IN THE LABORATORY

Radioautograph Technique With C^{14}

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Over the course of the past 6 months a method has been developed for taking radioautographs with C^{14} which provides the intimate contact required between tissue section and film and makes it possible to develop the film and stain the section while they are in contact. With reasonable care the developer can be kept from affecting the tissue, and the stain can be kept out of the emulsion. Thus, normal histochemical procedures can be used on a tissue section either before or after the radioautograph has been taken. It may be noted that the present technique is not merely another histochemical method; rather, it now makes possible the use of most histochemical methods in conjunction with radioautographs. Thus, for example, if prestained tissue is used, the radioactivity can be precisely located by selective leaching of the various biochemical fractions.

Although similar methods for making radioautographs with β-emitting isotopes have been proposed by Evans (*2*) and Pelc (*4*), following the work described by Gross and Leblond (*3*), none of these methods satisfies the desirable criteria of independence of staining and development. In Evans' method, the tissue shields the exposed portion of the film from the developer; and the emulsion, as well as the tissue, is stained. In Pelc's method the film is developed uniformly, but no method of staining has been used.[3]

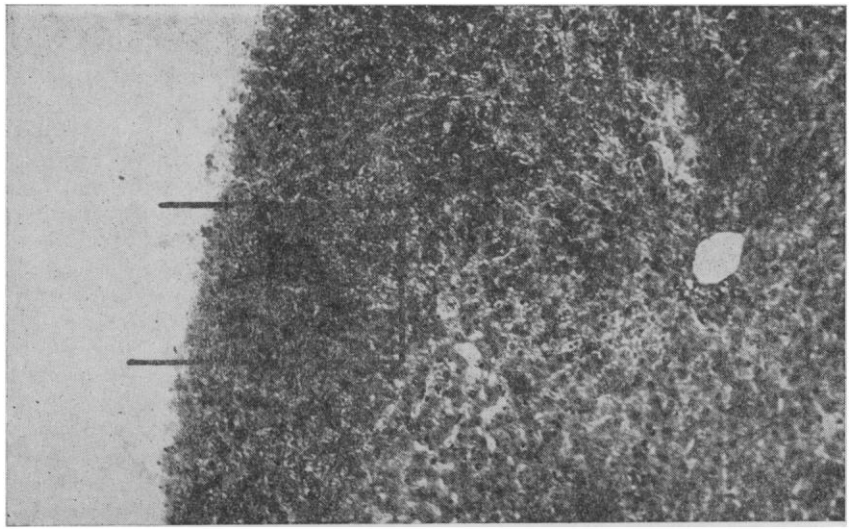

Fig. 1. Rabbit liver. A 10-μ paraffin section (×110), prestained with hematoxylin and eosin; Type M stripping film; exposure, 25 days.

Deane, Nesbett, Buchanan, and Hastings (*J. cell. comp. Physiol.*, 1947, **30**, 255) have shown that the glycogen is deposited at the periphery of the section under experimental conditions similar to those described here. The radioactive glycogen at the edge of the tissue shows up as the black band at the left edge of the tissue. The individual silver grains can be seen best by careful examination of the background fog to the left of the tissue. The large black spots within the tissue section are stained nuclei, and the gray background is cellular tissue. The differentiation between tissue and autograph is clear, of course, in the microscope, where the colored stains show up.

In brief, the present method requires stripping film, a 10-μ emulsion which, with its 7-μ base, can be stripped from a heavy supporting film. A tissue section is mounted on a glass slide, the stripping film is cemented, base side down, onto the tissue, and the radioautograph is made. After development, the film and tissue slice are

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[3] Since this article was submitted for publication, Leblond, Percival, and Gross (*Proc. Soc. exp. Biol. Med.*, 1948, **67**, 74) have proposed the use of prestained tissue which is coated first with celloidin and then with fluid photographic emulsion.

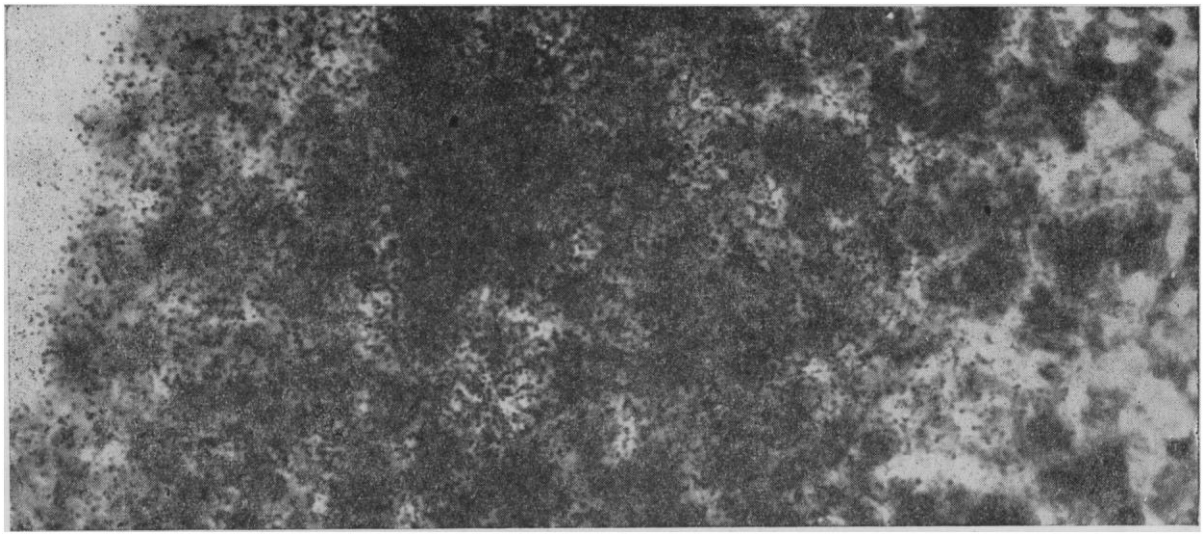

FIG. 2. High power of portion of outlined section of Fig. 1 (magnification, × 600).

For this photograph the silver granules are in focus. Since the granules are separated from the tissue section by 7μ or more, it is not possible at this magnification to bring both section and autograph into focus simultaneously. Nevertheless, the large, blurred, black nuclei may be distinguished from the particulate silver. In the section shown, normal vacuolated liver cells without C^{14} are seen on the right; in the middle is the tissue with the active carbon; and to the left is the stripping film itself with minimal fog.

removed together from the slide and cemented, this time emulsion side down, onto another glass slide. With the tissue now uppermost, the film base protects the emulsion from stain.

Since the primary purpose of the investigation was to work out a satisfactory technique for making radioautographs with C^{14}, it was first necessary to prepare a tissue slice with enough activity to enable us to take autographs in a reasonable period of time. For this purpose, slices of rabbit liver from an animal starved for 48 hrs were incubated *in vitro* with $NaHC^{14}O_3$, in the presence of a pyruvate substrate. This method is a modification of that previously described (*1*). After 2 hrs of incubation, the slices were removed from the medium, and fixed

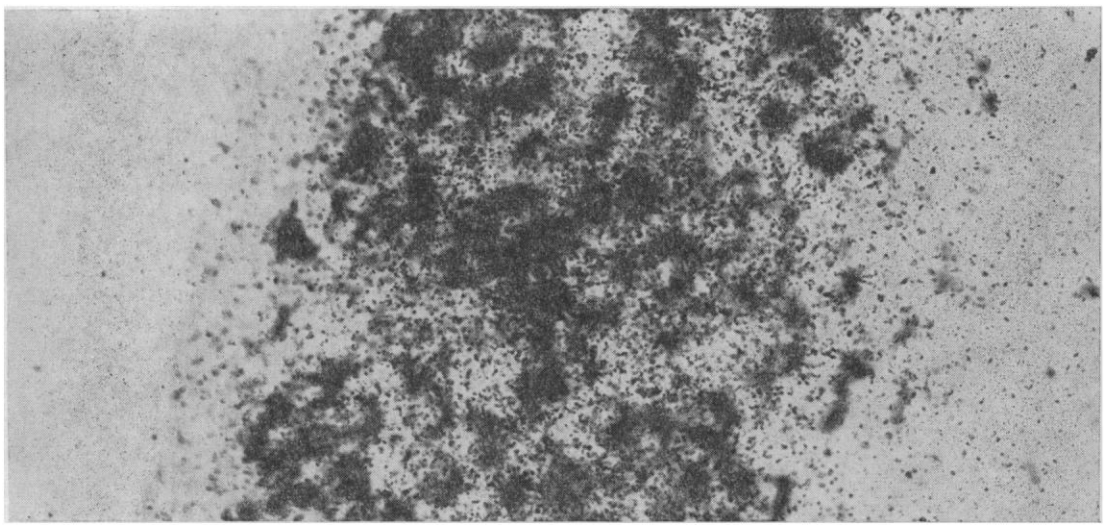

FIG. 3. Liver section (× 400), prestained by Feulgen Bauer method for glycogen.

The cloudy black effect of the histochemical staining for glycogen may be seen superimposed by the particulate black of the photographic image and effectively synchronizing with it. From left to right on the film may be seen the stripping film with mild fogging effect, image synchronizing with cells, and normal liver counterstained with basic fuchsin.

in Rossman's fluid. Sections were cut at 2-, 5-, and 10-μ thickness.[4]

Early experiments indicated that stripping film would give us the intimate contact necessary for high resolution, but the stripping film available commercially was too slow to enable us to work with our tissue sections. Burt H. Carroll, of the Eastman Kodak Company, was kind enough to supply us with stripping film coated with Eastman Type M X-ray emulsion. Of the emulsions tried, Type M gave the highest resolution compatible with the high sensitivity that was needed. Agfa Reprolith stripping film, a commercial product, is very satisfactory when tissue containing large amounts of radioactivity is used. Details of experiments with other emulsions and elements, along with a full description of our technique, will be described in a later publication.

The method is as follows: Paraffin is removed from the section with xylol, the section then being washed in absolute alcohol and allowed to dry. Sufficient 1% celloidin to cover the section is added with a Wright pipette.[5] The stripping film is removed from its base and applied, emulsion side uppermost, over the liquid celloidin-covered tissue section. The film is then pressed over the section until the celloidin is dry. A piece of fine, hard filter paper protects the emulsion from direct contact with the fingers, and great care is taken that the displaced celloidin does not run over onto the emulsion. The filter paper is removed, the emulsion is covered with a guard slide, and the sandwich wrapped round with Scotch tape and exposed under 12.5 lbs/in^2 of pressure. All the operations of development, fixing, and washing are done in a test tube 1″ in diameter. The film is never removed from the test tube; the solutions alone are changed. Distilled water is used in all operations, including washing, and all precautions necessary for fine development are observed. After the final washing, if the film with its firmly attached section is not detached from the glass slide, gentle traction will make the removal complete. The film, now emulsion side down, is attached to another glass slide with Kodalith stripping cement, allowed to dry, and treated like an ordinary section. The thin film base and the cement protect the emulsion from stain.

Figs. 1, 2, and 3 show examples of radioautographs taken with this technique.

References

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4. Pelc, S. R. *Nature, Lond.*, 1947, **160**, 749.

[4] We are grateful to F. M. Sinex and Miss Frances Nesbett, of the Department of Biological Chemistry, for preparing the liver slices and carrying out the incubation, and to E. W. Dempsey, of the Department of Anatomy, who has occasionally assisted in preparing the tissue sections.

[5] Since this article was submitted, we have observed some sections in which the celloidin apparently caused a uniform dense fog. Experiments are under way to determine the exact cause of this fog.

A Method for Making Small Rubber Articles for Laboratory Use

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By the simple method described here one can make reliable, elastic, thin-walled rubber balloons, of various shapes, for sensitive kymograph recording of gastric and doudenal movements, etc., as well as thin-walled rubber tubing of short lengths and various shapes.

The apparatus and chemicals required are: 60% Latex,[1] molds, wide-mouthed bottles, glass cylinders, rack to hold molds, sulfur chloride solution (SCL Merck), and a drying oven.

The molds are made of glass blown to the shape and size desired, with a stem for holding them. For making the balloons, a sufficient amount of Latex solution is poured from the stock can into a suitable short, cork-stoppered, wide-mouthed bottle. For the rubber tubing, a tall glass cylinder fitted with a cork stopper is required. The clean glass mold is dipped three times into the Latex solution, care being taken to avoid the creation of air bubbles. The mold should not be allowed to touch the container or any object after dipping. The surplus solution is then drained off for a moment or two, after which the mold is revolved slowly by hand until an even layer of the Latex is attained. It is then placed in a drying oven maintained at 60–80° C. The oven can be improvised by utilizing a wooden or tin box, about 18″ square, containing a 150-watt electric lamp. When first dipped, the Latex solution is an opaque milky white. When dry the color fades and the Latex becomes transparent. At this stage, it is ready for vulcanizing. A few drops of sulfur chloride are poured into a wide-mouthed bottle (for the balloons) or a cylinder (for the tubing) which is immediately corked to contain the vapor until one is ready to insert the mold. The stem of the mold is securely inserted into the hole of a second cork which fits the bottle or cylinder, and thus the mold may be suspended in the bottle or cylinder containing the vulcanizing fluid (SCL). The mold must be so suspended that it is exposed only to the vapor. One minute or more will suffice to vulcanize a mold coated as described above. If a heavier coating of Latex is applied to the mold, by repeated dipping after the first coat has dried, a slightly longer time is required for vulcanization. As soon as the mold is removed, the vulcanizing bottle, or cylinder should be firmly stoppered again to preserve its contents. The vulcanized article is now dipped in talc and gently rolled off the mold. It should be soft and elastic so that, when stretched, it returns to its original shape. If the mold is left too long in the vulcanizing vapor, it will stiffen and become brittle. A little practice in timing will bring good results.

[1] Obtainable from General Latex & Chemical Corporation, 600 Main Street, Cambridge, Massachusetts.