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Scope of Report: The tremendous number of articles in popular magazines and newspapers concerning the impact of atomic energy and radiation research on our present day society are evidence of the importance attached to these relatively new fields of scientific endeavor. It is important therefore, that some information concerning the biological effects of radiation, the use of radioisotopes in biological research and the utilization of radioisotopes by medical scientists be included in the high school biology curriculum. This report presents some experiments and demonstrations involving the use of radioisotopes or involving other types of radiation studies which would be suitable for use in the high school. Also included in the report are sources of materials and equipment as well as additional sources of information. Some general considerations, such as certain do's and dont's and other precautions, are presented and the value of radioisotopes as research tools is discussed.

Demonstrations Described in the Report: A demonstration involving the use of seed irradiated at different levels of radiation with x-rays and with neutrons is presented in detail. Information concerning the availability of irradiated seed for high school use is presented.

The procedure and materials involved in the demonstration of the preparation of a gross autoradiograph of a plant are described in detail. An autoradiograph of a bean plant is included in order to illustrate the kind of results to be expected from such a demonstration.

A demonstration involving the use of a radioisotope in determining the blood volume of an animal is discussed. The principle involved in the demonstration is presented and the problems of materials and problems involved in procedure are discussed.

ADVISER'S APPROVAL James H. Zant

RADIATION STUDIES

IN

HIGH SCHOOL BIOLOGY

By

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Bachelor of Science

Eastern New Mexico University

Portales, New Mexico

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RADIATION STUDIES

IN

HIGH SCHOOL BIOLOGY

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Report Advisor

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TABLE OF CONTENTS

LIST OF FIGURES

INTRODUCTION

The tremendous number of articles in popular magazines and newspapers concerning the impact of atomic energy and radiation research on our present day society are evidence of the importance attached to these relatively new fields of scientific endeavor. It is important therefore, that some information concerning the biological effects of radiation, the use of radioisotopes in biological research and the utilization of radioisotopes by medical scientists be included in the high school biology curriculum. It is believed that a list of experiments involving the use of small amounts of radioisotopes or other types of radiation studies together with a list of some basic concepts of radiation biology, which could be included in the high school biology course, might be of some value to high school biology teachers. It is for this reason that the present work is undertaken.

Radioisotopes are being used more and more in high schools. Various agencies such as the Atomic Energy Commission and the National Science Foundation are providing a variety of training programs designed to help high school teachers become better prepared to meet the challenge of this new and rapidly developing scientific era. In 1952, the New York City Public Schools conducted an in-service course, Radioisotopes-A New Aid to High School Science Teaching. More than 200 science teachers attended this course. One of the outcomes of the course was the drafting of a set of 20 experiments suitable for use in the fields of high school biology, physics and chemistry.[1]

There are also other examples of the use of radioisotopes in high schools,[2] and since radioisotopes are now generally available in small quantities ("exempt" amounts in accordance with present policies of the Atomic Energy Commission[3]) for educational purposes, we can expect increased use of them as more and more teachers become informed as to their possible uses and potential values as tools of research.

The experiments and demonstrations included in this report are suggested as being satisfactory for use in high school biology, and interested teachers and students could undoubtably devise additional experiments and variations of those listed to satisfy their own interests and local situations with reference to the availability of equipment and supplies.

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1. S. Schenberg, Laboratory Experiments with Radioisotopes For High School Science Demonstrations (Washington, 1953), p. V.
2. G.L. Glasheen, "What Schools are Doing in Atomic Energy Education," School Life, 1953, Supplement to Vol. 35, pp. 152-159.
3. Federal Register (Washington, 1956), Vol. 21, No. 6, Title 10, Part 30, pp. 213-217.

GENERAL CONSIDERATIONS IN THE USE OF RADIOISOTOPES

Atomic radiation, as such, is not a new phenomenon for our present knowledge indicates that cosmic rays from outer space have been plunging into the atmosphere and bombarding the earth since time immemorial. These naturally produced and naturally occurring rays bring about the transmutation of atmospheric nitrogen into radioactive C^{14} which becomes bound in the form of CO_2 and finds its way into the bodies of plants and thence into the tissues of all animals. This C^{14} exists in the body of each individual and gives off rays at the rate[4] of about 100,000 counts per minute per person. In addition to this, K^{40} in naturally occurring potassium is radioactive and there are a host of other naturally radioactive substances which produce "background radiation" to which the bodies of all living things are constantly subjected. The biological effects of these unseen, unfelt, and until fairly recent times, undetected rays are in general harmful to living things, but their total effects are insignificantly small, though not entirely understood by man.

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4. G. W. Morgan, "Protective Precautions in the Handling of Radioactive Materials," <u>Proceedings of the Auburn Conference on the Use of Radioactive Isotopes in Agricultural Research</u> (Alabama, 1948), pp. 54-69.

Yes, atomic radiation is not new but man's knowledge of it is fairly new and his ability to "artificially" induce radioactivity is even newer. It is an interesting paradox that these rays, which by their very nature avoided detection by man for so long, have now become useful tools of research because of the ease with which they may be detected by the instruments of man's creation. The ability of man to detect atomic radiation by the use of instruments or film enables him to acknowledge the presence of the radioisotope producing the radiation even in extremely small quantity and thus to use various radioisotopes as valuable tracers in his researches. The uses to which such radioisotopes can be put are limited only by the ingenuity of man and by the problems involved in their safe use.

While it is emphasized that any amount of radiation, however slight, is harmful to man, it is also emphasized that the increased radiation to which a worker would be exposed in performing experiments using radioactive isotopes as tracers, provided he uses the proper techniques, would be less than the increased radiation to which he would be exposed if he were to undergo a change in altitude of 7 to 10 thousand feet.[5] Therefore a scientist

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5. Morgan, p. 69.

would no more refrain from using a radioactive isotope as a tracer in an experiment than he would refrain from taking a vacation in the Rocky Mountains merely because at the higher altitude he would be exposed to slightly greater radiation than at the lower elevations. The point I wish to make is that our attitude should be that it is safe to use radioisotopes at the low levels of radiation involved in tracer research, but since any amount of radiation however slight is harmful, we should take every opportunity to minimize the danger and to protect the body from unnecessary exposure.

The following do's and dont's are quoted from a mimeographed letter from G. I. Gleason, Abbott Laboratories, Oak Ridge Division, and should be given careful consideration:

Do:

1. Exercise care to avoid contamination and ingestion or inhalation of the radioactive materials, since they may prove harmful if mishandled.

2. Minimize excessive exposure of persons by use of shielding and/or distance from the radioactive material. If possible, use of tongs and tweezers in handling is recommended.

3. Keep radioisotopes in labeled containers and place under lock and key when not in use.

Don't:

1. Possess or use at any one time more than a total of ten such quantities, as listed in Section 30.72 of the above regulation.

2. Effect an increase in the radioactivity of any of these individual quantities by adding other radioactive material thereto, by combining radioisotopes from two or more quantities, or by altering them in any other manner so as to increase the radiation therefrom.

3. Administer externally or internally, or direct the administration of any part of these quantities to a human being for any purpose.

4. Add or direct the addition of any part of these quantities to any food, beverage, cosmetic, drug, or other product designed for ingestion or inhalation by, or application to, human beings.

5. Include any part of these quantities in any device, instrument, or apparatus intended for use in diagnosis, treatment or prevention of disease in human beings, or animals, or otherwise intended to affect the structure of any function of the body of human beings or animals.

The dont's as listed above, are also a part of the provisions of the code of the Federal Regulations, Title 10, Part 30, "Licensing of Byproduct Material." A copy of this regulation may be obtained upon request from the Isotope Extension, Division of Civilian Application, Oak Ridge, Tennessee.

In addition to the above do's and dont's, the following precautions are also important:

1. Require the student to check his hands with a Geiger counter after performing operations involving the use of radioactive material to insure that none of the material has come in contact with the skin. Hands should always be washed after an experiment.

2. To prevent contamination of the "work space" in the laboratory, handling of radioisotopes should be carried out on washable trays or plastic mats or on layers of disposable paper. This is important not only because of the health factor, but also because a contaminated laboratory interferes with detection of low levels of radiation in experiments.

3. Thoroughly wash all pieces of apparatus that have come into contact with the radioactive material after each experiment.

4. All contaminated disposable material should be deposited in a can provided for the purpose.

In the high school in which the Physical Science Department already possesses some type of radioactivity demonstrator or Geiger-Mueller counter, the problem of equipment will not be too acute. Since the Biology Department will not require the counter very often, it is suggested that such equipment could be shared by all science departments.

If the school does not possess the necessary radiation monitoring equipment, it is suggested that such equipment be purchased for joint use by the physics, chemistry and biology departments.

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6. S. Schenberg, pp. 50-51.

The following list is presented to give the teacher an idea of some of the equipment which is available:

Nuclear Instrument and Chemical Corporation
223 West Erie Street
Chicago 10, Illinois.

> Model 1613A "Classmaster" Radioactivity Demonstrator. This is a count rate meter with a range of 0-15,000 counts per minute. Cost with accessories--$169.50. Cost of replacement counter tube--$11.50. (quote as of May 15, 1954.)

Tracerlab Inc.
130 High Street
Boston 10, Mass.

> S U 4C Radioactivity Demonstrator. This is a count rate meter with a range of 0-2500 counts per minute. Cost with accessories--$179.50. Cost of replacement counter tube--$17.50. (quote as of March 1954).

Other contacts for equipment:

Radiation Counter Labs., Inc.
5122 West Grove Street
Skokie, Illinois

El-Tronics Inc.
5th and Noble Sts.
Philadelphia, 23, Pa.

Nucleonic Co. of America
497 Union Street
Brooklyn 31, New York.

Berkeley Division
Beckman Instr. Inc.
2200 Wright Ave.
Richmond, Calif.

Radioisotopes in "exempt" amounts may be procured without AEC authorization from several companies including the first two listed above. The "package deal" as presented by Abbott Laboratories, Oak Ridge Division is as follows:

<u>Beta-Pack</u>. Price $5.00 <u>Air Parcel Post-paid</u>.

10 Micro-c. P-32 Half-Life 14.3 days.
50 Micro-c. S-35 Half-Life 87 days.

Gamma-Pack. Price $12.00 Air Express Prepaid.

10 Micro-c.	I-131	Half-Life	8 days.
50 Micro-c.	Cr.51	" "	27.7 ".
1 Micro-c.	Co-60	" "	5.3 Years.
10 Micro-c.	P-32	" "	14.3 days.

Jumbo Pack. Price $18.00 Air Express Prepaid.

10 Micro-c.	P-32 NaH_2PO_4	Half-Life	14.3 days.
10 " "	I-131 NaI	" "	8 " .
50 " "	Cr-51 Na_2CrO_4	" "	27.7 " .
10 " "	Rb-86 RbCl	" "	19.5 " .
10 " "	Na-22 NaCl	" "	3 years.
1 " "	Co-60 $Co(NO_3)_2$	" "	5.3 " .
1 " "	Zn-65 $ZnCl_2$	" "	250 days.
50 " "	S-35 Na_2SO_4	" "	87 days.
1 " "	Ca-45 $CaCl_2$	" "	180 days.

To minimize the spill-hazard until time of actual use, the isotopes are furnished in the dry state contained in small screw-cap vials. Upon receipt, the evaporated samples may be dissolved in the desired quantity of water and dispensed.

Further inquiries concerning these isotopes should be sent to the following address:

Goeffrey I. Gleason
General Manager
Abbott Laboratories
Oak Ridge Division
Oak Ridge, Tennessee.

It should be noted that since some of these isotopes have a half-life of only a few days, the isotopes should be used soon after they are received. On the other hand, the long-lived Co-60 serves as an excellent standard for counting equipment.

EXPERIMENTS AND DEMONSTRATIONS

The booklet, Laboratory Experiments With Radioisotopes for High School Science Demonstrations contains 20 experiments, 4 of which are in the field of biology. It may be purchased from the Superintendent of Documents, U. S. Government Printing Office, Washington 25, D. C. The cost of the booklet is 30 cents and its purchase is recommended.

The following experiments and demonstrations are such that they could easily be conducted in high school and are believed to be such that the educational results will be well worth the effort.

Experiment I. Biological Effects of Radiation. This demonstration requires no special equipment and therefore could be conducted in any high school.

Purpose. To observe the differential effects of various levels and types of radiation on plants.

Materials. Irradiated seeds. Planter boxes. Seed irradiated at different levels of radiation may be obtained from a variety of sources. Perhaps the colleges and universities could best provide this service but if the teacher is unable to obtain irradiated seed from them, seed may be obtained from either of the following two sources:

A. Brookhaven National Laboratories. In spite of the fact that during a major part of the year the facilities at Brookhaven are being used 24 hours a day, seven days a week merely to keep up with research commitments, they have agreed to irradiate seed on a limited scale and at certain times of the year for use by high schools. When this service is performed for schools and other non-profit organizations, there is no cost to the school.[7] The following steps should be followed in sending seeds to Brookhaven for irradiation:

1. How to Send Seed. Send sorghum seed in separate, small, heavy manilla envelopes with about 50-200 seed to the envelope. Write on each envelope the level of irradiation desired, i.e. with neutrons, 35,000, 25000, 20,000, 15,000, and 10,000 rem, and control (not irradiated), and with x-rays the same levels of radiation as listed for neutrons.

2. When to Send. Since the facilities at Brookhaven are not as heavily taxed in the late spring or early fall, and since it takes about 6 weeks to treat the seed and have it back in the hands of the sender (The seed must be stored at Brookhaven until its radioactivity has been dissipated), it is suggested that the seed be mailed in

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7. Seymour Shapiro, Dept. of Biology, Brookhaven Nat'l. Laboratory, Associated Universities, Inc., Upton, L.I., N.Y. (Personal Communication), Feb. 7, 1957.

the late spring with the request that it be irradiated and returned in the early fall for planting. The seed should be sent to the address listed in footnote 7.

B. Irradiated Seed From Oak Ridge. Instead of irradiating different material for each request, the Oak Ridge Laboratory has established a policy of sending, upon request, irradiated barley seed. They include with the seed a 2 page mimeographed letter of instructions and information. These may be obtained without cost by sending the request to the following address:

T. S. Osborne
UT-AEC Agricultural Research Program
P. O. Box 142
Oak Ridge, Tennessee.

Method. Plant the seeds about 1 inch deep and in rows about 3 inches apart as shown in Figs. 1 and 2. Record any information desired such as, number of seeds planted, date planted, per cent of germination at each level of radiation, average height at each level of radiation, etc. The best time to measure the height of the plants is about 15 days after planting.

Observations. In addition to observable differences in germination and rate of growth, the plants at the upper levels of neutron radiation may also show chlorophyll aberrations. Some of the plants in the range of 20,000 to 35,000 rem of neutron irradiation may have white strips down 1 or more leaves.

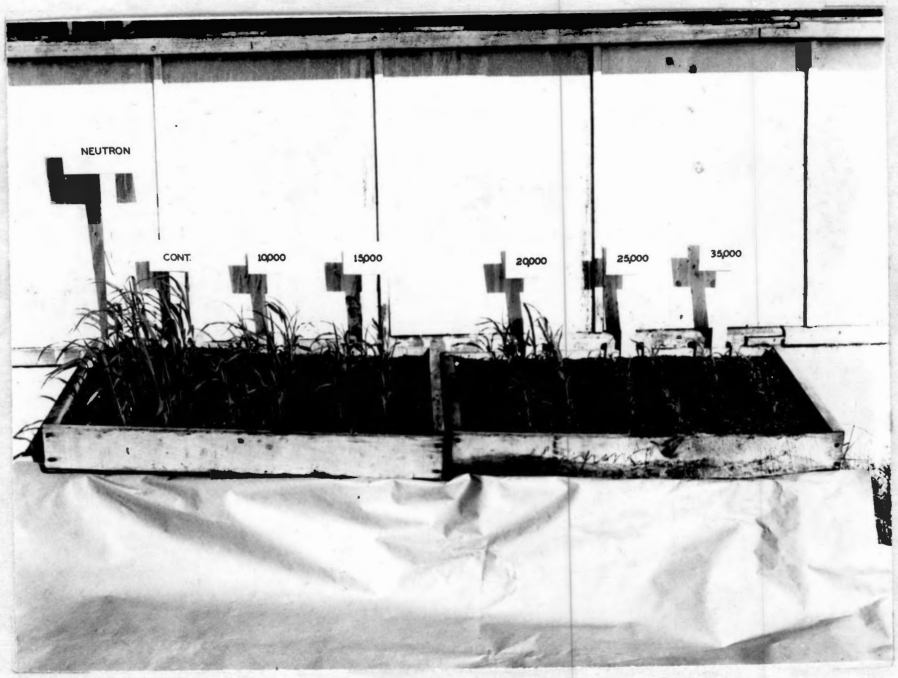

Fig. 1. Plants from Seed Irradiated with Neutrons at Brookhaven.

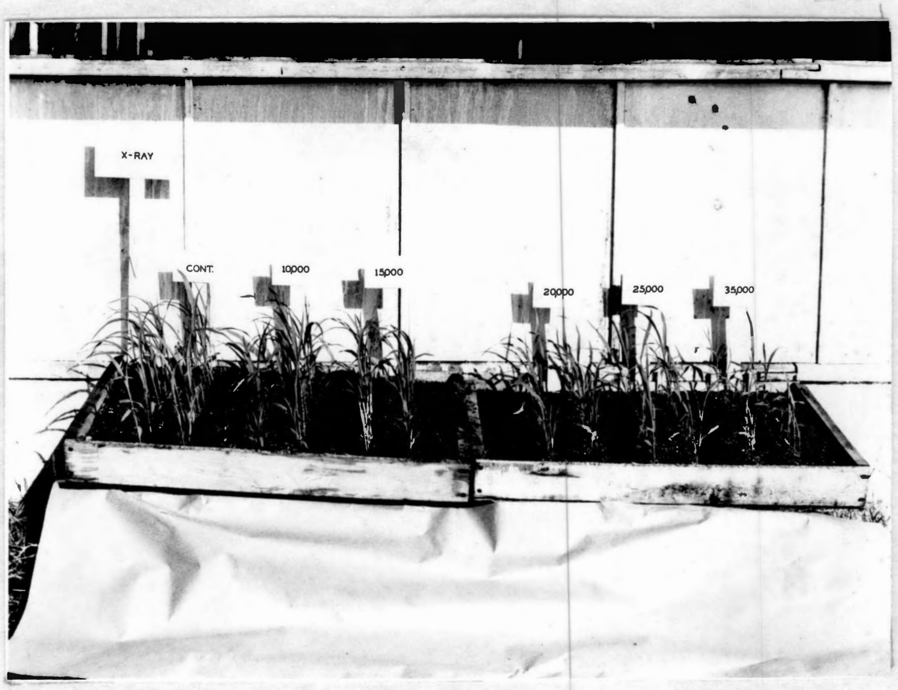

Fig. 2. Plants from Seed Irradiated with X-rays at Brookhaven.

In discussing the levels of radiation with students, one might point out that a dental x-ray machine delivers about 5 roentgens of radiation to the skin of the patients jaw.[8] A single large dose of more than 800 r of radiation delivered to the entire body of man would inevitably result in death.[9]

Experiment II. Preparation of a Gross Autoradiograph.

Purpose. To demonstrate that a radioisotope will reveal its presence by exposing film. To show that a plant will take up a mineral through its roots and that the mineral will become distributed through its leaves and stems.

Materials. 50 microcuries S^{35} (Na_2SO_4); 2 bean or tomato plants, 5 or 6 inches high; 2, 200 ml flasks; Geiger Counter; home-made press (Figs. 3 & 4) for holding the film and pressed plant; No-screen X-ray film; Glossy high-contrast paper; black paper.

Method. Remove the soil from the roots of two plants. Wash the roots carefully. Test all parts of the plants for radioactivity. Place 150 ml of tap water in each of the 200 ml flasks. Add 50 microcuries of S^{35} to one flask

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8. National Academy of Sciences, Nat. Research Council, The Biological Effects of Atomic Radiation--A Report to the Public (Washington, 1956), p. 12.
9. Ibid., p. 21.

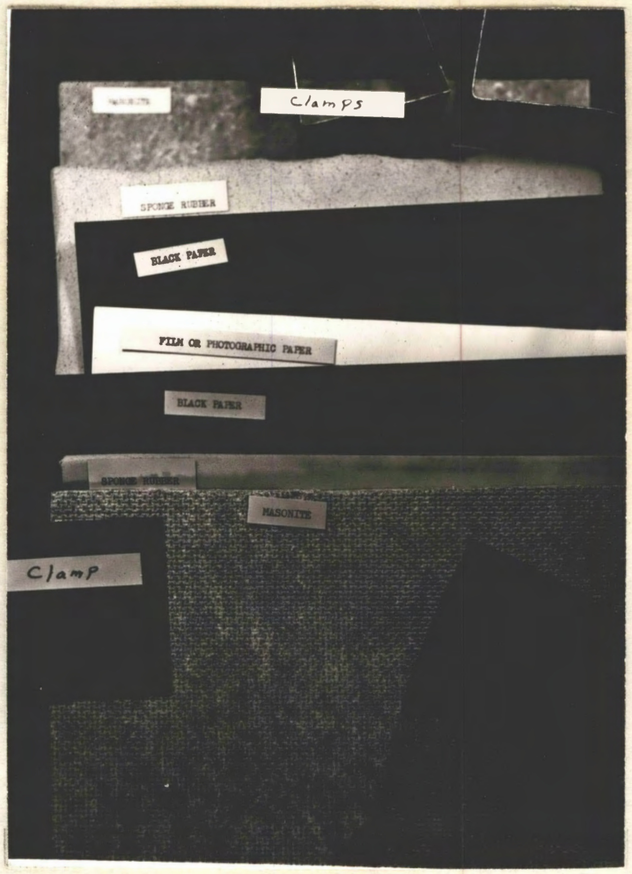


Fig. 3. Press for Holding Plant in Contact with Film in the Making of a Gross Autoradiogram. The press is 8 x 10 inches and is easily constructed. It consists of two sponge rubber pads about 1 inch thick, two pieces of masonite, and 4 clamps fashioned of sheet metal. The parts of the press are shown staggered in order to illustrate the position of the film and the plant between the two pieces of black paper.

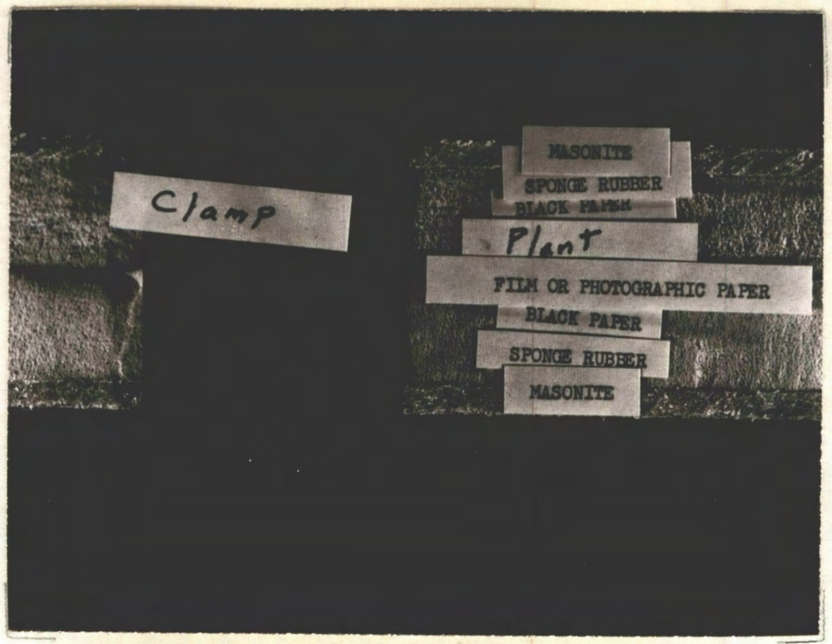


Fig. 4. Edgewise View of Press. The clamp is shown in place and the relative positions of the parts of the press are indicated.

Fig. 5. (p. 18) Gross Autoradiogram of Bean Plant. This is a contact print made from the original x-ray film which was left in contact with the plant for 3 weeks. The plant was tagged with C-14.

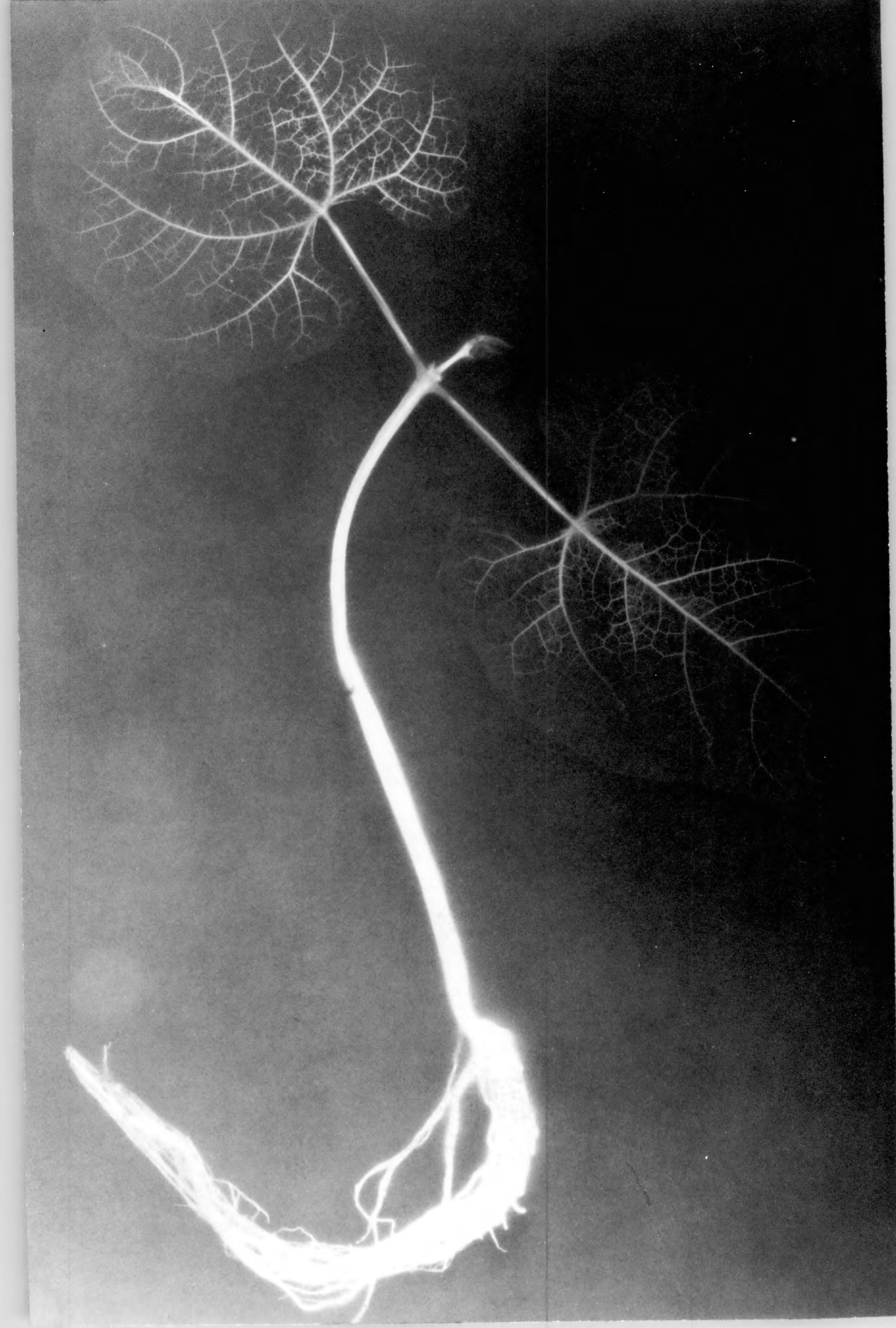
and do not add any radioactive sulfur to the control. Determine the radioactivity (in counts per minute) of the solution of the isotope. Place the experimental plant and the control plant in their respective flasks. The plants should be left in the flasks for at least 6 hours (overnight would be better).

Remove the plants and wash the roots in tap water. Press the plants between layers of absorbent paper for an hour or more. Check the activity in the pressed plants with a Geiger Counter.

In the darkroom, place the pressed plant directly on the film. Place the film and plant between the black papers in the press as shown in Figures 3 & 4. The press should be wrapped in black paper and left for 2 to 4 days or longer before removing the film for development.

Observations. If the film is left in contact with the plant for sufficient time, the radiation from the isotope should result in the production of an image of the plant on the film (Fig. 5.). The control plant should not result in exposure of the film thus indicating that it was the S^{35} which resulted in the formation of the image.

Experiment III. The Use of a Radioisotope in Determining the Volume of Circulating Blood in an Animal. This demonstration is believed to be of sufficient interest and simplicity that it could be presented in a high



school biology laboratory. Since the writer has not had actual first-hand contact with the performance of such a demonstration, no detailed procedure is presented. The following discussion of the principle involved and the problems of setting up a proper procedure might serve as a guide for interested teachers or students:

Principle. The principle of this demonstration is simple and could best be presented by stating the basic procedure. A known volume of a solution containing the isotope is checked with the Geiger Counter and the counts per minute recorded. The isotope is then injected into the bloodstream of the animal and allowed to circulate for a few minutes. A blood sample is then taken from the animal and the activity of the blood is checked with the Geiger Counter. The volume of blood is then computed on the basis of the principle of the dilution of the radio-isotope as follows:

$$\text{Vol. Blood (in cc)} = \frac{\text{Total counts/min. for isotope}}{\text{Counts/min/cc of blood}} - \text{Volume isotope added}$$

Problems of Materials. Indications are that Cr-51 (Na_2CrO_4)[10] would be satisfactory for use in this demonstration. Perhaps a rat or rabbit would be the most satisfactory animal to use for the demonstration. The size of the hypodermic syringe to be used will depend upon

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10. K. Sterling and S. J. Gray, "Determination of Circulating Red Cell Volume in Man by Cr-51," J. Clin. Invest., 1950, Vol. 29, p. 1614.

the amount of the isotope to be injected (perhaps .2 cc of the isotope would be satisfactory for a rat.[11]).

Problems of Procedure. The main problems involved in the procedure are as follows: (1) The problem of injecting the isotope into the blood stream without losing any of the isotope into the surrounding tissue. (2) The problem of ensuring that all factors are essentially the same when the radiation count from the blood is taken as when the radiation count of the isotope was determined.

Interpretation of the Results. If a rat is used for the demonstration, the volume of blood in the rat is about 8-10% of its body volume.[12] This estimate might be used in determining the relative accuracy of the results. The accuracy of the determination will hinge upon such factors as the extent to which the isotope becomes equally distributed throughout the blood, and the extent to which it is diffused out of the bloodstream. In spite of these factors, Cr-51 is reported to give results of sufficient accuracy.[13]

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11. W. S. Newcomer (Personal Communication).
12. Ibid.
13. Sterling et al., op.cit., p. 1614.

SUMMARY

It is becoming increasingly important to include some concepts of radiation research in the modern high school biology curriculum. Some high schools have already begun to use limited amounts of radioisotopes in demonstrations and experiments. The availability of "exempt" amounts of radioisotopes without AEC authorization will permit other high schools to make use of these "tools of research" in the education of students for this atomic age.

Sources of radioisotopes and of other supplies are listed. Some general considerations, such as certain do's and dont's and other precautions, are presented and the value of radioisotopes as research tools is discussed.

Information concerning the availability of irradiated seed for high school use is presented. A demonstration involving the use of seed irradiated at different levels of radiation with x-rays and with neutrons is presented in detail.

The procedure and materials involved in the demonstration of the preparation of a gross autoradiograph of a plant are described in detail. An autoradiograph of a bean plant is included in order to illustrate the kind of results to be expected from such a demonstration.

A demonstration involving the use of a radioisotope in determining the blood volume of an animal is discussed.

The principle involved in the demonstration is presented and the problems of materials and problems involved in procedure are discussed.

Reference is made to additional sources of information and to other experiments and demonstrations listed in published works.

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Publication: "A Report of the Use of Field Trips in the Teaching of Conservation in High School Biology," Conservation Handbook, Published by the National Association of Biology Teachers, 1955.