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AUTORADIOGRAPHY

Muriel E. Johnston and Patricia W. Durbin

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AUTORADIOGRAPHY*

Muriel E. Johnston and Patricia W. Durbin

Autoradiography (Boyd¹) began in 1896 when Becquerel placed uranyl sulfate on a photographic plate and obtained by radioactive emission an exact image of the crystal, and it has progressed from a clever scientific trick into a valuable research tool. The principle of the technique--the formation of an image within a photographic emulsion by radioactivity from a closely-placed object--has not changed, but there has been great technical improvement in resolution of the image. Recent rapid progress of autoradiographic studies has been due in large part to the ready availability of photographic emulsions having specifically desirable characteristics. Since the publication of Volume II of Medical Physics, an excellent book by Boyd,¹ and review articles by Doniac, Howard, and Pelc,² Fitzgerald,³ and Norris and Woodruff⁴ have appeared dealing with the technical and historical aspects of the applications of autoradiography.

There are 2 distinctive techniques for producing autoradiograms: the "temporary contact" autoradiograms, and the "maintained contact" autoradiograms. The former are obtained by temporarily placing the material to be autoradiographed in contact with a photographic emulsion but maintaining a separation of the object and film in all steps following exposure. The "maintained contact" autoradiograms, on the other hand, call for the intimate and permanent contact between the object being autoradiographed and its photographic medium.

"Temporary contact" autoradiograms demonstrate relatively gross

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localization of radioactivity in an object, and they may be made with almost any type of film. Because "temporary contact" autoradiograms ordinarily involve separation of object and emulsion after exposure, there are great problems in realignment and in determination of the exact localization of the activity with respect to its site of origin in the object. Techniques have been devised that permit precision realignment of the object and emulsion, even after the separate processing of each.

"Temporary contact" autoradiograms are usually made with high speed x-ray films. Films that have been used for such studies include Gevaert's super chrom 32⁰, Dentus rapid x-ray, and no-screen x-ray; Eastman Kodak's no-screen medical x-ray, industrial x-ray films, medium contrast lantern slide plates, and dental x-ray; Ansco's no-screen x-ray; Agfa's Printon film; and Ilford's x-ray film. It is possible to generalize that contact autoradiograms made on these films preclude highly specific localization of radioactive material because the developed silver grains are of large size and the emulsions are thick. These disadvantages may be overcome to some extent by using slow, fine-grained films such as Gevaert's Lippmann emulsion, Kodak's maximum resolution plates, or Kodalith ortho.

"Maintained contact" autoradiograms can be prepared by mounting specimens directly on films of varying thickness. Usually nuclear emulsion films are used, and sometimes photographic plates of lantern slide, special contrast, or nuclear track quality. Another method is to cover the specimen, mounted on a microscope slide, with a piece of stripping film, such as nuclear emulsion or x-ray film; or by an application of melted emulsion. The latter is achieved by pouring or dipping until the desired thickness is achieved. The best detailed images are being obtained

by the techniques that use film to cover the object under study. Special nuclear films are manufactured by 3 major suppliers, Eastman Kodak in the United States, and Ilford, and Kodak, Ltd. in Great Britain. A most comprehensive discussion of the characteristics and properties of emulsions used for autoradiographic studies has been prepared by Boyd.¹ This reference also lists in detail autoradiographic techniques that had been developed up to 1954.

Good resolution is essential for histological localization of radioactive material, and today's concept of resolution calls for localization in terms of a single cell--often a specific structure within a cell. The best conditions for obtaining high resolution are the use of very thin emulsions of small grain size and the maintenance of close contact between object and film. The nuclear emulsions cited above are very thin (generally 10 microns or under), and grain size is exceedingly small. Such emulsions permit a substantial advancement toward the precise cellular localization so long sought.

Photographic density, which is proportional to the concentration of radioactivity in the specimen and to the duration of exposure, is being exploited to provide quantitative information for studies of intermediary metabolism and dynamics of systems. Two primary techniques, densitometry and grain counting, are being used for quantitative biological studies. The former involves the use of electronic or mechanical equipment, though it is possible to prepare graded density scales capable of differentiation by the human eye. Grain counting, on the other hand, demands the careful application of human eyes well adapted to microscope work. The sources of error in grain counting are numerous, but accurate and reproducible

results have been achieved by maintaining control of the many variable side factors. These factors include emulsion thickness, tissue thickness, the space between tissue and emulsion, and the developing and staining procedures. With the use of a microdensitometer, suitable radioactive standards, and controlled exposures, "temporary contact" autoradiograms have been successfully applied to the measurement of the concentration of radioactivity in thick specimens. This technique has proved most valuable for material that must be preserved nearly intact or when the distribution of radioactivity is highly uneven, e.g., in specimens of bone from human beings exposed to radium.

The many technical improvements in nuclear emulsions have not solved all the numerous problems inherent in applying the technique of autoradiography to biological investigations. The chemical nature of the radioactive material being studied will determine the choice of preparative techniques for each individual case so that loss or movement of the radioactivity can be avoided at every step in the procedure (fixation, embedding, photographic development, and staining--if staining is undertaken prior to exposure and photographic development). The emulsion must be carefully selected to obtain the best autoradiograms of the particle emitted. Dosages of the radioactivity must be worked out to levels that will be compatible with normal functions, i.e., no radiation effects, and still allow obtention of an image within a reasonable period of time. Special staining procedures have been devised because the gelatin of photographic emulsions has a high affinity for many common stains. In order to hold down the production of artifacts, extreme cleanliness and care must be observed at all times in handling both the specimen and the emulsion (this includes using distilled water, and filtering all solutions).

Control preparations, i.e., without radioactivity present and processed identically with the radioactive specimens, will help to identify artifacts. Continuous care is essential when the final interpretations of the work are dependent upon the counting of the developed silver grains or their specific localization.

A survey of the current literature (see bibliographies by Boyd¹, Passalacqua⁵, and Johnston⁶,) immediately shows the extensive use being made of the autoradiographic technique as an adjunct in the study of biological processes and disease states. The applications are many and varied; they cover synthesis, renewal, breakdown, effectiveness of therapy, and a host of other processes that make up normal and abnormal metabolism. Among the radioisotopes whose physical and biological properties have lent themselves well to autoradiographic studies in human tissues, the following are representative: gold-198 (colloidal), iodine-131, yttrium-90 (colloidal), carbon-14, iron-59, phosphorus-32 (as NaPO_4 and colloidal CrPO_4), thorium-230, sulfur-35, natural uranium, and lutetium-177. The range of medical applications in which these and other isotopes have been employed includes: metabolic studies of normal tissues; skin penetration by various compounds; cancerogenic and cancerostatic properties of various compounds, including some in colloidal form to restrict their movement to other body areas; studies of disease states, such as goiter, and Grave's disease and Hansen's disease; synthesis of various compounds by the body, particularly studies of deoxyribonucleic acid.

Long-term animal studies are possible using larger quantities of isotopes than are presumed safe for human beings. Investigations of the distribution and subsequent redistribution of various labelled elements

and compounds are being undertaken. Mineralization studies of bone and teeth, normal growth studies of these structures, and the effects of feeding various compounds, such as fluorine, have been reported. The importance of certain trace minerals in dietary situations can be defined. The specificity of drugs for certain body areas can be demonstrated. The effects of large (therapeutic) dosages of radioisotopes upon neoplastic and normal tissues can be elucidated. As a consequence of these studies, the uses of certain radioisotopes are being extended to human medical studies.

Experimental dentistry has also made good use of autoradiographic techniques.⁷ Studies have been made of the penetration of various radioactive materials into the enamel and dentin of extracted teeth, the deposition of isotopes into and around filling materials, the penetration of radioisotopes at margins of acrylic restorations made by compression and non-compression tecnics, deposition in teeth of radioisotopes used in treatment of a disease state, the natural radioactivity of human teeth, and the process of calcification itself. As in other fields, much remains that can be elucidated by autoradiographic studies.

Allied medical fields are also utilizing autoradiography as a technique to broaden their knowledge. Some of this work includes the tagging of parasites, such as filarial larvae and hookworms, and of parasite vectors, such as larval and adult mosquitos; the labelling of pharmacological compounds, for example, penicillin, dihydrostreptomycin, and tetracycline; and the labelling of virus particles. These citations all serve as illustrations of situations in which the technique of autoradiography has been and can be utilized.

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