THE APPLICATION OF RADIOACTIVE ISOTOPES TO BIOLOGICAL RESEARCH

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GENERAL INFORMATION

Since the war, and the accompanying development of the uranium chain-reacting pile, radioactive isotopes of most of the elements that enter into biological processes can be made in relatively large quantities. The following elements of biological interest are now available in the radioactive form: hydrogen, carbon, sodium, phosphorus, sulfur, zinc, arsenic, bromine, rubidium, strontium, molybdenum, iron, iodine, mercury, bismuth, etc. Most of these have half-life periods of sufficient length to allow experiments of at least several weeks' duration.

The application of radioactive isotopes to the solution of biological problems is becoming increasingly evident. This trend is of sufficient magnitude to demand attention and, as a result, an Advisory Committee for the Division of Biology and Medicine was created within the scope of the U. S. Atomic Energy Commission. This committee is concerned with the relations of the American scientist to atomic energy, and its creation marks the beginning of the humane era of atomic energy.

The aims of the Division of Biology and Medicine have been summarized by John Z. Bowers,[1] assistant to the Director, Division of Biology and Medicine, USAEC, as follows:

1. Radioactive isotopes will be supplied to qualified investigators at a minimum cost.[2]
2. Ready consultation will be provided on problems relating to atomic energy by members of a regional staff, or other qualified personnel.
3. A program is being arranged for the training of personnel in atomic energy as related to biology and medicine in which individuals will obtain adequate financial support and excellent training opportunities at regional laboratories, established institutions or universities. This will include agriculture and its allied sciences.
4. Encouragement of research in the various fields will be realized by directly or indirectly supporting such projects.
5. Unusual facilities at regional laboratories will be made available for research work using the tools available there.

Here is an opportunity for the expansion of a new and invaluable research tool into the fields of biology. In terms of practical returns members of the U.S.A.E.C. have estimated that radioactive isotopes may be instrumental in increasing farm production in this country by $240,000,000 annually.

TYPES OF PROBLEMS

The unique advantages which radioisotopes offer in the solution of difficult problems in biology are due to the relative ease with which it is possible to follow the transfer and transformations of atoms and molecules within biological systems. Examples of the types of problems which are being investigated by means of radioactive isotopes follow:

1. Fertilizer placement studies to determine:
   a. The loss of the applied fertilizer by leaching, etc.
   b. The combination of the applied fertilizer in an unavailable form with other constituents of the soil.

[1] Symposium on the Use of Radioactive Isotopes in Agriculture, Ala. Polytech. Inst. and Oak Ridge Inst. of Nuclear Studies, Auburn, Alabama, December 17-21, 1947.

[2] Inquiries concerning acquisition of radioisotopes should be directed to: Dr. Paul C. Abersold, Chief, Isotopes Division, USAEC, Oak Ridge, Tennessee.

c. The uptake of the applied fertilizer elements as separate and distinct from the uptake of the natural fertilizer elements present in the soil.

2. Mechanics and conditions of absorption and accumulation of various elements by plants.
3. Rate and sequence of steps in the biosynthesis of organic compounds.
4. The mechanics of energy release within biological systems.
5. Therapeutic treatment of hyperthyroidism by selective uptake of injected radioactive iodine by the overactive thyroid tissue.
6. The fate of transfused blood by labeling blood components prior to transfusion.
7. The determination of gas exchange in connection with problems of aeroembolism ("bends") encountered at high altitudes.

The general procedure in the handling of radioactive isotopes in such problems involves the preparation of the isotope for introduction into the system to be studied, the formulation of a suitable means for introduction into the system, the separation and determination of the radioactive labeled compounds in the various biochemical fractions. In this manner the course of a given element in the biological system can be adequately traced. The radioactivity of the "tracer atom" employed is the means of its identification and quantitative determination.

Isotope tracer work is not an end in itself. It is a tool with unique possibilities which should be employed with judgment and applied where its qualities are unique to the solution of the problem at hand.

HANDLING ISOTOPES

The ionizing radiations emitted by the radioactive isotopes are the secret of their usefulness, but at the same time they are also a potential health hazard to the user and are to be treated with respect if one wishes to continue in the field. It is true that much important work can be done without exceeding the tolerance range set down by the health physicists as the quantities of radioactive materials employed are often considerably below that which may be regarded as a therapeutic dose. Nevertheless any competent biologist knows that when an ionizing radiation traverses his genes, there exists the possibility of ion formation, or fragmentation, of the genic molecules. Mutations are known to be possible as a result of direct radiation of hereditary material. Since by experiment we have learned that such mutations are predominantly deleterious we look upon unnecessary exposure as undesirable. However, as undesirable as it may be to expose oneself to ionizing radiations it is impossible entirely to escape them. Cosmic rays constantly bombard the earth with energies of the correct magnitude to convert carbon atoms into radioactive isotopes which, in the case of organisms, are then in place in the tissues. It is estimated that many thousands of radioactive carbon atoms already exist in each human body. Also, there are other radioactive materials, such as radioactive gases and other radioactive contaminants of the uranium-thorium series, present everywhere to be unescapably accumulated. The general cosmic ray and radon-thoron background results in an exposure of approximately 0.0001 r. per 8-hour day.

For those engaged in the handling of radioactive materials it is desirable to know the safe tolerance levels for the various kinds of radiation. The following standards have been set:

1. x and gamma radiation: 200 mr per day (International Congress of Radiobiology in 1934)
2. x and gamma radiation: 100 mr per day (American Advisory Committee on x-ray and Radium Protection)
3. x and gamma radiation: 100 mr per day (Clinton National Laboratories, Oak Ridge, Tennessee)
4. beta radiation: 100 mr e. p. (Clinton National Laboratories)

The roentgen is defined in terms of the absorption of secondary electron energy produced in air by x and gamma radiation. For biological effects it became necessary to convert this standard to energies absorbed by tissue and consequently an equivalent unit, the r. e. p. (roentgen equivalent physical) was proposed. It is an intensity of radiation such that it may be absorbed at the rate of 83 ergs per gram of tissue. One r. e. p. becomes a roentgen if the radiation is x or gamma and absorption takes place in air. An alternate definition is the dose of radiation which produces 1.615 x 10^{12} ion pairs per gram of air (in a microscopically small area at the site concerned).

It is generally considered that a dosage of 100 mr/day for several years will not produce visible damage to man, but as dosage to hereditary mechanism is additive or cumulative it may be undesirable to receive this exposure for an extended period. There is some evidence, according to Morgan[3] who in turn refers to unpublished work by E. Lorenz and L. O. Jacobson that a male (human) should not receive during his lifetime an accumulated total body dose in excess of 1,000 roentgens, and a female should not exceed 100 r.

The maximum body dose for a male is placed at 1,000 roentgens because at this level injury may approach that inflicted by other excesses, i.e., smoking, drinking, etc. Quoting the same authors, there is evidence from animal experiments to "indicate that the ovary is one of the most sensitive body organs to radiation and that there may be an increased incidence of ovarian tumors beginning with an accumulated dose of 100 roentgens of total body radiation."

There is almost universal agreement among the workers in the field of health physics that the fiber electroscope is the one most practical instrument for overall "health" monitoring.. It is very sturdy and maintains its calibration. It is also the cheapest instrument, and in many laboratories it is used for quantitive radioactive measurement in tracer experiments.

While handling isotopes in the volatile state extreme precautions are necessary, for fixation, by breathing or otherwise, of one ten-millionth of a curie of a long lived alpha emitter in the body, may approach fatality. Alpha emitters are not yet released by the U.S.A.E.C. for tracer work, but this may serve as a caution for those using beta and gamma emitters.

Considerable attention should be given to the design of a laboratory in which radioactive materials will be handled. Experience will show that several rooms should be available; i.e., one containing a good hood and used for preparation of isotopes for use; a second room, in plant work a greenhouse, in which isotopes are applied to the experimental material; a third room, or part of the first room, should be devoted to the preparation of the materials for "counting" and a separate room should house the radioactive assay or "counting" equipment. This seems necessary if one is to continue working in this field for an extended period. Absolute cleanliness is essential for it may be that accumulation of radioactive contaminations will ruin experiments before they become a serious health menace.

Operations of dilution and other handling of radioisotopes should be carried out on metal (preferably stainless steel) trays in which absorbent material has been placed. Pipetters (rubber bulb type) and curved pipettes should be used in order to avoid direct exposure to solutions being pipetted. Stainless steel or soapstone sinks in good repair should be used. It is the practice never to dispose of, via the sink, any materials which are significantly richer in radioactivity than the general background. For other material burial grounds, removed from water supplies, other refuse, etc., are recommended. Waste materials of short half-life may be accumulated and stored in a protected place until activity is dissipated. It it the obligation of all users of radioisotopes to furnish proof of competence in handling isotopes and evidence of knowledge of protective measures. In fact, each institution contemplating use of materials or equipment emitting any of the damaging radiations should have

[3] K. Z. Morgan (Director, Health Physics Division, Clinton National Laboratories), Chem. & Eng. News 25(51) :3794-3798. 1947.

a competent "health physicist" to advise in their proper usage.

CHARACTERISTICS OF RADIOACTIVE ISOTOPES

Radioactive isotopes emit ionizing particles of various kinds and energies. Most are beta or gamma radioactive. Either kind can be used in biological work. It is even possible to use two tracers simultaneously and identify each separately if one is gamma radioactive while the other is beta radioactive. Beta rays can be easily screened out with a thin layer of lead while the gammas penetrate the lead to reach the counter selectively.

The amount of radioactive material which can be prepared in a single bombardment in the "pile" varies considerably. Phosphorus is one of the highest. In total radioactivity 500 millicuries are available in a single unit quantity. One millicurie is equivalent to 3.7×10^{10} disintegrations per second. One thousandth of this amount is an extremely valuable amount. The approximate specific activity (millicuries per gram) at which the biologically important elements are available, varies from less than 1 to over 700.

For biological purposes those elements which have half-lives of from one to several weeks are easiest to use. If the half-life is longer the number of disintegrations per second is less and detection becomes more difficult, while if the half-life is shorter intensity of disintegration is higher but the useful duration of radioactivity is shorter. As the presence of the radioelement is detected by the ionizing radiations which accompany disintegration it would be desirable to have high activity over an extended period; however, these two characteristics do not occur together. This is best exemplified by radiocarbon. C^{11} has a half-life of 21 minutes; its usable span is a matter of hours but detection is relatively easy because of the rapid decay, (and the energy of the radiation emitted). C^{14} however has a half-life of 5100 years; hence, the rate of disintegration is low (in addition, the energy of the ionizing radiation is also low). These two characteristics both attribute to the difficulties involved in the use of radiocarbon (C^{14}). Special equipment for handling this isotope is in the process of development in a number of laboratories, but none is available at the present.

The particular peculiarities of each radioelement create minor problems in detection and measurement, but since the physical principles involved in the detection of ionizing radiations are well understood it is merely a problem of bending existing facts to fit special cases.

The number of radioactive isotopes now available through the Isotopes Division of the U.S.A.E.C. number over a hundred. A current catalogue of available isotopes is maintained, giving the half-life, the nature and energy of the ionizing radiation, the nature and size of the target material, the millicuries of activity in the irradiated sample, the specific activity, and the price.

This is a glorious achievement. The vision and careful planning of those in the policy making division of the U.S. A.E.C. have provided the American scientist with golden opportunities, usually well in advance of his immediate needs. The stimulating effects of these newly opened horizons in research technique, coupled with the achievement in production of radioactive isotopes has created almost unlimited opportunities and consequently released a veritable flood of research activity.

The only barriers to opportunity in this field are training in proper usage of radioactive materials, training in halth protection, and the necessary space, materials, and time for work. In general, it can be said that reception of "atomic energy" and its ramifications has been unusually well appreciated and understood, thanks to the foresight of its creators. There has been immediate adoption and absorption by biologists with the most gratifying preliminary results. In dollars and cents a $240,000,000 increase in the annual output of American farms, which is not an impossible goal, will be a great inducement for the use of radioactive isotopes to enrich the American way of life.