15736

Microscopic Historadiographic Technic for Locating and Quantitating Radioactive Elements in Tissues.

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Accurate quantitative microscopic studies of tissue and cellular distribution of radioactive elements which decay with emission of alpha particles may be made quite easily by the technics to be described. The essential points in the technic are (1) the use of fine-grained silver-bromide emulsions with selective response to alpha radiation,[1,2] (2) the mounting of tissue sections permanently on the emulsion at the beginning of the exposure, (3) examination of such preparations at 400 diameters in dark-field illumination for determination of number of alpha particle tracks per unit area of emulsion, and (4) examination of such preparations at 1000-2000 diameters for determination of the precise cellular location of the radioelement by tracing straight tracks through the emulsion to their points of origin in the tissue.

The following technics have been evolved in the study of radium F (polonium) which decays with emission of alpha particles and very low intensity gamma rays. The polonium is administered in physiological saline buffered with $NaHCO_3$. Tissues are fixed in buffered neutral 4% aqueous HCHO for 48 hours. Bones are decalcified in 5% aqueous HCOOH. Tissue blocks are dehydrated in acetone, cleared in gasoline, embedded in paraffin in a vacuum oven and sectioned at 5 μ. The ribbons are floated off cold water onto the photographic emulsion (Eastman alpha particle plate No. 329,489). All manipulations of the undeveloped photographic plates are carried out in a room illuminated by the appropriate safelight. The preparation is dried quickly in a current of air and is placed in a light-tight container for the remainder of the exposure period. At the end of the exposure period the paraffin is removed by placing the plate in xylene for 5 minutes and the plate is dried in a current of air. The plate is developed in D-19, hardened in SB-4, and fixed in F-5 (Eastman Kodak formulae) at room temperature after which it is washed in filtered tap water for 2 hours. The plate may be stained immediately or dried and stored for staining later. The plate is stained in freshly prepared Weigert's acid-iron hematoxylin for 2 minutes, washed in tap water 5 minutes, dehydrated in 3 changes of acetone, cleared in 1:1 acetone-xylene and in 3 changes of xylene. A drop of xylene-clarite is placed on the tissue and the preparation is covered with a glass coverslip.

Crude quantitation of the relative distribution of polonium in tissues may be obtained by microdensitometer analysis of the developed emulsion. A much more precise local analysis is obtained by counting the number of particle tracks per unit area of emulsion. The amount of polonium present is directly proportional to the number of tracks when the experimental conditions are constant. Determination of relative distribution is thus reduced to comparison of number of tracks. The actual content of polonium per unit of tissue can be calculated from decay constant, length of exposure, spatial relationship of emulsion and tissue, volume of tissue, etc., but this calculation is not ordinarily necessary.

In addition to the quantitative aspects, there is another feature of great interest to the biologist. This is at present limited to the alpha ray emitting elements. In preparations in which the tissue is mounted permanently on the emulsion at the beginning of the exposure, one can locate the point of origin of the alpha particle with great precision. This depends upon the extremely shallow depth of focus at high magnifications (500-2000×). By turning the fine ad-

1 Yagoda, H., *Am. Mineralogist*, 1946, **31**, 87.

2 Yagoda, H., *Am. Mineralogist*, 1946, **31**, 462.

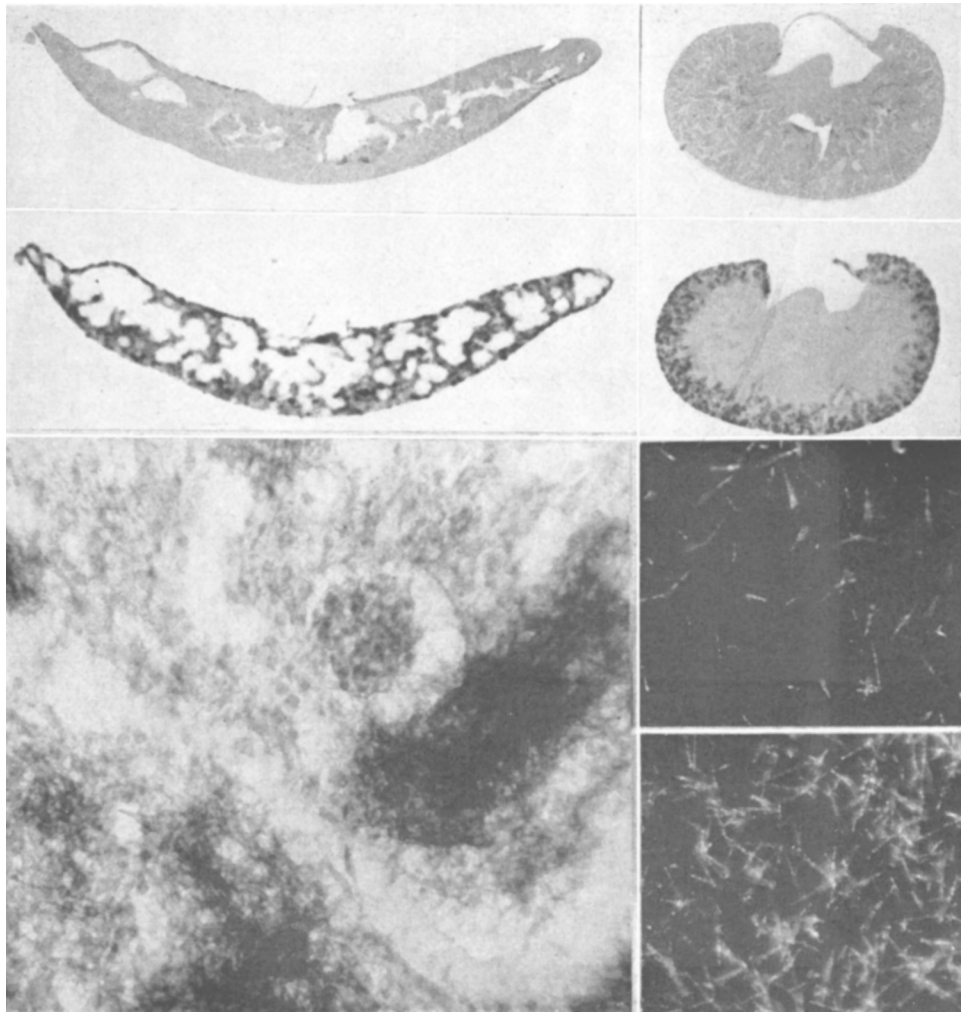

PLATE I.

1. Photomicrograph of mouse spleen containing polonium, hematoxylin, and eosin. × 5.
2. Temporary contact historadiograph prepared from (1). × 5.
3. Photomicrograph of mouse kidney containing polonium.
4. Temporary contact historadiograph prepared from (3).
5 and 6. Dark field views of alpha particle tracks from an area of low and medium polonium content (salivary gland). × 400.
7. Photomicrograph of permanent tissue-photographic emulsion preparation of mouse kidney containing polonium showing renal cortex with extremely dense areas of alpha particle tracks beneath distal convoluated tubules. ×500.

justment knob of the microscope, one can trace each alpha particle track from its end to its beginning and thus to the precise point in the tissue from which it originated. It is thus possible to determine whether the parent atom was located in cytoplasm or nucleus of a given cell. The cellular distribution of radioelements which decay solely with emission of beta or gamma radiations probably cannot be studied by such methods.

Macro images for low power photomicrography are best prepared by one of two technics in which the tissue is not mounted on the emulsion permanently. The tissue ribbon may be mounted on a glass slide, deparaffinized in xylene, coated with celloidin, and placed in contact with the photographic emulsion. At the end of the exposure the glass slide and the photographic plate are separated, the plate being developed and the

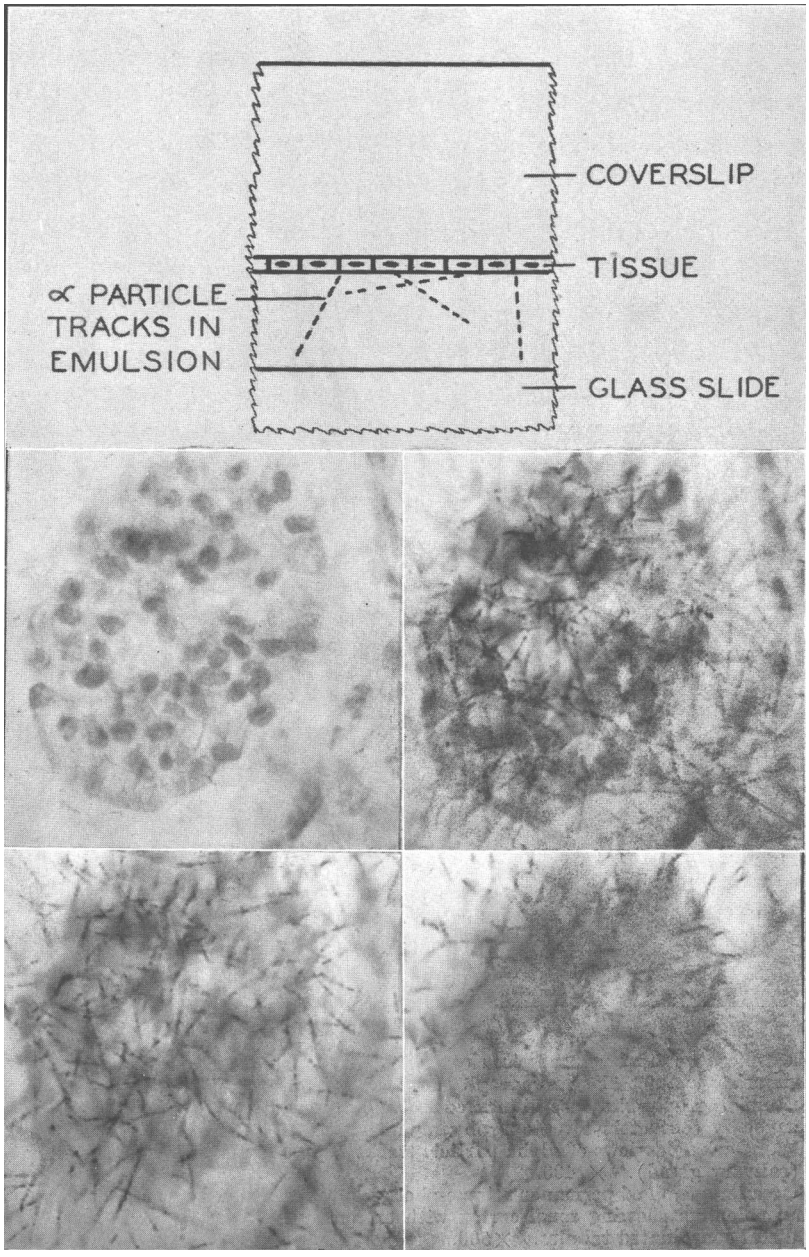

PLATE 11.

1. Schematic drawing of permanent tissue-photographic emulsion preparation.

2, 3, 4, 5. Photomicrographs of renal glomerular tuft and underlying photographic emulsion at successively deeper focus. Permanent tissue-photographic emulsion preparation. Hematoxylin and eosin stain. × 1000.

tissue slide being stained by any one of the many ordinary methods after removing the celloidin with acetone. Equally satisfactory and in many cases superior historadiographs are obtained if the plane-surfaced paraffin block, which remains after the sections are cut, is placed in contact with the photographic plate for preparation of the historadiograph. The image is compared with the stained section last cut from the block.