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AUTORADIOGRAPHY AND THE PARTICLE PROBLEM

G. E. Stapleton, W. D. Gude, H. I. Kohn, J. C. Kile, P. C. Tompkins

A small group within the Division has been concerned recently with methods for detecting and demonstrating radioactive particles within animal tissues, chiefly the lungs. A portion of the work has been devoted to (1) review of pertinent literature and (2) adapting the already well-known technique of autoradiographing to a rapid and sure method of finding the particles in tissue and determination of location of a particle with respect to the lung structure.

The application of radioautography to biologic problems has been presented in a manuscript by Axelrod, which will appear in the new edition of "Handbook of Microscopic Technique," by McClung.

The most used technique for location and demonstration of radioactivity in tissues consists simply of placing sensitive fine grain film over a section known to contain a radioactive element. After suitable exposure blacking appears on the developed firm, as a result of the passage of ionizing particles through the emulsion.

For beta particles emitting isotopes the most suitable film is X-ray film (Ansco nonscreen, nonduplitized). For alpha and other heavy particles, the individual tracks are visible and much studied by use of the nuclear particle films, developed quite recently by Eastman. When developed, the film can be superimposed on the stained section and the location of the active deposits determined. The resolution depends on the range of the ionizing particles; that is, low-range alphas and betas give rather good resolution, whereas hard betas give rather poor resolution and gammas presumably none.

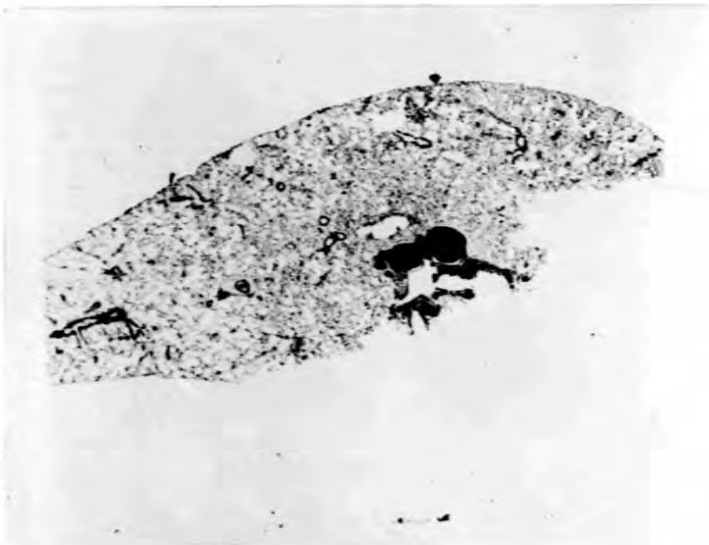
The problem of radioautography in locating active deposits in tissue falls into two distinct categories; (1) location of active material, where many deposits are present, giving some outline to the pattern on the film, usually resulting in a blackened image which can be easily oriented with respect to the stained section, (2) location of minute deposits, or even single particles, where the blackened image is only a small spot or several spots, not permitting any orientation unless the film is mounted with special care.

The two types of radioautographs can best be shown by actual photographs; Figure 1 - A is the photograph of a stained lung section; B is the radioautograph. The deposits are widespread enough to give some outline to the radioautograph. C, on the other hand, is a film exposed to a section which contained only one radioactive particle and obviously no orientation is possible except by special technique.

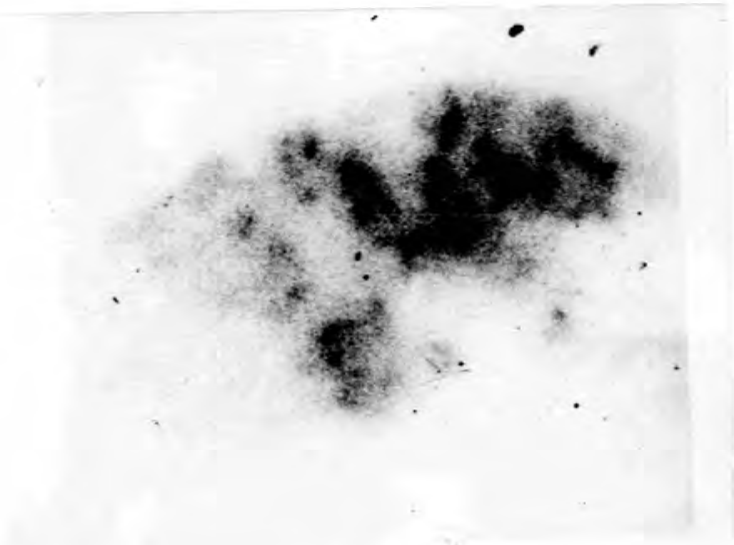
Since all the work in the location of radioactive particles falls in the second category it was necessary to devise a method for orienting radioautograph and stained sections. It seemed plausable to attempt to produce a photographic image of the section on the same film on which the radioautograph had been produced by the active material within the section. Moreover it was necessary to produce the photographic image on the film while it was firmly attached to the slide bearing the section so that no slipping of one over the other would occur during manipulation. It was also necessary that the photographic process would produce a dull image which would not mask the blackening produced by the radioactive material.

Figure II is a diagram of the elements constituting an individual mount for radioautographing. After the exposure of film to the section containing the activity, the whole device was removed to a contact printer

FIGURE I

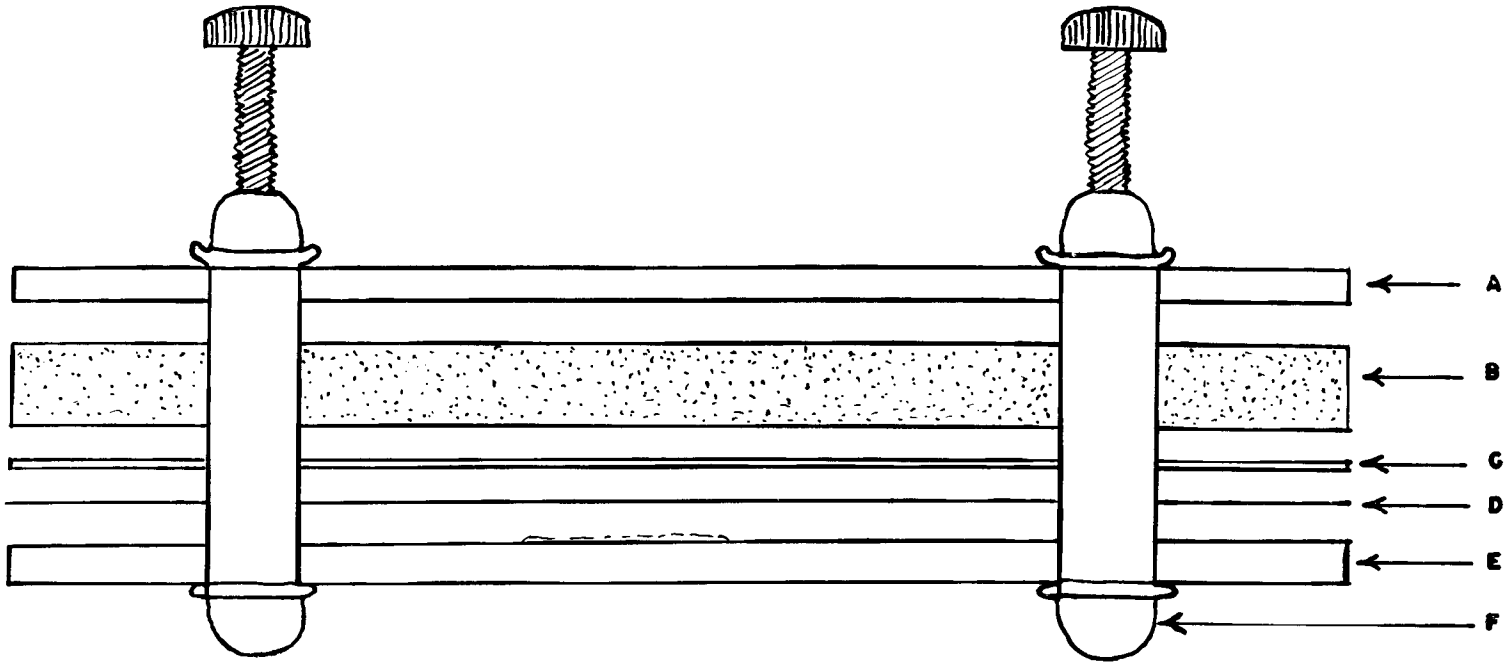


A



B

FIGURE 11



A MICROSCOPE SLIDE 1 x 3 In.
B CORK PAD
C X-RAY FILM
D CELLOPHANE ABSORBER
E MICROSCOPE SLIDE WITH SECTION
F BULLDOG CLAMP

Drawing # 7193

or other source of light and, with the slide masked in order that the entire film would not be blackened, a predetermined light exposure was made through the section-bearing slide onto the X-ray film. A few trial runs with X-ray film so exposed gave the desired effect, which is a dull shadow of the section with a sharp black spot or spots indicating the location of the radioactive particle or particles.

After removal of the section and slide, another photograph of the section was made on fast film, (not X-ray film) using the section as a photographic "negative."

A comparison of the two photos, after enlargement, will reveal the location of the particle in the section.

Preparation of Tissues

Some animals were injected with radioactive particles and the following procedure used for the preparation of the tissues. The animals were killed by ether anesthesia. The lungs with part of the trachea attached, were removed and placed in absolute ethyl alcohol for a period of 16-18 hours or overnight. Absolute alcohol was used, rather than usual fixatives, because the possibility of leaching out of radioactive compounds is thereby reduced to a minimum. Using a scalpel, slices were made through the lung tissue to divide the lung into cuts varying in thickness between 3 - 8 mm. No attempt was made to keep all divisions of tissue in one plane, and the lengths of the sections varied between 1.5 - 5 cm . However, where junctions of bronchi and trachea were encountered, an effort was made to cut these tubes in a longitudinal plane so as to expose the greatest area of tissue.

Slices of tissue were placed in fresh absolute ethyl alcohol for 4 - 6 hours. Rough scanning for radioactivity was made by passing slices under a

mica-window Geiger-Muller tube. Tissues not showing activity were stored in absolute alcohol. Those showing signs of activity were carried through the following procedure:

Absolute alcohol - 2 hours

Xylol - 2 changes of 1 hour - 30 minutes each

Tissue mat 56° - 58° C. - 4 hours

Change after first 15 minutes

" " next 45 minutes

" " " 60 minutes

" " " 120 minutes

Tissues were blocked and cut at 50 micra. Sections were mounted individually on slides containing albumin-glycerin, and again checked for activity.

Radioautographic Technique

The sections previously shown to contain a radioactive particle or particles were counted roughly under the Geiger-Muller tube and the number of particles emitted per unit time determined. On the basis that 10^6 disintegrations per square centimeter will produce observable blackening on X-ray film, the exposure time was estimated.

After exposure the well-clamped holder with its component parts was removed and the image of the section produced.

The film was developed in Eastman X-ray developer for five minutes at 68° C. and then washed in a stop bath (acetic acid) and fixed in X-ray fixer. (Some workers prefer to use Eastman D-72 which requires but one minute developing time). Our experience indicates satisfactory results can be obtained by either developing procedure.

A photograph of the stained section was then made using the section and

slide as a negative. With some enlargement (6 times or so) the two photographs could be compared for location of the particle.

The radioautograph could be compared directly with the stained section under a low-power microscope after a means of orienting radioautograph and section was available.

The staining procedure used was as follows:

Xylol - 5 minutes

Xylol - 5 minutes

Absolute alcohol - 5 minutes

95% alcohol - 3 minutes

70% alcohol - 2 minutes

50% alcohol - 2 minutes

Water distilled - 5 minutes

Harris' Hematoxylin - 3 minutes
Containing 4% acetic acid,
diluted 1 part acidified stain
to 3 parts water.

Water, tap - rinse

Lithium carbonate - 2 minutes

Water, tap - 2 minutes

70% alcohol - 2 minutes

Eosin - 1 minute
1% in 90% alcohol

95% alcohol 4 rinses of 30 sec. ea.

Absolute alcohol 2 rinses of 30 sec. ea.

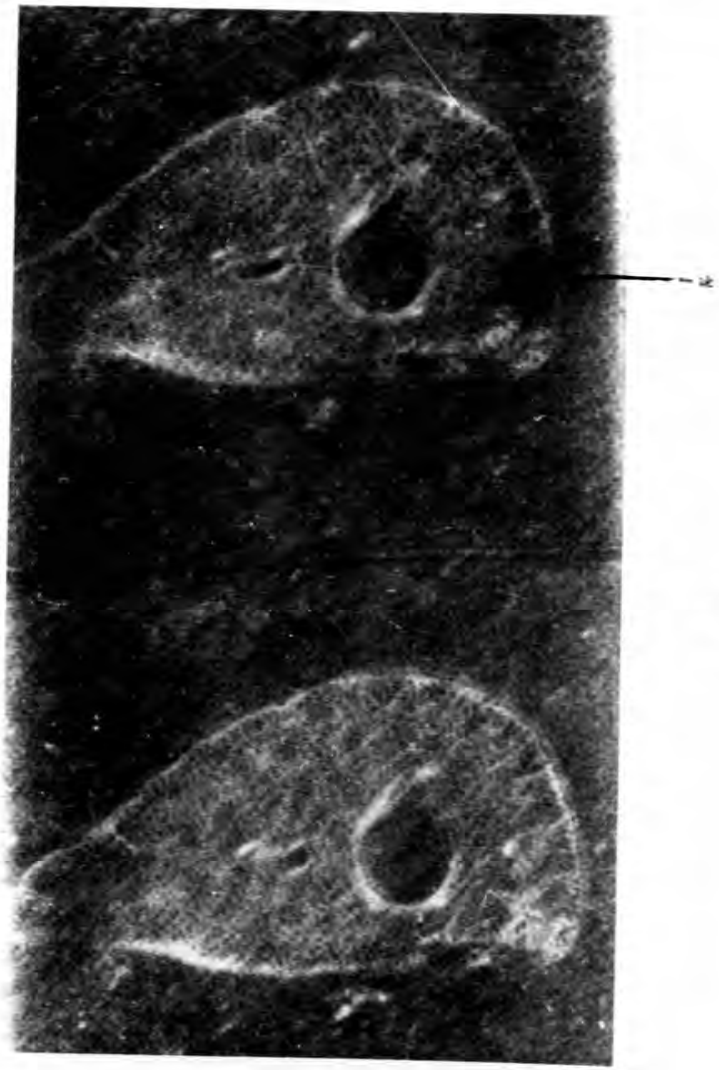
Carbol-xylol - 5 minutes
25% phenol in xylol

Xylol, 2 changes of 30 min. ea.

Mount in clarite

Typical photographs obtained by the prescribed methods are shown in Figure III.

FIGURE III



* - DEPOSITED RADIOACTIVITY

Assay of Animals for Contained Particles

Several animals were actually injected with radioactive particles, a suspension containing a few particles was forced into the trachea with a syringe. After the injection the animals were killed and the lungs removed and the prescribed technique used. The photos used in Figure III are from these animals.

After we had assured ourselves that the technique would give us the desired results, a small scale assay was made of animals found in the general region where there was some probability that radioactive particles would have been breathed.

The result of a search for particles in the lungs of ten animals was entirely negative, no indication of radioactivity having been found in any. This conclusion was confirmed by combining the solutions in which the sections had been fixed with the total lung tissue of the animal, wet ashing in fuming HNO_3 and evaporating either the total sample or an appropriate aliquot in a Coors capsule. In no case did the experimental capsule differ by more than three times the standard deviation of the background count. The tissue ash weights and counting times were selected so that it was established with certainty that no lung contained as much as 0.002 μc of a β-emitter whose radiation is about 0.7 Mev. No examination of the rest of the carcass was made.

A tabulation of the animals used and location where obtained was prepared by Gude and is presented on the next page.

Animal	Location
1. Crow	75 yards south of building 706-A
2. Muskrat	Immediately west of building 205
3. Snipe (woodcock)	On hill across Bethel Valley Road from location of pile
4. Cat	Behind cafeteria building
5. Kitten	Behind cafeteria building
6. Rat	In field, 100 yards south of 706-A
7. Rat	In field, 50 yards east of 706-A
8. Skunk	In field, 50 yards east of 706-A
9. Rabbit	In field, 125 yards east of 105
10. Rabbit	In field, 75 yards east of 706-A