TECHNICAL PAPERS

Preparation of Radioautographs of Tissues without Loss of Water-Soluble P^{32}

Margaret W. Holt,[1] Russell F. Cowing, and Shields Warren[2]

New England Deaconess Hospital, and Harvard Cancer Commission, Boston, Massachusetts

A study of techniques for use in preparation of radioautographs with animal tissues which contain radioactive elements in water-soluble form has been made. Conventional histological methods of fixation, cutting, and mounting the tissue result in the loss of a large fraction of the radioactive element before the tissue section is ready for exposure to the photographic emulsion. A recent investigation (*2*) presents evidence which indicates that essentially all of the P^{32} remaining in animal tissues that have been handled by the usual methods is that which has been incorporated in the tissue as newly formed nucleic acids. However, 2 hr after the administration of labeled phosphate to a mature rat the amount of the phosphate that is present as desoxyribosenucleic acid represents only 0.14% in the liver and 4.81% in the intestinal mucosa of the total inorganic phosphorus incorporated in these organs (*1*). The present investigation describes procedures which make it possible to use the radioautographic technique for a more complete study of the distribution of P^{32} in tissues. The objective of the study has been to devise simple methods to prepare the tissue section and mount it on the photographic emulsion without encountering any appreciable loss, or shift in distribution, of the radioactive element.

A recent report by Russel, Sanders, and Bishop (*3*) describes the use of a freezing substitution technique to study the distribution of P^{32} in plant tissues. In this technique the tissue is frozen at $-170°$ C and then transferred to absolute alcohol saturated with basic lead acetate to precipitate the phosphate *in situ* as lead salt during dehydration. While this technique is reported by these investigators to give good results for plant tissues, a somewhat similar technique employed by us with animal tissues has failed to give entirely satisfactory results. Rat tissues were fixed in alcohol-formalin which was saturated with basic lead acetate; this was followed by dehydration with the usual alcohol series, each alcohol solution being saturated with basic lead acetate. Measurements of these solutions for radioactivity showed that very little was lost from the tissue; however, stained sections and radioautographs indicated that the phosphate was being precipitated around the periphery of the tissue, which also hardened and became difficult to cut. Freezing before treatment with a lead salt was not tried.

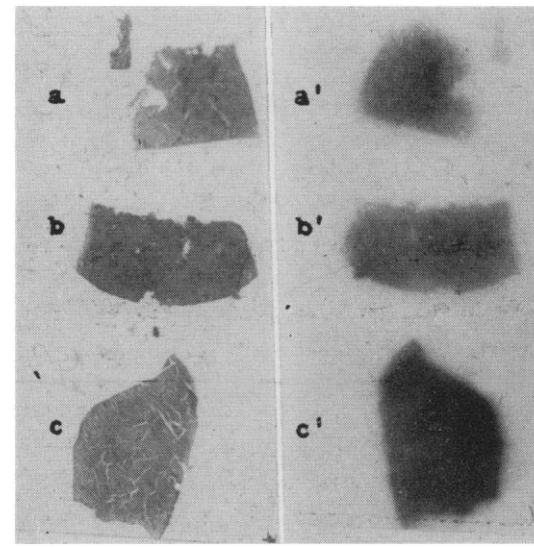

FIG. 1. Stained sections (a, b, c) and corresponding radioautographs (a′, b′, c′) of these sections from three adjacent pieces of the liver of a rat injected with P^{32}; the three sections were fixed and dehydrated by methods A, B, and C respectively as described in the text. (Enlarged approximately 4 ×.)

A freezing and vacuum dehydration technique (method A) for preparing tissues containing P^{32} was tried and the resulting radioautographs have been compared with those obtained from tissues prepared by two other methods.[3] Fig. 1 shows radioautographs and stained sections made from adjacent pieces of the liver of a 96-g rat which had been injected subcutaneously with 0.75 mc of carrier-free P^{32} 2 hr before sacrifice. The section shown in Fig. 1a was prepared by method A, which consists of immersion of the tissue in isopentane cooled to $-170°$ C, followed by vacuum dehydration. Following freezing the tissue was kept at $-70°$ C under a pressure of 0.1 mm Hg for a period of about 48 hr; the tissue was then allowed to come slowly to room temperature in the vacuum. Infiltration of the tissue was accomplished by placing it in liquid paraffin at 40° C under a pressure of 20 mm Hg for 2 hr. The histological results obtained with this precedure were good and were reproducible. The section shown in Fig. 1b was prepared by method B with the following series: alcohol-formalin (9: 1), 2 hr; 95% alcohol, 1 hr; absolute alcohol, 1 hr; absolute alcohol-xylol (1: 1), 1 hr; cedarwood oil, 1 hr; cedarwood oil, over-

[1] Atomic Energy Commission Postdoctoral Research Fellow in the Biological Sciences.

[2] This work was done under contract from the Office of Naval Research and the Atomic Energy Commission. The authors wish to express their appreciation to Mrs. Kathryn Haley for her assistance.

[3] Radioactive phosphorus supplied by the Oak Ridge National Laboratory and obtained on allocation from the U. S. Atomic Energy Commission.

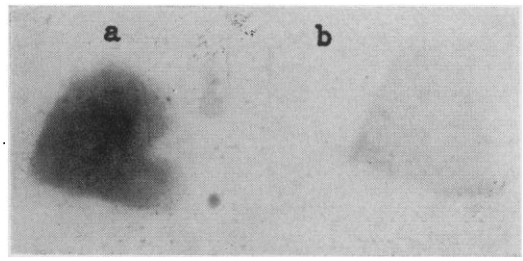

FIG. 2. Radioautographs of two successive sections of rat liver cut from the same block and exposed simultaneously; a differs from b in having no contact with water during the procedure of mounting the tissue on the slide. (Enlarged approximately 4 ×.)

night; clearing in xylol and embedding in paraffin. The series (method C) used for the section shown in Fig. 1c was as follows: alcohol-formalin (9:1), 2 hr; three changes of dioxane (2 hr, 1 hr, 1 hr); followed by the usual paraffin infiltration and embedding. The method of cutting and mounting sections from paraffin blocks was the same with all three sections shown in Fig. 1 and will be described. All three sections were mounted on the same slide and the slide was used for making the radioautographs, which are also shown in Fig. 1 (a′, b′, and c′). The exposure was made by clamping the slide against the emulsion of an Eastman Kodak NTB3 Nuclear Track Plate. The exposure time was 48 hr and was started 14 days after the injection of the animal. This newly developed emulsion is very satisfactory for obtaining radioautographs with P^{32} because of its high sensitivity to high energy beta particles. It can be seen that the amount of P^{32} retained by the tissue, as judged by the radioautographs, is not markedly different in the three methods of fixation and dehydration. However, measurements were made of radioactivity present in the solutions used in preparing tissues by method B and it was found that roughly 25% of the activity present in the tissue was lost to the solutions. This loss was less for method C (i.e., dioxane is preferable to the use of alcohols for dehydration), and in method A (the freezing and vacuum dehydration technique) no loss was possible. These three methods were chosen specifically to minimize the loss of P^{32}. While no data are available, the use of other methods (in particular the use of acid fixatives or prolonged periods of contact with solutions) would probably result in greater loss.

The method of cutting and mounting the sections was found to be very important. It is again desirable to avoid, if possible, contact with all solutions which will extract the P^{32}. This has been accomplished as follows: The sections are cut at the usual thickness of about 8 μ. Before cutting each section, the surface of the block is coated with a thin layer of melted paraffin. After this has cooled for a few seconds the section is cut; allowing the proper cooling time will avoid compression of the paraffin or curling, which occurs if the time is either too short or too long. This layer of paraffin serves as a backing for the section and has avoided the difficulties with curling and folding of the section which occur with a 8-μ section which has no backing and has not been floated on water for flattening. The section may then be mounted directly on a slide and the paraffin removed with xylol or not, as desired. The sections shown in Fig. 1 and in Fig. 2 were cut and mounted in this way on slides coated with a small amount of egg albumen; the slides were placed in the oven (40° C) for a few minutes before removal of the paraffin with xylol. In order to avoid the possibility of chemical action of the tissue on the emulsion, a thin piece of cellophane was placed between the tissue and the emulsion before they were clamped together. Fig. 2 shows radioautographs obtained from two successive sections cut from the same block of frozen, dehydrated liver and exposed simultaneously to the same plate (this was also a 48-hr exposure to an NTB3 emulsion). The two sections were different only in the following respect: after cutting, section 2b was placed on a dish of water with the tissue in contact with the water surface for a period of time judged to be similar to that used in the regular procedure when the ribbon is floated on water before mounting. The marked difference in the density of the radioautographs in Fig. 2a and 2b demonstrates the loss of a large fraction of the P^{32} by floating the section on water.

References

1. LAWRENCE, JOHN H. and HAMILTON, J. G. *Advances in biological and medical physics.* New York: Academic Press Inc., 1948. Vol. I, p. 430.
2. LEBLOND, C. P., STEVENS, C. E., and BOGOROCH, R. *Science,* 1948, **108**, 531.
3. RUSSEL, R. SCOTT, SANDERS, F. K., and BISHOP, O. N. *Nature,* Lond., 1949, **163**, 639.

On the Relationship of Blood Group A to Rh Immunization and the Occurrence of Hemolytic Disease of the Newborn

S. P. Lucia, Marjorie L. Hunt, and John C. Talbot

Division of Medicine, Subdivision of Preventive Medicine, University of California Medical School, San Francisco

In the course of an analysis of immunological data collected from 11,649 pregnant women during the years 1943–1948 inclusive, there was observed a larger number than expected of individuals of blood group A among the sensitized Rh-negative women who bore infants afflicted with hemolytic disease of the newborn. The question was then raised as to whether a relationship could be established between the presence of group A in the red blood corpuscles and an increased incidence of response to immunization on the part of an Rh-negative woman bearing an Rh-positive fetus; and secondly, whether the presence or absence of hemolytic disease of the newborn could in part be related to the ABO blood group of the Rh-positive infant.

The ABO blood group frequencies of the sensitized Rh-negative women and their offspring were compared with the distribution of ABO blood groups in a random sample of the population of this area. In order to determine