree of sterility in the native population as a result of the released sterile males.

One can assume that, in some programme area where the release of sterile insects is under way, it will be possible to find an ecologically isolated area where immigration and emigration do not occur, or are so minimal as to be ignored. This area must be small enough to ensure that native and released populations can be monitored within the available resources of the ongoing programme.

In this area, one would want to evaluate the results in terms of the generation time of the Mediterranean fruit fly rather than in days or weeks. In such a case, one would need an estimate of the generation time of this insect. It may vary from location to location but, for this example, one would assume that the egg stage lasts for 6 days, the larval stage for 10 days, the pupal stage for 8 days and the preoviposition time for adult females (the time from emergence of an adult female to the time she deposits eggs) for 6 days. Then the life-cycle (or generation time) would be 30 days. (For future reference, the life-cycle would be about 1 month, which seems reasonable, and would result in about 12 generations per year in tropical areas.) The reason for establishing the generation time is that one wants to use Knipling's generation model. To do this, one can average all the data obtained on the basis of a generation time, no matter at which interval of time it is taken (daily, bi-weekly, weekly, etc.).

In ecologically isolated areas one would establish trimedlure baited traps to monitor the population for one or two generations (months) before any releases of sterile insects are made into the area. The traps should supply data on the number of native males per trap. By averaging all the values obtained during one generation, one should produce an estimate of the relative density of the males during that time. If this is done for two generations, one would have the change in density from one generation to the next. Since the sex ratio of this insect is one male:one female, one would also have an estimate of the relative number of females, even though one may not know where these females are or be able to trap them. However, at least one produces an estimate of relative density and can look at the change in density over generations.

If this approach provides sufficient numbers for analysis, one would then consider the release of sterile insects on a routine basis for four to six generations. The released males should respond to the traps and could be caught and counted. Since the released males have been marked with a fluorescent dye, it would be possible

to establish the ratio of released to native males. With this simple ratio alone, or more sophisticated procedures such as the Lincoln index approach, it would then be possible to make estimates of the absolute number of males (and females) in the population — one of the requirements of developing the sterile male technique. Continuing such monitoring during the whole time of the releases would permit determination of the actual change in density which occurs. One would assume that the releases have been successful and that the density has been reduced greatly during the experimental trial. One can, of course, follow this change in density by monitoring the trimedlure traps.

At the same time, it would be necessary to monitor the sterility induced in the native population by the released males. This could be done by collecting egg samples and assaying the degree of hatch. Again, all this data on sterility could be averaged for generation times so that they can be used along with the density data in analysis.

The relationship of interest is the theoretical one established by Knipling for the mode of action of SIT

$$F_1 = P \times (1 - S) \times R \qquad \text{or} \qquad R = F_1/(P \times (1 - S))$$

where P is the relative (or absolute) density in one generation, F_1 is the relative (or absolute) density in the next generation, S is the decimal degree of sterility induced by the sterile males into the wild females within a generation and R is the generation growth rate.

From monitoring the relative density of this population over several generations, including two generations prior to release and four to six after release, one has the F_1 and P values to use in the above equations. One also has the S values from the egg hatchability data and can calculate the R values both prior to release and during release. The calculated values of R are not for some laboratory colony of the insect or the result of theoretical mathematical calculations; they are the actual values of a field population subjected to a control strategy, the values that occurred in the 'real world', and are needed to understand and develop SIT more systematically and efficiently. If this methodology can be used, such experimentation would, for the first time, establish data on such growth rates for this insect or other insects. These results do not mean that other growth rate values could not be measured in other locations or on other host products. However, additional studies would continue to accumulate data and lead to information on the growth rates of different hosts at different locations.

Another area of interest in the improvement of the SIT approach is the competitiveness of the released insects or their efficiency once released into the native population. Normally, one measures the competitiveness of sterilized males in cage experiments and defines this competitiveness as the difference between the expected value (e.g. 90% sterility with a 9:1 ratio) and the observed value (as measured in the hatchability of eggs from females mated to sterile males). This type of informa-

tion is obviously of value as an indicator of quality and for decisions on the use and release of mass reared and sterilized insects. However, it does not tell us anything about the effectiveness, efficiency or competitiveness of these released insects against the native population in the field.

The field experimentation described above can be used to analyse the effectiveness of released males against the native population. First, since ratios of released to native males can be established, it is possible to compare the degree of sterility that would be expected from these ratios with that actually observed in the native females over several generations. Second, it is possible, once the above calculations have been made on the growth rates, to predict the density trend expected in subsequent generations from the relative density, sterility and growth rate observed in one generation ($F_1 = P \times (1 - S) \times R$). Using this information to predict the outcome of a field experiment with different levels of competitive males (100% to 10% and several values in between) would provide the predictive curves that reduce density too rapidly (males considered to be more competitive than they actually were) or too slowly (males considered to be less competitive than they actually were). The curve fitting most closely to the observed density trend would be a good estimate of the actual performance of the released males in practical problem situations.

For an example of determining absolute density, one should review an experiment planned and conducted by the Insects Affecting Man and Animals Research Laboratory at Gainesville on the release of sterile males against *A. albimanus* in El Salvador, Central America. This group found a relatively small population of this mosquito breeding in almost complete isolation. It was planned to release sterile males for about 4 months to determine whether SIT would have any effect on the natural population. The first problem was to estimate how many males would have to be released over time. This involved estimating the total population of mosquitoes in the test area. Fortunately, the larval breeding sites of the mosquitoes in this area could be identified and the total breeding area estimated by measurements. From larval dipping records, the average number of pupae per square metre in the larval breeding areas was determined. Multiplying this number by the total square metres of the area produced an estimate of the total number of pupae in the area. Since half the pupae were males and half were females and since the pupal stage required 2 days for development, it was possible to estimate the number of males and the number of females emerging each day from the breeding areas, i.e. the total number of pupae divided by 4. Since it was planned to release males daily, this was the estimate needed. However, to further confirm these estimates, some release–recapture experiments were carried out in the area. From these experiments, the total number of adults in the area was estimated. The total number of adults in the area is not the same as the number emerging each day. However, using the life history approach and some assumptions on adult survival, it is possible to compare the two approaches. For every 200 adults (100 males and 100 females) emerging each day, one can expect a calculable total number at any particular time, assuming a contin-

uously breeding population, and an estimate of the average daily survival for the adults: total adults is the summation over the product of the number of adults emerging per day and their average daily survival.

In the experiments, the group obtained values of 6500 males and 6500 females emerging daily at the time releases were started, using the method of pupal counting in the breeding areas, and 6600 males and 6600 females emerging daily, using the mark–release–recapture method. The results of the two methods were surprisingly similar. The number of males or females of all ages present in the area was 22 000–24 000.

Without this type of information on the total number of insects in an area it is impossible to estimate the number of insects needed in a release programme. There is also the trial and error approach. For example, with no idea of the actual number of insects in a given area, one can always release only sterile insects and see what happens. If it is possible to collect wild females and assay them and the eggs they produce for fertility or egg hatchability, then it is possible to determine the impact of the releases on the wild population. Sterility levels that are too low would be the result of the release of too few insects, insects of poor quality or poor release techniques. However, this trial and error method is a poor approach, since without sufficient means of quantifying the results of the releases there is no way of determining the cause of negative results. Negative trials can be very discouraging and lead to the conclusion that the method does not work rather than to the proper conclusion that there was insufficient data to be able to evaluate the new approach properly.

In sterile male research and development, use of relatively simple methods of estimating total population numbers has been very successful. In the screwworm programme in the southeast USA, it was necessary to estimate the total number of individuals before building a screwworm production factory. Earlier mark–release–recapture studies had provided the information that screwworm populations generally occurred in numbers of a few hundred per square kilometre. An estimate of the number of screwworms in the state of Florida (to confirm this figure) was made in the following way. The total number of domestic animals in the state was obtained from the County Extension Services. In the files of the United States Department of Agriculture (USDA), some earlier data existed on the percentage animals with cases of screwworm in various locations at different times of the year. From screwworm biology research it was concluded that 20 adult flies would normally result from one screwworm case. Using this information, the estimated number of cases of screwworm for all domestic animals could be multiplied by 20 for an estimate of the total population. Such an estimate would probably not be adequate if it were not in agreement with earlier estimates from mark–recapture–release studies and confirmed by initial tests that showed that the release of a known number of flies (based on the estimates) did indeed result in the expected level of sterility in the wild population.

A similar approach was used by G.C. LaBrecque and colleagues at the Insects Affecting Man and Animals Research Laboratory in estimating the number of stable

flies on the Island of St. Croix (218 km^2) prior to experiments on the release of sterile insects there. It was impossible to count all the flies on the island or to put together the resources for mark–release–recapture on the entire island. It was, however, possible to take counts of the number of flies on the legs of selected and representative domestic animals in the various herds, and to estimate the total number of domestic animals on the island. Multiplying these two values would provide an estimate of the total number of flies on the animals. However, stable flies only feed on animals once or twice a day. At other times, they are not on the animals and fly around or rest on vegetation. There seemed to be no easy way of estimating the number of flies not on the animals. Researchers then did some experiments in large outdoor cages in their laboratory. They placed a beef animal together with a large, but known, number of stable fly adults in a cage. Every hour during the day they made counts of the number flies on the animal. The results of their studies showed that for every fly on the animal, there were 55 not on the animal, resting somewhere in the cage. The assumption was then made that the number of flies on animals represented only about 1.8% of the total population. It would be difficult to confirm this figure for wild populations. However, since these researchers were conducting an experiment on sterile insect releases, it was possible to use this estimate in determining whether the sterility attained at the start of releases approached that predicted from the known number of steriles released. The initial degree of sterility found in wild females was similar to that predicted.

Although such methods only provide estimates of wild populations, they have been useful in designing successful sterile insect release experiments and programmes. Perhaps more work should be done on these practical means of estimating total populations. One could develop simple or very complex models of how new control methods such as the sterility principle may work, but without this basic field data on pest populations one cannot turn theory into practice or improve the efficiency of an ongoing programme.

V–4.5.3. Population growth rates

One should look at a concept that would help understand the population growth rates under natural conditions, and when these are subjected to control by SIT. The point has already been made that the actual change in the density of a population (R_a) is simply the number of individuals in a generation (F_1) divided by the number of individuals in the previous generation (P)

$$R_a = F_1/P$$

This occurs when no control methods are applied. However, if a control method is applied, a different situation arises. The potential growth rate of the population (R) can be calculated as the actual change in density (R_a or (F_1/P) multiplied by the

effect of the control technique on the fertility of the females (1 − S) (where S is the degree of sterility caused))

$$R = F_1/(P \times (1 - S))$$

Another form of this equation is

$$R = R_a/(1 - S) \text{ or } R_a = R \times (1 - S)$$

The actual change in density (R_a) is a function of the potential growth rate (R) and the degree of control of fertility (1 − S). The actual density can only decrease when control of the fertility overcomes the potential to increase (R). This may seem to be a simple point of little consequence. However, if one examines the implications with examples, one sees how important the concept is.

It is important that more data become available on the growth rate of populations under actual field conditions in problem areas. Experiments on the release of sterile insects could provide this type of data if the tests are run correctly. From the formulas above, it should be noted that one is dealing with parameters that are normally measured in field experiments, i.e. the density (F_1 and P); these density estimates can be either relative or absolute. Many trapping and survey methods provide estimates of the relative density of a population; these could be used in specific situations. If one traps wild females, separates them from released females and then assays them for fertility, one could obtain an estimate of the degree of sterility induced into the natural population by the released insects. For example, if the actual change in density observed from one generation to the next was 0.5 (a 50% reduction) and the degree of sterility induced into the population was 90%, then the potential growth rate of the population at that time would be fivefold (0.5/0.1).

Another interesting aspect of the dynamics of populations can be studied in this manner when a successful sterile insect release programme is run. Ecologists talk of those factors regulating population growth that are density dependent. The concept is that, at higher densities, populations are regulated by density dependent factors which limit the potential of the population to grow, i.e. the actual growth rate is reduced. In fact, populations can reach an equilibrium in which the number of insects in a generation is the same, or no greater, than the number in the preceding generation, i.e. the actual growth rate is one. Or, in the words of the ecologist, the 'carrying capacity' has been reached. If this population is reduced below the carrying capacity, it is expected that the potential growth rate will increase if the factors regulating it are density dependent. Then, one could expect that a successful sterile insect release experiment, in which the density is reduced through the action of the sterile releases, should allow density dependent factors to come into play and show an increased growth rate, even though the density is being reduced. Following a population reduction caused by the release of sterile insects against two species of mosquito, just such

an effect was shown — an increase in the growth rate, with increasing control and decreasing density.

Two experiments that were run with mosquitoes — one with *Culex quinquefasciatus* and the other with *A. albimanus* — by the Insects Affecting Man and Animals Research Laboratory illustrate what can be accomplished in this way. It should be remembered that in both cases these experiments were run with natural populations, but in areas that were essentially isolated from other populations. The two experiments involved the release of sterile males into natural populations for several generations. During releases, the populations were monitored for relative density and the degree of sterility induced into the natural females by the releases. With *C. quinquefasciatus*, the density was monitored with ovitraps. Egg rafts were collected daily and counted. With *A. albimanus*, the biting rate of female mosquitoes was monitored twice each week. In the two experiments, females were captured and returned to the laboratory, where they were held for egg deposition. Hatching of the resulting eggs was determined. The data obtained were averaged for periods matching the generation times of these species; a summary of the results appears in Table V–IV.

It should be noted that the experiments listed in the table were relatively long. For *A. albimanus*, the life-cycle requires about 3–4 weeks, so with six generations one is considering a test lasting for 4–6 months. For *C. quinquefasciatus*, the generation time is about 3 weeks, so testing continued for about 3 months in the summer of 1968 and 4 1/2 months in the summer of 1969. Such experiments require time, personnel and funds. However, they are worth the effort, providing data on the feasibility of the sterile release technique and the dynamics of the populations of insects under suppression.

Obviously, the release of sterile insects had an influence on these natural populations, overcoming their reproductive capacity and reducing their density. The average number of *A. albimanus* larvae per square metre decreased from 62.42 to 0.73, a reduction in density of 98.8%. The number of egg rafts of *C. quinquefasciatus* decreased from 144 to 58 (a 60% reduction) in 1968 and from 228 to 8 (a reduction of 96%) in 1969.

However, the data also tell us much about the dynamics of these populations and the requirements for conducting a successful SIT programme against these types of insect in the future. Unfortunately, each insect species is different and one cannot transfer data from one species to another. However, the methodology for learning more about the dynamics of any species is present in SIT itself.

The growth rates calculated for the two mosquito species in Table V–IV should be reviewed and note be taken of the similarity of the results in the two tests with the same species as well as the differences between the two species. When releases were begun against *A. albimanus*, the natural population did not show good potential growth rates. In generations 1 and 2 it should be observed that the growth rates were less than 1 (0.6 and 0.5). This is very helpful in obtaining positive results from the

TABLE V–IV. RESULTS OBTAINED WITH *A. albimanus* AND *C. quinquefasciatus*

Generation	Average No. of larvae/m^2	Average degree of sterility in population	Growth rate
	A. albimanus		
1	62.42	0	0.6
2	39.83	0.23	0.5
3	12.92	0.56	0.9
4	5.27	0.56	0.7
5	1.51	0.79	2.3
6	0.73	0.81	—
Generation	Average No. of egg rafts/day	Average degree of sterility in population	Growth rate
	***C. quinquefasciatus* (first year, 1968)**		
1	144	0.65	0.9
2	44	0.80	5.4
3	48	0.88	10.0
4	58	0.93	—
	***C. quinquefasciatus* (second year, 1969)**		
1	228	0.50	1.3
2	146	0.81	5.4
3	151	0.93	4.5
4	47	0.91	2.1
5	9	0.91	9.9
6	8	0.98	—

release of sterile insects, as has been shown with the theory of SIT. Attention is drawn to the fact that these growth rates did not increase very much, i.e. they were still less than 1 in generations 3 and 4, even though the density of the population had been reduced by about 90% by generation 4. In generation 5, the growth rate finally rose above 1, to a value of 2.3. This is obviously an increase, but it is still not a high growth rate for a mosquito which has the ability to produce a large number of eggs per female. This population showed some increase in the potential growth rates as the density was reduced, suggesting that density dependency played some role in the dynamics of this population. However, it was not as large an effect as might be expected from the high biotic potential of this species. In practice, a species limited to low growth rates (particularly rates of less than 1) would require the release of fewer sterile insects. This information is extremely important to those considering this approach in experimentation or programmes.

The results obtained with *A. albimanus* should be contrasted with those obtained with *C. quinquefasciatus*. In both test years, the *C. quinquefasciatus* populations had low growth rates when the release experiments were started (0.9 and 1.3); in other words, the population had reached equilibrium at the carrying capacity. The reduction in density caused by the release of sterile insects resulted in a dramatic increase in the potential population growth rates in the next generation, to a little over 5. (A fivefold potential rate of growth is a high rate of growth in field populations and can cause tremendous increases in density over a few generations.) In this case, the release of sterile males overcame this expectation and actually reduced the density in the second generation (by 36% in 1968 and 70% in 1969). It should be noted that in both years the continued reduction in density in subsequent generations by the release of sterile insects resulted in an increase in potential growth rates (to about 10) when the density was extremely low. In 1969, less favourable environmental conditions during generations 4 and 5 reduced the potential growth rates, even though they were still above 2.

This type of experimentation provides information on the dynamics of populations that is not only lacking at the present time but is also needed for further development of the feasibility of SIT, and for its success. It should be remembered that it was difficult to apply Knipling's model, since one had so little information on the absolute density of the insects and their potential growth rates under natural field conditions. SIT is also difficult to apply to practical problem areas because it has to be applied to the total population over large areas.

In the experiments described above, the starting ratios of sterile to fertile insects were relatively low. With *A. albimanus*, they were only about 2:1. The same was true for the experiments with *C. quinquefasciatus*. Normally, one would not expect such ratios to accomplish population reduction. One could use the experiment with *A. albimanus* to illustrate how the sterile releases worked as well as the theory of SIT using the formulas given above. The experimenters determined that there were about 7000 normal males emerging daily in their area at the start of releases

and that they released about 13 000 sterile males into the area. From the data presented above, it can be seen that the population growth rate was only about 0.6. The predicted trends in density can be calculated if assumptions on the competitiveness of the released males are made. The experimenters actually made calculations on the degree of competitiveness and reported that it was about 25%. Thus

$$F_1 = P\left(1 - \frac{N}{n + P}\right) 0.6$$

$$F_1 = 7000\left(1 - \frac{(13\,000 \times 0.25)}{(13\,000 \times 0.25 + 7000)}\right) 0.6$$

$$F_1 = 2868$$

According to these calculations, the density should be reduced from 7000 males emerging per day to only 2868, a reduction in density of about 40%. The results tabulated above for the *A. albimanus* experiment show a 36% reduction to the first generation, from 62.42 to 39.83 larvae/m^2. To ensure that the predicted results conform to those observed, it was necessary to use a competitiveness factor of 0.25. This is not an unreasonable factor, since it is difficult to release males that are fully competitive from the standpoint of quality, vigour and mating ability and to disperse them equally with the wild males in the mating environment.

The theory of SIT has been demonstrated with practical examples using mosquitoes. If, one wonders why mosquitoes were used (a species for which the technique has not been put into use operationally) rather than screwworms, fruit flies or some other insects, the reasoning is simple: these insects have already been eliminated from areas or prevented from establishing themselves by SIT. However, data on the growth rates for these populations have neither been published nor are they available. For example, elimination of the screwworm from Curacao was accomplished with the Knipling model. He predicted that this insect would be eliminated in four to six generations. He used his knowledge of the density of screwworms with the assumption that the population would grow at a fivefold rate. His predictions were validated in actual releases there. However, data are still not available from natural populations in which the potential growth rates of populations of screwworms have been measured.

Finally, the complexity and sophistication of SIT should be reiterated. The total population in large areas must be considered. If less than the total area and population is used, then the movement and migration of the insect must be understood and barriers established. In the screwworm eradication programme in the southwestern USA, reinvasion of the US was to be prevented originally by an 83 km barrier of releases along the US–Mexican border. In this large programme, it did not take long to learn that screwworms can move distances much greater than 83 km,

so the barrier zone was increased to 483 km. Research on movement and migration as well as analysis of the reinvasion of the western USA showed clearly that screwworms are capable of movement exceeding 333 km.

V–4.5.4. Methods, numbers and timing of releases

The methods of releasing insects have been varied, depending on the insect, the size of the area and the type of habitat. Screwworm releases have been made by air because of the large areas to be treated. Insects are packed in release boxes, which are dropped by aircraft through machines designed to drop them at the required rate. Fruit flies are also generally released in paper bags by aircraft. In each case, it is necessary to determine the drop rate so as to arrive at the proper number of insects in a given area. Tsetse fly releases have been made from the ground. The release pattern must be developed for each insect to fit its behaviour, mating habits and location. The number of insects released must be sufficient to establish the desired ratio allowing for the actual effectiveness of the released males in searching out the wild females. The timing of releases is dependent on the insect, its survival rate in nature and, of course, its development cycle. The criteria for all these factors must be developed in small scale trials prior to the programme.

V–4.6. Quality control and handling of released insects

This is an area of utmost importance to the successful implementation of SIT. Insects of poor quality cannot perform satisfactorily in natural environments. In some cases, use of poor quality insects can be overcome by increasing the numbers released. This option is not always effective. The quality of insects can be lost in the colonization, rearing, sterilization and handling procedures. However, quality is probably one of the most important aspects of SIT.

There are many things that can be done to ensure the quality of the released insects. Not only does one need a strong, vigorous, highly productive colony to supply the stock for growing the insects to be released, but the insects that are released must also be of the same high quality. There are also many tests for measuring the quality of the colony as well as that of the released insects. Samples of released insects should be taken at the time of release to determine the loss in quality, rearing, sterilization, handling and transportation. The quality of insects in the rearing facility can be very different from that 1 day later, after a long trip to the release site or after release from the ground or air.

Testing methods can include crosses to determine the fertility and competitiveness of both colony and released insects. Insects can also be held for survival tests and be tested on flight mills to compare their flying ability to standards. The mating success of males and females, as well as the number of eggs per female and their hatchability, can be compared to standards. The size and weight of the reared insects

can also be followed routinely. All these methods serve as useful indicators of the quality of the reared and released insects and could well lead to the detection of potential problems before they become critical or disastrous. Nevertheless, the final answers are to be found in the results in the field where the programme is being conducted.

Unfortunately, standardized tests for insect quality for SIT have not yet been developed. These were first attempted by the Animal and Plant Health Inspection Service, USDA, for the Mediterranean fruit fly. Details are available from this group for those interested in this important development. Essentially, the group described standardized testing and criteria with standardized equipment for such factors as pupal size, per cent adult emergence and flight ability, stress and sex ratio. Additional developments are required for this and other species.

The screwworm programme developed a routine in which the stock strain used for rearing was replaced periodically. From time to time, questions were raised about whether the programme was actually working effectively. It was difficult to determine whether the problem lay with the quality of the insects released or whether individuals in the wild population had developed different behavioural or mating patterns. From a theoretical point of view, it is possible that the release of sterile insects could lead to the selection of survivors that would not be mated by the strain reared for years 'in captivity' because of their behavioural differences. A similar argument could be made that by moving the release programme into a new area, such as happened with the screwworm programme, different strains of the test insect would be encountered which would not intermate with the old colony strain. There are still many questions of this type that cannot be answered simply. The screwworm programme adopted an approach of periodically replacing (or, when possible, developing a new strain in the rearing factory to replace) the old strain. A new strain was established from captured wild flies at different locations within the release area. Once the new colony was self-sustaining, it would replace the old factory strain as the source stock for the programme.

Although successful release programmes can be developed and run with the information available, there is still much to be learned about the biology, ecology and dynamics of populations and the quality and behaviour of the individuals within these populations.

V–4.7. Evaluation techniques for SIT programmes

An effective SIT programme depends on a successful monitoring programme. Monitoring generally involves systems which follow the density of the insect population and the degree of sterility caused in that population throughout the programme.

Density can be monitored in a variety of ways, but usually involves a trapping system or some other means (trimedlure or other attractants for fruit flies, or the number of cases on sentinel animals or in herds of animals for screwworms) of fol-

lowing the changes in the relative number of individuals. The system differs with each insect. Scientists have a tendency to overemphasize the weakness and limitations of any survey or trapping system, while programme specialists tend to accept the results of the system. In any case, a trained field biologist is required to interpret the results obtained from the density survey methods. No density survey system is perfect in reflecting the true number of insects present at any particular time, therefore it is important that the best method be accepted and standardized. Improvements in survey techniques can be incorporated into the programme, but incorporation must include a method of translating the information from the old methods so that data from both the old and the new methods can be used to follow the density changes.

One of the serious limitations of insect survey techniques is their inability to sample insects when populations are at very low densities, the most important time for gathering information on managing or eliminating populations. What does it mean when traps collect no insects? Obviously, collecting no insects does not necessarily mean that there are no insects in the environment. Are the traps in the wrong location or habitat or among the wrong hosts? How many traps are needed per hectare or field? How long must a trap remain empty to assume that there are no insects in that location? Only experience and judgement can answer such questions at this time. There are no simple answers to this problem, yet it is of utmost importance in managing problem insect populations at low densities or in eliminating them.

Monitoring the degree of sterility in the natural population is an essential requirement for a SIT programme. It may not be possible to monitor the entire programme area for sterility, but the monitoring of hot spots or troublesome areas may provide information indicating insufficient numbers or poor quality of released insects and make all the difference between success or failure. Generally, adult females or eggs can be collected from wild populations, taken to a laboratory and the hatchability of eggs measured. With tsetse flies, it is possible to determine whether the wild females have mated with the released sterile males by dissecting and observing their reproductive organs. Mated females can be identified by mating scars or, in some insects, by the presence of spermatophores.

The increasing availability of microcomputers is a helpful tool in the storage, retrieval and analysis of monitoring data, particularly in large programmes. Systems for the common storage, transfer and accessibility of all the data are being developed. Software is available for mapping the programme areas and for superimposing the data on these maps. Such capability will allow more rapid data transfer and analysis.

V-5. CASE STUDIES

V-5.1. Examples of ongoing and/or successful programmes

V-5.1.1. Screwworm

V-5.1.1.1. Reasons for selecting this species

The screwworm was the species on which the original concepts of SIT were developed and proved. This insect was chosen because of the high losses it inflicted, its geographical limitations and its low populations. It was a serious problem to livestock production in the southeastern and southwestern USA, spreading to much of the southern central portion of the country during the summer time. The costs of control were high. Ranchers hired personnel to ride their ranges to find and treat screwworm cases; even then, some animals died. The screwworm occurs in relatively low density (hundreds rather than hundreds of thousands per square kilometre). Livestock producers were organized into producer groups which were willing to support the programme once research had shown the possibilities. In the USA, the screwworm problem existed in defined, although large, geographical areas. In the southeast of the country, the population was confined to central and southern Florida during the winter, even though it spread north as far as the Carolinas in the summer. In contrast to some other agricultural insect problems, where elimination of one species does not reduce treatment costs because of other insects, control of the screwworm solved a problem and saved ranchers' money.

V-5.1.1.2. General life-cycle

The screwworm is a dipteran insect with a sex ratio of 1:1. The insect is capable of strong flight and movement over hundreds of kilometres. Adults cause no economic damage. Males feed on carbohydrates for energy and cluster at mating sites. Females search out animals and lay a large number of eggs in any wound. They continue to lay eggs in these wounds as they enlarge. The larvae feed on live flesh in the wound. They then drop to the ground to pupate, with new adults emerging to continue the life-cycle. In favourable tropical and subtropical climates, they continue to develop throughout the year. They do not overwinter in unfavourable climates — reinfestation requires reinvasion from flight, movement on air currents or movement of infested animals.

V-5.1.1.3. Available control methods other than SIT

Insecticidal smears or powders can be applied to the infested wounds of animals. Treatment requires surveying all the animals for screwworm infestations

and individual treatment of the animals. In quarantine, animals can be run through insecticidal dipping vats containing a material such as Coral. An attractive bait containing Swass-lure and an insecticide can be applied by aircraft to reduce the number of screwworm in highly infested areas prior to the release of sterile males.

V–5.1.1.4. Research and development of the SIT strategy

The strategy was first conceived in the late 1930s in Texas by Knipling. Laboratory trials on sterilization by X and gamma radiation were started in the late 1940s and completed in the early 1950s. Complete sterility could be induced into both males and females by exposing either the pupal or adult stages to radiation, with no serious effects on the longevity, vigour or mating ability. Tests with different ratios of sterile to fertile males competing for mating with normal females in cages further confirmed the mating vigour of sterilized males. Field trials that released both sterilized males and females were attempted on the Island of Sannibell, Florida. The trials were inconclusive because of migration from the nearby mainland of Florida. Even though methods of mass producing screwworms for release were inadequate, continual progress has been shown in rearing on artificial diets, sterilization procedures for large numbers and aircraft release techniques. A large scale trial on the isolated Island of Curacao off the coast of Venezuela successfully demonstrated the principle of SIT for the first time under actual field conditions by eliminating the population there in four to six generations, as predicted by Knipling in his model. Since that time, there have been continual improvements in rearing, including diets and handling techniques, sterilization procedures and equipment, distribution techniques and programme management, so that recent programmes have been able to rear, sterilize and release 400–500 million insects per week. Knowledge of the biology, ecology and movement of screwworm populations has been increased by the execution of large scale programmes.

V–5.1.1.5. Status

Following the successful demonstration of the concept on the Island of Curacao, considerable interest and support have been shown by livestock producing groups in the southeast USA for an eradication programme in their area. The screwworm population was a self-sustaining, distinct and isolated one. An eradication campaign was considered and planned in spite of the fact that all the potential problems may not have been anticipated. The programme was started in 1957 and elimination achieved within 18–24 months. This successful programme led to a programme for the southwestern USA. The problem was quite different in this area because the screwworm existed in Mexico and reinvasion had to be prevented along the border between the two countries. The southwestern programme was begun in 1962 and, initially, met with considerable success, although some serious problems

also had to be faced. It is not possible in this Manual to describe all of the successes and problems encountered. For interesting details, the student is referred to a Symposium on Screwworm Eradication that appears in Miscellaneous Publication No. 60 of the Entomological Society of America. Successful reduction of screwworm in the southwestern USA led to a co-operative programme with Mexico, starting in 1977. Again, problems and crises arose, but the screwworm has now been eliminated from both the USA and Mexico and is confined to a barrier release zone south of Mexico. At the same time, a campaign was conducted for the elimination of the screwworm from the Island of Puerto Rico. This programme has also been successful.

In 1988, a screwworm outbreak was reported in the Libyan Arab Jamahiriya. This was the first time that this insect had become established outside of the Americas. An international programme has been launched to eradicate this outbreak before it spread to all of Africa, the Middle East and southern Europe. The flies were reared and sterilized in Mexico and then flown to Libya, where they were released throughout the infested area for 9 months (a total of about 1.3 billion sterile flies were used). Over a period of approximately 18 months, the programme has successfully eradicated the screwworm from Libya.

V-5.1.1.6. Future

The overall hope of people involved in the screwworm problem is that the programme can be moved down through Central America and that a new barrier zone can be established in Panama to prevent the reinvasion of areas freed from this insect. As research is continued it is hoped that further improvements in technology will be made. Since the inception of the programme in the southeastern USA, much has been learned. One meaningful advancement in the technology was the development of a highly effective additional control component — an attractive bait that provided further technology to be combined with SIT. It is hoped that future research will provide genetic means of sexual separation of the sexes prior to rearing and release. The almost unbelievable success of the screwworm programme led to the development of similar approaches for other insects. Each insect problem is different and requires original thought and development for implementation. The potential of this new approach to the management of insect problems from the area wide point of view needs to be seriously considered.

V-5.1.2. Fruit flies

V-5.1.2.1. Reasons for selecting these species

Tropical fruit flies are important pests of many fruits and some vegetables. The major species include the Mediterranean fruit fly (*Ceritidis capitata* (Wied.)), the olive fruit fly (*Dacus oleae* (Gmelim)), the Mexican fruit fly (*Anastrepha ludens*

(Loew)), the oriental fruit fly (*Dacus dorsalis* Hendel), the melon fly (*Dacus cucurbitae* Coq.) and the Queensland fruit fly (*Dacus tryoni* (Foggart)). The severity of damage to fruits requires many insecticide applications and extensive quarantines to prevent entry into non-infested areas. The costs of eliminating introduced species of fruit flies have been vast. Use of SIT offered great potential when combined with insecticidal bait sprays for eliminating introduced populations, eradicating existing populations and protecting non-infested areas.

V–5.1.2.2. General life-cycle

In cool regions, the Mediterranean fruit fly winters as a pupa or an adult, but in warmer regions it can reproduce throughout the year, resulting in 12 or more generations per year. Eggs are deposited in the fruit; a single female may lay up to 800 eggs. Larvae burrow in the pulp and then pupate in soil within 3–6 cm of the surface. The whole generation time is only about 30 days under optimum conditions.

V–5.1.2.3. Available control methods other than SIT

The most commonly and easily employed control technique used to reduce the numbers prior to release is a bait toxicant spray. The attractant in the spray may differ for various species of fruit flies. Currently, the toxicant most commonly used is malathion. A single bait spray should kill 90–99% of the adults present.

V–5.1.2.4. Research and development of the SIT technology

SIT technology is well developed for a variety of fruit fly species, including the sterilizing dose, the mass rearing technology, handling, and aerial and ground release methods. The Mexican programme against the Mediterranean fruit fly produced up to 500 million flies per week in the mass production facility. Island populations of the melon and oriental fruit fly in the Pacific region have been eradicated. A non-isolated population of the Queensland fruit fly has been suppressed. Several investigators have shown that non-isolated populations of the Mediterranean fruit fly can be suppressed.

V–5.1.2.5. Status

SIT technology has been used as a quarantine against the Mexican fruit fly. Along the USA–Mexican border, release of sterile insects has replaced use of pesticides to protect against invasion by this pest. The programme is very successful. SIT technology has been used in California against invasion by fruit flies. Recently, a large scale programme in Mexico eliminated the Mediterranean fruit fly and

prevented its northern movement. A programme in Guatemala against the same species is under way. The melon fly has been eradicated from most of Okinawa, Japan.

V–5.1.2.6. Future

It is anticipated that large scale programmes against fruit flies will continue to increase. The possibility of eliminating the Mediterranean fruit fly from Central America and Panama is under consideration. Programmes should develop in other areas such as North Africa.

V–5.1.3. Tsetse flies

V–5.1.3.1. Reasons for selecting these species

Tsetse flies are a serious problem in a wide area of Africa across the central part from east to west. They cause annoyance by their bites but, more importantly, they transmit trypanosomiasis to both people and domestic animals. Large areas in Africa have been difficult to develop, particularly for animal production, because of the presence of this group of insects. A variety of approaches to control and eliminate the tsetse fly has been developed. Such methods include use of insecticides and clearing of vegetation to remove the tsetse breeding habitat. Both methods, although effective, have disadvantages, either in the destruction of valuable trees and bush or the widespread application of pesticides to the environment. With both types of control it is difficult to remove the last segments of a population surviving after treatments. SIT provides the possibility of adding an effective, new and different control strategy. Integration of several control methods into management and elimination approaches offers great potential.

V–5.1.3.2. General life-cycle

Tsetse flies differ from most other insects in their development, life-cycle and reproduction and represent a unique system in the insect world. The eggs and larvae are retained in the reproducing female until the larvae have completed development. The larvae are then deposited into protected environments, where they crawl into soil and pupate. The number of progeny produced by a single female is low compared with other insects; progeny are produced individually. The life-cycle is about 2 months or more. Pupae in protected places in the ground require about 30 days to complete development. Males feed on carbohydrates for energy and require blood meals. The females require blood and about 15 days after emergence produce the first individual for larviposition. Additional individuals are produced singly at intervals of 10 days or more. Thus, the whole life-cycle requires about 60 days. The long life-cycle and low reproductive capacity of tsetse flies would appear to make them

poor candidates for SIT. For example, although tsetse females are relatively long lived, the slow reproductive process keeps the reproductive rate low. Some females may live long enough to produce six to eight progeny. It is generally accepted by tsetse specialists that the reproductive rate seldom approaches or exceeds 2. Difficulties in producing large numbers of testse flies are balanced by the low reproductive capacity of these species.

V–5.1.3.3. Available control methods other than SIT

Bush and vegetation clearing has been used for tsetse control. Destruction of favourable habitats or repeated area wide applications of residual pesticides control or eliminate these flies. Recently, trapping devices for the riverine species of tsetse have been shown to be effective for control. They are conical, blue traps to which the tsetse are attracted and caught, or rectangular pieces of blue cloth impregnated with an insecticide. Current work on attractants for the savannah type tsetse species suggests that attractive trapping devices may also be available. Use of these new devices for lowering the populations of tsetse fly followed by the release of sterile insects has been demonstrated to be an effective approach.

V–5.1.3.4. Research and development of the SIT strategy

Development of SIT against tsetse flies started in the early 1960s, following the successful programme against screwworms in the southeastern USA. However, its progress depended on the colonization of tsetse species and the development of effective methods of rearing large numbers of these flies. Excellent progress has been made in colonization and rearing and many species can now be maintained in the laboratory. Various methods of rearing them on animals, primarily rabbits or goats, porcine, bovine and freeze dried blood through artificial membranes, have been developed. Sterilization of tsetse flies with radiation or chemicals has been demonstrated. In Africa, research trials in the United Republic of Tanzania, Nigeria and Burkina Faso have shown that species of tsetse could be reared, sterilized and released in sufficient numbers to cause the density reductions predicted. In these early trials, immigration into treatment areas influenced the results.

V–5.1.3.5. Status

In both Nigeria and Burkina Faso, programmes have been completed using SIT after population reduction with attractant traps for the elimination of tsetse flies from large areas. Eradication has been demonstrated.

V-5.1.3.6. Future

Integration of the SIT methodology with improvements in conventional control methods such as insecticides and improved attractive toxicant devices, along with improvements in the rearing and release methods for more species of tsetse, suggests that this approach will continue to be developed and used. Tsetse specialists have amassed a large body of information on the ecology, biology and population dynamics of a variety of tsetse species. Regional rearing centres are envisioned in Africa. The approach is to be developed and used further.

V-5.1.4. Pink bollworm

V-5.1.4.1. Reasons for selecting this species

The pink bollworm was one of the early lepidopterous insects to be studied relative to the development of SIT. It is a serious pest of cotton in many parts of the world. It is an established pest in Texas and its movement westwards into New Mexico, Arizona and California created a serious problem for cotton production. SIT offered potential for the management and suppression of existing populations and the prevention of further spread.

V-5.1.4.2. General life-cycle

Adult moths are seldom seen because they are night fliers. They have been captured at altitudes of 900 m and can migrate long distances with favourable winds. The female can lay eggs within a day or two after emergence. She can produce up to 200–400 eggs, but lays them either singly or in groups of 5–10 all over the cotton plant. The eggs hatch and the larvae bore into squares or bolls. Pupation occurs in the upper 6 cm of soil. The generation time, under optimum conditions, is about 1 month. In cool, dry weather with a decreased food supply larvae can enter 'diapause' as resting larvae in small cocoons in partially opened bolls, lint, stored seed or soil.

V-5.1.4.3. Available control methods other than SIT

These are largely cultural and chemical control methods.

V-5.1.4.4. Research and development of the SIT strategy

Early research has shown that the pink bollworm can be sterilized. The dosages required were high, up to 400 Gy; further work has shown that the dosage can be reduced to 200 Gy. Field cage tests have indicated that the sterilized pink bollworms

were sufficiently competitive to suppress reproduction. The first pilot test was unsuccessful, probably due to migration of the adults. However, the problem of protecting the San Joaquin Valley in California from pink bollworms has been approached by releasing sterile insects. The sterilized moths were released on 25 000–50 000 hectares of cotton in the southern portion of the valley, normally at the rate of 100–200 moths per hectare per week. Traps using synthetic attractants were used to monitor the population. If native males were captured, the release was increased to about 2000 moths per hectare per week. Firm establishment of the pink bollworm has not occurred since the programme began in 1968.

V–5.1.4.5. Status and future

The quarantine programme and research and development on SIT and F_1 sterility are to continue.

V–5.1.5. Cotton bollweevil

V–5.1.5.1. Reasons for selecting this species

The bollweevil is one of the most serious pests of cotton. Its occurrence has required the use of large and repeated applications of pesticides. In some cases, such use may result in increased problems with other pests. A solution to bollweevil control could reduce the use of pesticides and the seriousness of some other pest problems. The bollweevil is such an important pest of cotton that use of SIT and integrated methods for its management have received high priority.

V–5.1.5.2. General life-cycle

The bollweevil overwinters in the adult stage in any kind of shelter. Adults straggle out of hibernation at the time when the cotton first appears (March) until early or late June, feeding on the tender terminal growth. Weevils prefer to attack the blossom buds or squares when they are 6 days old. They eat holes in them and lay eggs in the holes. Usually, they lay one egg per hole and one per square. Later in the season, they lay eggs in bolls, but squares are preferred. A female may lay from 100 to 300 eggs. The eggs hatch and the larvae feed inside the square, or boll pupation occurs inside the hollowed out cavity in which it has fed. The emerging adult eats its way out of the square or boll. Generation time can be completed in 25 days. There can be 2–3 or 8–10 generations per year; however, the straggling emergence of adults from hibernation renders the generations indistinct.

V-5.1.5.3. Available control methods other than SIT

There are a variety of control methods for use against the bollweevil. These include insecticides, attractive pheromone toxic baits and cultural practices. The availability of such a large number of control practices led to a pilot study of the combination of these methods with SIT.

V-5.1.5.4. Research and development of the SIT technology

The difficulty in sterilizing the bollweevil, either with radiation or chemicals, without seriously reducing the competitiveness of the adults limited the development and evaluation of SIT for this insect. Because of reduced vigour and competitiveness, emphasis was placed on a combination of control methods to suppress, manage or eliminate this insect. From 1971 to 1973, a pilot study was conducted in the mid-south USA that attempted complete suppression or elimination of this insect. The technology included three main approaches. Insecticides were applied in autumn to kill as many adults as possible before they entered hibernation. Up to four insecticide applications were used. The purpose was to reduce the population of overwintering individuals as much as possible. When adults emerged in the spring, pheromone trapping with the aggregating and sex pheromone, 'grandlure', was used to reduce the population further. The third component in the programme was the release of sterilized weevils. Although there was some disagreement on the results of the study, Knipling and his colleagues concluded that the study demonstrated the successful application of this new technology to the elimination of the bollweevil from a central core area in a non-isolated region very favourable to the development of the bollweevil.

V-5.1.5.5. Status and future

Large scale experimental trials and programmes started in North and South Carolina in the USA are continuing to demonstrate the successful application of this combination of new control technologies and SIT for the bollweevil.

V-5.2. Examples of programmes showing considerable research development

V-5.2.1. Stable flies and house flies

V-5.2.1.1. Reasons for selecting these species

Stable flies and house flies are important pests of people and animals. The eggs, larvae and pupae of both species develop in decaying organic matter, including the waste and excrement of humans and animals. House flies cause annoyance by

their presence and mechanically transmit diseases. Stable flies require blood meals and are a problem because of this behaviour. Their bites are extremely annoying to people and animals and the potential of mechanical transmission of a disease is of concern. Stable flies cause a reduction in weight gain in livestock and can drive people from preferred recreational areas. The two species have been studied in the laboratory and the field for many years and are easy to colonize and rear. The need for additional methods of control and extensive knowledge of their behaviour have made them good candidates for SIT. Their presence in extremely large numbers over large geographical areas and their ability to fly long distances or move on air currents have presented some problems to the immediate applicability of SIT technology for their control. However, the potential of combining SIT with ongoing control programmes or developing chemosterilant baits for treatment of natural populations has offered good possibilities.

V-5.2.1.2. General life-cycle

Although there are specific differences in the life-cycle and location of the major breeding sources of these two species, one can, in general, combine a description of their developmental cycle. Eggs are deposited in decaying organic matter. Each female can lay hundreds of eggs in her lifetime. Larvae crawl to the soil close by and pupate. The life-cycle under optimum conditions requires 2–3 weeks. Populations are characterized by high reproductive potentials, but under field conditions the growth potentials are greatly reduced by natural biological and environmental control factors. Studies to date have shown a growth rate no greater than about tenfold for house flies and three to fivefold for stable flies under natural field conditions.

V-5.2.1.3. Available control methods other than SIT

There are a variety of control methods for these types of flies. Source reduction — removal of the wastes in which these flies breed — has always been recommended, although its implementation may not always be good or sufficient. Management of wastes to preserve natural parasites and predators is now recommended in animal production. A number of insecticides can be applied in a variety of ways, including residual treatments, space sprays, aerial and ground applications, insecticidal baits, fogging devices, etc. The greatest problem in controlling house flies with insecticides is their resistance to widely used insecticides. Area wide control and management programmes, other than refuse removal provided by municipal authorities, are difficult to implement because of the lack of organizations.

V-5.2.1.4. Research and development of the SIT technology

Extensive use of the house fly as an experimental animal and studies of possible methods for its control led to a great deal of research on sterilization being carried out from the mid-1950s onwards. Sterilizing doses, with either radiation or chemicals, were developed along with related information. Several thousand compounds were screened and both female and male sterilants were discovered. The effective male sterilants were too toxic and hazardous for use. However, the principle of using sterilizing agents rather than killing agents for the treatment of natural populations was proved in field tests on islands and mainland areas. SIT technology for house fly control has not been put into practical use. For stable flies, developments have been made in the rearing methodology, sterilization and release methods for SIT. A large scale field trial on the Island of St. Croix (US Virgin Islands) demonstrated that SIT could be combined with insecticide applications and even innundative releases of parasites to reduce the island population of stable flies to near extinction.

V-5.2.1.5. Status and future

Further development of the use of SIT and related technology awaits the existence of large area wide programmes for the control and management of these insects.

V-5.2.2. Ticks

The worldwide importance of ticks as a pest of animals and people and their role in the transmission of a large number of diseases made them an early object of SIT development. Although sterilization techniques were developed and data accumulated, field trials with ticks in the Middle East region were not encouraging. Since the potential of sterile hybrid ticks as a genetic approach to tick control or management with the *Boophilus* species is covered in Section V-2.4 of this Manual, it is not repeated here.

V-5.2.3. Mosquitoes

V-5.2.3.1. Reasons for selecting these species

Mosquitoes have been the scourge of mankind for centuries. They have adapted to all types of habitat, from the clear snow melt waters of the Arctic to the hot, polluted waters of the tropics. They occur in such large numbers when conditions are right that their presence alone causes problems. Their significance comes from the deadly and devastating diseases they transmit. Organized programmes exist, either for protection from annoyance (as in the USA and other developed countries) or for the elimination of major diseases (such as malaria). These programmes

need new methods of control to combat such serious problems. Several important species of mosquito have served as excellent experimental material for years. Much is known of their biology and ecology. For all these reasons, a great deal of research has been done on sterilization, genetic control and field trials of SIT and related technology. The major limitations to the development of SIT are the immense number of mosquitoes that can be present in a natural population and the large number of species and species complexes that are of concern.

V–5.2.3.2. General life-cycle

Because of the many species of mosquito that are serious problems throughout the world, it is difficult to summarize them all or even list the species and types. This is left to the many texts on the subject. From the control point of view, the interesting, but oversimplified, grouping of types of mosquito can be done on the basis of their type of development. Some species are considered to be permanent water breeders. Eggs are laid on water that is more or less permanent and hatch within a few days, with no quiescent stage of eggs that can survive for long periods before hatching. Examples of this type of development are found in anopheline mosquitoes, which transmit malaria, and *Culex* mosquitoes, which transmit encephalitis. In general, for these mosquitoes eggs hatch in a few days and the larval forms continue development in water; the pupae also remain in the water. Males and females emerge to fly over the surrounding area. Movement and migration of mosquitoes vary with species, from short distances to many kilometres. Males feed on nectar and carbohydrates, but do not take blood. They are relatively short lived, but some individuals live up to 3–4 weeks. Females generally require blood to develop their eggs and feed on a variety of animals. In tropical areas, where they can develop year round, a generation time can be completed in 2–4 weeks, so many generations can be produced each year. In northern climates, there may be only one generation a year. The development and behaviour of different moquitoes are quite variable. However, other mosquitoes have a mode of development that makes the application of SIT more complicated. Eggs (such as those of the genera *Aedes* and *Psorophora*) are laid on damp soil. Eggs can lay dormant for months or even years until rains or flooding again supply the necessary water for the development of immatures. These types of mosquito make the release of sterile or genetically altered insects much more difficult.

V–5.2.3.3. Available control methods other than SIT

Over 100 years of research and control operations have led to a large number of methods of mosquito control; these can be grouped into categories. There are methods of protecting individuals from the bite itself, including screening, protective clothing and shelters, and repellents for personal use. The requirement of water for

the development of immature stages has led to a variety of ways of reducing mosquitoes, e.g. ditching, draining, water level control and clean-up of discarded containers and tyres. This approach is generally referred to as source reduction. Biological control agents play a large role in regulating the mosquito density naturally. Manipulation of one of these agents, mosquito fish, has been used for years in some locations. Two bacteria are now available commercially for use against mosquito larvae. Insecticides are available for application from the ground or the air as space sprays and residual treatments against both adults and larvae. Growth regulating material is available for use against larvae. Larvae can also be controlled by applying surface film agents. There are many methods of mosquito control and many types of application, equipment and formulation. Organizations directed primarily towards mosquito control exist in many countries.

V–5.2.3.4. Research and development of the SIT strategy

The literature on research and development of sterilization, SIT, chemosterilants and genetic mechanisms is too extensive to be covered here. It includes both laboratory and field studies and trials. A short summary of field trials of the SIT approach is given. In the late 1950s, the first trials of the release of sterile male mosquitoes were attempted in Florida. Releases of *Anopheles quadrimaculatus* in a small area in central Florida and *Aedes aegypti* in northern Florida were both unsuccessful in demonstrating the effectiveness of SIT. These negative trials either resulted from poor quality insects or the fact that the colonized mosquitoes were behaviourally different from the native, target mosquitoes. Following these original trials, various field trails in other countries were either unsuccessful or successful in the sense that they showed that sterility could be introduced into the native populations with a reduction in density. In India, a large project demonstrated that radiation sterilized males released in small villages did induce sterility in wild *Culex* mosquitoes. It also showed that genetic mechanisms such as cytoplasmic incompatibility and translocations could be induced in natural populations of this type of mosquito as well as *A. aegypti*. The field results were clouded by the immigration of mosquitoes from outside the test area and usable release technology was not developed. In Florida, release of radiation and chemically sterilized males into a population of *C. quinquefasciatus* on a small island demonstrated the successful reduction in and elimination of the island population. In El Salvador, a larger release programme of chemically sterilized *A. albimanus* males was similarly successful in a small isolated area. Increasing the size of the trial in another area that was not isolated again showed the capability of reducing populations, but immigration of mosquitoes was a problem.

The programme in El Salvador successfully used a male only strain for releases. This particular strain of mosquito was created by geneticists from a strain in which resistance to an insecticide was linked to sex, so that the males were resis-

tant to the insecticide while the females were susceptible. The females could thus be killed as they hatched from the eggs.

V-5.2.3.5. Status and future

Currently, no programmes on SIT or release of genetically altered mosquitoes are in use. The existence of many organized control programmes against both pest and disease carrying mosquitoes suggests that such technology would be helpful to execute programmes. However, mosquito problems generally do not exist as a single species problem; several species with different behavioural characteristics and breeding habitats are usually involved. Mosquitoes occur in large numbers, their distribution is not uniform and they move over large distances. Management of such populations by techniques based on sterility or genetic mechanisms has yet to be developed.

V-6. LABORATORY EXERCISES

V-6.1. Insect sterilization using gamma rays and chemosterilants

Use of sexually sterile males in the area wide management of insect problems is a technique that has been demonstrated for several important species. These laboratory exercises are designed to acquaint the student with some of the basic procedures necessary to initiate these investigations.

The following eight experiments require approximately 24 days to complete when starting with insect eggs. When adult insects of the right stage and age are ready, approximately 10 days are required, although certain manipulations have to be carried out every day. Each single experiment requires about 10 days to complete, but day to day attention is not necessary. Organization is essential. Plan to spend a full 4 hour laboratory period each day during the 10 day period if all the experiments are to be done at one time.

MATERIALS

The materials needed for all eight experiments are given below; the methods and procedures are given separately for each experiment.

(1) House fly and mosquito colonies
(2) Small cages and all the equipment necessary for handling the insects
(3) A cobalt or caesium source
(4) Chemosterilants

(5) The usual laboratory glassware, and other equipment
(6) A microapplicator
(7) House fly saline: 14.52 g of NaCl, 0.36 g of KCl, 0.29 g of $CaCl_2$ and 2.5 g of dextrose per litre of solution and 25 mL of 0.04M phosphate buffer of pH7.0
(8) Ringer's solution: 0.1 g of KCl, 0.0135 g of $CaCl_2$, 0.0120 g of sodium bicarbonate ($NaHCO_2$) and 0.75 g of NaCl; dissolve in distilled water to make 100 mL of solution.

PURPOSE

To determine the dosage of gamma radiation that produces 100% sterility in male and female house flies, as measured by the dominant lethals induced in the sperm or eggs produced, and also whether infecundity in the females is obtained when house flies are irradiated at the pupal stage.

PROCEDURE

Expose several hundred house fly pupae, between 2 and 3 days old, to each of the following dosages of radiation: 5, 10, 20, 30, 60 and 90 Gy. Place 100 pupae from each dose level in emergence cages. Allow the adults to emerge and separate the sexes after a period of 12 hours. When 3 days old, place 30 irradiated males from each group in cages containing 25 virgin females. Also place 25 irradiated females from each group in cages with 30 virgin males that are 3 days old. A control cage consists of 30 virgin males and 25 virgin females (all 3 days old). The five additional males in each cage are included to ensure that the males will not have to mate twice. Supply the cages with food and water. Check adult mortality daily. The females are allowed to oviposit for a period of several hours on day 7. Float the eggs from each group. Place about 300–400 eggs each in Petri dishes on moist, black cloth until all the eggs are plated out. Record the number of eggs in each dish. Determine the percentage hatch after 24 hours. To be truly precise, the female should be egged individually, even though this is tedious. The above method provides reliable results.

After the females have egged, it is important to check them for insemination as this may alter the results; unmated females often lay a few eggs. The procedure is relatively simple. The anaesthetized female is placed on her back, the abdomen is depressed with a blunt probe and the extruded ovipositor is gently pulled off with jeweller's forceps. In most instances, the spermathecae and other reproductive glands remain with the ovipositor. The severed tissue is then placed in a drop of modified Ringer's solution on a slide, and the spermathecae and ducts are dissected from the adjoining tissue under X10 to X15 magnification. The spermathecae and ducts are then placed in another drop of Ringer's solution on a slide, covered with

a cover slip and examined with a compound microscope. If the spermathecae are still whole, firm pressure on the cover slip with the tip of the probe will rupture them and any sperm present can easily be seen.

As in all experiments of this type, a control is run concurrently. Plot the lethality percentage obtained, as reflected in the hatch data, against the dose for both sexes.

SCHEDULE

Day 1 — morning: irradiate the pupae
— afternoon: place 100 pupae from each dose level into emergence cages
Day 2 — separate the flies by sex and place them in separate cages
Day 4 — set up the mating cages for each group
Day 7 — collect and count the eggs; determine the insemination rate
Day 9 — determine the hatch and plot data
Day 2 to day 7 — check the adult mortality in each cage.

QUESTIONS

(1) What effect did radiation have on the females? Explain.
(2) At what dosage was 100% dominant lethality produced in the males?
(3) Was there any effect on longevity at the higher dosages? If so, explain.

V-6.2. Effect of radiation on the sexual competitiveness of male house flies

PURPOSE

To determine whether irradiation with 60 Gy produced any deleterious effect in male house flies that would prevent them from being sexually competitive with non-irradiated males.

PROCEDURE

House fly pupae, 65–72 hours old, are exposed to a sterilizing dosage of 60 Gy. Newly emerged adult males and females are isolated before mating. Irradiated males, non-irradiated males and non-irradiated females at ratios of 1:1:1, 2:1:1 and 5:1:1 are placed in cages containing food and water and allowed to mate. To determine whether the males are sterile, a check cross of irradiated males and non-

irradiated females is run, and a cross of non-irradiated males and females is run concurrently to establish a natural sterility baseline. The crosses are made as follows:

Irradiated males	Non-irradiated males	Non-irradiated females	Ratio
—	5	5	0:1:1
5	5	5	1:1:1
10	5	5	2:1:1
25	5	5	5:1:1
5	—	5	1:0:1

The females are introduced into the mating cages 24 hours after the males.

The adults are allowed to mate; several days later, CSMA® larval medium[1] is placed in the cages and the females are allowed to oviposit. All eggs from each individual cage are floated in water, and 100 eggs, randomly selected, are placed on a square of moistened cloth. The cloth is placed in a Petri dish and 24 hours later the percentage hatch is determined. The eggs from each cage are evaluated as described in the schedule.

SCHEDULE

Day 1 — morning: the house fly pupae are irradiated at 60 Gy; this is done by the instructor

Day 2 — morning: all the flies that have emerged overnight are discarded because mating may have occurred; the irradiated and normal males and females that emerge in the morning are isolated by the instructor before copulation

— afternoon: the correct ratios of virgin irradiated and virgin non-irradiated males are placed in competition cages with adequate food and water; the females are held separately for 24 hours to allow all the males to become oriented in the cages

Day 3 — afternoon: introduce the females into the competition cages

Day 8 — afternoon: collect and count random samples of 100 eggs from each cage

Day 9 — afternoon: observe the eggs with a dissecting microscope to determine the number of hatched eggs; calculate the percentage sterility.

[1] Standard house fly larval medium manufactured by the Ralston Purina Company, St. Louis, MO, USA.

QUESTION

Is a dosage of 60 Gy effective for sterilization?

V–6.3. Effect of a chemosterilant on the reproductive potential of house flies

PURPOSE

To determine the effect of various concentrations of a chemosterilant, in this case tepa, on male and female house flies. The insects are treated in various ways — topically, by injection or through residues — and the efficacy of the various treatments is compared.

PROCEDURE

(a) Topical application: Groups of 30 unmated males and 25 unmated females (all 1 day old) are treated topically by means of a microapplicator at the following concentrations of the chemosterilant tepa in an acetone solution: 0.1, 0.2, 0.4, 0.6 and 0.8 μg/μL. The control group is treated with the solvent only. After treatment, the flies are placed in holding cages containing food and water. Twenty-four hours later, 25 virgin females are added to the cages containing the treated males, and 30 unmated males are added to the cages containing the treated females. When the females are 6 days old, they are allowed to oviposit over a period of 3 hours. The checks for egg hatch and insemination are carried out in the same way as described in Section V–6.1. Plot the percentage lethality, as indicated by the hatch, versus the concentration of the chemosterilant for both sexes.

(b) Injection: Using a microapplicator, the same number of male and female flies is injected with the chemosterilant tepa and dissolved in a modified Ringer's saline solution at the following concentrations: 0.1, 0.2, 0.4, 0.6 and 0.8 μg/μL. Two controls are used: one in which only the males are injected with the modified saline solution and another in which the females are also injected with it. The rest of the test is carried out in exactly the same way as in item (a).

(c) Residue application: Pint ($\pm$ ½ litre) jars are treated on their interior with 0.5, 1, 2.5, 5 and 10 mg/cm^2 of tepa and kept for 24 hours. Groups of flies, as described in item (a), are anaesthetized, placed in Petri dishes and covered with a card. When the flies have recovered from the carbon dioxide treatment, they are allowed to ascend into the inverted jars by removing the card. After an exposure period of 1 hour, the flies are permitted to escape from the jars into holding cages

containing food and water. On day 3, 30 virgin males and 25 virgin females are introduced into the respective cages for the crosses. The rest of the experiment is handled in the same way as in item (a).

QUESTIONS

(1) Compare the percentage dominant lethals obtained by the different application methods of the chemosterilant. Explain any differences observed. Which treatment is the most effective?
(2) What effect does the sex of the fly have on the results of the treatment? Why does it have this effect?

V–6.4. Effect of a chemosterilant on the sexual competitiveness of male house flies

PURPOSE

To determine whether any deleterious effect on sexual competitiveness is produced by the chemosterilant in the males, thereby reducing the impact of introducing such sterilized males into a natural population.

PROCEDURE

Newly enclosed adult male flies are offered fly food, containing 0.25% tepa, for 3 days. The treated males are confined, together with normal males, in ratios of 1:1, 2:1 and 5:1. Five females are introduced into each of the mating cages 1 hour later. To determine if the males are sterile, cross the treated males and normal females. Cross the normal males and normal females concurrently to establish a natural sterility baseline. Two replicate experiments for each ratio are set up using the following number of flies:

Sterile males	Normal males	Normal females
—	55	
5	—	5
5	5	5
10	5	5
25	5	5

The flies are allowed to mate; 3 days later, moistened CSMA larval medium is placed in the cages and the females are allowed to oviposit. The eggs from the individual cages are floated in water and 100 egg samples from each cage, selected randomly, are placed on moistened black cloth patches. On the following day, the number of hatched eggs is determined.

SCHEDULE

Day 1 — morning: the instructor prepares the treated diet; tepa is incorporated into the fly diet as acetone solution, the diet is left to dry overnight in the hood and then repulverized

Day 2 — afternoon: the instructor separates the flies by sex upon emergence to ensure that no mating occurs

— afternoon: students set up the cages with treated or untreated food and introduce the separated flies

Day 5 — afternoon: students remove the flies from the cages and make proper crosses

Day 9 — morning: the instructor initiates the egging procedure for competition tests

— afternoon: students collect the eggs and prepare 100 egg samples from each test cage

Day 10 — afternoon: students check the hatched eggs collected on the previous day and calculate the percentage sterility.

QUESTIONS

(1) What is the effect of increasing the ratio of treated males to normal males?
(2) Is the effect greater or smaller than in the theory? Explain.

V–6.5. Effect of a chemosterilant on the sexual competitiveness of male mosquitoes

PURPOSE

To determine if male *Ae. aegypti* mosquitoes sterilized by contact with a chemosterilant are sexually competitive with non-sterile mosquitoes.

PROCEDURE

One day old adults are confined for 4 hours in a tepa treated glass jar. After exposure, the treated males, the normal males and the normal females are confined together in ratios of 1:1:1, 2:1:1 and 5:1:1. To determine if the males are sterile, cross the treated males and normal females. Cross the normal males and normal females concurrently to establish a natural sterility baseline. Two replicate experiments for each ratio are set up using the following numbers of mosquito:

Sterile males	Normal males	Normal females
—	25	25
25	—	25
25	25	25
50	25	25
125	25	25

The mosquitoes are allowed to mate for 4 days, after which time guinea pigs are offered to the females for blood feeding. The guinea pigs are placed in the mosquito cages for 1 hour. Two days later, pint jars lined with blotting paper and containing 250 mL of tap water are placed inside the cages for oviposition. One week later, the jars are removed and samples of at least 300 eggs are cut from the paper and flooded; then the hatching ratio is determined.

SCHEDULE

Because the experiment is of such a prolonged nature, the instructor performs the initial phase in the laboratory.

Day 1 — morning: the instructor sets the eggs in order to obtain adults of the proper age

Day 8 — morning: the instructor separates the pupae from the larvae and later separates the newly emerged adults by sex to ensure that no mating occurs

Day 10 — morning: the instructor exposes the virgin adults to tepa treated jars for 4 hours

— afternoon: the instructor makes the ncessary mating crosses

Day 14 — morning: students feed the mosquitoes on guinea pigs, since a blood meal is necessary for oviposition

Day 16 — afternoon: students collect the mosquito eggs

Day 23 — afternoon: students collect the egg samples and check the hatching ratios.

QUESTIONS

(1) What is the effect of increasing the ratio of treated males to normal males?
(2) How does this effect compare with the results obtained in Section V-6.4 with house flies?

V-6.6. Effect of sterilizing treatments on the reproductive functions of house flies

PURPOSE

To determine: (1) whether a female has become inseminated, (2) the insemination rate of a sterile male compared with that of a normal insect, and (3) whether the sperm from a sterilized male remains motile in the female.

PROCEDURE

The female house fly is anaesthetized with carbon dioxide. It is then immobilized by placing the dorsum on a strip of masking tape fastened to a card. The abdomen is depressed with a blunt probe and the extruded ovipositor is slowly pulled off with jeweller's forceps. In most instances, the spermathecae and other reproductive glands remain with the ovipositor.

The severed tissue is then placed in modified Ringer's solution on a depression slide, and the spermathecae and ducts are dissected from the adjoining tissue under X10 to X15 magnification. The spermathecae and ducts are then placed in a drop of Ringer's solution on a slide, covered with a cover slip and examined with a compound microscope. If the spermathecae are still whole, firm pressure on the cover slip with the tip of a probe will rupture them and any sperm present will readily be visible in the ruptured area.

This experiment is particularly useful in determining the number of females inseminated in a wild population, as well as the number of times a male is capable of inseminating a female.

V-6.7. Effect of sterilizing treatments on the chromosomes of house flies

PURPOSE

To determine whether irradiation or treatment with a chemosterilant has any effect on the chromosomes. For this purpose, a simple technique has been developed

whereby consistently good chromosome preparations from the gonads of adult female house flies can be obtained.

PROCEDURE

(1) The tissue of the reproductive organs is dissected and placed on a 1.0% hypotonic solution of sodium citrate for 10 minutes
(2) The tissue is soaked in modified Carnoy's fixative for 5 minutes
(3) The tissue is briefly washed in 45% glacial acetic acid
(4) The tissue is placed in a drop of 45% glacial acetic acid on a glass slide
(5) The tissue is covered with a siliconized cover slip and a piece of filter paper
(6) Gentle pressure is applied to the filter paper to squash the tissue; then the cover slip is gently tapped
(7) The slide is placed on dry ice for 30 minutes
(8) The cover slip is removed with a chilled razor blade
(9) The slide is immersed in 95% ethyl alcohol in a coplin jar for 5 minutes
(10) A drop of Gurr's Natural Orcein and fast green stain is placed on the tissue while the slide is still damp
(11) The tissue is covered with a siliconized cover slip
(12) Excess stain is removed with filter paper
(13) The preparation is now temporary, but it will last for months if the cover slip is ringed with warm balsam Parafilm; to make the slide permanent, ignore step (13) and proceed to step (14)
(14) The slide is placed on dry ice again for 1–8 hours
(15) The cover slip is removed with a chilled razor blade
(16) The slide is bathed in 95% ethyl alcohol for 5 minutes and the excess alcohol is allowed to drain
(17) The slide is transferred to absolute ethyl alcohol for 1 minute and the excess alcohol is allowed to drain
(18) A drop of euparal is placed on the tissue
(19) A non-siliconized cover slip is applied to the tissue and excess euparal is absorbed with filter paper
(20) The preparation is allowed to dry for 14 hours before use.

V–6.8. Effect of anoxia on the radiation sterilization of insects

An inadequate supply of oxygen during the radiation sterilization of insects frequently results in incomplete sterilization and unpredictably erratic sterilization. Anoxia can occur as a result of overcrowding in air tight containers for relatively long periods of time. When anoxia exists, cell division in proliferating tissues slows down or comes to a halt, a state which tends to nullify the sterilizing effects of radiation.

PURPOSE

To show that, for insects in the state of anoxia, larger dosages of gamma radiation are necessary to cause sterility than for insects with an adequate supply of oxygen.

MATERIALS

(1) House fly pupae (7500), 1 day from emergence
(2) Gastight containers (canisters, small jars, plastic bags)
(3) Cylinders of carbon dioxide, nitrogen and oxygen
(4) Cages (15)
(5) Adult fly food and water
(6) Petri dishes (15)
(7) Filter paper of the same size as the Petri dishes
(8) Three binocular microscopes
(9) Three lengths of rubber tubing for the gas
(10) Plastic bags
(11) Pressure reducing valves.

PROCEDURE

Divide the house fly pupae into 15 lots of 500 flies each. Make three groups of five lots each, one group for each type of gas. Work with one gas at a time. Place the pupae in unsealed gas tight containers and put the containers in a plastic bag. Fill the bag with gas (5 L/min for 5 minutes, and then 1 L/min for 25 minutes). Remove the gas hose and close the bag opening. Place the lids on the gas tight containers without disturbing the gas concentration and use different exposures for the five lots within a group, as shown below:

Group 1 lot (pupae)	Group 2 lot (pupae)	Group 3 lot (pupae)	Irradiation (Gy)
1	1	1	10
2	2	2	20
3	3	3	30
4	4	4	40
5	5	5	50

After irradiation, place the pupae in cages with food and water and a properly prepared label, stating the kind of gas used and the radiation dose. Five days after irradiation, the emerged flies should be egged. Place the eggs on moist filter paper in Petri dishes and label. Twenty-four hours after incubation, count the hatched and unhatched eggs and determine the percentage hatch.

QUESTIONS

(1) Was the dose response linear or exponential for each of the various gases?
(2) Which gas caused the greatest depression of fertility? Which gas caused the least?
(3) What are the ways of preventing anoxia when a large number of insects is being irradiated?

Part VI

GLOSSARY OF SOME BASIC TERMS AND CONCEPTS

Absorbed dose. The mean radiation energy imparted by ionizing radiation to a unit mass of the irradiated material. Expressed in rads, grays or joules per kilogram (see **units**).

Absorber. The layer of matter inserted between the source and the detector, thereby causing a reduction in the radiation transmitted.

Absorbing event. An interaction, such as photoelectric absorption, the **Compton effect** or **pair production,** by which photon energy is transferred to matter.

Abundance. The relative concentration of the individual isotope in a mixture of isotopes of the same chemical element.

Activation analysis, neutron. A method for determining trace amounts of a chemical element, based on the assay of one or more radioisotopes produced by neutron bombardment (see also **neutron activation equation**).

Activity. The amount of a radioactive substance expressed as the number of disintegrations taking place per unit time, expressed in becquerels or curies (see **units**). *The count rate is a non-absolute measure of activity dependent on the counting efficiency.*

Alpha particle, α particle. An alpha particle comprises two neutrons and two protons (a helium nucleus), ejected at high speed from a disintegrating alpha active nucleus.

Amplifier. A device whose output is a magnified function of its input signal, drawing its power from a source other than the signal. In counting contexts, it is usually an electronic unit.

Annihilation. The reaction of a particle and its anti-particle, whereby they both cease to exist as such. For instance, a positron is annihilated together with an electron, and two photons of 0.51 MeV energy are created in their place.

Anti-coincidence circuit. An electronic circuit that rejects pulses arriving at two detectors in coincidence, i.e. within a very short interval of time, typically 1 μs.

Atomic number, Z. An integer that expresses the positive charge on the nucleus of an atom and hence defines the position of a chemical element in the periodic table. **Z** is equal to the number of protons in the nucleus of any atom that belongs to element number **Z**.

Attenuation coefficient, total. The fractional decrease in the number of particles or photons per unit distance traversed in a medium as a result of interactions in that medium (see Section II–1.5). This concept includes absorption and **scattering.**

Attenuation factor. The ratio of the photon intensity after traversing a layer of matter to its intensity before, for example, matter placed in the path of a radiation beam for radiation protection purposes (a reduction factor).

Auger electron. The fast electron ejected as the result of the interaction between an X ray photon and an orbital (valence) electron, resulting in non-radiative transition of an atom to a lower excited electronic energy state (see **isomeric transition**).

Autoradiography. The method for recording the two dimensional distribution of radioactive material in an object (or a thin section thereof). Performed by placing the surface of the object in contact with photographic emulsion which, by blackening on development, indicates where particles have been emitted by radioactive nuclei.

Average liftime, see **turnover time.**

Background. Signals not emanating from the tracer but from the surroundings, sample holder, etc. which the measuring system does not discriminate from the tracer signals. The background count rates may be determined with a blank (zero tracer) sample in place. The net count rate of the tracer in any sample is obtained by subtracting the background from the gross sample count rate.

Bandhead. The optical spectra of molecules appear as groups of closely spaced and partly overlapping lines; these groups are called bands. At one end, the band rises to a relatively high intensity peak that falls off sharply to zero; this is called the bandhead. The synonym is band edge.

Becquerel (Bq). The SI derived unit of **activity**, being one radioactive disintegration per second.

Beta particle, β particle. A β^- particle is a high speed (negatively charged) **electron** ejected from a nucleus during radioactive decay. A β^+ particle is a high speed positron (positively charged electron) ejected from a nucleus during radioactive decay.

Biological half-life, see **half-life, biological.**

Body burden, permissible. The maximum acceptable amount of a given radionuclide that may be continuously contained in the human body.

Bremsstrahlung. The photon radiation emitted by fast moving charged particles that are sharply decelerated or deflected by an electric or magnetic field. (The original German word means, literally, braking radiation.)

Carrier. (1) A given substance added in a ponderable amount to a trace amount of test substance to facilitate chemical or physical separation or manipulation; and (2) a ponderable amount of stable element mixed with a radioisotopic tracer of that element.

Carrier free. The designation for a radioisotope of a given element prepared by transmutation and which is free, *or practically free*, of any stable isotope of that element.

Cerenkov radiation. The light emitted when a charged particle travels through a medium at a speed that exceeds the speed of light in that medium.

Channels ratio method. A method of correcting for quench in **liquid scintillation counting** by establishing a relationship between: (1) the increased ratio of lower to higher energy pulses due to quenching; and (2) the reduced counting efficiency due to quenching.

Closed system. A kinetic system in which no transfer of matter (or energy) to or from its surroundings takes place.

Coincidence circuit. An electronic circuit that rejects single pulses and passes a one pulse signal only when it receives two pulses (one from each of two detectors) within a specified short interval of time, e.g. 10^{-6} seconds (i.e. *simultaneously* or in coincidence).

Compton effect. The interaction (*elastic, incoherent scattering*) between a (high energy) photon and a free or loosely bound electron, whereby the photon suffers a change in direction and a loss in energy, and the electron gains an amount of energy equivalent to that lost by the photon (see **scattering**).

Compton region. The lower energy part of a gamma spectrum caused by photons that undergo a Compton interaction in the detector and then escape total absorption.

Contamination. The dirtying or soiling of objects (e.g. the body, clothes, samples, equipment) with undesired (radioactive) material.

Continuous spectrum. A spectrum that exhibits no detailed structure (lines or bands), but represents an unbroken sweep of intensity or activity over the wavelength or energy range considered.

Cosmic ray. The radiation originating outside the Earth and its atmosphere, capable of producing ionizing events in interactions with matter.

Counting efficiency, counting yield, ϵ. The fraction of disintegrations counted. Expressed as a percentage, it is equal to the number of counts registered by the counter per 100 disintegrations in the source (see Section III–2.1.).

Count rate. The number of counts registered per unit of time, e.g. counts per second (counts/s).

Cross-section. A value expressing the probability of a given reaction occurring, for example, between a thermal neutron and a specified atomic nucleus. The dimensions of cross-section are area, expressed in units of the order of 10^{-28} m^2 (10^{-24} cm^2); a special unit, the barn (b), is often used (see **units**).

Curie (Ci). The special unit of **activity** which is being superseded by the becquerel. The curie is defined as: 1 Ci = 3.7×10^{10} disintegrations/second (dis/s) = 3.7×10^{10} Bq.

Daughter nucleus. The nucleus to which a given radioactive nucleus is transformed when it decays.

Dead time. The period following a discharge during which an ionizing particle entering the sensitive volume of a GM tube will not initiate a new pulse (see also **resolving time**).

Decay constant. The fraction of the (large) number of radioactive atoms of a given radionuclide that decays in unit time.

Decay scheme. A diagram depicting the process(es) involved in the decay of a given radionuclide. The half-life, the branching ratios, the energies and the type of particle(s) and/or photon(s) as well as the daughter nuclide(s) involved in the transition(s) are shown in schematic form (see Section II–1.5).

Density thickness, see **mass per unit area.**

Differential discriminator. The electronic circuit, used in pulse height analysers, which only passes pulses above a certain minimum (voltage) value and below a higher value. Pulses above and below the limits are rejected.

Discrete (energy) spectrum. A spectrum consisting of a number of well resolved lines.

Discriminator, pulse height. The electronic circuit designed to pass all pulses above a certain minimum (voltage) value and to reject all those below the limit.

Dose equivalent. The product of an **absorbed dose**, D, **quality factor**, Q, and N, the product of other modifying factors, i.e.: H = DQN.

Effective half-life, see **half-life, effective.**

Einstein's equation. A quantitative relationship expressing the transformation of mass to energy and vice versa (see Section II–1.2).

Electron. A small particle having a rest mass of 9.107×10^{-28} g, an atomic mass of 1/1837 of a hydrogen atom, a diameter of 10^{-12} cm and carrying one elementary unit of positive or negative charge. The positively charged electron is called the positron, while the negatively charged electron is usually termed the electron (the term negatron is rarely used). (See also **beta particle**.)

Electron capture (EC). The mode of radioactive decay of an atom in which its nucleus captures one of its orbital electrons, whereby a **proton** in the nucleus is transformed to a **neutron** and a neutrino is emitted.

Electronvolt (eV). A unit of energy equal to the energy acquired by a singly charged particle when it is accelerated in a vacuum through a potential difference of 1 V (see **units**).

Enrichment (atom per cent excess). The abundance of a given stable isotope in a labelled sample minus the abundance of that isotope in nature.

Epicadmium neutrons. Neutrons with energies greater than those readily absorbed by cadmium (greater than approximately 0.5 eV).

Excitation. The transition of a nucleus, an atom or a molecule to an energy level above that of its ground state.

Exposure. The absolute value of the total charge of ions of one sign produced in air when all the electrons (positrons and negatrons) liberated by the photons (usually X rays or gamma rays) in a volume element of air of unit mass are completely stopped in air. Expressed in units of coulomb per kilogram (C/kg) or, more commonly, röntgen or gray (see **units**).

External standard. (1) A radioactive source permanently installed in a liquid scintillation counter in such a way that, by unshielding it, the source can be brought to irradiate the sample for the purpose of evaluating counting efficiency; (2) any suitable radioactive source that is defined accurately and can be used as a standard to calibrate a measuring system.

Film badge. A small photographic film in a light tight envelope worn by personnel to register exposure to ionizing radiation.

First order kinetics. A process in which the rate of change of the variable is proportional to the magnitude of the variable present at any time. If the variable is P, then: $dP/dt \propto P$, i.e. $dP/dt = kP$, where k is termed *the first order rate constant*. This behaviour is common to many processes, e.g. **the radioactive decay law**, many biological and open compartment processes (see Eqs (II–1) and (II–14)). The integrated equation is of the form $P = P_0e^{-kt}$, giving the basis for constants of the type **half-life** (q.v.), **half value thickness** (q.v.).

Fission. The splitting of a heavy nucleus into two (or rarely more) lighter nuclei of about equal masses, whereby one or two neutrons and a relatively large amount of energy (including some gamma radiation) are released. Fission is usually preceded by neutron capture.

Fluence, particle. The time integral of particle flux, being the number of particles incident per unit area during a given time interval.

Fluorescence. The **luminescence** that exists only as long as the energy source is exciting the phosphor. (Phosphorescence is **luminescence** that continues after the exciting source is removed.)

Flux, particle. The number of particles incident per unit area and per unit time. It is identical with the product of the particle density and the average speed.

Gamma photon, γ photon. A gamma photon is an electromagnetic energy packet emitted at the speed of light from an atomic nucleus.

Gamma radiation. The electromagnetic radiation emitted in the process of nuclear transition or particle annihilation.

Gas amplification, gas multiplication. The production of secondary ionization by primary ions (electrons) produced in a gas filled detector across which the voltage is sufficiently high (regions III and IV in Fig. III–2), thus adding to the total charge collected.

Geiger–Müller tube, GM tube. A gas filled detector across which the voltage is so high that gas amplification has reached saturation (region IV in Fig. III–2).

Geometry. A term used loosely to designate the arrangement in space of the various components of an experimental or measuring system. This designation includes the positions of the source, detector and any intervening absorber. The solid angle around the source that is irradiated or measured is also sometimes indicated, e.g. '2π geometry'.

Glove box. An area in which one can handle small quantities of radioisotopes and avoid contamination of the hands. It comprises a dust tight box, fitted with windows and gloves, in which manipulations with hazardous alpha or beta active material can be carried out.

Gray (Gy). The SI derived unit of **absorbed dose** of **ionizing radiation**. It is defined as being equal to 1 joule of energy absorbed per kilogram of matter undergoing irradiation (see **units**).

Ground state. The lowest energy level of an atom, molecule or nucleus (with respect to the emission of photons); it is used to designate the normal stable state in some contexts.

Half-life, biological. The period of time during which a given biological organism physiologically eliminates half the amount of a given substance that has been introduced into it when the rate of elimination is approximately exponential (see **first order kinetics**).

Half-life, effective. In a biological organism, the time taken for the activity of a radioisotope to decrease to half its value as a result of both radioactive decay and physiological elimination when the rate of removal is approximately exponential (see **first order kinetics**).

Half-life, radioactive. For a single radioactive decay process, the time required for the (radio)activity to decrease to half its value by that process.

Half-value layer, half-value thickness. The thickness of a given material which, when introduced into the path of a given beam of radiation, reduces the value of a specified radiation quantity by half. (In general, the thickness that reduces by half the intensity of a beam of photons of given energy.)

ICRP. International Commission on Radiological Protection, Sutton, Surrey (UK).

ICRU. International Commission on Radiation Units and Measurements, Washington, DC (USA).

Infinite thickness. The saturation thickness of solid samples with respect to the **self-absorption** of beta particles from a given isotopic tracer. The **count rate** of a given sample material of infinite thickness is independent of variations in thickness and is proportional to the activity concentration in that material.

Integrated dose. The dose obtained by summing up all the individual dose contributions over a period of time.

Internal conversion (IC). A process whereby an atomic nucleus, that would otherwise emit a gamma photon, de-excites by interacting with one of its own orbital electrons (usually in the K, L or M shell), the electron being ejected at high velocity. The ejected electron (termed conversion electron) has a kinetic energy, which is the difference between the transition (de-excitation) energy and the binding energy.

Internal standard. A known amount of tracer activity (in becquerels, i.e. dis/s) added in a non-quenching form to a liquid scintillation sample in order to determine the counting efficiency of the **tracer** in that sample.

Inverse square law. A law stating that the intensity of the radiation emanating uniformly over the full solid angle (4π) from a source in a vacuum *decreases* proportionally and monotonically with the square of the distance from the source, i.e. inversely proportional to the square of the distance.

Inverse tracer dilution. A method of isotopic dilution analysis in which a known amount of **trace** of natural isotopic composition is added to a sample containing a **tracee** that is labelled.

Ion exchange resin. An artificial solid material that can adsorb ions and in solution exchanges these with other ions of the same sign.

Ionization. The production of ion pairs (of which one may be an **electron**).

Ionization chamber. A device which detects **ionizing radiation** (particles which produce primary or secondary ionization) by means of the ionization produced in a volume of gas. The sensitive volume of the instrument is filled with air or other suitable gas and the voltage across the electrodes is high enough for all the ions formed to be collected on the electrodes, but not so high as to cause gas amplification (q.v.). It can measure high and low dose rates and, for biological measurements, can be designed to give *tissue equivalent* measurements.

Ionizing radiation. Particles or photons of sufficient energy to produce ion pairs (of which one may be an electron) on passing through matter.

Ion pair. Most often an ion pair is formed by the division of a neutral atom into a free electron (negative ion) and an atomic residue (positive ion).

ISO. International Organization for Standardization, Geneva (Switzerland).

Isomer, nuclear. Nuclides having the same **mass number** and **atomic number**, but occupying different nuclear energy states.

Isomeric transition (IT). The decay with a measurable half-life of an **isomer** (in a metastable state) to an **isomer** of lower energy. De-excitation may occur by emission of a gamma photon or an internal conversion electron, with emissions of X rays and/or Auger electrons.

Isotope dilution. A method of analysis in which a known amount of **tracer** is added carrier free or together with a known amount of **tracee** (carrier) to a sample containing a **tracee** of natural isotopic composition.

Isotopes. Nuclides having the same **atomic number** (i.e. the same chemical element), but different mass numbers (i.e. same **Z**, different **N**).

K capture, see **electron capture.**

LET (linear energy transfer), restricted linear stopping power, L_Δ. The LET of charged particles in a medium is the energy loss due to collisions with energy transfers less than some specified value Δ per unit distance traversed by the particle. It is recommended that Δ be expressed in electronvolts (eV) (L_∞ = linear collision stopping power).

Liquid scintillation counting. A method of counting radiation, especially beta particles of low energy, by mixing the sample with an organic solvent containing an organic scintillator (i.e. an organic compound that transforms part of the energy dissipated into a flash of light). The light flashes emitted are registered by photomultiplier tubes.

Luminescence. The property of some substances of emitting light in response to **excitation** (excluding incandescence). Radiofluorescence, i.e. luminescence in a phosphor because of the prompt release of the energy absorbed from ionizing radiation, is termed scintillation (q.v.). Absorption of such energy with subsequent release only after illuminating or heating the phosphor is termed radiophotoluminescence or radiothermoluminescence, respectively, and forms the principle underlying the operation of certain types of dosimeters.

Mass number. The total number of protons and neutrons ($Z + N$) in the nucleus of any given nuclide. The mass number, A, is the nearest whole number to the atomic weight (mass) of the nuclide.

Mass per unit area. A parameter used for specifying the thickness of the **absorber** that, for a given radiation, is independent of the absorbing material itself over a wide range. It is obtained by multiplying the absorber thickness, i.e. the path length through the absorbing medium, by the density of the medium. There is no agreement on a name for this, it being called variously: surface density, density thickness, area density, mass thickness and, simply, thickness. The dimensionally descriptive title is used in this Manual.

Mass spectrometer. An instrument that separates (ionized) atoms or molecules according to mass (actually mass divided by ionic charge), these being focused on to electrodes and registered electrically. The record is a spectrum indicating the relative intensity as a function of mass divided by the charge of each species.

Mass thickness, see **mass per unit area.**

Monoenergetic radiation. The radiation that comprises photons or particles all having the same energy.

Natural isotope. The naturally occurring nuclide of any given chemical element. A natural isotope is either: (1) stable; (2) of very long half-life; or (3) the descendant of a very long lived precursor.

Natural uncertainty. Statistical deviations from a mean value of activity due to the natural randomness in time inherent in the decay of radioactive nuclei (see Section III–2.2).

Net count rate. The **count rate** of a radioactive sample minus the **count rate** of the background (with or without a blank sample in place for the background measurement, as appropriate).

Neutron. A nuclear particle with no electric charge and a mass approximately the same as that of a **proton**. Free neutrons are unstable, and decay with a lifetime of about 13 min to ^{1}H (i.e. **proton**) and a β^- **particle**.

Neutron activation analysis, see **activation analysis.**

Neutron activation equation. An equation relating the **activity** produced to the neutron flux, cross-section, number of target nuclei and duration of neutron bombardment.

Neutron number, N. The number of neutrons incorporated in the nucleus of a given nuclide.

Normal distribution, Gaussian distribution. Symmetrical arrangement of replicate values that deviate randomly on either side of a mean value. This bell shaped distribution is described mathematically by the Gaussian equation.

Nucleon. One of the types of particles, comprising protons and neutrons, constituting an atomic nucleus. Hence, a commonly used name for protons or neutrons belonging to a nucleus.

Nuclide. Any given atomic species characterized by: (1) the number of protons, **Z**, in the nucleus; (2) the number of neutrons, **N**, in the nucleus; and (3) the energy state of the nucleus (in the case of an **isomer**).

Open compartment. A kinetic compartment that is free to exchange matter (or energy) with its surroundings.

Open system. A system of kinetic compartments containing at least one open compartment.

Pair production. The transformation of a high energy photon into an **electron** and a positron.

Photocathode. A device that liberates electrons when struck by photons of light.

Photoelectric absorption. A process by which an atom completely absorbs a **photon**. The energy of the **photon** is transferred to an (inner) orbital electron that is liberated from the atom.

Photospectrometer (emission spectrometer). An instrument that disperses the light from a source and produces a spectrum indicating relative intensity as a function of wavelength.

Pocket dosimeter. A robust electrometer type instrument designed to be worn in the pocket to register the integrated dose of penetrating (photon) radiation to which a person has been exposed (see Section III–1.2.1).

Point source. A source of radiation with dimensions that are small compared with the distance from the source to the point of observation.

Poisson distribution. The (asymmetrical) statistical arrangement of replicate observations of the (relatively small) number of events (for example, radioactive disintegrations) that occur when the number of 'tries' included in the single observation is extremely great and the probability of the event occurring in any one 'try' is small but constant. In the case of Poisson distribution, the standard deviation of the observed number of events can be mathematically predicted; it is equal to the square root of the mean number of events observed.

Primary ionization. The ionization produced by primary particles on interaction with matter. In counting tubes, it is the ionization produced by incident radiation, without gas amplification.

Proportional region. Pertaining to a gas filled detector, it is the high voltage region in which the size of the output pulse is proportional to the amount of input energy, i.e. the region in which the amplification is linear (see Section III–1.2.4).

Proton. A nuclear (elementary) particle with an atomic weight (mass) of approximately one unified atomic mass unit, carrying one elementary unit of positive charge.

Pulse height. The magnitude, usually measured in terms of the maximum voltage, of an electronic pulse.

Pulse height analyser. An instrument that uses voltage level discriminators to sort electronic pulses according to height (i.e. maximum voltage).

Pulse size. A more general term, sometimes synonymous with **pulse height** and sometimes referring to the area under a pulse.

Quality factor, Q. A factor, dependent on **LET** (i.e. linear collision stopping power), by which the absorbed dose of a given type of radiation is multiplied in order to obtain a quantity that expresses **dose equivalent**, H, used for *radiation protection*. Q is formulated currently as a continuous function of the linear collision stopping power (see also **LET**).

Quenching gas. The gas that is added to a **GM tube** to prevent multiple or continuous discharge.

Quenching of light. In liquid scintillation counting, the reduction in light output from the scintillator due to chemical inhibition (chemical quenching) and/or chromatic absorption (colour quenching).

Rad. The special unit of **absorbed dose** of **ionizing radiation** which is being superseded by the gray. The rad is defined as: 100 rad = 1 J/kg = 1 Gy.

Radioactive decay law. A law of nature that may be stated as follows: the probability of a radioactive atom decaying within a unit of time is a constant, characteristic of the particular radionuclide, but independent of its age and surroundings (see Section II–1.3).

Radioactivity, see **activity.**

Radiocarbon dating. A method of dating objects whose life was terminated hundreds or thousands of years ago. The method is based on: (1) the natural abundance of ^{14}C in the past; (2) the known radioactive half-life of ^{14}C; and (3) the fact that, on death, an organism ceases to exchange carbon with its surroundings (see Section II–1.4).

Radiotoxicity (as opposed to chemical or biological toxicity). The quality of a radionuclide that is poisionous on entry into the body of a person, an animal or other living organism because of the ionizing radiation emitted by the nuclide.

Range. The distance that a particle penetrates a given substance before its kinetic energy is reduced to such a level that it no longer causes ionization. As used for alpha and beta particles of a given (maximum) energy, the maximum distance they penetrate a given substance in a specified direction (since β^- particle tracks, in particular, are tortuous).

Rate constant. The fractional rate of reaction in **first order kinetics** (q.v.).

Rem. The unit of **dose equivalent** used for radiation protection purposes only (see Table I–I).

Resolving time. Relating to a detector and a counting system, in particular a GM counter, it is the short period of time that elapses after the detector has been activated by a particle or **photon**, before the counting system is capable of registering another particle or **photon**. It comprises the **dead time** of the detector and any further delay due to gradual **pulse size** recovery and delays in the electrons (see Section III–1.2.5 and **dead time**). Since in a well designed system the latter is relatively small, the resolving time usually approaches the **dead time.**

Röntgen (R). The special unit of **exposure**. There is no derived SI unit for this quantity, and the interrelationship is given in terms of coulomb per kilogram of air: $1\ R = 2.58 \times 10^{-4}$ C/kg (exactly).

Scattering (see also **Compton effect**). The change in direction of a particle or **photon** caused by a collision or interaction with another particle, an atom or a system. (1) If the momentum and kinetic energy of the incident and scattering particle are conserved, the process is termed **elastic scattering**. (2) If some of the total kinetic energy raises a target atom or nucleus to a higher energy state, the process is termed **inelastic scattering**. (3) If the scattering centres act such that the scattered particles bear a phase relationship to one another, **coherent scattering** results; for example, a crystal lattice scatters particles in such a way that a diffraction pattern results. (4) If no phase relationship exists, the **scattering is incoherent**; for example, **Compton scattering** (see **Compton effect**). (5) If the scattering angle is less than 90°, the term **forward scattering** is used; if it is greater than 90°, the term **back scattering** is used. (6) A photon or particle may undergo **single or multiple scattering**.

Scintillation. A flash of light produced in a phosphor by an ionizing event.

Scintillation crystal. A crystal in which a *significant* fraction of the energy absorbed from **ionizing radiation** is re-emitted as light in the visible or near UV region.

Scintillator. Any phosphor used for the detection or measurement of **ionizing radiation** by the scintillation produced.

Secondary ionization. The ionization produced by ions or particles resulting from primary ionization. For example, in counting tubes, the primary ions formed by incident radiation are accelerated at high voltages to cause *secondary ionization* in a filling gas; the effect is termed gas amplification.

Self-absorption. The absorption by a radioactive sample of some of its own radiation, whereby the number of externally detectable particles or photons is reduced.

Semi-conductor radiation detector. A solid state detector in which an ionizing ray produces electron, 'hole' pairs (the functioning thus being analogous to that of a gas filled ionization chamber). When cooled in liquid nitrogen (to reduce the thermal noise), a semi-conductor detector has a high power of photon energy resolution.

Sievert (Sv). Equal to 100 rem (see **units**).

Specific activity. Pertaining to a radioactively labelled sample, it is the amount of tracer activity per unit amount of **tracee**. Expressed in curies per mole, becquerels per mole, curies per gram, etc.

Stable isotope. A naturally occurring isotope, with no observable radioactivity.

Steady state. With respect to a specified substance, the characterization of a dynamic system in which the input and output rates of the substance are equal.

Technical uncertainty. Statistical deviations in replicate observations as a result of instrumental and operational variations and/or errors.

Thermal neutrons. Neutrons that are in thermal equilibrium with their surroundings, i.e. having kinetic energies corresponding to the temperature of the surroundings.

Total absorption peak (as opposed to the **Compton region**). A peak in a photon energy spectrum arising from events in which the detector completely absorbs the energy of the **photon**.

Total attenuation coefficient, see **attenuation coefficient.**

Tracee. The test object, element or compound that the investigator is endeavouring to trace.

Tracer. A substance (e.g. an isotope) that is mixed with or fixed to the **tracee** in order to follow its translocation or to identify its location.

Tracer equilibrium. The stage (real or extrapolated) at which the **tracer** has become completely and uniformly mixed with the **tracee**, so that the **specific activity** has become the same throughout the system.

Transfer rate of exchange. The amount of a given substance transferred per unit time in both directions during the process of exchange between two compartments.

Turnover time. Pertaining to a given substance in a steady state system, it is the time needed for the input (and thus the output) to equal the total amount of the substance in the system.

Units. To facilitate the conversion of units into SI equivalents, the following table has been appended to aid the reader. (Factors are given exactly or to a maximum of four significant figures.)

The SI base units are the metre (m), kilogram (kg), second (s), ampere (A), kelvin (K), candela (cd) and mole (mol)
(See **Some Basic Symbols and Units Used in this Manual**)

Multiply		*by*	*to obtain*
Radiation units (see Table I–I)			
becquerel	1 Bq	$(= 2.7027 \times 10^{-11}$	Ci)
disintegrations per second	1 dis/s	$= 1.00 \times 10^{0}$	Bq
curie ($= 3.7 \times 10^{10}$ dis/s)	1 Ci	$= 3.70 \times 10^{10}$	Bq
röntgen	1 R	$= 2.58 \times 10^{-4}$	C/kg
gray	1 Gy	$(= 1.00 \times 10^{0}$	J/kg)
rad	1 rad	$= 1.00 \times 10^{-2}$	Gy
		$(= 1.00 \times 10^{-2}$	J/kg)
rem (in radiation protection only)		dimensions of J/kg	
Mass			
unified atomic mass unit (1/12 of the mass of ^{12}C)	1 u	$= 1.661 \times 10^{-27}$	kg
pound mass (avoirdupois)	1 lb	$= 4.536 \times 10^{-1}$	kg
ounce mass (avoirdupois)	1 oz	$= 2.835 \times 10^{1}$	g
ton (long) (= 2240 lbm)	1 ton	$= 1.016 \times 10^{3}$	kg
ton (short) (= 2000 lbm)	1 short ton	$= 9.072 \times 10^{2}$	kg
tonne (= metric)	1 t	$= 1.00 \times 10^{3}$	kg
Length			
statute mile	1 mile	$= 1.609 \times 10^{0}$	km
yard	1 yd	$= 9.144 \times 10^{-1}$	m
foot	1 ft	$= 3.048 \times 10^{-1}$	m
inch	1 in	$= 2.54 \times 10^{-2}$	m
mil ($= 10^{-3}$ in)	1 mil	$= 2.54 \times 10^{-2}$	mm
Area			
hectare	1 ha	$= 1.00 \times 10^{4}$	m^2
mile (statute mile)^2	1 mile^2	$= 2.590 \times 10^{0}$	km^2
acre	1 acre	$= 4.047 \times 10^{3}$	m^2
yard^2	1 yd^2	$= 8.361 \times 10^{-1}$	m^2
foot^2	1 ft^2	$= 9.290 \times 10^{-2}$	m^2
inch^2	1 in^2	$= 6.452 \times 10^{2}$	mm^2
barn	1 b	$= 1.00 \times 10^{-28}$	m^2

Multiply		*by*	*to obtain*
Volume			
yard^3	1 yd^3	$= 7.646 \times 10^{-1}$	m^3
foot^3	1 ft^3	$= 2.832 \times 10^{-2}$	m^3
inch^3	1 in^3	$= 1.639 \times 10^{4}$	mm^3
gallon (British or imperial)	1 gal (Brit.)	$= 4.546 \times 10^{-3}$	m^3
gallon (US liquid)	1 gal (US)	$= 3.785 \times 10^{-3}$	m^3
litre	1 L	$= 1.00 \times 10^{-3}$	m^3
Force			
dyne	dyn	$= 1.00 \times 10^{-5}$	N
kilogram force	1 kgf	$= 9.807 \times 10^{0}$	N
poundal	1 pdl	$= 1.383 \times 10^{-1}$	N
pound force (avoirdupois)	1 lbf	$= 4.448 \times 10^{0}$	N
Density			
pound mass/inch^3	1 lbn/in^3	$= 2.768 \times 10^{4}$	kg/m^3
pound mass/foot^3	1 lbn/ft^3	$= 1.602 \times 10^{1}$	kg/m^3
Energy			
British thermal unit	1 Btu	$= 1.054 \times 10^{3}$	J
calorie	1 cal	$= 4.184 \times 10^{0}$	J
electronvolt	1 eV	$\cong 1.602 \times 10^{-19}$	J
erg	1 erg	$= 1.00 \times 10^{-7}$	J
foot-pound force	1 ft-lbf	$= 1.356 \times 10^{0}$	J
kilowatt-hour	1 kW·h	$= 3.60 \times 10^{6}$	J
Temperature, energy/area time			
Fahrenheit, degrees −32	°F−32	$\frac{5}{9}$	°C
Rankine	°R	$\frac{5}{9}$	K
1 Btu/ft^2·s		$= 1.135 \times 10^{4}$	W/m^2
1 cal/cm^2·min		$= 6.973 \times 10^{2}$	W/m^2

SOME BASIC SYMBOLS AND UNITS USED IN THIS MANUAL

(see Glossary, Part VI)

Symbol	Description	Dimensions and/or units
Z	atomic number, i.e. proton number	
A	mass number	
A_r	relative atomic mass	unified atomic mass unit (u)
E	energy of nuclear transformation	electronvolt (eV) or megaelectronvolt (MeV)
M, M_{Ca}, M_{CaSO_4}	gram-atomic or gram-molecular mass	gram (g)
N_A	Avogadro's constant (number)	$N_A = 6.022 \times 10^{23}$ mol^{-1}
$T_{1/2}$	radioactive half-life	time, e.g. year (a), day (d), hour (h), minute (min), second (s)
λ	radioactive decay constant	inverse time, i.e. s^{-1}
t	time in general	time, i.e. s, etc.
T	counting time (duration of)	time, i.e. s, etc.
C	accumulated counts in time T	counts
R (= C/T)	count rate, including background or blank	counts per second (counts/s) counts per minute (counts/min)
R_b	count rate of background or blank	counts/s, counts/min
R_s	count rate of sample	counts/s, counts/min
ϵ	counting efficiency = counting yield	
A*	activity	becquerel (Bq), curie (Ci) (becquerel = disintegrations per second (dis/s))
SA	specific activity of a radioisotope	activity per gram or mole (e.g. kBq/g, TBq/mol, mCi/g, dis·min^{-1}·mg^{-1}, etc.)
	or atoms % excess of stable isotope	
S	amount of tracee (substance being traced)	mass (i.e. gram) or mole
$T_{1/2,\ biol}$	biological half-life (physiological elimination)	time, e.g. s, etc.
$T_{1/2,\ eff}$	effective half-life (including effects of physiological elimination and radioactive decay)	time, e.g. s, etc.

ISBN 92-0-101792-8