14 Radioisotope techniques

R. J. SLATER

14.1 WHY USE A RADIOISOTOPE?

When researchers contemplate using a radioactive compound there are several things they have to consider. First and foremost, they must ask the questions: is a radioisotope necessary, is there another way to achieve our objectives? The reason for this is that radioisotope use is governed by very strict legislation. The rules are based on the premise that radioactivity is potentially unsafe (if handled incorrectly) and should therefore only be used if there are no alternatives. Then, once it is decided that there is no alternative, the safest way of carrying out the work needs to be planned. Essentially this means using the safest isotope and the smallest amount possible.

But why do we use radioisotopes in the first place? There are very good reasons; here are some of them. Firstly, it is possible to detect radioactivity with exquisite sensitivity. This means that, for example, the progress of a chemical through a metabolic pathway or in the body of a plant or animal can be followed relatively easily. In short, much less of the chemical is needed, and the detection methods are simple. Secondly, it is possible to follow what happens in time. Imagine a metabolic pathway such as carbon dioxide fixation (the Calvin cycle). All the metabolites in the cycle are present simultaneously so a good way to establish the order of the metabolism is to add a radioactive molecule (e.g. ^{14}C-labelled sodium bicarbonate) and see what happens to it. Thirdly, it is possible to trace what happens to individual atoms in a pathway. This is done for example by creating compounds with ^{14}C in specific locations on the molecule. Fourthly, we can identify a part or end of a molecule, and follow reactions very precisely. This has been very useful in molecular biology, where it is often necessary to label one end of a DNA molecule (e.g. for techniques such as DNA footprinting, a method for investigating sequence-specific DNA-binding proteins).

Finally, there is a use that seems obvious after you have heard it; but you may never have thought of it yourself until now. In chemistry and biochemistry we are used to chemical reactions where one compound is turned into another. We can identify and measure ('assay') the reactants and products and learn something about the reaction. But what if the product of the reaction is identical to what we start with? You may have guessed the example already: DNA replication. To study this we need some method for detecting the product of the reaction, and this is often done with isotopes.

Having understood why we need a radioisotope we now need to understand what radioactivity is and how to use it. Read on.

14.2 THE NATURE OF RADIOACTIVITY

14.2.1 Atomic structure

An atom is composed of a positively charged central nucleus inside a much larger cloud of negatively charged electrons. The mass of an atom is concentrated in the nucleus, even though it accounts for only a small fraction of the total size of the atom. Atomic nuclei are composed of two major particles, **protons** and **neutrons**. Protons are positively charged with a mass approximately 1850 times greater than that of an electron. The number of protons present in the nucleus is known as the **atomic number** (Z), and it determines what the element is, for example six protons is carbon. Neutrons are uncharged particles with a mass approximately equal to that of a proton. The sum of protons and neutrons in a given nucleus is the **mass number** (A). Thus

$$A = Z + N$$

where N is the number of neutrons present.

Since the number of neutrons in a nucleus is not related to the atomic number, it does not affect the chemical properties of the atom. Atoms of a given element may not necessarily contain the same number of neutrons. Atoms of a given element with different mass numbers (i.e. different numbers of neutrons) are called **isotopes**. Symbolically, a specific nuclear species is represented by a subscript number for the atomic number, and a superscript number for the mass number, followed by the symbol of the element. For example:

$${}^{12}_{6}\mathrm{C}\ {}^{14}_{6}\mathrm{C}\ {}^{16}_{8}\mathrm{O}\ {}^{18}_{8}\mathrm{O}$$

However, in practice it is more conventional just to cite the mass number (e.g. ^{14}C). The number of isotopes of a given element varies: there are three isotopes of hydrogen (^{1}H, ^{2}H and ^{3}H), seven of carbon (^{10}C to ^{16}C inclusive) and 20 or more of some of the elements of high atomic number.

14.2.2 Atomic stability and radiation

In general, the ratio of neutrons to protons will determine whether an isotope of an element is stable enough to exist in nature. Stable isotopes for elements with low

Table 14.1 **Properties of different types of radiation**

Alpha	Beta	Gamma, X-rays and Bremsstrahlung
Heavy charged particle	Light charged particle	Electromagnetic radiation (em)
More toxic than other forms of radiation	Toxicity same as em radiation per unit of energy	Toxicity same as beta radiation per unit of energy
Not penetrating	Penetration varies with source	Highly penetrating

atomic numbers tend to have an equal number of neutrons and protons, whereas stability for elements of higher atomic numbers requires more neutrons. Unstable isotopes are called **radioisotopes**. They become stable isotopes by the process of **radioactive decay**: changes occur in the atomic nucleus, and particles and/or electromagnetic radiation are emitted.

14.2.3 Types of radioactive decay

There are several types of radioactive decay; only those most relevant to biochemists are considered below. A summary of properties is given in Table 14.1.

Decay by negatron emission

In this case a neutron is converted to a proton by the ejection of a negatively charged beta (β) particle called a **negatron** (β^-):

Neutron → proton + negatron

To all intents and purposes a negatron is an electron, but the term negatron is preferred, although not always used, since it serves to emphasise the nuclear origin of the particle. As a result of negatron emission, the nucleus loses a neutron but gains a proton. The mass number, *A*, remains constant. An isotope frequently used in biological work that decays by negatron emission is ^{14}C.

$$^{14}_{6}C \rightarrow {}^{14}_{7}N + \beta^-$$

Negatron emission is very important to biochemists because many of the commonly used radionuclides decay by this mechanism. Examples are: ^{3}H and ^{14}C, which can be used to label any organic compound; ^{35}S used to label methionine, for example to study protein synthesis; and ^{33}P or ^{32}P, powerful tools in molecular biology when used as nucleic acid labels.

Decay by positron emission

Some isotopes decay by emitting positively charged β-particles referred to as **positrons** (β^+). This occurs when a proton is converted to a neutron:

Proton → neutron + positron

Positrons are extremely unstable and have only a transient existence. Once they have dissipated their energy they interact with electrons and are annihilated. The mass and energy of the two particles are converted to two γ-rays emitted at 180° to each other. This phenomenon is frequently described as **back-to-back emission**.

As a result of positron emission the nucleus loses a proton and gains a neutron, the mass number stays the same. An example of an isotope decaying by positron emission is ^{22}Na:

$$^{22}_{11}\text{Na} \rightarrow {}^{22}_{10}\text{Ne} + \beta^{+}$$

Positron emitters are detected by the same instruments used to detect γ-radiation. They are used in biological sciences to spectacular effect in brain scanning with the technique **positron emission tomography** (PET scanning) used to identify active and inactive areas of the brain.

Decay by alpha particle emission

Isotopes of elements with high atomic numbers frequently decay by emitting **alpha** (α) **particles**. An α-particle is a helium nucleus; it consists of two protons and two neutrons ($^{4}\text{He}^{2+}$). Emission of α-particles results in a considerable lightening of the nucleus, a decrease in atomic number of 2 and a decrease in the mass number of 4. Isotopes that decay by α-emission are not frequently encountered in biological work although they can be found in instruments such as scintillation counters and smoke alarms. Radium-226 (^{226}Ra) decays by α-emission to radon-222 (^{222}Rn), which is itself radioactive. Thus begins a complex decay series, which culminates in the formation of ^{206}Pb:

$$^{226}_{88}\text{Ra} \rightarrow {}^{4}_{2}\text{He}^{2+} + {}^{222}_{86}\text{Rn} \rightarrow\rightarrow\rightarrow {}^{206}_{82}\text{Pb}$$

Alpha emitters are extremely toxic if ingested, due to the large mass and the ionising power of the α-particle.

Electron capture

In this form of decay a proton captures an electron orbiting in the innermost K shell:

$$\text{proton} + \text{electron} \rightarrow \text{neutron} + \text{X-ray}$$

The proton becomes a neutron and electromagnetic radiation (X-rays) is given out.

Example:

$$^{125}_{53}\text{I} \rightarrow {}^{125}_{52}\text{Te} + \text{X-ray}$$

Decay by emission of γ-rays

In some cases α- and β-particle emission also give rise to γ-rays (electromagnetic radiation similar to, but with a shorter wavelength than, X-rays). The γ-radiation has low ionising power but high penetration. For example, the radiation from ^{60}Co will penetrate 15 cm of steel. The toxicity of γ-radiation is similar to that of X-rays.

Example:

$^{131}_{53}\mathrm{I} \rightarrow {}^{131}_{54}\mathrm{Xe} + \beta^- + \gamma$

14.2.4 Radioactive decay energy

The usual unit used in expressing energy levels associated with radioactive decay is the **electron volt**. One electron volt (eV) is the energy acquired by one electron in accelerating through a potential difference of 1 V and is equivalent to 1.6×10^{-19} J. For the majority of isotopes, the term **million** or **mega electron volts** (MeV) is more applicable. Isotopes emitting α-particles are normally the most energetic, falling in the range 4.0 to 8.0 MeV, whereas β- and γ-emitters generally have decay energies of less than 3.0 MeV. The higher the energy of radiation the more it can penetrate matter and the more hazardous it becomes.

14.2.5 Rate of radioactive decay

Radioactive decay (measured as disintegrations per minute, d.p.m.) is a spontaneous process and it occurs at a rate characteristic of the source, defined by the rate constant (λ, the fraction of an isotope decaying in unit time, t^{-1}). Decay is a nuclear event so λ is not affected by temperature or pressure. The number of atoms disintegrating at any time is proportional to the number of atoms of the isotope (N) present at that time (t). Clearly, the number of atoms N, is always falling (as atoms decay) and so the rate of decay (d.p.m.) falls with time. Also, the slope of the graph of number of unstable atoms present, or rate of decay (d.p.m.) against time, similarly falls. This means that a graph of radioactivity against time shows a curve, called an **exponential decay curve** (Fig. 14.1). The mathematical equation that underpins the graph shown is as follows:

$$\ln N_t/N_0 = -\lambda t \qquad (14.1)$$

where λ is the decay constant for an isotope, N_t is the number of radioactive atoms present at time t, and N_0 is the number of radioactive atoms orginally present. You will notice the natural logarithm (ln) in the equation; this means if we were to plot log d.p.m. against time we would get a graph with a straight line and a negative slope (gradient determined by the value of λ).

In practice it is more convenient to express the decay constant in terms of half-life ($t_{½}$). This is defined as the time taken for the activity to fall from any value to half that value (see Fig. 14.1). When N_t in equation 14.1 is equal to one-half of N_0 then t will equal the half-life of the isotope. Thus

$$\ln 1/2 = -\lambda t_{1/2} \qquad (14.2)$$

$$\text{or } t_{1/2} = 0.693\lambda \qquad (14.3)$$

The values of $t_{½}$ vary widely from over 10^{19} years for lead-204 (^{204}Pb) to 3×10^{-7} seconds for polonium-212 (^{212}Po). The half-lives of some isotopes frequently used in biological work are given in Table 14.2. The advantages and disadvantages of working with isotopes of differing half-lives are given in Table 14.3.

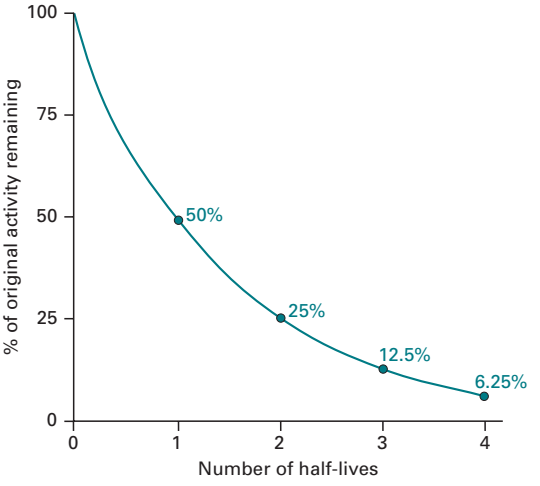

Fig. 14.1 Demonstration of the exponential nature of radioactive decay.

Example 1 **THE EFFECT OF HALF-LIFE**

Question The half-life of ^{32}P is 14.2 days. How long would it take a solution containing 42 000 d.p.m. to decay to 500 d.p.m.?

Answer Use equation 14.3 to calculate the value of λ. This gives a value of 0.0488 days^{-1}. Then use equation 14.1 to calculate the time taken for the counts to decrease. In this equation $N_0 = 42\,000$ and $N_t = 500$. This gives a value for t of 90.8 days. (You can check that this is right by doing an estimate calculation in your head; it is roughly 6 half-lives to get from 42 000 to 500.)

14.2.6 Units of radioactivity

The Système International d'Unités (SI system) uses the becquerel (Bq) as the unit of radioactivity. This is defined as one **disintegration per second** (1 d.p.s.). However, an older unit, not in the SI system and still frequently used, is the **curie** (Ci). This is defined as the quantity of radioactive material in which the number of nuclear disintegrations per second is the same as that in 1 g of radium, namely 3.7×10^{10} (or 37 GBq). For biological purposes this unit is too large and the micro-curie (mCi) and millicurie (mCi) are used. It is important to realise that the units Bq and Ci refer to the number of disintegrations actually occurring in a sample not to the disintegrations detected, which generally will be only a proportion of the disintegrations occurring. Detected decays are referred to as **counts** (i.e. counts per second or c.p.s.).

Table 14.2 **Properties of radioisotopes commonly used in the biological sciences**

Property	^{3}H	^{14}C	^{35}S	^{32}P	^{33}P	^{125}I	^{131}I
$t_{1/2}$	12.3 years	5730 years	87.4 days	14.3 days	25.4 days	59.6 days	8.04 days
Mode of decay	β	β	β	β	β	X (EC) and Auger electrons	γ and β
Max β energy (MeV)	0.019	0.156	0.167	1.709	0.249	Auger electrons 0.035	0.806
ALI[a]	480 (Mbq)[b]	34 (Mbq)	15 (Mbq)	6.3 (Mbq)	14 (Mbq)	1.3 (Mbq)[c]	0.9 (Mbq)[c]
Maximum range in air	6 mm	24 cm	26 cm	790 cm	49 cm	>10 m	>10 cm
Shielding required	None	1 cm acrylic	1 cm acrylic	1 cm acrylic	1 cm acrylic	Lead 0.25 m or lead-impregnated acrylic	Lead 13 mm
γ dose rate (μSv h^{-1} from 1 GBq at 1 m)	–	–	–	(β dose rate 760 μSv, 10 cm from 1 MBq)	–	41	51
Čerenkov counting	–	–	–	Yes	–	–	–

Notes: [a]Annual limit on intake, based on a dose limit of 20 mSv using the most restrictive dose coefficients for inhalation or ingestion.
[b]Bound ^{3}H.
[c]Based on dose equivalent limit of 500 mSv to thyroid.

Table 14.3 **The advantages and disadvantages of working with a short-half-life isotope**

Advantages	Disadvantages
High specific activity (see Section 14.3.3) makes the experiment more sensitive	Experimental design; isotope decays during time of experiment
Easier and cheaper to dispose of	Cost of replacement for further experiments
Lower doses likely (e.g. in diagnostic testing of human subjects)	Frequently need to calculate amount of activity remaining

For quick reference, a list of units and definitions frequently used in radioisotope work is provided in Table 14.6 at the end of the chapter.

14.2.7 Interaction of radioactivity with matter

α-Particles

These particles have a very considerable energy (3–8 MeV) and all the particles from a given isotope have the same amount of energy. They react with matter in two ways: they cause **excitation** (energy is transferred from the α-particle to orbital electrons of neighbouring atoms, these electrons being elevated to higher orbitals, but eventually fall back, emitting energy as photons of light) and they ionise atoms in their path (the target orbital electron is removed, thus the atom becomes ionised and forms an ion-pair, consisting of a positively charged ion and an electron). Because of their size, α-particles have slow movement and double positive charge. They cause intense ionisation and excitation and their energy is rapidly dissipated. Despite their initial high energy, α-particles frequently collide with atoms in their path and so the radiation is not very penetrating (a few centimetres through air).

Negatrons

Negatrons are very small and rapidly moving particles that carry a single negative charge. They interact with matter to cause ionisation and excitation exactly as with α-particles. However, due to their speed and size, they are less likely than α-particles to interact with matter and therefore are less ionising and more penetrating. Another difference between α-particles and negatrons is that negatrons are emitted over a range of energies. Negatron emitters have a characteristic energy spectrum (see Fig. 14.5b below). The **maximum energy level** (E_{max}) varies from one isotope to another, ranging from 0.018 MeV for ^{3}H to 4.81 MeV for ^{38}Cl. The difference in E_{max} affects the penetration of the radiation and therefore the safety measures that are required: β-particles from ^{3}H can travel only a few millimetres in air, whereas those from ^{32}P can penetrate over 1 m of air. Therefore radiation shields are needed when working with ^{32}P.

γ-Rays and X-rays

These rays (henceforth collectively referred to as γ-rays for simplicity) are electromagnetic radiation and therefore have no charge or mass. They cause excitation and

ionisation. They interact with matter to create secondary electrons that behave as per negatron emission.

Bremsstrahlung radiation

When high atomic number materials absorb high energy β-particles, the absorber gives out a secondary radiation, an X-ray, called **bremsstrahlung radiation**. For this reason, shields for ^{32}P use low-atomic-number materials such as acrylic.

14.3 DETECTION AND MEASUREMENT OF RADIOACTIVITY

There are three commonly used methods of detecting and quantifying radioactivity. These are based on the ionisation of gases, on the excitation of solids or solutions, and the ability of radioactivity to expose photographic emulsions (i.e. autoradiography).

14.3.1 Methods based upon gas ionisation

If a charged particle passes through a gas, its electrostatic field dislodges orbital electrons from atoms sufficiently close to its path and causes ionisation (Fig. 14.2). The ability to induce ionisation decreases in the order

$$A > \beta > \gamma \ (10\,000 : 100 : 1)$$

If ionisation occurs between a pair of electrodes enclosed in a suitable chamber (Fig. 14.2) a pulse (current) flows. Ionisation counters like those shown in Fig. 14.2 are sometimes called **proportional counters** ('proportional' because small voltage changes can affect the count rate). The Geiger–Müller counter (Figs. 14.3, 14.4a) has a cylindrical-shaped gas chamber and it operates at a high voltage. This makes the instrument less dependent on a stable voltage, so the counter is cheaper and lighter.

Example 2 COUNTING WITH IONISATION COUNTERS

Question Using the information described in Sections 14.2.3 and 14.2.7, what types of radiation can be detected by an ionisation counter?

Answer The counters will detect α-particles and medium- to high-energy β-particles; they will detect γ-radiation, but with lower efficiency. They will not detect tritium because the β-particles have very low energy and will not pass into the gas chamber.

In ionisation counters, the ions have to travel to their respective electrodes; other ionising particles entering the tube during this time (the so-called 'dead time') are not detected and this reduces the counting efficiency.

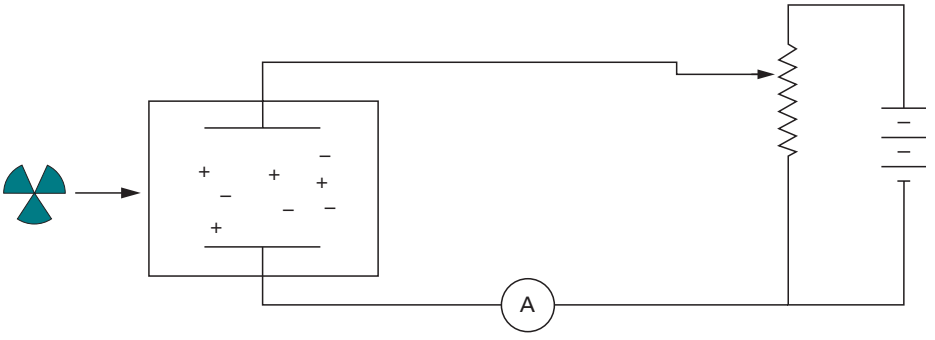

Fig. 14.2 Detection based on ionisation.

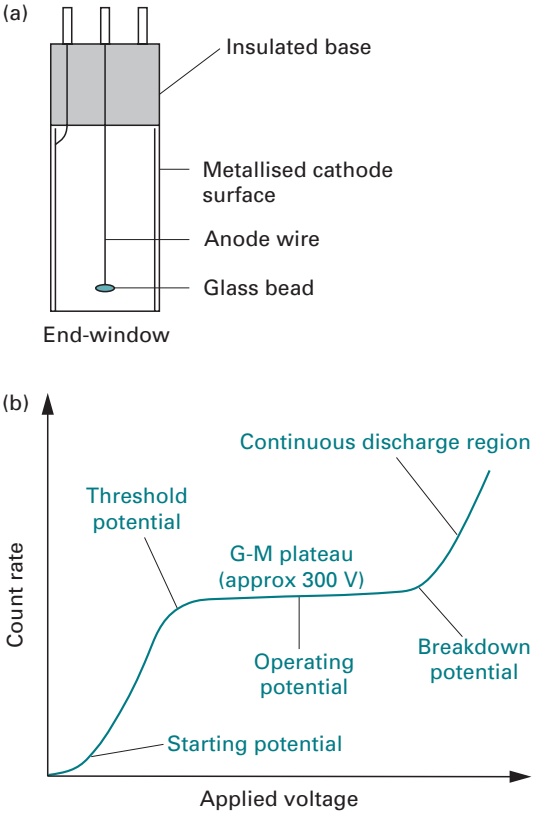

Fig. 14.3 (a) The Geiger–Müller (G–M) tube and (b) the effect of applied voltage on count rate.

Ionisation counters are used for routine monitoring of the laboratory to check for contamination. They are also useful in experimental situations where the presence or absence of radioactivity needs to be known rather than the absolute quantity, for example quick screening of radioactive gels prior to autoradiography, checking that

Fig. 14.4 (a) Geiger–Müller bench monitor; (b) liquid scintillation counter; (c) sample rack for liquid scintillation counter. (Reproduced with permission from LabLogic; instrument shown is the Hidex 300SI.)

Example 3 **THE EFFECT OF DEAD TIME**

Question What do you think will happen to the counting efficiency of a Geiger–Müller counter as the count rate rises?

Answer The efficiency will fall since there will be an increased likelihood that two or more β-particles will enter the tube during the dead time.

a labelled DNA probe is where you think it is (and not down the sink!) or checking chromatographic fractions for labelled components.

14.3.2 Methods based upon excitation

Radioactive isotopes interact with matter in two ways, ionisation and excitation. The latter effect leads an excited atom or compound (known as a **fluor**) to emit photons of light. The process is known as **scintillation**. When the light is detected by a photomultiplier, it forms the basis of scintillation counting. Essentially, a photomultiplier converts the energy of radiation into an electrical signal, and the strength of the electric pulse that results is directly proportional to the energy of the original radioactive event. This means that two, or even more, isotopes can be separately detected and measured in the same sample, provided they have sufficiently different emission energy spectra. The mode of action of a photomultiplier is shown in Fig. 14.5a, and the energy spectrum of a β-particle emitter in Fig. 14.5b.

Types of scintillation counting

There are two types of scintillation counting, which are illustrated diagrammatically in Fig. 14.6. In solid scintillation counting the sample is placed adjacent to a solid fluor (e.g. sodium iodide). Solid scintillation counting is particularly useful for γ-emitting isotopes. This is because they can penetrate the fluor. The counters can be small hand-held devices with the fluor attached to the photomultiplier tube (Fig. 14.5.a), or larger bench-top machines with a well-shaped fluor designed to automatically count many samples (Fig. 14.6.a).

In liquid scintillation counting (Fig. 14.6b; see also Figs. 14.4b, c), the sample is mixed with a scintillation fluid containing a solvent and one or more dissolved fluors. This method is particularly useful in quantifying weak β-emitters such as ^{3}H, ^{14}C and ^{35}S, which are frequently used in biological work. Scintillation fluids are called 'cocktails' because there are different formulations, made of a solvent (such as toluene or diisopropylnaphthalene) plus fluors such as 2,5-diphenyloxazole (PPO), 1,4-bis(5-phenyloxazol-2-yl)benzene (nicknamed POPOP, pronounced as it reads: 'pop op') or 2-(4′-*t*-butylphenyl)-5-(4″-bi-phenyl)-1,3,4-oxydiazole (butyl-PBD). Cocktails can be designed for counting organic samples, or may contain detergent to facilitate counting of aqueous samples.

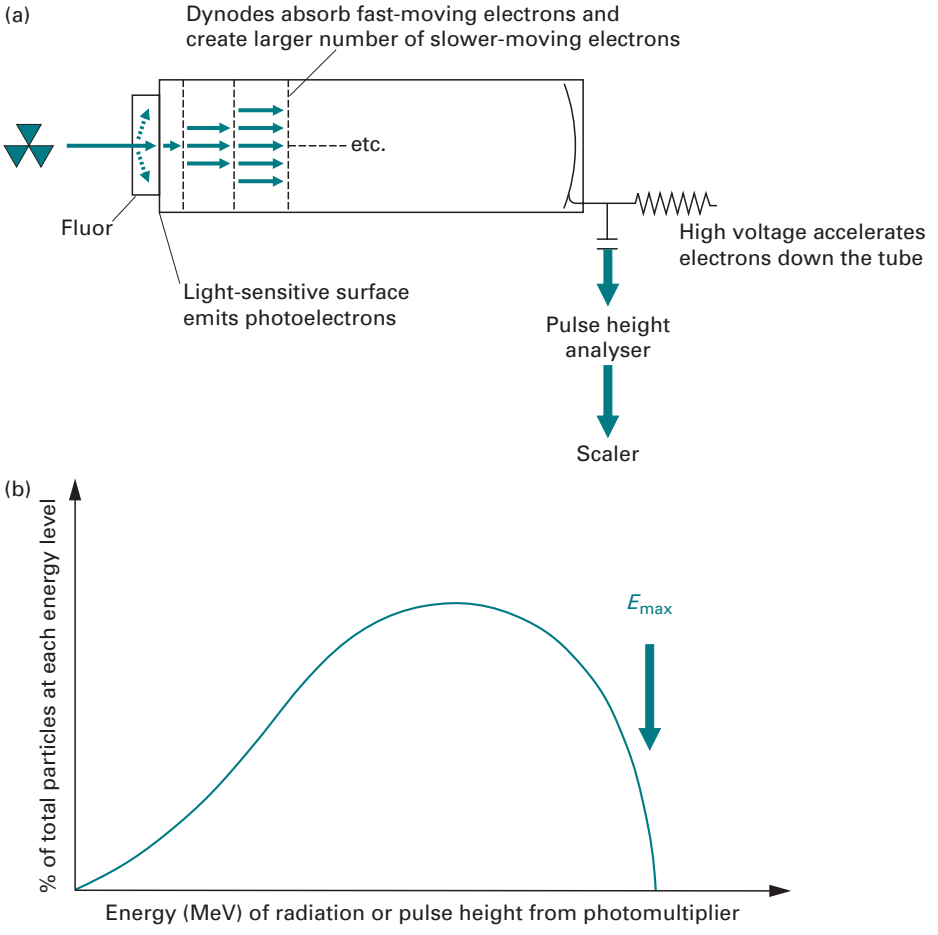

Fig. 14.5 (a) The mode of action of a photomultiplier and (b) the energy spectrum of a typical β-emitter.

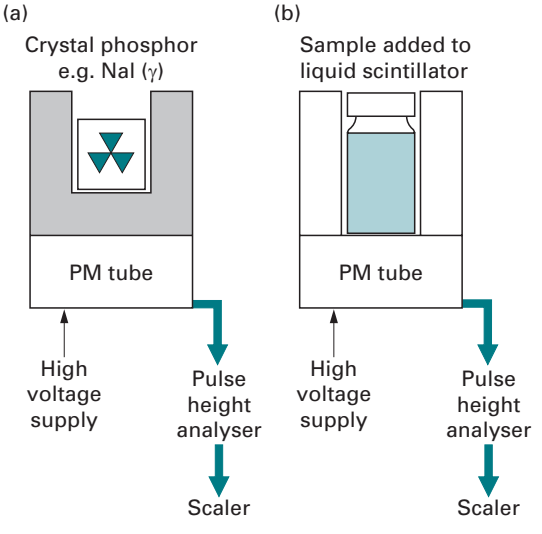

Fig. 14.6 Diagrammatic illustration of (a) solid and (b) liquid scintillation counting methods.

Advantages of scintillation counting

Scintillation counting is widely used in biological work and it has several advantages over gas ionisation counting:

- fluorescence is very fast so there is effectively no dead time
- counting efficiencies are high (from about 50% for low-energy β-emitters to 90% for high-energy emitters)
- the ability to count samples of many types, including liquids, solids, suspensions and gels
- the general ease of sample preparation
- the ability to count separately different isotopes in the same sample (used in dual-labelling experiments)
- highly automated (hundreds of samples can be counted automatically and built-in computer facilities carry out many forms of data analysis, such as efficiency correction, graph plotting, radioimmunoassay calculations, etc.).

Disadvantages of scintillation counting

No technique is without disadvantages, so the following have to be considered or overcome in the design of the instruments:

- cost of the instrument and cost per sample (for scintillation fluid, the counting vials and disposal of the organic waste)
- potentially high background counts; this is due to photomultiplier noise but can be compensated for by using more than one tube (noise is random, but counts from a radioactive decay are simultaneous, the coincident counts only are recorded)
- 'quenching': this is the name for reduction in counting efficiency caused by coloured compounds that absorb the scintillated light, or chemicals that interfere with the transfer of energy from the radiation to the photomultiplier (correcting for quenching contributes significantly to the cost of scintillation counting)
- chemiluminescence: this is when chemical reactions between components of the samples to be counted and the scintillation cocktail produce scintillations that are unrelated to the radioactivity; modern instruments can detect chemiluminescence and subtract it from the results automatically
- phospholuminescence: this results from pigments in the sample absorbing light and re-emitting it; the solution is to keep the samples in the dark prior to counting.

Using scintillation counting for dual-labelled samples

Different β-particle emitters have different energy spectra, so it is possible to quantify two isotopes separately in a single sample, provided their energy spectra can be distinguished from each other. Examples of pairs of isotopes that can be counted together are: ^{3}H and ^{14}C, ^{3}H and ^{35}S, ^{3}H and ^{32}P, ^{14}C and ^{32}P, ^{35}S and ^{32}P. The principle of the method is illustrated in Fig. 14.7, where it can be seen that the spectra of two isotopes (referred to as S and T) overlap only slightly. By setting a pulse height analyser to reject all pulses of an energy below X (threshold X) and to reject all pulses of an energy above Y (window Y) and also to reject below a threshold of A and a

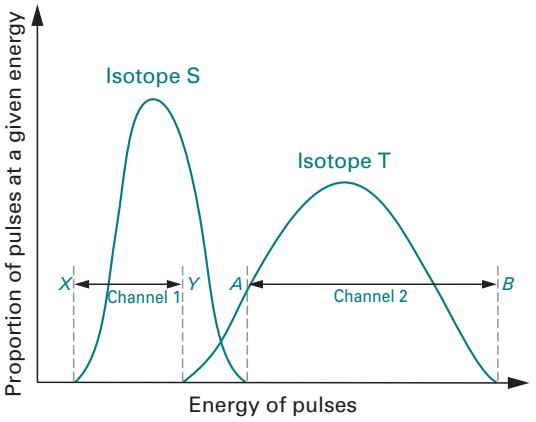

Fig. 14.7 Diagram to illustrate the principle of counting dual-labelled samples.

window of *B*, it is possible to separately count the two isotopes. A pulse height analyser set with a threshold and window for a particular isotope is known as a channel. Modern counters operate with a so-called **multichannel analyser** that records the entire energy spectrum simultaneously. This greatly facilitates multi-isotope counting and in particular allows the effect of quenching on dual-label counting to be assessed adequately.

Dual-label counting has proved to be useful in many aspects of molecular biology (e.g. nucleic acid hybridisation and transcription), metabolism (e.g. steroid synthesis) and drug development.

Determination of counting efficiency

When we detect radioactivity we usually need to know the actual rate of decay (the d.p.m.) (see Section 14.2.6). This is because we want to record and publish data that are independent of the types of equipment used to do the detection. To calculate d.p.m. we need to know the efficiency of counting. What's more, in liquid scintillation counting we have to contend with quenching. Samples may vary in nature so the levels of quenching may vary from one to the next. Therefore the efficiency of counting needs to be determined for every sample.

One way to do this is to use an internal standard (called a **spike**). The sample is counted (and gives a reading of, say, *A* c.p.m.), removed from the counter and a small amount of standard material of known disintegrations per minute (*B* d.p.m.) is added. The sample is then recounted (*C* c.p.m.) and the counting efficiency of the sample calculated:

$$\text{counting efficiency} = [100(C - A)/B]\% \qquad (14.4)$$

Carefully carried out, it is the most accurate way of correcting for quenching. On the other hand, it is tedious since the process has to be done for every sample. Therefore automated methods have been devised; however, these all use the internal standard as the basis for establishing the parameters.

As a sample is quenched, the efficiency of light production falls, and it therefore creates an illusion that the radiation has a lower energy. The principle is straightforward: observe how the energy spectrum shifts as the efficiency falls, store this relationship in a computer, and then analyse the energy spectrum of the sample to determine the efficiency. However, there is a catch: different isotopes have different energies so we need to be sure that we are measuring the effect of quench and not just the fundamental differences in isotopes. This is resolved by using an 'external standard' source of radioactivity built into the counter. It's called an external standard because it is placed just outside the sample vial by a mechanical device in the instrument. The quenching in the sample is observed by counting this standard, the external source is then moved away, and the experimental sample counted. This is done for every sample in turn and the counter prints out corrected d.p.m. automatically. To set all this up a standard curve using a set of quenched standards is counted: the absolute amount of radioactivity in the standard is known and therefore the efficiency of counting can easily be determined. These are the data that are stored in the computer as a standard curve.

Some instruments (e.g. the Hidex 300 SL, shown in Figs. 14.4, and 14.8) on the market calculate counting efficiency in a totally different way. The counters use three photomultiplier tubes. The mathematics of the process are beyond the scope of this

Example 4 EXTERNAL STANDARD EFFICIENCY CALCULATIONS

A scintillation counter analyses the energy spectrum of an external standard, it records a point on the spectrum (the quench parameter, QP) and assesses the shift in spectrum as the efficiency falls. The efficiency of detecting ^{14}C in a scintillation counter is determined by counting a standard sample containing 105 071 d.p.m. at different degrees of quench (by adding increasing amounts of a quenching chemical such as chloroform). The results look like this:

c.p.m	QP
87 451	0.90
62 361	0.64
45 220	0.46
21 014	0.21

Then an experimental sample gives 2026 c.p.m. at a QP of 0.52. What is the true count rate?

Firstly, the counting efficiency of the quenched standards needs to calculated; the efficiency is the ratio of the c.p.m. to the d.p.m. ($\times 100$ if expressed as a %). Then the efficiency is plotted against the QP. The QP for the experimental sample (0.52) is put into the curve and the efficiency read (in this case 48%). This is then used to calculate the true d.p.m.: $2026 \times 100/48$. The answer is 4221 d.p.m.

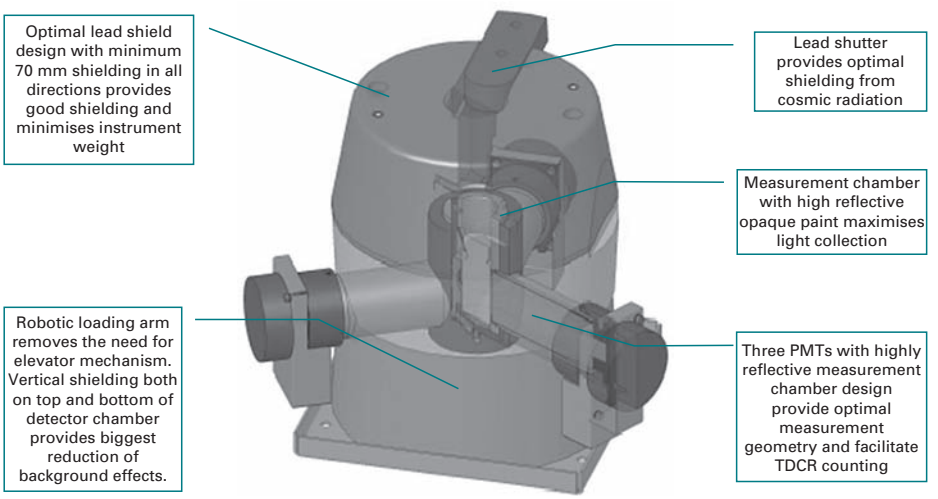

Fig. 14.8 The arrangement of photomultiplier tubes in a liquid scintillation counter. (Reproduced with permission from LabLogic; instrument shown is the Hidex 300SL.)

chapter but the chances of either three or two tubes detecting a signal is affected by the extent of quench. Therefore the ratio of triple to double coincidence is related to the counting efficiency. Such counters are smaller, and do not require a built-in radioactive source.

Sample preparation

For solid scintillation counting, sample preparation is easy and only involves transferring the sample to a glass or plastic vial (or tube) compatible with the counter. In liquid scintillation counting, sample preparation is more complex and starts with a decision on the type of sample vial to be used (glass; low-potassium glass, with low levels of ^{40}K that reduce background count; or polyethylene, cheaper but not re-usable). Vials need to be chemically resistant, have good light transmission and give low background counts. The trend has been towards mini-vials, which use smaller volumes of scintillation fluid. Some counters are designed to accept very small samples in special polythene bags split into an array of many compartments; these are particularly useful to, for example, the pharmaceutical industry where there are laboratories that do large numbers of receptor binding assays. Accurate counting depends on the sample being in the same phase as the counting cocktail. As described above the scintillation fluid should be chosen as appropriate to aqueous or organic samples.

If colour quenching is a problem it is possible to bleach samples before counting. Solid samples such as plant and animal tissues may be counted after solubilisation by quaternary amines such as NCS solubiliser or Soluene. Not surprisingly these solutions are highly toxic and great care is required.

Radioactive compounds are often separated by HPLC. The output of an HPLC instrument can be connected to a flow cell system where scintillation fluid is added

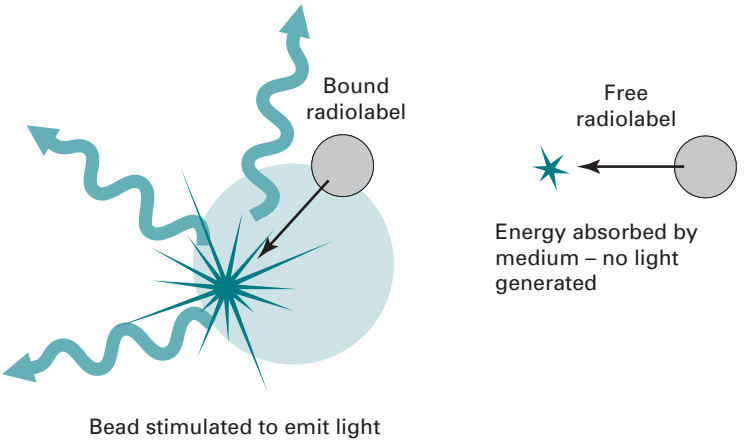

Fig. 14.9 The concept behind SPA. (Reproduced by courtesy of Amersham Biosciences.)

to the effluent prior to entering a detector. This significantly increases automation and reduces waste.

Čerenkov counting

The **Čerenkov effect** occurs when a particle passes through a substance with a speed higher than that of light passing through the same substance. If a β-emitter has a decay energy in excess of 0.5 MeV, then this causes water to emit a bluish white light usually referred to as Čerenkov light. It is possible to detect this light using a typical liquid scintillation counter. Since there is no requirement for organic solvents and fluors, this technique is relatively cheap, sample preparation is very easy, and there is no problem of chemical quenching. Table 14.2 indicates which isotopes can be counted this way.

Scintillation proximity assay

Scintillation proximity assay (SPA) is an application of scintillation counting that facilitates automation and rapid throughput of experiments. It is therefore highly suited to work such as screening for biological activity in new drugs. The principle of SPA is illustrated in Fig. 14.9. The beads for SPA are constructed from polystyrene (or sometimes other materials) that combine a binding site for a molecule of interest with a scintillant. You need to remember that some types of radiation do not travel far, in particular β-particles from weak energy emitters such as ^{3}H and ^{14}C. If molecules containing such radioisotopes are in solution with a suspension of SPA beads, the radiation does not stimulate the scintillant in the beads and cannot be detected efficiently by a scintillation counter. This is because the radiation is absorbed by the solution; it does not reach the scintillant. If, on the other hand, the radioisotope becomes bound to the bead, it is close enough to stimulate the scintillant in the bead, so light is given out and the isotope is detected.

Table 14.4 **Advantages of scintillation proximity assay**

Versatile: use with enzyme assays, receptors, any molecular interactions
Works with a range of appropriate isotopes such as ^{3}H, ^{14}C, ^{35}S and ^{33}P
No need for separation step (e.g. free from bound ligand)
Less manipulation therefore reduced toxicity
Amenable to automation

There are many applications of this technology such as enzyme assays and receptor binding, indeed any situation where we want to investigate the interaction between two molecules. Take receptor binding as an example. In this case a receptor for a particular ligand (such as a drug or hormone) is attached to the SPA beads. The ligand is radiolabelled and mixed with the beads. Any ligand that binds will stimulate the scintillant and be counted. If the researcher wishes to investigate chemicals that might interface with this binding (which is the mode of action of many medicines), they can be added at increasing concentration to study the effect and, for example, determine optimum dosage (see also Section 16.3.2).

A summary of the advantages of SPA technology is shown in Table 14.4.

14.3.3 Methods based upon exposure of photographic emulsions

Ionising radiation acts upon a photographic emulsion or film to produce a latent image much as does visible light. This is called **autoradiography**. The emulsion or film contains silver halide crystals. As energy from the radioactive material is dissipated the silver halide becomes negatively charged and is reduced to metallic silver, thus forming a particulate latent image. Photographic developers show these silver grains as a blackening of the film, then fixers are used to remove any remaining silver halide and a permanent image results.

It is a very sensitive technique and has been used in a wide variety of biological experiments. A good example is autoradiography of nucleic acids separated by gel electrophoresis (see Fig. 14.10).

Suitable isotopes

In general, weak β-emitting isotopes (e.g. ^{3}H, ^{14}C and ^{35}S) are most suitable for autoradiography, particularly for cell and tissue localisation experiments. This is because the energy of the radiation is low. The sample must be close to the film, the radiation does not spread out very far and so a clear image results. Radiation with higher energy (e.g. ^{32}P) give faster results but poorer resolution because the higher-energy negatrons produce much longer track lengths, exposing a greater surface area of the film, and result in less discrete images. This is illustrated in Fig. 14.10, showing autoradiography with three different isotopes.

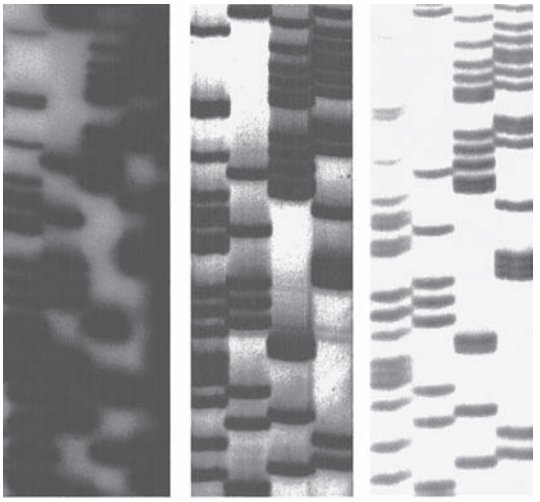

Fig. 14.10 Three autoradiographs showing the use of different radioisotopes in DNA sequencing. The isotope with the highest energy (^{32}P) leads to the poorest resolution because the radiation spreads out further, making the DNA bands appear thicker. The lowest energy radiation (from ^{35}S) gives the best resolution. (Reproduced with permission from M. W. Cunningham, A. Patel, A. C. Simmonds and D. Williams (2002), *In vitro* labelling of nucleic acids and proteins, in *Radioisotopes in Biology,* (2nd edn), R. J. Slater (ed.), Oxford University Press, Oxford.)

Choice of emulsion and film

Autoradiography emulsions are solutions of silver halide that can be made to set solid by the inclusion of materials such as gelatine. This can be used for example for autoradiography of microscope slides. X-ray film is the alternative and is used for gels (as shown in Fig. 14.10). Films differ in sensitivity; advice on what to use is provided by the manufacturers.

Direct autoradiography

In direct autoradiography, the X-ray film or emulsion is placed as close as possible to the sample and exposed at any convenient temperature. Quantitative images are produced until saturation is reached. The shades of grey in the image are related to a combination of levels of radiation and length of exposure until a black or nearly black image results. Isotopes with an energy of radiation equal to, or higher than, ^{14}C (E_{max}= 0.156 MeV) are required. The higher the energy the quicker the results.

Fluorography

If low-energy β-emitters are used it is possible to enhance the sensitivity several orders of magnitude by using fluorography. A fluor (e.g. PPO or sodium silicate) can be used to enhance the image. The β-particles emitted from the isotope will cause the fluor to become excited and emit light, which will react with the film. This has been used for example for detecting radioactive nucleic acids in gels. The fluor is infiltrated into the gel following electrophoresis; the gel is dried and then placed in contact with a preflashed film (see below).

Intensifying screens

Intensifying screens are used when obtaining a fast result is more important than high resolution. It is useful for example in gel electrophoresis or analysis of membrane filters where high-energy β-emitters (e.g. ^{32}P-labelled DNA) or γ-emitting isotopes (e.g. ^{125}I-labelled protein) are used. The intensifying screen consists of a solid phosphor, and it is placed on the other side of the film from the sample. High-energy radiation passes through the film, causes the phosphor to fluoresce and emit light, which in turn superimposes its image on the film. The reduction in resolution is due to the spread of light emanating from the screen.

Low-temperature exposure

When intensifying screens or fluorography are used the exposure should be done at low temperature. This is because the kinetics of the film's response are affected. The light is of low intensity and a back reaction occurs that cancels the latent image. Exposure at low temperature (−70 °C) slows this back reaction and will therefore provide higher sensitivity. There is no point in doing direct autoradiography at low temperature as the kinetic basis of the film's response is different.

Preflashing

The response of a photographic emulsion to radiation is not linear and usually involves a slow initial phase (lag) followed by a linear phase. Sensitivity of films may be increased by preflashing. This involves a millisecond light flash prior to the sample being brought into juxtaposition with the film and is often used where high sensitivity is required or if results are to be quantified.

Quantification

Autoradiography is usually used to locate rather than to quantify radioactivity. However, it is possible to obtain quantitative data directly from autoradiographs by using digital image analysis. Quantification is not reliable at low or high levels of exposure because of the lag phase (see preflashing above) or saturation, respectively. Preflashing combined with fluorography or intensifying screens create the best conditions for quantitative working.

14.4 OTHER PRACTICAL ASPECTS OF COUNTING RADIOACTIVITY AND ANALYSIS OF DATA

14.4.1 Self-absorption

Self-absorption is primarily a problem with low energy β-emitters: radiation is absorbed by the sample itself. Self-absorption can be a serious problem in the counting of low-energy radioactivity by scintillation counting if the sample is particulate or is, for instance, stuck to a membrane filter. Automated methods for calculating counting

efficiency in a scintillation counter will not correct for self-absorption effects. Particulate samples should be digested or otherwise solubilised prior to counting if quench correction is required.

14.4.2 Specific activity

The specific activity of a radioisotope defines its radioactivity in relation to the amount of material, expressed by units such as Bq mol^{-1}, Ci $mmol^{-1}$ or d.p.m. $mmol^{-1}$. It is a very important aspect of the use of radioisotopes in biological work because the higher the specific activity the more sensitive the experiment. This is because the higher the specific activity the smaller the quantities of labelled substance that can be detected. The highest specific activities are associated with isotopes with short half-lives, since the rate of decay per unit mass (or mol) is higher.

Sometimes, it is not necessary to purchase the highest specific activity available. For example, enzyme assays *in vitro* often require a relatively high substrate concentration and so specific activity may need to be lowered. Consider the example below (for definitions of units, see Table 14.6 at the end of the chapter):

[^{3}H]Leucine is purchased with a specific activity of 5.55 TBq $mmol^{-1}$ (150 Ci $mmol^{-1}$) and a concentration of 9.25 MBq 250 mm^{-3} (250 mCi 250 mm^{-3}). A 10 cm^3 solution of 250 mM and 3.7 kBq cm^{-3} (0.1 mCi cm^{-3}) is required. It is made up as follows:

- 10 cm^3 at 3.7 kBq cm^{-3} is 37 kBq (1 mCi), therefore pipette 1 mm^3 of stock radioisotope into a vessel (or, to be more accurate, pipette 100 mm^3 of a $\times 100$ dilution of stock in water).
- Add 2.5 cm^3 of a 1 M stock solution of cold leucine, and make up to 10 cm^3 with distilled water.

Note that there is no need in this case to take into account the amount of leucine in the [^{3}H]leucine preparation; it is a negligible quantity due to the high specific activity.

If necessary (e.g. to manipulate solutions of relatively low specific activity), however, the following formula can be applied:

$$W = Ma[(1/A') - (1/A)] \qquad (14.5)$$

where W is the mass of cold carrier required (mg), M is the amount of radioactivity present (MBq), a is the molecular weight of the compound, A is the original specific activity (MBq $mmol^{-1}$), and A' is the required specific activity (MBq $mmol^{-1}$).

14.4.3 Statistics

The emission of radioactivity is a random process. The spread of results forms a normal distribution. The standard deviation can be calculated very simply (using mathematics devised by Poisson) by taking a square root of the counts:

$$\sigma = \sqrt{\text{total counts taken}}$$

Essentially this means that the more counts we take the smaller the standard deviation is as a proportion of the mean count rate. Put simply, the more counts measured the more accurate the data.

Example 5 MAKING UP A SOLUTION OF KNOWN ACTIVITY

Question One litre of [^{3}H]uridine with a concentration of 100 mmol cm^{-3} and 50 000 c.p.m. cm^{-3} is required. If all measurements are made on a scintillation counter with an efficiency of 40%, how would you make up this solution if the purchased supply of [^{3}H]uridine has a radioactive concentration of 1 mCi cm^{-3} and a specific activity of 20 Ci mol^{-1}, 0.75 TBqmol^{-1}?
[NB: M_r uridine = 244; 1 Ci=22.2 × 10^{11} d.p.m.]

Answer This problem is similar to the leucine example given above. Correcting for the 48% counting efficiency: 50 000 c.p.m. is 125 000 d.p.m. Multiplying this by 10^3 for a litre gives a d.p.m. equivalent to 56.3 μCi (125 × 10^6/22.2 × 10^5 = 56.3 μCi). Given 20 Ci mol^{-1}, work out how many moles there are in 56.3 μCi (56.3/20 × 10^6 = 2.815 mmoles). 100 000 mmoles of uridine are required in a litre; from the molecular mass this is 24.4 g. The 2.815 mmoles from the radioactive input is only 0.685 mg and so can effectively be ignored. The answer is, therefore, 56.3 mm^3 (56.3 μCi, 2.08 MBq) of [^{3}H]uridine plus 24.4 g of uridine.

Consider these simple examples for a series of 1 min counts:

counts = 100	$\sigma = \sqrt{\text{total counts}} = 10$	σ is 10% of the mean
counts = 1000	$\sigma = \sqrt{\text{total counts}} = 33$	σ is 3% of the mean
counts = 10 000	$\sigma = \sqrt{\text{total counts}} = 100$	σ is 1% of the mean

It is common practice to count to 10 000 counts or for 10 min, whichever is the quicker, although for very low count rates longer counting times are required. Another common practice is to quote mean results plus or minus 2 standard deviations, since 95.5% of results lie within this range.

Example 6 ACCURACY OF COUNTING

Question A sample recording 564 c.p.m. was counted over 10 min. What is the accuracy of the measurement for 95.5% confidence?

Answer 5640 counts were recorded (564 × 10); the square root is 75. Therefore the range is 5640 ± 150 for 95.5% confidence, or 564 ± 15 c.p.m., an acceptable level of accuracy.

14.4.4 The choice of radionuclide

This is a complex question depending on the precise requirements of the experiment. A summary of some of the key features of radioisotopes commonly used in biological

Table 14.5 **The relative merits of commonly used radioisotopes**

Isotope	Advantages	Disadvantages
^{3}H	Relative safety	Low efficiency of detection
	High specific activity possible	Isotope exchange with environment
	Wide choice of positions in organic compounds	Isotope effect
	Very high resolution in autoradiography	
^{14}C	Relative safety	Low specific activity
	Wide choice of labelling position in organic compounds	
	Good resolution in autoradiography	
^{35}S	High specific activity	Short half-life
	Good resolution in autoradiography	Relatively long biological half-life
^{33}P	High specific activity	Lower specific activity than ^{32}P
	Good resolution in autoradiography	Less sensitive than ^{32}P
	Less hazardous than ^{32}P	Cost
^{32}P	Ease of detection	Short half-life affects costs and experimental design
	High specific activity	
	Short half-life simplifies disposal	High β energy so external radiation hazard
	Čerenkov counting	Poor resolution in autoradiography
^{125}I	Ease of detection	High penetration of radiation
	High specific activity	
	Good for labelling proteins	
^{131}I	Ease of detection	High penetration of radiation
	High specific activity	Short half-life

work is shown in Table 14.5. The key factors in the decision are often based on safety, the type of detection to be used, the sensitivity required (see section 14.4.2) and the cost. For example ^{33}P may be chosen for work with DNA because it has high enough energy to be detected easily, it is safer than ^{32}P and its half-life is short enough to give high specific activity but long enough to be convenient to use.

Although they undergo the same reactions, different isotopes may do so at different rates. This is known as the isotope effect. The different rates are approximately proportional to the differences in mass between the isotopes. This can be a problem in the case of ^{1}H and ^{3}H, but the effect is small for ^{12}C and ^{14}C and almost insignificant for ^{33}P and ^{32}P. The isotope effect may be taken into account when choosing which part of a molecule to label with ^{3}H.

14.5 SAFETY ASPECTS

The greatest practical disadvantage of using radioisotopes is the toxicity: they produce ionising radiations. When absorbed, radiation causes ionisation and free radicals form that interact with the cell's macromolecules, causing mutation of DNA and hydrolysis of proteins. The toxicity of radiation is dependent not simply on the amount present but on the amount absorbed by the body, the energy of the absorbed radiation and its biological effect. There are, therefore, a series of additional units used to describe these parameters.

A key aspect of determining toxicity is to know how much energy might be absorbed, just as too much sun gives you sunburn and potentially skin cancer. The higher the energy of the radiation the greater the potential hazard. The **gray** (Gy), an SI unit, is the unit used to describe this; 1 Gy is an absorption of 1 J kg^{-1} of absorber. The gray (Gy) is a useful unit, but it still does not adequately describe the hazard to living organisms. This is because different types of radiation are associated with differing degrees of biological hazard. It is, therefore, necessary to introduce a correction factor, which is calculated by comparing the biological effects of any type of radiation with that of X-rays. The unit of absorbed dose, which takes into account this weighting factor is the **sievert** (Sv) and is known as the equivalent dose. For β-radiation Gy and Sv are the same, but for α-radiation 1 Gy is 20 Sv. In other words α-radiation is 20 times as toxic to humans as X-rays for the same energy absorbed. Clearly, from the point of view of safety, it is advisable to use radioisotopes with low energy wherever possible.

Absorbed dose from known sources can be calculated from knowledge of the rate of decay of the source, the energy of radiation, the penetrating power of the radiation and the distance between the source and the laboratory worker. As the radiation is emitted from a source in all directions, the level of irradiation is related to the area of a sphere. Thus the absorbed dose is inversely related to the square of the distance (the radius of the sphere) from the source; or, put another way, if the distance is doubled the dose is quartered. A useful formula is:

$$\text{dose}_1 \times \text{distance}_1^2 = \text{dose}_2 \times \text{distance}_2^2 \qquad (14.6)$$

The relationship between radioactive source and absorbed dose is illustrated in Fig. 14.11. The rate at which dose is delivered is referred to as the dose rate, expressed in Sv h^{-1}. It can be used to calculate your total dose. For example, a source may be delivering 10 m Sv h^{-1}. If you worked with the source for 6 h, your total dose would be 60 mSv. Dose rates for isotopes are provided in Table 14.2.

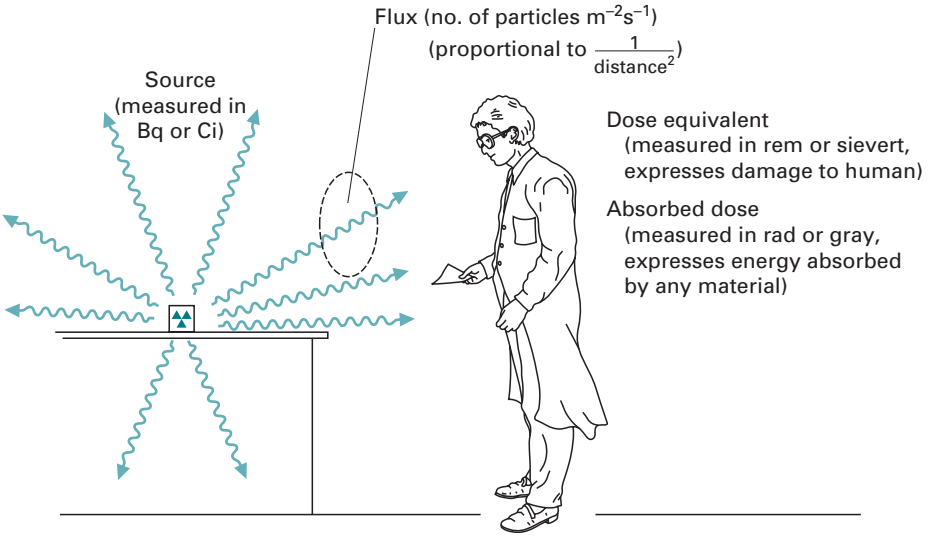

Fig. 14.11 The relationship between radioactivity of source and absorbed dose.

Example 7 CALCULATION OF DOSE

Question A 1 mCi source of ^{125}I gives a dose of 10 mSv h^{-1} at 1 cm. What will be the dose rate at 5 cm?

Answer Using the formula above $10 \times 1^2 =$ new dose $\times 5^2$.
Therefore the new dose is $10/25 = 0.4$ mSv h^{-1}.

Currently the dose limit for workers exposed to radiation is 20 mSv in a year to the whole body, but this is rarely ever approached by biologists because the levels of radiation used are so low. Limits are set for individual organs. The most important of these to know are for hands (500 mSv per year) and for lens of the eye (150 mSv per year).

Dose limits are constantly under review and, although limits are set, it is against internationally agreed guidelines to work up to such a limit, that is we are not allowed to assume that all is satisfactory if the limit is not exceeded. Instead, the ALARA principle is applied, to work always to a dose limit that is As Low As Reasonably Achievable. Work that may cause a worker to exceed three-tenths or one-tenth of the dose limit must be carried out in a controlled area or a supervised area, respectively. In practice, work in the biosciences rarely involves a worker receiving a measurable dose. Supervised areas are common but not always required (e.g. for ^3H or ^{14}C experiments). Controlled areas are required only in certain circumstances, for example for isotope stores or radioiodination work. A potential problem, however, in biosciences is the internal radiation hazard. This is caused by radiation entering the body, for example by inhalation, ingestion, absorption or puncture. This is a likely source of hazard where work involves open sources (i.e. liquids

Table 14.6 **Units commoly used to describe radioactivity**

Unit	Abbreviation	Definition
Counts per minute or second	c.p.m.	The *recorded* rate of decay
	c.p.s.	
Disintegrations per minute or second	d.p.m.	The *actual* rate of decay
	d.p.s.	
Curie	Ci	The number of d.p.s. equivalent to 1 g of radium (3.7×10^{10} d.p.s.)
Millicurie	mCi	Ci$\times 10^{-3}$ or 2.22×10^{9} d.p.m.
Microcurie	μCi	Ci$\times 10^{-6}$ or 2.22×10^{6} d.p.m.
Becquerel (SI unit)	Bq	1 d.p.s.
Terabecquerel (SI unit)	TBq	10^{12}Bq or 27.027 Ci
Gigabecquerel (SI unit)	GBq	10^{9}Bq or 27.027 m Ci
Megabecquerel (SI unit)	MBq	10^{6}Bq or 27.027 μCi
Electron volt	eV	The energy attained by an electron accelerated through a potential difference of 1 volt. Equivalent to 1.6×10^{-19} J
Roentgen	R	The amount of radiation that produces 1.61×10^{15} ion-pairs kg^{-1}
Rad	rad	The dose that gives an energy absorption of 0.01 J kg^{-1}
Gray	Gy	The dose that gives an energy absorption of 1 J kg^{-1}. Thus 1 Gy=100 rad
Rem	rem	The amount of radiation that gives a dose in humans equivalent to 1 rad of X-rays
Sievert	Sv	The amount of radiation that gives a dose in humans equivalent to 1 Gy of X-rays. Thus 1 Sv=100 rem

and gases); most work in biology involves manipulations of radioactive liquids. Control of contamination is assisted by:

- complying with local rules, written by an employer
- conscientious personal conduct in the laboratory
- regular monitoring
- carrying out work in some kind of containment.

A useful guide for internal risks is the annual limit on intake (ALI). The ingestion of one ALI results in a person receiving a dose limit to the whole body or to a particular organ. Some ALIs are shown in Table 14.2. Management of radiation protection is similar in most countries. In the USA, there is a Code of Federal Regulations. In the UK there is the Radiaoactive Substances Act (1993) and the Ionising Radiations Regulations (1999). Every institution requires certification (monitored by the Environmental Protection Agency in the USA or the Environment Agency in the UK) and employs a Radiation Protection Advisor.

When planning to use a radioisotope consider the following:

(1) Is a radioisotope necessary?
If the answer is no, then a non-radioactive method should be used.

(2) Which isotope to use?
Ideally the one with the lowest energy that can deliver your needs.

When handling radioisotopes the rules are to:

- Wear protective clothing, gloves and glasses
- Use the smallest amount possible
- Keep radioactive materials safe, secure and well labelled
- Work in defined areas in a spill tray
- Monitor your working area frequently
- Have no foods or drinks in the laboratory
- Wash and monitor hands after the work is done
- Follow all local rules such as for the dispensing of stock and the disposal of waste
- Do not create radioactive aerosols or dust

and for penetrating radiations (e.g. ^{32}P and λ-emitters):

- Maximise the distance between yourself and the source
- Minimise the time of exposure
- Maintain shielding at all times.

14.6 SUGGESTIONS FOR FURTHER READING

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