RADIOACTIVE TRACERS

1. Introduction

Radiochemical tracers, compounds labeled with radioisotopes, have become one of the most powerful tools for detection and analysis in research, and have become widely used in clinical diagnosis. A molecule or chemical is labeled using a radioisotope either by substituting a radioactive atom for a corresponding stable atom in the compound, such as substituting ^{3}H for ^{1}H, ^{14}C for ^{12}C, ^{32}P or ^{33}P for ^{31}P, and ^{35}S for ^{32}S, eg, or by adding a radioactive atom to a molecule, such as iodinating a protein or peptide using ^{125}I. In some cases, radioactive labeling results in substituting an atom using a noncorresponding, but chemically similar, radioactive isotope, such as replacing ^{16}O with ^{35}S.

Radiometric detection technology offers high sensitivity and specificity for many applications in scientific research. The radioactive emission of the labeled compound is easily detected and usually does not suffer from interference from endogenous radioactivity in the sample. Because of this unique property, labeled compounds can be used as tracers to study the localization, movement, or transformation of molecules in complex experimental systems.

The use of radioactive tracers was pioneered by Georg von Hevesy, a Hungarian physical chemist, who received the Nobel Prize in 1943 for his work on radioactive indicators (1). Radioisotopes have become indispensable components of most medical and life science research strategies. In addition, the technology is the basis for numerous industries focused on the production and detection of radioactive tracers. Thousands of radioactive tracers have been synthesized and are commercially available. These are used worldwide in tens of thousands of research laboratories.

2. Properties

Any radioactive nuclide or isotope of an element can be used as a radioactive tracer, eg, chromium-51 [14392-02-0], cobalt-60 [10198-40-0], tin-110 [15700-33-1], and mercury-203 [13982-78-0], but the preponderance of use has been for carbon-14 [14762-75-5], hydrogen-3 [10028-17-8] (tritium), sulfur-35 [15117-53-0], phosphorus-32, and iodine-125 [14158-31-7]. More recently phosphorus-33 has become available and is used to replace sulfur-35 and phosphorus-32 in many applications. By far the greater number of radioactive tracers produced are based on carbon-14 and hydrogen-3 because carbon and hydrogen exist in a large majority of the known natural and synthetic chemical compounds.

The properties of some commonly produced and used radioactive isotopes are listed in Table 1. The half-lives, and therefore the specific activities, of these nuclides vary over many orders of magnitude. It is this variation, coupled with variation in decay energy, which determines the suitability of a nuclide for the various applications and detection strategies. For example, techniques requiring the highest sensitivity of detection utilize ^{32}P, ^{33}P, ^{35}S, or ^{125}I. In addition, physical properties, along with the chemical form and biological properties, determine the approaches used for safe handling and waste disposal. The radioactive isotopes having short half-lives, eg, ^{32}P or ^{33}P, do not pose a serious

disposal problem because these can be held for decay and ultimately disposed of as cold waste. On the other hand, the disposal of ^{14}C and ^{3}H low level radioactive waste has become a problem for generators in the United States owing either to the unavailability of operating disposal sites or to the cost of disposal.

3. Syntheses

Syntheses of radioactive tracers involve all of the classical biochemical and synthetic chemical reactions used in the synthesis of nonradioactive chemicals. There are, however, specialized techniques and considerations required for the safe handling of radioactive chemicals, strategic synthetic considerations in terms of their relatively high cost, and synthesis scale constraints governed by specific activity requirements.

The radioactive isotopes available for use as precursors for radioactive tracer manufacturing include barium [^{14}C]-carbonate [1882-53-7], tritium gas, [^{32}P]-phosphoric acid or [^{33}P]-phosphoric acid [15364-02-0], [^{35}S]-sulfuric acid [13770-01-9], and sodium [^{125}I]-iodide [24359-64-6]. It is from these chemical forms that the corresponding radioactive tracer chemicals are synthesized. The [^{14}C]-Carbon dioxide, [^{14}C]-benzene, and [^{14}C]-methyl iodide require vacuum-line handling in well-ventilated fume hoods. Tritium gas, [^{3}H]-methyl iodide, sodium borotritide, and [^{125}I]-iodine, which are the most difficult forms of these isotopes to contain, must be handled in specialized closed systems. Sodium [^{35}S]-sulfate and sodium [^{125}I]-iodide must be handled similarly in closed systems to avoid the liberation of volatile [^{35}S]-sulfur oxides and [^{125}I]-iodine. Adequate shielding must be provided when handling [^{32}P]-phosphoric acid to minimize exposure to external radiation.

A multistep synthesis is strategically designed such that the labeled species is introduced as close to the last synthetic step as possible in order to minimize yield losses and cost. Use of indirect reaction sequences frequently maximizes the yield of the radioactive species at the expense of time and labor.

Many applications in tracer technology require products of high specific activity, ie, compounds having a high degree of substitution of specific atoms with radioisotopes. For many labeled compounds nearly 100% labeling can be achieved at one or more locations in a molecule using ^{14}C, ^{3}H, ^{32}P, ^{33}P, or ^{35}S, eg, using carrier-free precursors. The mass quantities of high specific activity compounds are usually low, even for those required for commercial production. Micro or semimicro synthetic methods are thus used in the manufacturing process.

Synthetic chemical approaches to the preparation of carbon-14 labeled materials involve a number of basic building blocks prepared from barium [^{14}C]-carbonate (2). These are carbon [^{14}C]-dioxide; [^{14}C]-acetylene; [U–^{14}C]-benzene, where U = uniformly labeled; [1- and 2-^{14}C]-sodium acetate, [^{14}C]-methyl iodide, [^{14}C]-methanol, sodium [^{14}C]-cyanide, and [^{14}C]-urea. Many complicated radiotracers are synthesized from these materials. Some examples are [1-^{14}C]-8,11,14-eicosatrienoic acid [3435-80-1] from [^{14}C]-carbon dioxide, [ring-U–^{14}C]-phenylisothiocyanate [77590-93-3] from [^{14}C]-acetylene, [7-^{14}C]-norepinephrine [18155-53-8] from [1-^{14}C]-acetic acid, [4-^{14}C]-cholesterol [1976-77-8] from [^{14}C]-methyl iodide, [1-^{14}C]-glucose [4005-41-8] from sodium [^{14}C]-cyanide, and

[2-^{14}C]-uracil [626-07-3], [27017-27-2] from [^{14}C]-urea. All syntheses of the basic radioactive building blocks have been described (4).

The introduction of tritium into molecules is most commonly achieved by reductive methods, including catalytic reduction by tritium gas, [$^{3}H_2$], of olefins, catalytic reductive replacement of halogen (Cl, Br, or I) by $^{3}H_2$, and metal [^{3}H] hydride reduction of carbonyl compounds, eg, ketones and some esters, to tritium-labeled alcohols (5). The use of tritium-labeled building blocks, eg, [^{3}H]-methyl iodide and [^{3}H]-acetic anhydride, is an alternative route to the preparation of high specific activity, tritium-labeled compounds. The use of these techniques for the synthesis of radiolabeled receptor ligands, ie, drugs and drug analogues, has been described in detail in the literature (6,7).

Iodination of organic compounds using iodine-125 gives radiotracers that are in most cases modified forms of the compound being traced. Iodine is found in only a relatively small number of the naturally occurring compounds of interest. The radiotracer and the unlabeled parent substance must be determined to behave identically before acceptance of any derived data is valid. In the case of thyroxine, which is a naturally occurring iodine-containing substance, labeling with ^{125}I is achieved by exchange with [^{125}I]-NaI.

Noniodine-containing substances that are to be iodinated must have a moiety that can be iodinated directly, eg, phenol, imidazole, pyrimidine, etc, and can react with such reagents as [^{125}I]-KI_3, [^{125}I]-NaI–lactoperoxidase, [^{125}I]-ClI, etc. An alternative method for iodination is the use of the reactive Bolton-Hunter reagent [60285-92-9], ie, [^{125}I] iodinated *p*-hydroxyphenylpropionic acid *N*-hydroxysuccinimide ester. Proteins are most readily labeled with iodine and in most cases their properties are unaffected by iodination. The [^{32}P]-Phosphorus and [^{35}S]-sulfur are introduced mostly through the use of biosynthetic techniques acting upon [^{32}P]-phosphate and [^{35}S]-sulfate. Such reagents as [^{32}P]-PCl_5, [^{32}P]-$POCl_3$, [^{32}P]-PCl_3, [^{32}P]-P_2S_5, [^{35}S]-H_2S, [^{35}S]-Na_2SO_4, [^{35}S]-Na_2SO_3, [^{35}S]-KSCN, and [^{35}S]-P_2S_5 have been prepared and are either available routinely as synthetic precursors or as custom syntheses from the principal radiochemical suppliers.

Biosynthetic techniques utilizing enzymes isolated from plants, animals, or microorganisms have made possible the synthesis of many labeled compounds of biological interest. Enzymes are uniquely suited for such syntheses because the enzymes are biological catalysts that act with high specificity and at low substrate concentrations. The enzymes can be used for the synthesis of many high specific activity tritiated nucleosides, nucleotides, nucleotide sugars, and leukotrienes, as well as most ^{32}P- and ^{33}P-labeled nucleotides and ^{35}S-labeled nucleotide analogues. Many of these products have been synthesized at near-theoretical specific activity using multistep enzymatic procedures. Enzymes used in the production of radioactive tracers are isolated from various sources depending on the specific enzyme of interest. Bacteria, eg, *Escherichia coli*, sea urchin sperm, carrots, bull testicles, rat liver, and human blood are some of the varied sources. Some of these enzymes have been cloned in bacteria, making isolation and purification less complex than using natural sources. Some of the enzymes used for labeled compounds production are available from commercial suppliers, eg, Boehringer Mannheim (Indianapolis, Indiana), Sigma Chemical Company (St. Louis, Missouri), and Pharmacia (Uppsala, Sweden).

Microbiological procedures that exploit the ability of bacteria and photosynthetic algae to incorporate exogenous labeled precursors, eg, $^{14}CO_2$, $^{35}SO^{2-}_4$, and $^{32}PO^{3-}_4$ can be used to label complex molecules in cells, eg, proteins and nucleic acids, which are then processed to give labeled constituents, eg, uniformly labeled ^{14}C-amino acids, ^{14}C-nucleotides, ^{14}C-lipids, and ^{35}S-amino acids (8).

Even higher organisms can be used for the production of labeled compounds. Plants, tobacco, or *Canna indica*, eg, when grown in an exclusive atmosphere of radioactive carbon dioxide, [$^{14}CO_2$], utilize the labeled precursor as the sole source of carbon for photosynthesis. After a suitable period of growth, almost every carbon atom in the plant is radioactive. Thus, plants can serve as an available source of ^{14}C-labeled carbohydrates (9).

4. Purification

The small synthetic scale used for production of many labeled compounds creates special challenges for product purification. First, because of the need for use of micro or semimicro synthetic procedures, the yield of many labeled products, eg, high specific activity tritiated compounds, is often low. In addition, under such conditions, side reactions can generate the buildup of impurities, many of which have chemical and physical properties similar to the product of interest. Also, losses are often encountered in simply handling the small amounts of materials in a synthetic mixture. As a consequence of these considerations, along with the variety of tracer chemicals of interest, numerous separation techniques are used in purifying labeled compounds.

For products having relatively low specific activity, such as some compounds labeled with ^{14}C that are synthesized on the scale of several millimoles, classical organic chemical separation methods may be utilized, including extraction, precipitation, and crystallization. For separation of complex mixtures and for products having high specific activity, eg, those labeled with tritium, ^{125}I, and ^{32}P, chromatographic methods utilizing paper, thin layers, or columns packed with silica and ion-exchange resins are more useful. For many applications, the method of choice is high pressure liquid chromatography (HPLC) using columns packed with resins designed for ion exchange and normal phase chromatography, or materials designed for reverse-phase separations. The HPLC method has the advantages of high resolution and speed; however, it requires costly chromatography columns and instrumentation.

5. Decomposition

Decay products of the principal radionuclides used in tracer technology (see Table 1) are not themselves radioactive. Therefore, the primary decomposition events of isotopes in molecules labeled with only one radionuclide–molecule result in unlabeled impurities at a rate proportional to the half-life of the isotope. For ^{14}C and ^{3}H, impurities arising from the decay process are in relatively small amounts. For the shorter half-life isotopes, the relative amounts of these

impurities caused by primary decomposition are larger. For multilabeled tritiated compounds, the rate of accumulation of labeled impurities owing to tritium decay can be significant. This increases with the number of radioactive atoms per molecule.

More problematic for suppliers and users of tracer chemicals is the decomposition of the labeled compounds caused by radiolysis during storage. This phenomenon is the result of the dissipation of the energy released in the surrounding media by decay of a radionuclide forming reactive species, eg, free radicals, which can cause chemical bond cleavage in other labeled molecules in the surrounding microenvironment. This mechanism of decomposition is usually referred to as secondary decomposition and is the most significant factor affecting the stability of tracers labeled with weak β-emitting radionuclides, eg, tritium.

A related mechanism of degradation involves the direct interaction of the radioactive emission with other tracer molecules in the preparation. This phenomenon is likely to occur in high specific activity compounds stored at high radiochemical concentrations in the absence of free-radical scavengers.

Many tracer chemicals are inherently unstable even as the unlabeled forms. Susceptibility of a chemical to hydrolysis, oxidation, photolysis, and microbiological degradation needs to be evaluated when designing suitable storage conditions for the labeled compound. Factors that reduce radiolytic degradation, such as dispersal in solution, are apt to increase chemical degradation or instability.

A great deal of empirical information has been developed on storage conditions and additives that reduce the rate of decomposition of tracer chemicals. In general, low storage temperatures, dispersal in solution to reduce radiochemical concentration, and the addition of free-radical scavengers and antioxidants, eg, ethanol, benzyl alcohol, ascorbic acid, and mercaptoethanol among others, have been shown to stabilize many classes of ^{3}H-, ^{14}C-, ^{32}P-, and ^{35}S-labeled compounds (10,11). The choice of stabilizer often needs to be assessed against possible interference with the use of the tracer chemical. For example, high concentrations of ethanol inhibit enzymatic systems used with ^{32}P- and ^{35}S-labeled nucleotides. For these products alternative stabilizers, eg, Tricine (*N*-[2-hydroxy-1,1-bis-(hydroxymethyl)ethyl]glycine [5704-04-1]), have been used by commercial suppliers. Some novel proprietary formulations have been developed by manufacturers of tracer chemicals that allow the shipment of some ^{32}P-, ^{33}P-, and ^{35}S-labeled compounds at ambient temperatures.

6. Detection and Quantitation

The methods for detection and quantitation of radiolabeled tracers are determined by the type of emission, ie, β-, γ-, or X-rays, the cost of the tracer; the energy of the emission; and the efficiency of the system by which it is measured. Detection of radioactivity can be achieved in all cases using the Geiger counter. However, in the case of the radionuclides that emit low energy betas, eg, ^{3}H, large amounts of isotopes are required for detection and accurate quantitation of a signal. This is in most cases undesirable and impractical. Thus, more

sensitive and reproducible methods of detection and quantitation have been developed.

Liquid scintillation counting is by far the most common method of detection and quantitation of β-emission (12). This technique involves the conversion of the emitted β-radiation into light by a solution of a mixture of fluorescent materials or fluors, called the liquid scintillation cocktail. The sensitive detection of this light is affected by a pair of matched photomultiplier tubes in the dark chamber. This signal is amplified, measured, and recorded by the liquid scintillation counter. Efficiencies of detection are typically 25–60% for tritium; >90% for ^{14}C, ^{35}S, and ^{32}P; and 60–70% for ^{125}I. Since the efficiency can vary from sample to sample because of the variable presence of contaminants, eg, water in the sample, the efficiency needs to be measured for each sample. There are three methods for determining the efficiency of a sample: the internal standard approach, the pulse-height spectrum displacement approach, and the external standard approach. Most modern scintillation counters use the external standard approach, which involves placing a source of gamma radiation, eg, radium-226, near the sample. This will irradiate both the sample and the vial and produce Compton electrons, which will behave the same as the beta particles emitted by the radioactive sample. The efficiency of the measurement of the external standard will be proportional to the efficiency of the signal of interest, which allows the efficiency of the signal to be determined.

A lesser used technique for the detection and quantitation of β-emissions is planchette counting. A film of the sample on a planchette, a flat metal pan, is brought into proximity, but at a fixed distance, to a proportional counter. The emissions are measured and recorded. Typical efficiencies are ^{14}C, ~30–40%; ^{35}S, ~30–40%; and ^{32}P, ~50%.

Gamma cameras have become widely used in nuclear medicine to detect γ-emission. There are three main types of gamma cameras: planar cameras, single-photon emission computed tomography (SPECT) cameras, and positron emission tomography (PET) cameras.

The detection and quantitation of γ-emission from ^{125}I is accomplished by well counting. A thallium-activated sodium iodide crystal, having a well or drilled hole that contains the sample, converts the emission to light. The light is amplified, counted, and recorded using a photomultiplier tube. The efficiency for ^{125}I is typically 70%.

The β-emission of ^{32}P is energetic enough in its passage through water to emit light (Cherenkov effect). This emission can be measured by a photomultiplier tube with a typical efficiency of ~40%.

The nonquantitative detection of radioactive emission is sometimes required for special experimental conditions. Autoradiography, which is the exposure of photographic film to radioactive emissions, is a technique for locating radiotracers on thin-layer chromatographs (tlc), electrophoresis gels, tissue mounted on slides, whole-body animal slices, and specialized membranes (13). After exposure to the radiolabeled emitters, dark or black spots or bands appear as the film develops. This technique is especially useful for tritium detection, but is also widely used for ^{14}C, ^{35}S, ^{32}P, ^{33}P, and ^{125}I. Instrumentation is also available by which the location of radioactive spots or bands on gels and membranes can be imaged without use of film.

Gas-flow counting is a method for detecting and quantitating radioisotopes on paper chromatography strips and thin-layer plates. Emissions are measured by interaction with an electrified wire in an inert gas atmosphere. All isotopes are detectable; however, tritium is detected at very low (~1%) efficiency.

Other methods of sensitive detection of radiotracers have been developed more recently. Fourier transform nuclear magnetic resonance (nmr) can be used to detect ^{3}H (nuclear spin 1/2), which has an efficiency of detection ~20% greater than that of ^{1}H. This technique is useful for ascertaining the position and distribution of tritium in the labeled compound (14). Field-desorption mass spectrometry (fdms) and other mass spectral techniques can be applied to detection of nanogram quantities of radiolabeled tracers, and are well suited for determining the specific activity of these compounds (15).

Determination of the radiochemical purity of labeled compounds is usually carried out using various chromatographic techniques, eg, paper or tlc, or HPLC. These procedures involve separation of the radioactive components of a sample using an appropriate elution solvent, followed by quantitation of the radioactivity in the separated fractions or bands by gas-flow counting, liquid scintillation counting, or autoradiography.

7. Health and Safety Factors

Allowable external radiation doses are described in Ref. 16. Depending on the quantities used and type of operation, the more energetic emissions of ^{32}P (β-ray), ^{125}I (γ- and X-rays), and ^{51}Cr (γ-ray) may require appropriate shielding to minimize personnel exposure. The β-rays of ^{35}S, ^{14}C, ^{33}P, ^{45}Ca, and ^{3}H are of low enough energy to require no shielding because the radiation does not pass through skin. All isotopes, however, present toxicity problems if taken into the body. However, personal safety precautions are related to the relative quantities of radioactive materials handled. Basic laboratory procedures to be followed protect the user from oral ingestion, skin contact, self-injection, and inhalation. The use of closed systems; well-ventilated work areas, ie, hoods and glove boxes; disposable gloves; disposable lab coats, etc; and neat work habits provide a safe working environment (17). Personnel monitoring may include urine and exhaled breath (CO_2) analysis, thyroid uptake (^{125}I) radioassay, use of dosimeters for detecting radiation from ^{32}P, and contamination surveys using appropriate instruments. The choice of test and frequency of testing vary with quantities and use (18).

Radionuclides are also used as tracers in human subjects. When ingested or injected, radiation exposure issues can be different for the subject than for the experimenter. Since the more energetic emissions readily leave the body, the time of exposure for gamma tracers is usually short. However, the local tissues (and skin) containing the tracer act as a shield to the β-rays of low energy emitters, so that the damaging radiation is contained within the body. The exposure to this radiation will continue until the tracer is excreted from the body. Therefore low energy beta emitters pose a more serious risk to the subjects than to the experimenter, whereas the radiation exposure issues for high energy emitters are similar for both subjects and experimenters.

Some radionuclides, eg, fluorine-18, have a short half-life, so the rate that they decay will determine the total radiation exposure. However, other radionuclides, eg, ^{3}H have lengthy half-lifes, so that the rate of excretion of tracers from the body will generally be much faster than the half-life of the radionuclide. In this case, the rate of excretion of the tracer will generally be the factor that determines the length (and thus amount) of radiation exposure for subjects receiving radioactive tracers. The rate of excretion of tracer will vary greatly depending on the substrate containing the radionuclide. For example, the whole-body water pool in humans generally turns over at a rate of 9%/day. Thus a $^{3}H_2O$ tracer will be excreted at a rate of 9%/day, so this excretion rate will primarily determine the half-life of the radiation exposure. In contrast, ^{3}H labeled long-chain fatty acids will be incorporated into triglycerides in adipose tissue and as a result will take much longer to excrete from the body than labeled water. Thus, the total radiation exposure to a subject given ^{3}H labeled long-chain fatty acids will be much greater than if a comparably sized dose of $^{3}H_2O$ is given. (Some of the tritium in a $^{3}H_2O$ tracer will be exchanged with the hydrogen atoms of a wide range of molecules including long-chain fatty acids. This exchange is relatively small, but may contribute significantly to the overall radiation exposure because of the slow turnover.) Therefore, it is critical that the average time that the labeled compound stays in the body is accurately calculated and taken into consideration when computing the radiation exposure to the subject for radioactive tracers with a long half-life.

Protection of the environment from uncontrolled radioactive release is also a consideration in the use of radiotracers. The quantity and concentration of radionuclides that may be discharged into sewer systems is limited by regulations of the Nuclear Regulatory Commission (NRC). Similarly, airborne emission limits have been established by the NRC for nonrestricted areas. Limits of surface contamination must be established to provide a safe workplace for users (19). The application of the as low as reasonably achievable (ALARA) principle to the above draws on the creative talents of the user to regard the limits as nonapproachable barriers and not as tolerable maxima for discharge.

U.S. radiation protection guidelines are established by the National Council on Radiation Protection and Measurement (NCRP) and are based on the recommendations of the International Commission on Radiological Protection (ICRP). The National Research Council also sponsors a report from its advisory committee on the biological effects of ionizing radiations (20).

8. Uses

The most effective use of radiotracers has been in biomedical research. In the 1950s, radioactive tracers were widely used to measure metabolic pathways in vivo. However, with the development of suitable stable isotopes and advances in gas chromatography/mass spectrometry (gc/ms) technology, these original methods have largely been replaced with stable isotope methods (see Ref. 21 for many examples). Radioactive tracers are still used in some animal studies because of the convenience of measuring radioactivity. There are also a few specialized applications in humans where the traditional approaches are still

used. For example, in some studies many pathways need to be determined at the same time, and there are not enough stable isotopes to measure all of the parameters of interest. If one wants to measure acetate and palmitate oxidation simultaneously, one cannot label both acetate and palmitate with ^{13}C, because the oxidation of these two substrates will both result in the production of $^{13}CO_2$. To overcome this problem, one substrate could be labeled with ^{13}C and the other substrate could be labeled with ^{14}C, producing both $^{13}CO_2$ and $^{14}CO_2$, respectively, which allows one to distinguish the oxidation of the two substrates. Since palmitate is retained in the body for a much longer period of time than acetate, it would be preferable to use ^{14}C acetate and ^{13}C palmitate rather than ^{13}C acetate and ^{14}C palmitate in this example to reduce the radiation exposure.

The calories derived from food depend in part on the extent to which the food is taken up by the gut. Although the absorption of foods, eg, fat or sugar substitutes, by the gut can be quantitatively measured with stable isotopes, radioactive tracers are preferred in practice by the FDA. This is due in part to the fundamental difference in the units of measurement. The unit of radioactive tracer measurements is tracer concentration (dpm/mL), which can be converted to specific activity (dpm/mg) by dividing the tracer concentration (dpm/mL) by the unlabeled (tracee) concentration (mg/mL). By contrast, the unit of stable isotope tracer measurements is tracer to tracee concentration ratio (mg/mg), which can be converted to tracer concentration (mg/mL) by multiplying the tracer to tracee concentration ratio (mg/mg) by the unlabeled (tracee) concentration (mg/mL). The desired unit in most kinetic study applications is tracer to tracee concentration ratio, which is obtained directly with stable isotopes, but obtained with radioactive tracers only with the additional measurement of concentration. However, when looking at the absorption of foods by examining the amount of the ingested tracer that came out in feces, the desired unit is tracer concentration, which is obtained directly with radioactive tracers, but not stable isotopes. Hence, radioactive tracers are preferable in this particular application.

Although most of the earlier *in vivo* radioactive tracer methods are no longer used in humans, the advent of PET scanning has reinvigorated the use of radioactive tracers in biomedical research and has become widely used for some clinical applications. One advantage of PET scanning over previous radioactive tracer studies is that the half-life of the isotopes used in PET scanning is much shorter (eg, ^{11}C, half-life 20.3 min vs. ^{14}C, half-life 5730 years), which means that the total radiation exposure to the subject for a given dose of tracer is much smaller. On the other hand, the actual doses of tracer given with PET tend to be much larger than the doses of tracer required with older methods. However, the main advantage of PET scanning is the ability to obtain kinetic measurements in tissues from which biopsies cannot be readily obtained, such as brain and heart. Most of the new PET and SPECT applications are presented in Ref. 22, but a few examples will be described here briefly.

Sodium ^{11}C acetate PET imaging is used clinically to differentiate infarction from ischemia in cardiac tissue. Acetate is readily oxidized by viable cardiac muscle, so ^{11}C acetate will readily decay in ischemic tissue, but not in dead tissue. The ^{18}F fludeoxyglucose is a glucose analogue that is transported and phosphorylated, but is not metabolized further. As a result, the amount of ^{18}F

fludeoxyglucose in a tissue reflects the glucose utilization rate. When tissue becomes ischemic, anaerobic glycolysis is promoted, which results in an increase in the glucose utilization rate. As a result, when ^{18}F fludeoxyglucose and sodium ^{11}C acetate PET imaging are used in combination, it is possible to differentiate between healthy, ischemic, and infarcted myocardium. The ^{18}F fludeoxyglucose PET imaging is also used clinically to diagnose a wide range of cancers. Since the glucose utilization rate is elevated in tumors, more ^{18}F fludeoxyglucose will be taken up by the tumors than in normal tissue. Since the use of ^{18}F fludeoxyglucose PET imaging to diagnose cancer and assess myocardial perfusion is typically a reimbursable medical procedure, PET imaging has become a widespread clinical tool.

Many PET imaging methods have been developed to study brain function. The ^{18}F fludeoxyglucose can be used as mentioned above to assess cerebral glucose utilization rates, which will be altered in the presence of dementias, Parkinson's disease, and epilepsy. The ^{18}F flurodopa and ^{11}C raclopride PET imaging methods are used to assess the dopaminergic nervous system, and can detect differences caused by Parkinson's disease, psychosis, and epilepsy.

In addition to *in vivo* applications, there are a wide range of *in vitro* applications for radioactive tracers. For example, labeled drugs and ligands using ^{3}H, ^{14}C, and ^{125}I, are widely used for screening for potential new drugs. In this procedure, the labeled drug is incubated with a receptor preparation *in vitro* and the radioactivity bound to the receptor is quantitated using liquid scintillation counting. By including an unlabeled drug candidate compound in the mixture and measuring the extent of displacement or inhibition of the binding of the radioactive tracer, the potential pharmacologic activity of the candidate compound can be assessed. Thousands of chemicals can be tested using such receptor binding assays in a week employing automated, high throughput screening procedures.

In addition to being used for screening purposes for new drug development, labeled drugs and ligands are widely used by pharmaceutical companies for metabolic, bioavailability, and toxicological studies to support new drug applications for FDA approval.

Radiotracers have also been used extensively for the quantitative microdetermination of blood serum levels of hormones, proteins, neurotransmitters, and other physiologically important compounds. Radioimmunoassay, which involves the competition of a known quantity of radiolabeled tracer, usually ^{125}I or ^{3}H, with the unknown quantity of serum component for binding to a specific antibody that has been raised against the component to be determined, is used in the microdetermination of physiologically active materials in biological samples.

The determination of the presence of reverse transcriptase in virus-infected cells can be done using labeled nucleotide triphosphates. Reverse transcriptase is an enzyme capable of synthesizing deoxyribonucleic acid (DNA) from ribonucleic acid (RNA) and it is thought to play an important role in virus-mediated cell modification. This enzyme is also a marker enzyme for human immunodeficiency virus (HIV), the virus implicated in causing acquired immunodeficiency syndrome (AIDS). The procedure utilizes radiolabeled nucleotides with nonlabeled substrates to synthesize tagged DNA. The degree of radioactive incorporation reflects the reverse transcriptase activity.

The availability of high specific activity ^{32}P-, ^{33}P-, and ^{35}S-labeled nucelotides has been crucial in the development and wide application of techniques for labeling DNA and RNA, the pivotal targets of research in molecular biology and genetics. These labeled compounds are substrates for most of the enzymes that synthesize, modify, or degrade nucleic acids. Thus a multitude of approaches have been devised for incorporating the tracers into DNA and RNA fragments. The labeled DNA or RNA is usually used in one of two principal applications: structure or sequence analysis of the labeled fragment, or use as a probe to detect sequences in an unknown sample that are complementary to the labeled fragment. For example, the use of [γ-^{32}P]-adenosine 5′-triphosphate [2964-07-0] as a source of phosphate for end-labeling DNA is an integral part of the Maxam-Gilbert sequencing procedure. In this method, the [^{32}P] end-labeled DNA is selectively cleaved by reagents specific for the rupture of selected chemical bonds between the various base sequences. Gel electrophoresis of the reaction mixtures followed by autoradiographic detection of the fragments allows the determination of the sequence of the bases.

In another method for sequencing, the DNA is labeled at internal base positions by enzymes that replicate DNA strands in the presence of specific chain terminators, thus producing an array of labeled fragments that reflect the sequence of the DNA. The rest of the procedure is the same as described for the chemical cleavage method (23).

Similar procedures are in use for labeling DNA and RNA either internally or at terminal positions for use as probes for complementary sequences in samples immobilized on nylon or nitrocellulose membranes (24). In this procedure the membrane-bound DNA or RNA is exposed to a solution of the labeled probe, and then autoradiographed using X-ray film to determine the binding of the probe. Often the immobilized nucleic acid is prepared by blotting specifically processed and separated fragments from gel or agarose electrophoresis slabs to the membrane. Probing such a blot with specific labeled DNA or RNA fragments can be used to identify the location of genes in DNA or to generate patterns that are unique to organisms or even individuals. Thus, this technique forms the basis for DNA fingerprinting, a useful method for typing tissues for organ transplants, for determining maternity or paternity relationships of an offspring with contested parents, or for forensic analysis of blood and other bodily fluids (25).

Radioactive tracers also are used in agriculture. A test field containing a food crop is sprayed with either an organic fertilizer, pesticide, or fungicide that is laced with the appropriate radioactive tracer. Run off, leaching, or contamination of the water table can then be determined by measuring radioactivity in local ponds or rivers. Possible incorporation of these chemicals into the plants also is easily determined as is longevity or breakdown in the soil.

In the petroleum industry, the size of an underground oil deposit is determined by the injection of radiolabeled substances into a well head. The occurrence of radioactivity in the oil–water mixture, which is pumped out of adjoining wells, gives an indication of the pocket size of the oil deposit.

Concerns over safe handling of radioactive materials and issues around the cost and disposal of low level radioactive waste has stimulated the development of nonradiometric products and technologies with the aim of replacing radioactive tracers in research and medical diagnosis (26). However, for many of the

applications described, radioactive tracer technology is expected to continue to be widely used because of its sensitivity and specificity when compared with colorimetric, fluorescent, or chemiluminescent detection methods.

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DAVID L. CHINKES
The University of Texas Medical Branch

Table 1. **Properties of the Principal Radioactive Isotopes**[a]

Isotope	CAS Registry Number	Half-life, $t_{1/2}$	Specific activity, Bq/mmol[b]	Energy, MeV,[c] %	Decay product
^{3}H	[10028-17-8]	12.35 year	1.07×10^{12}	0.019, 100	^{3}He
^{14}C	[14762-75-5]	5730 year	2.31×10^{9}	0.156, 100	^{14}N
^{32}P	[14596-37-3]	14.29 day	3.36×10^{14}	1.71, 100	^{32}S
^{33}P	[15749-66-3]	25.4 day	1.89×10^{14}	0.249, 100	^{33}S
^{35}S	[15117-53-0]	87.44 day	5.51×10^{13}	0.167, 100	^{35}Cl
^{45}Ca	[13966-05-7]	163 day	2.95×10^{13}	0.257, 100	^{45}Sc
^{51}Cr	[14392-02-0]	27.70 day	1.73×10^{14}	0.320,[d] 10	^{51}V
^{125}I	[14158-31-7]	60.14 day	7.99×10^{13}	0.03, 20 0.02, 13 0.027,[e] 114 0.031, 27 0.035,[d] 7	^{125}Te

[a]Refs. 2 and 3.
[b]To convert Bq to Ci, divide by 3.70×10^{10}.
[c]Maximum energies for β^- emissions unless otherwise noted.
[d]γ-Emission.
[e]X-ray emission.