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HANDBOOK OF RADIOIMMUNOASSAY

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HANDBOOK OF RADIOIMMUNOASSAY

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PREFACE

Advances in physiology and medicine are usually preceded by and are the end results of breakthroughs in methodology. The past 15 years have witnessed such a breakthrough, with the advent of radioimmunoassay. As an adolescent suffering from giantism, radioimmunoassay has the size but not the experiences of an adult assay. Because of this, troubleshooting has become the most important aspect of a radioimmunoassay laboratory.

Although many books have been published on radioimmunoassay and its applications, not much emphasis has been given to the "hows" of radioimmunoassay at a level that can be translated into practice by relatively inexperienced radioimmunoassayists. This book is an attempt to fill this void. A part of the Handbook is devoted to the following general topics of importance: the safe handling of radioisotopes, the use of radiation detecting device, and the statistical evaluation of radioimmunoassay data. Nonconventional methods related to radioimmunoassay are also discussed.

In the section dealing with radioimmunoassay of individual compounds, the contributors were given much freedom in the format, presentation, and size of their chapters. The emphasis was on a clear, detailed description of the procedure making it possible for a relatively inexperienced radioimmunoassayist to set up the assay system with a minimum of difficulty.

It is the hope of the editor that this book will serve this purpose and will assist researchers in setting up radioimmunoassays for the compounds described with ease, safety, and efficiency.

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RADIATION SAFETY IN THE PERFORMANCE OF RADIOIMMUNOASSAY

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I. INTRODUCTION

The reason for including this chapter is not hard to discern: Radioimmunoassay (RIA) is performed by people. Even well-trained and technically competent people can grow careless or make mistakes. When this happens around radioactive materials serious problems can arise, and quickly. An example of this took place recently at a large, well-staffed laboratory. A visiting scientist, with excellent academic credentials and occupying a prestigious position, performed a routine radioimmunoassay. However, something went wrong, perhaps a flaw in technique, and when the assay was completed, 0.4 μCi of ^{125}I was lodged in the scientist's thyroid and considerably more of that radiotoxic material was scattered about the laboratory.

Therefore, this chapter will attempt to provide performers of radioimmunoassay work with both a description and a prescription: a description of the basic radiation hazards and a prescription for proceeding with an adequate program of radiation protection.

II. RADIATION PHYSICS

A. Fundamentals

For our purposes, in place of the word "physics" we can substitute the word "behavior." We will make only a short study of radiation and its behavior, just sufficient to acquire the basics. Then we can provide ourselves with the means necessary to provide adequate radiation protection. Before we consider the behavior of radiation, however, let us briefly inspect an atom itself, which is the birthplace of all radiation.

A useful and roughly accurate mental picture of the atom may be drawn if, for our easel, we use a basketball gymnasium or fieldhouse. If this represents the size of the atom itself, mostly empty space, and if the basketball at tip-off is the nuclear core or nucleus, containing 99% of the weight (actually the mass) of the gymnasium, we can complete our mental picture. Of course, we can render our picture more accurate, but unfortunately also more complex: make the basketball smaller, the gymnasium larger and more spherical, and fill it with dense smoke representing the cloud of electrons. For a more detailed study of the atom and the nucleus a number of references (1-5) are given at the end of this chapter.

As we shall see, the nuclear core or basketball will be our main concern as far as understanding radiation goes.

Radiation is a term that is used, or perhaps misused, in several ways. For us it is employed in two ways. First, we shall refer to small packets of energy called quanta or photons as radiation with no solid part, more precisely: massless, chargeless electromagnetic radiation. This radiation is called gamma radiation or gamma rays (γ rays). They are quite literally "shaken off" from the nuclear cores of certain unstable atoms in the process of radioactive decay much the same way drops of water are shaken off a duck's back. The nuclear core suffers disintegration, rearranges itself (the duck smoothes its ruffled feathers), and the photon or photons come flying off.

Since these photons, or packets of energy, have their origin in the nuclear core, they have much more energy in general than the more familiar x-rays, which originate outside the nuclear core. However, both are electromagnetic radiation and differ only in energy. The more energetic photons present problems for us in that we must provide ourselves with adequate radiation protection, as we shall discover later.

The second way we shall use radiation is to describe tiny particles such as electrons (betas) which are expelled from the nuclear cores of unstable atoms. In β decay the nuclear core also suffers disintegration, rearranges itself, and expels a particle electron. The particle electron weighs about 1/2000th that of the hydrogen atom and has a negative charge. More precisely its rest mass is 9.1×10^{-28} g and its charge is -1 basic unit. These properties of mass (loosely speaking, the weight) and charge will make easier our task of protecting against this type of radiation.

For purposes of completeness, it should be noted that other particles can come out of nuclear cores or nuclei during radioactive decay, but such cases do not concern us in radioimmunoassay work, since those nuclei we deal with (radionuclides) only involve γ rays and/or electrons, if by "involve electrons" we mean an electron may be expelled from the nuclear core or captured into it. The latter process is the inverse of expelling an electron, but we may use our analogy with the duck—now happily swimming; whose well-oiled feathers nevertheless permit a drop or two of the water to penetrate within. The electron drop is drawn into the nuclear core or "captured" and in there it takes part in the action going on within the nucleus. The vacancy created by the electron's sudden departure is soon filled from the atom's cloud of electrons. This gives rise to electromagnetic radiation, only now it is called x radiation or x-rays, since it originates outside the nuclear core. In general, it is not as energetic as γ rays so it will prove less difficult to protect against, as we shall see.

Thus, we restrict our use of the word radiation to two cases: first (Sec. IIB), the massless, chargeless electromagnetic energy emitted from the nuclear core (γ rays) or from the electron cloud outside that nuclear core (x-rays); second (Sec. IIC), the emission of an electron from the nuclear core (β rays).

B. Penetrating Radiation

For our limited purposes we consider only electromagnetic radiation, specifically γ rays and x-rays, as penetrating radiation, that is, able to penetrate much more deeply into a given medium (particularly a person's body) than nonpenetrating radiation such as an electron. The study of electromagnetic radiation and related phenomena occupies entire courses in physics. Here we review only the bare essentials; the interested reader is referred to additional works (6, 7) to do justice to what has been called physics' most precisely explained topic.

Several definitions and a couple of classical equations will provide us with the tools with which to analyze the behavior of penetrating radiation or indirectly ionizing radiation as it is also called for reasons which will become clear.

For a working definition we take ionization to mean that an electron has been forcibly separated from its parent atom and is free to travel about, the parent atom being left at least momentarily in a positively charged state. The freed or "ionized" electron usually has enough energy of motion to go some distance in the surrounding medium, and as it moves along, it in turn ionizes electrons belonging to other atoms. These secondary electrons, freed by the first or primary electron, are examples of the process of direct ionization: one charged particle exerts a force on another charged particle (usually one of the electrons of an atom), giving it energy with which it can break away from its parent atom.

We should keep in mind that the primary electron, which started the whole chain of events, was not produced in this way. Instead it was freed, or ionized, in an indirect way, not by another charged electron or indeed any charged particle, but rather by pure energy—a chargeless, massless packet of electromagnetic energy we have called x-ray or γ ray.

From our knowledge of wave motion we know that frequency f, wavelength L, and velocity c are related in a fundamental way:

$$fL = c \qquad (1)$$

For our work c is the speed of light and L is the wavelength region between 10^{-6} and 10^{-11} cm.

To this we add the basic equation for energy packets or quanta:

$$E = hf \qquad (2)$$

where E is the energy of the packet, from 100 eV to a few megaelectron-volts, and h is a very small number, 6.6×10^{-34} J-sec. It is called Planck's constant in honor of the German physicist who first brought forward the idea that radiation energy occurred only in discrete packets or quanta, in an attempt to explain certain experimental data involving electromagnetic radiation. This idea of packets of energy or quanta created a revolution in science, led to the development of modern or quantum physics, and even today is a mystery that has been called the "overarching feature of nature" (8).

Penetrating radiation may thus be identified and its behavior predicted once we know its energy, for the preceding equations immediately tell us its frequency and wavelength. More importantly, its interaction behavior with the material it passes through can be predicted once we know the energy. We can, by using certain tables and formulas, make an accurate estimate of how far our typical γ ray or x-ray will penetrate. We cannot, of course, tell how far any particular ray will penetrate, but we can state what the probability is that it will go such and such a distance before an interaction.

We bear in mind that these interactions are so-called single-shot interactions, characteristic of penetrating or indirectly ionizing radiation. No continual process is occurring; our radiation ignores the atomic electrons as it whizzes by them right and left. Only when it hits one, a "single shot" or "single bull's eye" is there an interaction. That interaction, apart from rare special effects, either tears loose an electron from its atom (and ends our energy packet's existence)–this is the photoelectric effect–or else resembles an auto collision where both electron and energy packet go limping off–this is the Compton effect, named after Arthur Holly Compton whose explanation of this process won him the Nobel Prize.

In summary we find that knowing the quantum or packet's energy is vital in dealing with indirectly ionizing radiation. Given that information we can then make precise predictions based on experimentally confirmed theory as to how far such radiation may be expected to penetrate a given material. We have also found that the "penetrating" property of such radiation is due to the fact that it will interact only in a single-shot way, slipping like a silent phantom through a host of electrons until it hits one, and only one.

A γ emitter commonly used in RIA is ^{57}Co. A second class of γ emitters that also emit β particles and are used in RIA consists of ^{125}I, ^{131}I, and ^{60}Co.

C. Nonpenetrating Radiation

Although a variety of particles could be included under this heading, for purposes of this study only electrons need be considered. As we saw in the preceding section, an electron has both mass (loosely speaking, weight) and charge. When expelled from within the nucleus, we call it a β particle, a β ray, or simply a β. Because a β particle is a charged particle it will interact with atomic electrons at short intervals along its journey through matter. There is a continual process going on. As we have seen in the preceding section a charged particle interacts with other charged particles by electrical forces. Such a particle can free or ionize atomic electrons directly. Such directly ionizing particles are called nonpenetrating radiation for that reason: "They do not penetrate." For β particles it is important to know the energy involved. Once this is known, along with the kind of material through which the particles are to pass, we consult tables or graphs and read off the expected range. In most processes that produce β rays, it is true there will be a spectrum range of energies; that is, the β particle may have any energy between barely measurable and some maximum energy characteristic of the particular process. However, it is sufficient to simply work with the mean or average energy for dosimetry and for shielding purposes. This is known once the isotope is known.

β emitters commonly used in RIA are ^{3}H and ^{14}C, both of which emit very low-energy β's. As mentioned previously, there is also a class of β-γ emitters used in RIA: ^{125}I, ^{131}I, and ^{60}Co.

In summary then we deal with two distinct kinds of particles: we have uncharged massless packets of energy, which are called quanta and which spend their energy in a single event—"one shot"—these are the indirectly ionizing particles and because of this type of interaction are penetrating radiation.

We also have the other kind of particles, charged electrons with mass, which spend their energy in a series of small events—"a steady dribble"—these particles, the β's, are directly ionizing particles and because of this type of interaction are nonpenetrating radiation.

III. RADIATION PROTECTION

A. Basic Principles

We have seen in RIA work that there are two kinds of radiation—penetrating γ's (mainly external hazards) and nonpenetrating β's (mainly internal hazards). The next step is to determine what, if anything, can be done to protect ourselves against them. Ionizing radiation, whether β or γ, transfers in varying degree unusable, and thus unwanted, energy to tissue. The theory of the mechanism of how radiation does this is incomplete (9, 10). The unwanted energy, if delivered in sufficient quantity to tissue, can and does cause harm. This injury may be to the body (somatic) and/or to future generations through damaged genes (genetic). The transferred radiation energy may dissociate an atom into positive and negative ions or free radicals which are highly reactive. Another possibility is that the transferred energy may excite (energize) the target molecule to the point of breakdown into other molecules.

As Morgan and Turner (9) point out, radiation damage in as few as 1 in 10^8 atoms of a cell can produce profound biological consequences. Therefore, it should not surprise us that relatively small radiation energy transfers, say of the order of 100 eV, may be involved in cell-killing (cytotoxic) interactions. This in turn leads us to the basic philosophy of radiation safety as stated in 1965 by the Federal Radiation Council (11):

> "Although radiation doses numerically equal to the RPG's (Radiation Protection Guidelines) may impose a risk so small that they can be accepted each year for a lifetime if there is significant benefit from the programs causing the exposure, they do not and cannot establish a line that is safe on one side and unsafe on the other. Rather, some risk of injury may exist at any level of dose and the risk continuously increases with dose."

Briefly, then, there is no threshold amount of radiation below which no damage will occur. There will always be <u>some</u> damage when there is radiation. Because radiation injury takes place when energy is transferred to matter, a useful quantity with which to measure such transfers is "energy per gram of matter." More precisely, if 62.5×10^6 MeV of energy is deposited in 1 g of matter, we call this an absorbed dose of 1 rad. From the standpoint of radiation safety this is a very large quantity of energy. In fact, we normally deal in units a thousand times smaller, the millirad. Since we are dealing only with β's and γ's, we will only briefly note that another quantity, also a unit of "energy per gram," is often used. This quantity is called the rem and takes into account the biological effect on tissue of doses from various radiations. This is done by means of modifying factors such as the quality factor and various distribution factors. This may be expressed mathematically by the following equation:

$$\underset{\text{(rem)}}{\text{Dose equivalent}} = \underset{\text{(rad)}}{\text{absorbed dose}} \times \text{quality factor} \times \text{distribution factor(s)} \qquad (3)$$

For example, the neutron quality factor ("how effective biologically") is about 10 times that for β's or γ's. The quality factor for both β's and γ's is taken as unity. Thus, we may, for practical purposes, disregard the differences in the two units, keeping in mind that most limits are expressed in rems, or actually in the much smaller millirems (mrem).

The limit is 1250 mrem/quarter for occupational exposure of the whole body, or at least certain designated critical organs, such as the gonads, lens of eye, and bone marrow (12). A higher limit is allowed for exposure of the hands and forearms, feet and ankles: 18,750 mrem/quarter. The basic rule for occupationally exposed persons may also be expressed mathematically:

$$\underset{\text{(lifetime)}}{\text{Whole body dose}} = 5(N - 18)\ \text{rems} \qquad (4)$$

where N is the individual's age in years at last birthday. Inspection of this rule shows that no one under 18 may be occupationally exposed to radiation (in fact, they are limited to 10% of that figure). It may also be seen that 5 rems/year is the limit. This may not be obtained at a rate greater than 3 rems/quarter. These limits are guidelines for radiation safety but as noted previously, they are not "threshholds," safe on one side while unsafe on the other. It should be kept in mind that while the occupational limit is 1250 mrem/quarter, a dose of 1000 mrem to a major portion of the bone marrow increases the risk of the occurrence of leukemia to about 1 in 50,000. Fortunately, nowhere near such exposures are expected at the levels of activity normally used in RIA work. Indeed, in a well-organized laboratory, even one using radiotoxic ^{125}I or ^{131}I in labeling, there is little reason for an exposure of even a few millirems. Therefore, we state as our summary that under proper conditions of preparation and precaution, RIA work may be performed quite safely. It is to these proper conditions that we now turn our attention.

It is assumed that RIA work and especially radioiodination will be done in a laboratory suited for the occasion and in conformity with all pertinent radiation safety regulations. The director of the laboratory is responsible for ensuring that all such requirements are met. The director should be satisfied that all workers are well experienced in RIA procedures or else they will be under the direct supervision of someone who is.

It might be necessary to invite an outside consultant to train the laboratory personnel if no experienced staff is available within the laboratory.

If radioiodination is to be done, it might even be advisable to secure advice from the local (radiological) health authorities regarding the safety problems involved, so that all necessary precautions may be taken. In particular, arrangements should be made to have regular thyroid counts taken after radioiodinations.

Most laboratories, regardless of size, will wish to do RIA work more than one time. Thus, a careful, well-thought-out approach will pay dividends later on in quality of work and in the knowledge that radiation hazards are being kept at a minimum.

In the so-called type C (or class 3) general clinical laboratory where RIA kits or radionuclides other than the iodine nuclides are used, no special precautions are necessary. Ordinary lab coats and gloves may be worn. In cases involving storage and use of large (millicurie range) activities of ^{125}I and ^{131}I for labeling, it would be best to have a more fully equipped laboratory, the so-called type B (or class 2) radioisotope laboratory (13):

Walls and ceilings:	Washable, hard nonporous paint.
Floor:	Linoleum, rubber tiles, vinyl—with rounded-off junctions between floor and walls.
Sinks:	Should be connected to main pipe, taps should operate by foot, knee, or elbow.
Ventilation:	Routes of entry and exit for ventilation of air should be defined under all conditions of use.
Hoods:	Regular airflow with no eddies; there should be no escape of air into the working place from the fume hood under typical operating conditions, including opening of windows, doors, suction of other fume hoods, etc.
Clothing:	Suitable to work done, should be clearly identified; should not be worn outside area; gloves to be worn when working with unsealed radioactive substances.

B. Safety Before RIA

1. Penetrating Radiation

Time, distance, and shielding are used as protection against γ radiation.

Time of exposure is kept to a minimum; distance from radioactive sources is kept as great as possible; and adequate shielding is used wherever necessary.

No smoking, eating, drinking, or applying cosmetics is allowed in the vicinity of work.

Mouth-pipetting should NEVER be done.

The first requirement to be met is that of good housekeeping. The laboratory should be cleaned and surveyed by appropriate means (meter and wipes) for radioactive contamination after each use. A periodic scrubbing of the floor and work area is highly recommended even when the lab is not in continual use.

All necessary equipment should be checked and put in good working order; this would include a calibrated meter, preferably with audible alarm. Decontaminant solution and disposable towels should be available in case of a spill. The basic rule for spills is to isolate and contain them. Isolation may be aided by working in a tray, and generous use of absorbent paper helps to contain any spill. The local laboratory's emergency procedures and regulations governing spills should be well known to the RIA worker, and should be complied with. For reasons of radiation safety or minimizing background for experimental accuracy, or both, RIA work, especially that involving radioiodination, should be done under an exhaust hood drawing at least 100 to 150 ft/min face velocity. For low-level activity with nominal toxicity hazards the Handbook of Laboratory Safety (14) recommends 100 ft/min. For greater activity or toxicity, the higher figure of 150 ft/min should be used. In preparation for all iodinations, a prescription of potassium iodide may be obtained and an appropriate amount taken sufficient to block the thyroid for a number of hours (Fig. 1). No precise rule can be applied validly for every individual. However, 3 to 5 mg of the solid (tablet) or 0.3 ml of the liquid is often used, taken about 2 hr before iodinations are begun.

Everything needed for RIA or labeling has already been placed within the shielded area in the hood: glassware, paper disposal cup, swabs, etc. Plastic-backed absorbent paper is put down everywhere practicable, with absorbent side up. Vacutainer tubes as opposed to flasks and beakers may be used for buffers and reagents (Fig. 2). All glassware carried into the iodination area should be disposable (Fig. 3).