A Constant Registry Autoradiographic Technic. (18329)

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During recent years several autoradiographic methods, particularly adapted for certain tissues and certain radioactive substances, have been developed. However, previous to the work of Hoecker and Roofe (1), none had been developed for bone which permitted exact correlation between the microscopic structure of bone and the alpha-particle track image of the radioactive substance in the bone after the bone section was removed during processing. In order to study the distribution and concentration of radium metabolically deposited in bone, it is necessary that certain very definite requirements be fulfilled by the autoradiographic technic. (1) It must permit the bone sections to be removed from the nuclear track emulsion during photographic processing and thus prevent the bone section from coming into contact with the processing solutions. (2) It must permit the bone sections to be removed from the nuclear track emulsion during the making of photomicrographs of the alpha-particle track pattern. (3) It must permit the bone sections to be returned exactly to their original positions on the nuclear track plate at any desired time.

The undecalcified bone sections are cut on a special apparatus (Fig. 1 of ref. 2) and then mounted on one end of 1 inch x 3 inch plastic cover slips* with Canada balsam. Before the bone sections are mounted, a rectangular section ¼ inch x 1 inch is cut out of the center of each cover slip in such a manner that the long axis of the cutout is parallel to the long axis of the cover slip. The purpose of this cutout is to concentrate the bending of the cover slip in the region of the cutout portion and thus relieve the strain on the bone sections when the cover slip is bent. Several small holes are punched in the end opposite the bone sections to facilitate attachment of the cover slip to the nuclear track plate by a special wax consisting of equal parts bees wax and rosin. Eastman nuclear track plates, type NTA, with an emulsion thickness of 50 μ are used. The plastic cover slip is securely clamped to the plate at its mid-point with the bone sections in contact with the emulsion. The wax is first melted directly onto the emulsion side of one end of the nuclear track plate with a pencil-type soldering iron; that end of the cover slip containing the small holes is then pressed down into the melted wax, and more of the wax is melted onto the top of the holes. When the bone sections are mounted in this manner on the nuclear track plate, they may be removed from the emulsion surface by lifting the end of the cover slip to which the bone sections are attached away from the nuclear track plate and thus bending the cover slip in the region of the rectangular cutout. When the cover slip is released, the bone sections will return exactly to their original positions if the temperature at the time they are returned is the same as at the time of the original contact. During exposure it is necessary for the bone sections to be in close contact with the emulsion. This contact is best achieved by placing a one inch cube of lead on top of that end of the cover slip containing the bone sections. It is desirable to obtain a more equal distribution of weight by placing a glass slide between the cover slip and the lead weight. The exposing autoradiographs are then placed in a light-tight box until time for development.

These plates are processed in a specially designed processing tank consisting of 3 separate compartments, each measuring 6⅛ inch x 2¾ inch x 7¾ inch. A new hard rubber

1. Hoecker, F. E., Roofe, P. G., *Radiology,* 1949, v52, 856.

2. Roofe, P. G., Hoecker, F. E., Vorhees, C. D., PROC. SOC. EXP. BIOL. AND MED., 1949, v72, 619.

* The plastic cover slips obtained from Charles F. Hubbs, New York City.

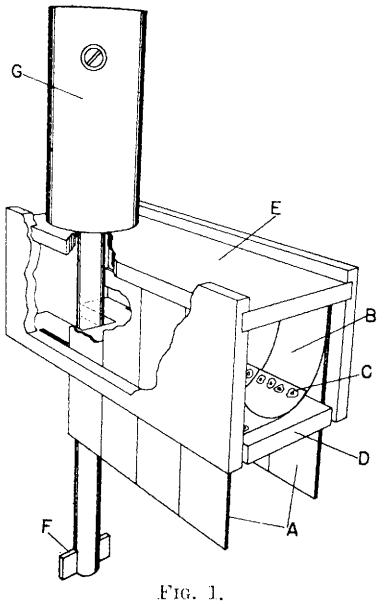

FIG. 1.
Autoradiograph processing unit consisting of nuclear track plate carrier and agitator; nuclear track plates and cover slips in position for processing. A. Immersed portions of the nuclear track plates. B. Bent plastic cover slip. C. Bone sections mounted on end of cover slip. D. Plate carrier. E. Base of motor-driven agitator fitted into grooves extending longitudinally in side pieces. F. Paddle of motor-driven agitator. G. Agitator motor.

storage battery case has been used satisfactorily. On the inner sides of each compartment are vertical projections extending from the bottom to a point 2½ inches below the top. Each compartment is filled with a processing solution up to a level just below the termination of the side projections. The plate carrier is supported just above the level of the processing solutions by the vertical side projections. The plate carrier (Fig. 1) consists of a flat rectangular piece of plastic of such dimensions as to allow it to fit down into the tank compartments and be supported by the side projections. Two slots, each ¼ inch from one of the longitudinal edges and parallel to it, extend from one end of the plate carrier to within 1½ inches of the other. The nuclear track plates fit into these slots during the processing. In the center of that end of the plate carrier into which the slots do not extend is cut a notch through which the shaft of a motor-driven agitator passes. In order to place an exposed nuclear track plate in one of the slots of the plate carrier, the attached plastic cover slip is bent away from the nuclear track plate to a position perpendicular to the plate. The plate is then placed in one of the slots in such a manner that its long axis is perpendicular to the carrier surface while the bent cover slip is held against the upper surface of the carrier, and one half of the track plate extends below. (Fig. 1). Thus the bone sections are protected from contact with the processing solutions by the plate carrier, and that half of the plate extending below the carrier is immersed in the solutions. A plastic side piece two inches high extends perpendicularly along each longitudinal edge of the plate carrier. A groove for the base of a motor-driven agitator extends the length of each side piece ½ inch below its top edge. The function of the side pieces is to help maintain the nuclear track plate in a vertical position during the processing and to support the base of the motor-driven agitator.

The agitator consists of a motor-driven plastic rod with a paddle attached to its free end. (Fig. 1). The small motor is mounted on a flat piece of plastic which fits into the grooves in the side pieces described above. When in position in the grooves the plastic base serves to prevent the nuclear track plates from slipping up because of the tension produced by the bent cover slips. When the plate carrier and agitator are in proper position in the tank, the rod and paddle of the agitator extend down three inches below the nuclear track plates.

The nuclear track plates are processed in accordance with the recommendations of Eastman Kodak Company. However, before the developed and cleared nuclear track plates are washed, they must be removed from the plate carrier. It is necessary that the bone sections neither come in contact with the wet, soft emulsion or be exposed to the running water. To prevent this, an open side tubing clamp is clamped over the nuclear track plate and cover slip immediately in front of the wax. The cover slip, which has thus far been held out of contact with the emulsion by the fingers, is raised to a perpendicular position

and attached to the topmost arm of the clamp. The attachment is accomplished by means of a small wire retainer made by bending both the small loop and the long end of the large loop of an ordinary paper clip down in the same direction until they are parallel to each other and perpendicular to the unbent, horizontal portion of wire. The wire of the small loop is then cut just above its lower curve. The ends of the two straight parallel wires of the cover slip retainer fit into small holes drilled through each end of the uppermost arm of the clamp. The cover slip is held between the clamp and the horizontal portion of the retainer.

The following is an enumeration of all the succeeding steps of the entire autoradiographic process:

1. Punch several small holes in one end of the cover slip. 2. Mount the bone sections on the other end of the cover slip. 3. Cut a rectangular section out of the center of the plastic cover slip. 4. Clamp the cover slip to the nuclear track plate and cement the end containing the small holes to the nuclear track plate with the special wax. 5. Remove the clamp. 6. Expose the track plate with a lead weight resting on that end of the cover slip on which the bone sections are mounted. 7. After the exposure, remove the lead weights and place the undeveloped autoradiographs in the plate carrier. 8. Fill the 3 compartments of the processing tank with developer, short stop, and acid fixer solutions respectively. 9. Process the plates. 10. Remove the processed autoradiographs from the plate carrier. 11. Prepare for washing by attaching the clamp and holding the cover slip perpendicular to the track plate with the cover slip retainer. 12. Wash the plates. 13. Remove clamp and cover slip retainer when the plates are dry.

The 3 requirements of a technique suitable for the production of autoradiographs to be used in the study of the distribution and concentration of a radioactive substance metabolically deposited in bone are fulfilled by this technique. (1) The attachment of the flexible cover slip containing the bone sections at one end with the special wax permits the bone sections to be removed from the portion of the nuclear track plate being processed. The bone sections are protected from contact with the processing solutions by the plate carrier. (2) The secure attachment of the cover slip to the nuclear track plate at one end also permits the bone sections to be removed from the nuclear track emulsion during the making of photomicrographs. (3) Since the cover slip is constantly attached to the nuclear track plate at one end, the bone sections mounted on the other end always return to exactly their original positions when desired.

The plate carrier, with a capacity for eight plates, and the motor-driven agitator prove to be valuable time-saving devices, for it is now possible to produce twice as many autoradiographs in a certain period of time as was formerly possible. This may be attributed to the capacity of the plate carriers and the actual reduction of the processing time, evidently due to superior agitation. Moreover, the quality of the autoradiographs is greatly improved, for no discoloration of the nuclear track plates is encountered.

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Histopathology of the Liver Following Histamine Infusion.* (18330)

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One of the important aspects of hepatic physiology is the study of effects wrought by changes in the circulatory conditions in the liver. The present report is part of a series of such studies in this laboratory and is con-

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