of other community groups to participate in the school health program, e.g., conferences and medical and/or educational presentations for teacher groups.

19. Interpreting school health program and unmet needs to the community and stimulating action if desirable.

Approved by the School Health Section Council of the American Public Health Association, November, 1947.

IF YOU HAVE NOT renewed your membership, turn to page 201 and send the blank with the necessary remittance to the secretary-treasurer. Volume 11 starts with the January issue; this is the last number to be mailed to those who have not paid their dues for 1949.

The Radioautographic Method in Biology and Medicine

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For centuries the chemical elements have been objects of scientific investigation. In 1896 Henri Becquerel discovered that some of the heavier elements were naturally radioactive, emitting alpha, beta and gamma radiation. This emission was studied extensively during the subsequent years. One of the significant advances was the announcement of artificial radioactivity by Curie and Joliot in 1934. It was soon established that all of the known chemical elements possess varying numbers of isotopes, many of which are artificially radioactive, emitting alpha, beta and gamma particles and positrons. The disintegration process is unique, for only one type of emission is known for each radioactive isotope.

The radiations emitted by the isotopes may be detected with Geiger counters, spectrometers, ionization chambers or by photographic methods. In 1924 Lacassagne and Lattes reported a modification of the photographic method which has been widely used in biology and medicine. It is simply a method of contact photography for detecting and recording radiations from radioactive isotopes. The photographs obtained are usually called radioautographs but some workers prefer autoradiographs or simply autographs.

In the usual applications of this method a solution containing a radioactive isotope is administered to the specimen (or patient) under examination. After a suitable period of time has elapsed portions of the specimens are removed and sectioned. The sections are placed in direct contact with a photographic plate and the combination bound together. Those portions of the specimen containing the radioactive element emit radiations which affect the photographic emulsion in the same manner as ordinary visible light or X-rays. After the development of the photographic plate that region of the emulsion which was adjacent to the radioactive portion of the sample will be darkened. The type and extent of darkening is a measure of the concentration of the radioactive material in that portion of the sample in contact with the plate. The radioautographic technique localizes the sources of radiation more closely than any other method of detection now available.

Choice of Isotopes

For best results an isotope should be chosen which will be localized in the specimen, and which possesses a half-life long enough to permit satisfactory completion of the histological and photographic work. For most of the isotopes in common use, such as P-32, I-131 or Na-24, exposures of one or two half-lives are sufficient for good results.*

The isotope chosen for the photographic work should not be soluble in any of the solutions used in the histological procedure. For example, P-32 is soluble in acid fixing agents, hence acetone or alcohol should be used instead. Some workers prefer to freeze the tissue and keep it frozen during sectioning and photography. This prevents loss of soluble material, but requires thick sections and does not give precise localization.

* In this paper the radioactive isotopes are indicated by the symbol of the chemical element, followed by the mass number, i.e. P-32, Phosphorus with a mass number of 32.

Specimen Sections

For best results thin sections, 5–10 μ,* are used. These are placed in close contact with the film. A thicker section will give good results only if the isotope emits soft beta or alpha radiations.

The sections may be placed on paraffined paper, and then on the film, or may be placed directly on the emulsion and fastened with tape. In some cases a small press is used to hold the film and section in close contact. After exposure the section and plate are separated and the plate developed. For comparison with the radioautograph the sections may be stained in the usual manner.

* This symbol is used to denote a *micron*, which is one thousandth of a millimeter.

Photographic Plates

The disintegration process for the particular isotope chosen will determine the type of photographic plate to be used. Plates are available with emulsions sensitive to X-rays, to visible light, to particular particles or to spectra. For beta and gamma emitters ordinary X-ray film is sensitive enough, but the emulsion contains large and irregular grains. Many workers recommend Agfa no-screen X-ray film. The spectroscopic and particle plates are finer-grained, but are less sensitive. Lantern slide emulsions and dental X-ray films are also suitable.

Alpha emitters show up very clearly on the special particle plates, appearing as short, straight lines. In many cases it is possible to locate the origin of a particular particle. Beta particles have shorter ranges than gamma, and hence are more effective. The beta energies are dissipated within the film emulsion, but only a small percentage of the gamma are utilized in the emulsion.

In some experiments it is desirable to use some method of alignment of photographic plate and section so that the plate and section may be studied together under low magnification by first focusing on the tissue and then on the autograph. A reference mark on the plate is usually sufficient.

Exposure Time

The time of exposure required depends on the amount of radioactive isotope concentrated in the section. In plant experiments the exposures may be as short as one hour. 3 μ sections of thyroid containing I-131 of activity 0.3 microcuries per gram, must be exposed from 5 to 8 days for satisfactory autographs.

Typical Investigations

One of the earliest radioautographic studies was that of D. I. Arnon, P. Stout and F. Sipos (*Am. J. Botany*, 27, 791, 1940). They studied the distribution of P-32 in tomato plants growing in an aerated nutrient solution. The isotope was added as a phosphate, Na_2HPO_4, with maximum concentration of radioactive phosphorus 28.5 microcuries per liter of nutrient solution. Leaves and fruit were removed from the plants 36 hours after the introduction of the labeled phosphorus. Radioautographs of the leaves were made with and without sectioning. The samples were laid upon paraffined paper and placed over no-screen X-ray film. The combination was bound in black paper and pressed together with a heavy glass plate. Exposures of one hour gave a clear picture of the P-32 distribution. Samples of green and ripe fruit were sectioned, placed on paraffined paper, and then on the film. Over-night exposure was necessary for good results. The radioautographs showed that phosphorus is found in the stems and conduction system of the leaves. They showed that the phosphate moves upward with the transpiration stream. The green fruit contained the greatest concentration of phosphorus, especially in the seeds.

Another modification of the basic process is illustrated by the use of K-42 in radioautography of a rat's brain. The isotope was administered by intraperitoneal injection in the form of a 5.44% aqueous solution of the chloride, in doses equivalent to 0.75 millicuries per 100 grams of body weight. After a lapse of from 12 to 18 hours to allow the system to reach equilibrium the brain of the rat was removed. Slices 3 to 4 mm. thick were fixed in absolute alcohol, infiltered with a 50% xylene-50% paraffin mixture, blocked and sectioned. The sections, 6–12 μ in thickness, were fixed to microslides, and a small strip of dental X-ray film bound to each slide under pressure, and exposed from 12 to 14 hours. H. F. Colfer and E. E. Essex (*Proc. Soc. Exptl. Biol. & Med.* 63, 243, 1946) found that the K-42 was homogeneously distributed throughout the brain, with a slight concentration in the cerebral and cerebellar cortex.

An interesting point in this experiment is that the radioautographs were less distinct if the tissues were cleared of xylene and paraffin before binding to the film. Other workers, using different tissues, however, have obtained satisfactory results on removing the paraffin and xylene.

Limitations of the Method

Perhaps the chief limitation of the radioautographic technique is that it is essentially qualitative and not quantitative. The radioautographs show where the isotope is located, but not how it was transferred to its location, or in what form it exists in the location. One method of quantitation by means of microdensitometric measurements has been devised by D. J. Axelrod and J. G. Hamilton (*Am. J. Pathol.* 23, 389, 1947). This may have wide application.

Another limitation is that the range of resolution for radioautography is the same as that obtainable by ordinary photomicrography. The method, then, does not yield any structural results not already known.

Since the use of the atomic bomb much has been written on the effects of radiations from radioactive materials. Some of these dangers are associated with the useful isotopes of radioautography. The beta emitters are usually less dangerous because the glass containers serve as sufficient screens, but beta and gamma emitters, such as I-131, should be handled with extension tongs and the material be kept shielded. The same precautions which are required in our nuclear laboratories should be utilized with the isotopes. This means that not only must proper care be taken by the experimenter, but that the dosage of tissue must be kept below tolerance limits for radiation damage.

Applications

The method described here offers a wide field for investigation by the student of biology or medicine. The techniques are not difficult to master. Frequent consultations with departments of physics or radiology are desirable to insure safety. Until the radioactive materials are available in simpler forms and certain of the hazards eliminated, it is not recommended that these projects be instituted in the high school laboratory.

The growing of plants in nutrient solutions has been well established on both a commercial and laboratory scale. There are many possible investigations similar to the P-32 work of Arnon, Stout and Sipos, using the different elements present in the various nutrient formulae. Since the method of hydroponics is applicable to a wide variety of plants, the radioautographic technique may be utilized in many different ways.

The field af invertebrates has been almost neglected in radioautographic studies. The distribution of the chemical elements in the bodies of the invertebrates, and the tracing of these elements during various stages of development, constitutes a vast project.

The isotopes for use in radioautographic experiments may be obtained through commercial supply houses, such as Tracerlab, or the Eastman Kodak Company, or through the Oak Ridge Laboratories. In each case a certificate of eligibility from the Atomic Energy Commission is required. This may be obtained quite readily by college departments.

A summary of the more significant results of radioautographic experiments is given below. It is impossible to include all of the results. Those cited have been selected to illustrate the use of a variety of isotopes.

Phosphorus—32

1. Upward movement of inorganic ions in cotton, willow and geranium. Experiments showed that the anions and cations of assimilated salts were transported to the aerial portions by the wood. P. Stout and J. R. Hoagland (*Am. J. Botany,* 26, 320, 1939).
2. Distribution of isotope in wax moth, mealworm, cockroach and firebrat. Autographs indicate that in the wax moth larva the radiophosphorus is found in the digestive tract, the silk glands and the ducts of the silk glands. In all four insect species the epithelium of the midintestine contained a large amount of the phosphorus. Most of the detectable phosphorus was in phosphoprotein combination, particularly in the newly formed tissues such as the gonads, gonoducts and wing buds. E. Lindsey and R. Craig (*Ann. Entomol. Soc. Am.,* 35, 50, 1942).
3. Deposition as phosphate in the skeletal structure. Rats were used as test animals. The P-32 was found not only in bone but in the bone marrow and soft tissue, such as liver. In the same experiment Sr-89 and Ca-45 were investigated. Both were found to be deposited almost completely in bone. The bones were placed directly on the film for the autographs. Rabbits and white mice were also used. C. Pecher (*Proc. Soc. Exptl. Biol. & Med.,* 46, 86, 1941).
4. Translocation studies using squash plants. Distribution and mode of distribution to the leaves, using aqueous Na_2HPO_4 containing labeled phosphorus. The tubules in the phloem were found to be the channels of distribution. The embryos of the seeds and interfascicular cambium in the stem were

regions of P-32 accumulation. R. N. Colwell (*Am. J. Botany,* 29, 798, 1942).

Iodine—131

1. Distribution in various kinds of normal thyroid and in cancerous thyroids. The isotope was administered orally as an aqueous solution of the sodium salt. Thyroidectomies were performed 48 to 55 hours after dosage. An almost uniform distribution of iodine throughout the normal thyroid was found. In a hyperplastic thyroid there was an accumulation of iodine in the undamaged colloid. Iodine was not deposited in cancerous tissue. J. G. Hamilton, M. H. Soley and K. B. Eichorn (*U. Cal. Pub. Pharmacol.,* I, 28, 339, 1940 and J. G. Hamilton, *J. Appl. Physics,* 12, 440, 1941).
2. Iodine metabolism in *Drosophila gibberosa.* B. M. Wheeler, (*Proc. Nat. Acad. Sci.* 33, 298, 1947).
3. Completion of thyroidectomy tests. Thyroidectomized rats were injected with 4 ml. sodium iodide, containing I-131, of dose activity 2 microcuries per ml. The regions of the trachea and esophagus bordering the thyroid were dissected out, and autographs made. Animals showing no visible thyroid tissue remaining showed the presence of unremoved tissue in the autographs. W. O. Reinhardt (*Proc. Soc. Exptl. Biol. & Med.,* 50, 81, 1942).

Sulfur—35

1. Distribution in wheat kernels. B. F. Harrison, M.D. Thomas and G. R. Hill (*Plant Physiol.* 19, 245, 1944).
2. Distribution in the skin of mustard gas containing labeled sulfur. Mustard gas was shown by radioautographs to penetrate the epidermis quickly, and accumulate in the hair follicles, sebaceous glands and walls of the blood vessels within the dermis. D. J. Axelrod and J. G. Hamilton (*Am. J. Pathol.* 23, 389, 1947).

Sodium—24

Location of radiosodium in *Nitella.* The protoplasm of *Nitella* was not harmed by the radioactive sodium provided the activity was under 1000 microcuries per liter. J. Mullins (*J. Cell. and Comp. Physiol.* 14, 403, 1939).

Zinc—65

Investigation of the concentration of radiozinc in tomato fruit and seeds. The seeds showed the greatest concentration. E. O. Lawrence (*Nuclear Physics and Biology,* Rutgers University Symposium, 1946).

Strontium—89

Deposition of radiostrontium in the skeletal structure. Following administration as a lactate the strontium accumulated in the neoplastic tissue and in the epiphyseal line where growth is proceeding. There was also some deposition in the callus. D. H. Copp and D. M. Greenberg (*J. Nutrition,* 29, 261, 1945).

Copper—64

Distribution of radiocopper in the chick embryo. E. Smith and P. Gray (*Anat. Record,* 99 (Suppl.), 52 (Abstr.), 1947).

Carbon—14

Existence in bone of this isotope of carbon in the form of a carbonate. W. Bloo, H. J. Curtis, F. C. McLean (*Science,* 105, 47, 1947).

THE WASHINGTON MEETING of 1948 will be reported as fully as possible in the columns of The American Biology Teacher; there are of course many features of a meeting that cannot be put on the printed page, and these can only be mentioned. But many of the papers will be printed in full and briefs or abstracts of the others will appear; reports of all official actions will be printed. The proof of the January issue must go to the printer too soon to permit much of the meeting to be reported in that number. Most of the "reporting" will be in the February number, and the March issue will feature as many as possible of the papers presented at the various program sessions.