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SOME BASIC INFORMATION ABOUT RADIOACTIVE TRACERS FOR PLANT SCIENTISTS

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The use of artificial radioactive isotopes as tracers in biological experiments has increased greatly since 1946 when these materials became available from the pile at Oak Ridge, Tennessee. It will increase even more during the next few years. Many plant scientists will be using this relatively new experimental tool for the first time within the next year or two, and even those who are not contemplating the use of tracers will wish to know more about them.

This paper is designed to provide a brief basic introduction to the subject for both of these groups, especially the latter one. It is not designed as a manual or set of laboratory directions for those actually beginning work with tracers. It would be desirable for anyone who is planning independent work with tracers first to work in a laboratory where the best techniques are being used. There are numerous "tricks of the trade" which cannot be learned satisfactorily from books or papers on the subject, or by trial and error experience.

History of Radioactive Tracers

The history of radioactive tracers is, of course, associated with the history of radioactivity in general. Natural radioactivity was discovered by Becquerel in 1896 while he was working with uranium potassium sulphate. About the same time several other important discoveries were made, which were essential for developing an understanding of radioactivity. Roentgen discovered X-rays in 1895, in 1897 Thompson discovered the electron, and in 1898 the Curies discovered radium and polonium. Rutherford discovered the alpha particle in 1904; in 1912 he and Bohr postulated the now-familiar structure of the atom. In 1919 Rutherford produced the first artificial transmutation, changing a few atoms of nitrogen into oxygen by bombarding them with alpha particles from a naturally radioactive substance. The Rutherford-Geiger counter for measuring radioactivity was produced in 1908, followed by the Geiger counter in 1913, and the well-known Geiger-Muller counter in 1928.

The first of the three distinct periods in the use of radioactive tracers in biology, or the period of natural radioactive tracers, began in 1923 when Hevesy (111) used radioactive lead (radium D) to study absorption and translocation in plants. Later, he also used radioactive bismuth (radium E). He attempted to avoid both radioactive injury and toxic effects by using very dilute solutions of the radioactive salts. The only other study of this period using plants was that of Lark-Horovitz (123), who investigated permeability by means of radioactive lead (thorium B). It is obvious why so little work was done with natural radioactive tracers. The naturally radioactive substances, except for K^{40}, which is very scarce, are not elements involved in normal plant metabolism. Their use would, therefore, give little information about normal physiological processes, except by rather dubious inference. In addition, they are rather toxic.

The second period in the use of radioactive tracers began about 1936 with the availability of artificial radioactive isotopes from the cyclotron, which followed

another series of important basic discoveries in nuclear physics. Many of these came in 1932. In that year, Chadwick discovered the neutron, Anderson the positron, Urey, Brickwedde and Murphy deuterium (heavy hydrogen), and Lawrence the deuteron. Cockcroft and Walton first used artificially accelerated nuclear particles, producing transmutation of lithium by artificially accelerated protons. Van de Graaf built his huge electrostatic generator for producing accelerated nuclear particles, and Lawrence outlined the principle of the cyclotron, whereby even greater acceleration could be secured. In 1934 Irene Curie and J. Joliot discovered artificial radioactivity, and three artificial radioactive elements were produced that year. By 1937, 190 radioactive isotopes had been produced by means of the cyclotron. The same year, a cyclotron was under construction in Lawrence's laboratory especially for the production of radioactive isotopes for use in biology and medicine. It was completed in 1939. The betatron developed by Kurst in 1941 has not been used extensively in the production of radioactive isotopes.

The first use of artificial radioactive tracers in plants was by Hevesy, Linderstrom-Lang and Olsen (113) in 1936, using radioactive phosphorus. In the 11-year period, 1936-1946, at least 64 papers were published involving the use of radioactive tracers in plants, exclusive of bacteria. It is beyond the scope of this paper to review this literature, but the distribution of the studies by years and types of use is presented in Table 1. Radioactive phosphorus was used in 33 studies, potassium in 15, sodium in 13, carbon in 11, bromine in 7, and rubidium in 6. Nitrogen (114,139), hydrogen (131), magnesium, copper, zinc, iodine, sulphur, iron and calcium have also been used.

The third period in the use of radioactive tracers dates from 1946 when radioisotopes were first made available from the Oak Ridge pile for non-military use. The first self-sustaining chain reaction pile was put in operation at the University of Chicago on December 2, 1942. However, subsequent developments were centered on the production of atomic bombs and supplying other military uses. It was not until June 14, 1946 that radioisotopes became available for tracer studies and similar non-military uses.

One major significance of the pile as a source of radioisotopes is that it is possible to produce them much more cheaply and in much larger amounts than by means of the cyclotron. For example, one millicurie of C^{14} produced by the use of cyclotrons would cost about $1,000,000 and would require the continuous operation of five cyclotrons for a year. In the first four months of production of C^{14} from the uranium pile over 50 millicuries were sold at only $50 per millicurie.

Radioactive Isotopes as Tracers in Plants

Radioisotopes have been used as tracers in plants principally in the study of the absorption, translocation, accumulation and general metabolism of mineral salts, and in the study of photosynthesis (Table 1). The reasons for such emphasis are obvious - the ease with which radioactive elements may be incorporated in mineral salts, or in carbon dioxide. As more organic compounds with radioactive carbon or other radioactive elements incorporated in them are produced, and when radioactive hydrogen becomes generally available, permitting the synthesis of radioactive water, it should be possible to apply radioactive tracer techniques to the study of practically every physiological process of plants. The nature of the intermediate steps in the process of photpsynthesis is already being clarified by the use of radioactive carbon. The same will probably soon apply to the mysteries of fat and protein metabolism and many other chemical processes in plants. In plant pathology, the tracers should provide new information in regard to host-parasite relations, fungicides and many other aspects of the field. The use of tracers in other phases of the plant sciences now appears to be more limited, but not impossible. Dr. Orlin Biddulph has said that "the possibilities of radioactive tracer used are limited only by the imagination."

Table 1. Number of papers involving the use of radioisotopes as tracers in plants, 1936 to 1947, inclusive, listed in accompanying bibliography

Year	Soils and absorption	Translocation	Photosynthesis	General metabolism and misc.	Total
1936	0	1	0	0	1
1937	0	2	0	2	4
1938	4	0	0	0	4
1939	2	3	2	5	12
1940	8	1	3	4	16
1941	2	1	4	1	8
1942	2	1	3	3	9
1943	0	1	0	0	1
1944	3	1	0	3	7
1945	0	0	0	1	1
1946	1	0	0	0	1
1947	4	1	1	1	7
Total	26	12	13	20	71

It should be emphasized that the importance of radioactive tracers lies in the ease with which they may be detected, in many cases without destroying the tissue involved, and often permitting detection and measurement which would be absolutely impossible by ordinary methods. In general, experiments are not set up differently than if ordinary substances were being used, the only difference being the methods of detection or measurement employed. These will be discussed later.

Stable Isotopes as Tracers in Plants

The use of non-radioactive isotopes, or stable isotopes, as tracers is beyond the scope of this review, but it may be well to consider them briefly. Not long after Urey and his associates discovered deuterium or heavy hydrogen in 1932, Schoenheimer and Rittenberg began using it as a biological tracer (1935). They and

other investigators have since done considerable work with deuterium, but in addition stable isotopes of carbon, nitrogen, oxygen and sulphur have been used. The use of stable isotopes as tracers has been limited because of certain disadvantages as compared with radioactive isotopes. Detection and measurement of the stable isotopes is a rather prolonged and laborious process involving the use of the mass spectrograph, although in the case of deuterium, thermal conductivity and the density or refractive index of combustion water can also be used. It is always necessary to destroy the tissue being tested, as the nitrogen, for example, must be reduced to elemental nitrogen, the carbon oxidized to carbon dioxide, and the potassium ashed. It is thus impossible to make a series of readings from a single specimen as can be done in some types of experiments with radioactive tracers. However, stable isotopes are usually preferable for long-time experiments, and besides, the radioactive isotopes of some biologically important elements have such short half-lives that it is practically impossible to use them as tracers.

Some Basic Facts about Radioactivity

In order to use radioactive tracers intelligently it is necessary to have in hand a few of the elementary concepts of nuclear physics. Shannon (29) provides an excellent general non-technical background, while Pollard and Davidson (24) provide a somewhat more technical and excellent discussion. Many of the other references in Section A of the bibliography also provide some information on nuclear physics and radioactivity, perhaps the most valuable for introductory purposes being the Enclycopedia Britannica and the papers of Livingood and Seaborg (21), Loofbourow (22) and Seaborg (26). The recent book on radioactive tracers in biology by Kamen (18) includes some basic information on artificial radioactivity, as well as a discussion of methods used in tracer studies. It should be read by all who wish to use radioactive tracers.

Symbols and terminology. The figures clustered around the symbol for a radioactive element may at first seem confusing, but they are quite simple. The subscript to the left is the atomic number, that is, the number of elementary units of positive charge on the nucleus or the number of electrons in the outer shells. The superscript to the right is the mass number, that is, the whole number nearest to the exact atomic mass of the element as determined by use of the mass spectrograph. Thus, ordinary potassium is ${}_{19}K^{39}$, naturally radioactive potassium is ${}_{19}K^{40}$, the heavy stable isotope is ${}_{19}K^{41}$, and artificially radioactive potassium is ${}_{19}K^{42}$. Since the atomic number is the same for all isotopes of any particular element it is often omitted. Sometimes an asterisk is used in place of the mass number for radioactive isotopes. Thus, radioactive potassium sulphate might be written either $K_2^{42}SO_4$ or $K_2{}^*SO_4$. The latter, of course, does not distinguish between the different radioactive isotopes of an element.

Instead of using the phrase "radioactive element" the contraction "radioelement" is frequently used. Similarly, it is quite common to use "radiophosphorus" instead of "radioactive phosphorus," for example.

Atomic particles. Scientists are generally familiar with the current concept of atomic structure, that is, one or more outer shells of electrons surrounding a nucleus composed of neutrons and protons. The neutron has no charge, and its mass is slightly larger than that of a proton.

The deuteron, or nucleus of heavy hydrogen (deuterium), is composed of one proton plus one neutron. The alpha particle, or helium nucleus, is composed of two protons plus two neutrons. The beta particles (electrons) are not stable constituents

of atomic nuclei, but are presumably formed and emitted when a neutron changes into a proton. The positive beta particles (positrons or positive electrons) are formed and emitted from a nucleus when a proton changes into a neutron. The positron usually exists only momentarily, uniting readily with an electron and forming a photon (a quantum of radiant energy) with the emission of about 1 million electron-volts of gamma radiation. A photon may also cause the production of an electron and a positron. The mesotron, or "heavy electron," is a component of cosmic rays, and is not yet shown to exist in or be produced by atomic nuclei. Table 2 presents an outline of the various atomic particles.

Radiations from radioactive substances. Both natural and artificial radioactive substances emit the following basic types of radiation:
1. Alpha rays or particles. These are helium nuclei, having a double positive charge and a mass four times that of a proton or hydrogen nucleus. They travel from 3 to 11 cm. from their source in air and are easily absorbed by various substances.
2. Beta rays or particles. These are high speed electrons from an atomic nucleus and are far more penetrating than alpha particles, in some instances being able to pass through several mm. of aluminum.
3. Gamma rays. These are very short electromagnetic waves, and may be regarded as high frequency X-rays. They usually accompany radioactive processes but are far more penetrating than beta particles, being able to pass through several centimeters of iron or lead. Unlike the charged alpha and beta particles they are not deflected by magnetic or electrical fields. Further information about these radiations are presented in Table. 3.

There are several additional types of radiations of lesser importance. 1. Positive beta rays consist of a stream of positrons instead of electrons. They are not found in naturally radioactive substances. 2. K-electron capture consists in the capture of an electron from the K shell by the nucleus. The K shell is the outer electron shell nearest the nucleus, and successive shells are labeled L, M, N, and so on. 3. Internal conversion. A process in which radiation from the nucleus of an atom is absorbed in the extra-nuclear structure of that same atom and converted by the photoelectric effect into kinetic energy of one of the extra-nuclear electrons. 4. Delta particles. Low speed electrons released from matter by alpha particles.

In addition to radiation, the naturally radioactive substances such as radium also emit radioactive matter in the form of emanations and active deposits. These are some of the inevitable dissociation products. The emanations are radioactive gases such as radon. The active deposits are non-gaseous radioactive substances which accumulate on nearby objects. These emanations and active deposits are not formed by the radioactive substances used in biological tracer work.

As time goes on, the activity of a radioactive substance, or the radiation from it, decreases. This decay follows an exponential curve, and the life of the radioactivity is theoretically infinite for all radioisotopes. It is, however, relatively simple to determine the half-life of a radioactive substance, that is, the time required for the initial radioactivity to be reduced to one-half. The half-lives of various artificially radioactive substances vary greatly. For example, the half-life of B^{12} is only 0.022 second while that of C^{14} is 5,100 years. Most radioactive substances have a long half-life. It must be kept in mind that the life of a radioactive substance is not just twice its half-life, but an infinite period due to the exponential nature of the decay. For example, P^{32} has a half-life of 14.3 days, but in twice that time it still has 28 percent of its initial activity left, and even after 100 days there is a fraction of a percent of its initial activity remaining.

Table 2. Information about sub-atomic particles. Modified from Shannon (29)

Particle	Mass	Charge	Source	Symbol
Electron	1/1846 of mass of H atom	-e	outer shells of atoms	$_{-1}^{0}e$
Beta particle	. .	-e	nucleus of radioisotopes	$_{-1}^{0}e$
Positron or positive electron	. .	+e	. .	$_{+1}^{0}e$
Mesotron	150 to 250 times that of the electron	-e, +e, 0	cosmic rays	
Proton	1.0076 atomic units	+e	nuclei of radioisotopes (the nucleus of ordinary H)	$_{1}^{1}H$
Neutron	slightly greater than a proton	0	nuclei of radioisotopes	$_{0}^{1}n$
Deuteron	2.0143 atomic units	+e	nucleus of heavy H	$_{1}^{2}H$
Alpha particle	4.0286 atomic units	+ 2e	nuclei of radioisotopes (He nucleus)	$_{2}^{4}He$

Units used in measuring radioactivity. The most common units of measurement related to radioactivity are as follows:

Curie - The amount of a radioactive substance giving the same number of disintegrations per unit time as one gram of radium, i. e., 3.71×10^{10} disintegrations per second. A millicurie is 1/1000 curie. A microcurie is 1/1,000,000 curie.

Half life - The time required for the disintegration rate of a radioactive isotope to decrease one-half its initial rate.

Half thickness - Thickness of absorbing material necessary to reduce the intensity of radiation by one-half. Usually applied only when absorption obeys the exponential law. Measured in centimeters of air or in mg./cm.^{2} of a given substance, often a metal such as aluminum.

Range - Distance radiation travels from source. Measured in the same units as half thickness.

Specific activity - Activity in millicuries determined from radioactivity measurements per milligram of substance determined chemically, relative to a milligram of radium.

Particle energy - The energy of radioactive particles (alpha, beta) usually expressed in millions of electron-volts (Mev) or kilo-electron volts (Kev). One Mev equals 1,000 Kev. An electron-volt is the amount of energy acquired by an electron in falling through a potential difference of one volt, or the charge of an electron (1.60×10^{-19} coulomb) times one volt ($=10^{7}$ ergs per coulomb), or 1.6×10^{-12} ergs. One Mev then equals 1.6×10^{-6} ergs, and one Kev 1.6×10^{-9} ergs.

Quantum energies - Used for measuring the energy of gamma rays or other electromagnetic radiation. In all atomic or molecular processes involving emission or absorption of energy the energy is transferred in separate portions or quanta, the magnitude of which is equal to a constant multiplied by the frequency. A photon is a quantum of free energy.

Table 3. Some characteristics of the common types of radiation from radioactive substances. Modified from Shannon (29)

Alpha rays	Beta rays	Gamma rays
Deflected slightly by magnetic and electrical fields, in such a direction as to show a positive charge	Deflected in a direction opposite to alpha rays or in the same direction if positive beta rays	Not deflected
Produce fluorescence	Feeble fluorescence	
Ionize gases 10,000 times as strongly as gamma rays	Ionize gases 100 times as strongly as gamma rays	Ionize gases
Poor penetrating power; stopped by 10 cm. of air or 0.1 cm. Al.	100 times more penetrating than alpha rays	10,000 times more penetrating than alpha rays
Speed from 1.45×10^{9} cm./sec. to 2.2×10^{9} cm./sec.	Speed from .36 to .99 that of light	Speed that of light
2 plus charge	1 minus or plus charge	No charge
Mass four times that of an H atom	Mass about that of an electron	No mass
A helium nucleus	Electron or positron	Very short electromagnetic waves; short X-rays

Radioactive Isotopes Suitable for Tracer Studies in Plants

Radioactive isotopes of all the elements have been produced, but some of these are not suitable for tracer studies because of their very short half lives. Of the elements important in plant metabolism only oxygen and boron have such short half-lives that they cannot be used at all. O^{15} has a half-life of 126 seconds, O^{19} a half-life of 31 seconds, and B^{12} a half-life of 0.022 second. Other radioelements have half-lives too short to permit their use in experiments extending over any period of time, especially if the source of the radioelements is some distance from the experimental laboratory. This is particularly true of C^{11}, N^{13}, Mg^{27}, Cl^{32}, and to a lesser extent of Na^{24}, K^{42}, and Cu^{64}. Information relating to the radioelements suitable for tracer studies in plants is presented in Table 4. In addition to the botanically important elements listed in this table, there are other suitable radioactive isotopes which might be employed occasionally in plants; such as F^{18}, Br^{82}, Si^{31}, Ba^{139}, Se^{75} and Ru^{86}.

Securing Radioactive Isotopes

Radioactive isotopes may be secured from laboratories which have cyclotrons, and some can still be secured only from this source. However, at the present time most radioactive isotopes can be secured in much greater quantities and more cheaply from the atomic piles operated by the U. S. Atomic Energy Commission, which periodically publishes a price list of isotopes available (65). Radioisotopes currently available without special order and which are suitable for plant tracer studies are checked in Table 4. The Atomic Energy Commission is now supplying also three stable isotopes; deuterium, B^{10} and O^{18}. To secure radioisotopes from the U. S. Atomic Energy Commission (those from the piles cannot be secured elsewhere) it is necessary to file a request with the Isotopes Division, U. S. Atomic Energy Commission, Box E, Oak Ridge, Tenn.

The isotopes from Oak Ridge are shipped in three different forms;

1. Fission products. These are mostly shipped in the form of mixed isotopes which usually require chemical separation and purification before use, although some are available as unmixed isotopes. These include no isotopes which would ordinarily be used as biological tracers.
2. Separated radioisotopes. These are of high enough purity for all ordinary tracer uses. Only C^{14}, I^{131}, P^{32}, S^{35} and Ca^{45} are available in this form.
3. Irradiated units. These are aluminum containers in which a specified quantity of target material has been sealed and irradiated in the pile. These are shipped without chemical processing. As several radioisotopes are usually present it is necessary to purify them before using.

In addition to any necessary purification, the isotopes must be incorporated in the desired compound and diluted and standardized. While the preparation of inorganic compounds containing the desired isotopes is not particularly difficult, the synthesis of organic compounds containing tracer elements is often very difficult, and sometimes living organisms must be used as synthetic agents. Except for radioactive gases, radioactive tracers are usually used in solution. It is important that exact concentrations be known if quantitative results are desired.

Since purification, synthesis and standardization are beyond the scope of the ordinary plant science laboratory, this must be turned over to a chemistry laboratory, or to a commercial radioactivity laboratory. Tracerlab, Inc., of Boston, performs these and other similar services, which are outlined in their bulletin (62). When isotopes are requested from Oak Ridge a request may also be filed to have them

Table 4. Information about radioelements most likely to be used as tracers in plants. (Adapted from Hamilton (12) and Radioisotopes Catalog and Price List (65))

Radio-element	Type of radiation	Half-life	Energy in Mev		Avail-ability	Mc. per unit	Price per unit or mc.
			Particles	Rays			
H^3	beta	20 y.	0.013	-	0	-	-
C^{14}	beta	5100 y.	0.145	-	s	1	$50/m.c.
N^{13}	beta+, gamma	9.93 m.	1.2, 0.92	0.28	0	-	-
Na^{22}	beta+, gamma	3 y.	0.58	1.3	0	-	-
Na^{24}	beta, gamma	14.8 h.	1.4	1.4,2.8	i	50	$12
Mg^{27}	beta, gamma	10.2 m.	1.8	0.9	0	-	-
P^{32}	beta	14.3 d.	1.69	-	s	1	$1.10/m.c.
S^{35}	beta	87.1 d.	0.17	-	s, i	1, 1	* , $33
Cl^{36}	beta	10^6 y.	0.66	-	i	0.005	$33
K^{42}	beta, gamma	12.4 h.	3.58, 2.07	1.51	i	130	$12
Ca^{45}	beta	180 d.	0.3	-	s, i	0.001	$4/m.c.;$33
Mn^{54}	K, gamma	310 d.	-	0.85	0	-	-
Fe^{59}	beta, gamma	44 d.	0.26, 0.46	1.1, 1.3	i	1	$33
Cu^{64}	beta, beta+	12.8 h.	0.58, 0.66	-	i	100	$12
Zn	beta+, K, e^-, gamma	250 d.	0.4 B+(1%) 1 K, e^-(99%)	1.14	i	20	$33
I^{131}	beta, gamma	8 d.	0.6	0.367, 0.08	s, i	130	$1.70/m.c.

K - K-electron capture; e^- - internal conversion electrons; h - hour; d. - day; m. - minute; y. - year; Mev - million electron volts; 0 - not available from Oak Ridge; s - separated radioisotopes; i - irradiation unit; mc - millicuries; * As H_2SO_4 - $2.40/m.c.; as Na_2S - $6.00/m.c.

sent directly to Tracerlab, or another similar laboratory, for processing before being sent on to the experimental laboratory.

In addition to supplying the radioisotopes in the forms mentioned, the Isotopes Division of the U. S. Atomic Energy Commission also performs service irradiations on substances sent to them by the user, and it supplies a limited number of labeled compounds. There is also a film badge service for radioactivity monitoring, which is quite inexpensive.

Securing radioisotopes is not a simple matter. Any investigator planning to use them should secure and read carefully the pertinent literature (50, 54, 55) issued by the Isotopes Division. Initial requests for radioisotopes must be made on Form 313 and supplementary requests on Form 335. A separate form must be filled out for each radioisotope desired. The forms, especially 313, are extremely detailed. In addition to exact specifications of the isotope, they ask for information such as: statement of intended uses, including syntheses to be performed; importance of the investigation; detection instruments to be used; name and experience of person who will supervise measurements; number and kind of organisms to be used; amount of activity per organism; proposed monitoring for health protection; proposed method of disposal of surplus and waste; list of publications on related studies; and name of journal in which the results are likely to be published. When the publication appears three copies of it must be sent to the Isotopes Division. The request is referred to the advisory committee nominated by the National Academy of Sciences, or one of its sub-committees.

When the request has been fully approved, the actual sale and shipment is handled by the Isotopes Office, Clinton National Laboratory, Monsanto Chemical Company, Oak Ridge, Tennessee. This should not be confused with the Isotopes Division of the U. S. Atomic Energy Commission.

At present, radioisotopes are supplied individuals only through qualified institutions. The Isotopes Division recommends (64) that each institution using radioisotopes set up a local radioisotopes committee which should approve all requests before they are sent to Oak Ridge, coordinate the use of the isotopes within the institution, effect economies in purchase of the isotopes, and make sure that adequate health precautions are provided. The committee should consist of a physicist, a chemist and a biologist, all of whom are acquainted with radioactivity, in addition to representatives of the specific departments or groups which will be using the isotopes.

It is obvious that there is little possibility of the radioisotopes getting into the hands of persons not qualified to use them, and also that an individual investigator has little chance of securing the isotopes unless the institution for which he is working decides to set up a coordinated program of research involving the use of the radioisotopes. These are undoubtedly very excellent safeguards.

The Detection and Measurement of Radioactivity

The procedures involved in experiments with radioisotopes are no different than those which would be used in similar experiments with stable substances, except for health precautions and for the methods employed in the detection and measurement of the radioactivity.

A variety of methods have been used in detecting and measuring radioactivity, all of them depending on the ionizing effects of the radiations. The instruments which have been used include the Lauritsen electroscope, ion chamber electroscopes, vibrating reed electrometers, cloud chambers, Geiger-Muller counters and photographic plates (radioautographs). Only the last two are used to any extent at all in biological tracer studies at the present time, so only these will be discussed.

Radioautographs. These provide the simplest method available for determining distribution of radioisotopes in plants. The tissue is placed in close contact with a photographic plate or film, and the radiation from the tissue exposes the negative. If properly done it is possible to secure remarkably good radioautographs.

It is, of course, necessary to prevent any light from reaching the film. All types of radiation (alpha, beta, gamma) are effective in producing radioautographs. While this method is primarily qualitative it may also give some quantitative information. The radioautographs may be enlarged or magnified. In this way semi-microscopic details may be observed, especially if a very fine-grained film has been used. Radioautographs can be made even with very low energy radioisotopes such as C^{14} (40).

Special films must be used to secure good radioautographs. These may be secured from the Eastman Kodak Company, Rochester, New York. Inquiries relating to them should be addressed to the Industrial Photographic Sales Division. The type of film to be used depends on the type of particles or rays emitted by the radioisotopes. Since no alpha particles are emitted by the isotopes ordinarily used as biological tracers, information on suitable films will be given here only for beta particles and gamma rays. In a letter to the writer, W. F. Swann of the Eastman Kodak Company, recommends the following films:

Beta particles: The type of film or plate recommended depends on the granularity and resolving power required. The following are suggested: Kodak Industrial X-ray Film, Type K; Eastman No-Screen X-ray film; or for finer grain Kodak Industrial X-ray Film, Type M. For very fine grain, Type V-O Spectroscopic Plates or Type 548-O Spectroscopic Plates are recommended.

Gamma rays: Kodak Industrial X-ray Film, Type K and Eastman No-Screen X-ray Film are the fastest materials for recording gamma rays. If finer grain, greater definition and higher contrast is desired, Kodak Industrial X-ray Film, Type A is recommended. This requires five times the exposure as that of Type K and No-Screen.

Geiger-Muller counters. The following equipment is necessary when Geiger-Muller counters are used: (1) the Geiger-Muller counter tube itself; (2) a suitable mount for reproducably introducing radioactive samples near the counter; (3) a lead shield for the counter to reduce cosmic and environmental background count. (This is not absolutely essential, but is of value, especially when the radiations to be measured are weak); (4) a regulated high voltage source for the counter; (5) an amplifier; (6) a scaling circuit and mechanical recorder, or a counting rate meter; (7) a standard cathode ray oscillograph, or a loud speaker, or headphones for visual or auditory monitoring of the counting process (optional), and (8) a stop watch or an electrical timer for accurate measurement of the counting period. Items 4, 5 and 6 are usually incorporated in one instrument, and sometimes items 7 and 8 are also included.

Items 1, 2, 4 and 6 can be constructed in well-equipped physics workshops, and items 5, 7 and 8 are usually on hand in a physics laboratory. Brewer and Bramley (35) have described home-made apparatus especially designed for use with plants. However, complete equipment is now available commercially from a number of companies which specialize in radioactivity equipment. Most investigators will probably find this commercial equipment more satisfactory and less expensive. Many of the firms which supply such equipment advertise regularly in SCIENCE and other scientific journals. A complete list of firms may be secured from the Isotopes Division, U. S. Atomic Energy Commission, Oak Ridge, Tennessee.

Geiger-Muller tubes come in a considerable variety of models, many of them for specialized uses, and cost from about $30 to $300. The mounts cost around $32, and the lead shields from $20 up. The scaling circuit and mechanical recorder with necessary auxiliary equipment, cost from $345 to $770, although the higher priced instrument includes also an electric timer with automatic cut-off. The complete

total cost for equipment would, then, range from a little less than $500 to around $1,400, depending on the type of equipment needed and chosen. A portable beta-gamma count rate meter, operating on batteries and complete with the Geiger-Muller tube and with headphones for auditory monitoring, is available for about $280. For some types of botanical tracer studies this instrument is more satisfactory than the more expensive scaling circuit outfits. In general, the first equipment ordered should have the greatest possible versatility; more specialized equipment can be added later after a greater familiarity with needs has been developed.

Basically, the Geiger-Muller counter tube is a cylindrical metal tube with a slender wire running through its center. This tube has a thin window of mica, glass or other substance, which permits radiation to enter without marked attenuation. The tube is filled with an inert gas such as argon, with not over 10 percent of some polyatomic gas added as a quenching agent. The following description of the operating principle of the Geiger-Muller counter is slightly modified from Korff (47):

"When an electron is accelerated through a gas by a sufficiently intense electrical field, the electron may acquire sufficient kinetic energy between successive collisions to ionize the gas molecules with which it collides. The electrons so produced are in turn accelerated and produce further ionization. This chain reaction leads to an amplification of 10^{10} to 10^{15} which is many times greater than any conveniently obtainable with an electronic amplifier. Another important advantage of this method of amplification is its comparatively great insensitivity to electrical and microphonic disturbances. A Geiger-Muller counter will respond only to the production of ionization within its sensitive volume and, since little or no further amplification is required, the associated electronic circuits can be made relatively insensitive to external electrical disturbances.

"A counter is composed, usually, of two coaxial electrodes. Common practice is to ground the outer electrode, the cathode, and connect the center electrode, the anode, which is a wire of small diameter, to a source of high voltage and an amplifier, followed by a scaling circuit and a mechanical recorder. The Geiger-Muller counter is generally filled with a noble gas, such as neon or argon, to a pressure of approximately 10 cm. plus 3-10% of a complex molecule gas which serves as a discharge quenching agent.

"The pulse produced by the counter is introduced into an electronic circuit which may amplify and reshape it. The pulse then goes to a scaling circuit, which causes some given fraction of the pulses to be recorded on a mechanical register. The scaling circuit is necessary as mechanical registers are not rapid enough to follow the counter impulse at high counting rates."

In purchasing counter tubes for biological tracer studies the proportional counters must be avoided. They are specially constructed to count alpha particles, and are useless for the detection of beta and gamma radiation, the only types produced by most tracer isotopes. Proportional counters are modified Geiger-Muller counters operating at a lower voltage.

Most Geiger-Muller counters are sensitive to radiation of as little as 10^{-5} microcuries. Because of such great sensitivity, Geiger-Muller counters have a residual counting rate even when no radioactive substances have been placed near them. This residual rate or minimum reading is known as the background count, and is due to cosmic rays or contamination of nearby objects with radioactive substances. The background count is usually between 10 and 60 per minute, except in laboratories where there has been considerable contamination due to previous use of radioisotopes.

The Geiger-Muller tube never responds to all of the ionization events taking place within its sensitive volume. It has a finite resolving time of the order of 10^{-4} sec. Ionization events occurring at smaller intervals will be missed.

Influence of Tracer Radioisotopes on Organisms

The question naturally arises whether the radioisotopes used in tracer studies may not affect the organisms in some such way as to invalidate the results secured by their use. It is generally agreed that the chemistry of the various isotopes of an element is not different enough to have any biological significance. Barnett (69) has suggested that radioactive tracers may have disturbing effects, but most of the notes which have been written on this subject (70-78) agree that radioisotopes in the amounts used for tracer studies have no disturbing physiological effects. Brooks (71) found that radioactive Na*Cl and K*Cl did not change the rate of respiration of Nitella. The activity of the samples was from 2.2 to 20 millicuries per liter. Mullins (76, 77) found that the radiation from Na* reduced permeability of Nitella when its concentration was over one millicurie per liter, but noted no effect whatsoever when the concentration was less than that. So far there is little evidence which indicates that radioactive isotopes have any physiological effects different from stable isotopes of the same element, in the concentrations employed in tracer studies. However, there is always the possibility that such differential physiological effects may be discovered.

Health Precautions

The disastrous effects of exposing the body to strong radiation from radioactive substances are now well known. Exposure to even comparatively weak radiation may produce skin erythema (sunburn), a reduction in the number of leucocytes, especially the polymorphonuclear ones, and possible mutations. Such results are dependent on both the intensity of the radiation and the duration of exposure to it. A normal daily safe dose of X-rays is regarded to be about 0.1 roentgens, and the same applies also to gamma rays. An exposure of one minute to beta rays from 100 millicuries of P^{32} will produce a definite effect on the skin, though it is limited to a thin surface layer. The effects of radioactivity on the human body are not yet thoroughly understood. However, there is probably no serious health hazard in the use of radioisotopes as tracers in biology. First, none of these radioisotopes commonly used as tracers produces the dangerous alpha particles or neutrons, and some of them do not produce gamma rays. Those which emit beta particles only are relatively safe. Second, there is less danger of serious injury because of the rather low concentrations and activities usually employed in tracer studies. Nevertheless, it is wise not to subject oneself to any longer exposure than absolutely necessary. Since the minimum amount of radiation producing mutations is not known it is perhaps desirable for those who have not completed their families to be particularly careful in handling radioisotopes.

The disposal of radioactive substances presents a serious problem, both from the standpoint of possible health hazards and contamination of experimental areas, which will result in high background counts in future experiments. A number of disposal methods have been suggested, but none is completely satisfactory. It is also important to avoid, insofar as possible, contaminating laboratory tables, apparatus, greenhouse benches, and the like by spilling or splashing radioactive solutions.

Anyone who is uncertain whether the radiation to which he is being exposed is safe may take advantage of the film badge monitoring service available (63, 65). The badges contain films the size of dental X-ray films and are attached to the clothing by means of a clip. After a week of wearing they are sent in for developing. The extent to which the films are exposed indicates the amount of exposure to radiation and whether it passed safe limits during the week. Pocket ionization chambers, now available for about $13, serve a purpose similar to the film badges*. These are, of course, of value principally in planning future activities, and do not warn against a serious overdose at the time. For such purposes the portable Geiger-Muller rate of count meters are very useful.

The greatest hazard in radioactive tracer studies is perhaps not the possibility of radiation injury, but of ordinary electrical shock. Geiger-Muller counters operate at high voltages, usually 800 volts or over. Carelessness or ignorance of the instruments may result in a serious or even fatal shock. However, if proper precautions are taken, neither the possibility of radioactive injury or shock should constitute a sufficiently serious occupational hazard to deter a biologist from conducting investigations with radioisotopes.

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Reading of the manuscript by members of the U. S. Atomic Energy Commission does not imply its approval by the Commission.

Selected Bibliography

Sections A and B of this bibliography are not complete, but include those publications which are likely to be most valuable to plant scientists who are interested in radioisotope tracer studies. Most of the definitely out of date reviews were not included in Section A, unless they were of historical interest. An effort was made to make Sections C and D as complete as possible through 1947.

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* Apparatus for reading and interpreting radiation as recorded in these chambers costs around $200.

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