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The Hydrophobic Effect in the Histology Laboratory

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Spectral analysis of aqueous staining solutions has shown that different sized aggregates of dye molecules are formed in the solutions. It was also shown that the hydrophobic effect may be the main contributor to the aggregation of dye molecules.[1] The polar region of the monomeric dye molecules may attract the very polar water molecules, while the hydrophobic portion stabilizes the surrounding water molecules. This allows for the formation of a stable shell of water around the dye molecules. This also allows other dye molecules to get closer to each other and cause a greater stabilization of the shells of water around them and a stronger hydrophobic bond between them, which results in the formation of dye aggregates.

The ability of dye aggregates to penetrate and interact with tissue components may be an important factor in determining the quality of stained tissue sections. The size of the aggregates and the hydrophobicity of the tissue molecules may contribute to the intensity of the penetration by dye molecules.[2] Larger aggregates may have stronger and more stable shells of water surrounding them; therefore, the tissue may repel the aggregates or form hydrophobic bonds and prevent penetration. The smaller aggregates and monomeric dye molecules may avoid the hydrophobic effects of the tissue, and therefore penetrate and interact with tissue components. The hydrophobic character of tissue sections may be dependent on the chemical component and the fixation of the tissue.

The hydrophobic effect may also play a role in the interaction of dye and tissue molecules. When loosely bound water surrounding protein molecules is removed, the protein molecules usually undergo some conformational change.[4] When dye molecules interact with tissue the water molecules may be displaced, and then the dye and tissue molecules may conform to each other and lock into stable positions with each other. It was also suggested that the conformational change due to water movement in tissue may be a cause for the attachment of debris to tissue sections.[2]

The hydrophobic effect may cause too much aggregation in staining solutions and result in the tissue not staining well with the solutions. The cause of such an occurrence may involve the water quality itself, exposure to a contaminant, change in temperature, the quality of mixing, or a combination of these occurrences. By eliminating or changing these occurrences, the staining quality most likely will improve. One may also try adding small quantities of ionic salts or alcohols which may reduce the hydrophobic effect in aqueous solutions.

Urea crystals are most efficient in breaking up hydrophobic bonds of proteins.[4] Urea crystals also break up dye aggregates by interrupting with the hydrophobic bonds.[3] Urea was added to poorly staining solutions of toluidine blue O, methyl green pyronin, and cresyl echt violet. There was some change in the intensity of the color of the stains. All three staining solutions showed improved staining quality when 0.2 gm to 1.0 gm of urea was added to 50.0 ml of the staining solutions. As larger concentration of urea crystals were added, it was found that good quality slides may be obtained in a fraction of the staining time it took without urea crystals.

The amount of urea crystals to add to the staining solution depends on the strength of the hydrophobic effect in staining solution. A convenient way to measure the hydrophobic effect may, some day, prove beneficial to the histotechnician who wishes to maintain high quality stained slides.

References:

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Health Hazard Study

The National Society for Histotechnology will participate with NIOSH in a study to determine the effects of formaldehyde, xylene and toluene on the health of histotechnologists. A pilot study has already been conducted with 90 histology technicians. Controls for this study were other hospital personnel. Results of this pilot study created enough significant interest and concern that the program has been extended to include additional histotechnologists. In order for this study to be effective nationally, NSH is urging all histologists attending the Symposium/Convention in Boston to take part in this health study and participate in an examination program. The study will be conducted during five days of the convention, beginning on Sunday. This will enable the participating physicians to examine a large number of histologists and gain significant information from a cross section of the country.

Kaye Kilburn, M.D., the Ralph Edgington Professor of Medicine at the University of Southern California, will direct the study. Examinations will be conducted at the NSH Symposium/Convention, Sheraton-Boston Hotel, on September 12-16, 1982. Each histology technician/technologist participating in this study will complete a questionnaire, receive a dermatology, respiratory and neurological examination, and will be sent a follow-up of their personal evaluation. NSH will receive a report of the results upon completion of the study. This USC program is being supported by NIOSH with the cooperation of scientists from Mt. Sinai Medical School of New York and from Stockholm, Sweden. Please join NSH in this joint venture and take part in a most significant study regarding our laboratory profession.

Muscle Quenching With Liquid Nitrogen and Graphite

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Rapidly frozen tissue is useful for enzyme localization using histochemical techniques and for identification of elements using energy dispersive microanalysis. These techniques require the tissue to be as free of ice crystal artifact as possible. To achieve this, the use of liquid nitrogen, combined with isopentane or other fluorocarbons as a quenching agent, has been employed. However, the hazards associated with isopentane and fluorocarbons are an undesirable feature, and when liquid nitrogen is used alone, the rate of cooling is insufficient to eliminate the formation of ice crystal artifacts.

Moline and Glenner[1] employed talcum powder to coat the tissue prior to freezing in liquid nitrogen. With this method the gaseous layer which forms around tissue when it is immersed in liquid nitrogen is prevented, thus eliminating the insulator effect which it produces. Noble and Challa[2] have utilized this method routinely for muscle biopsies and found it to be safe and relatively inexpensive.

With the development of energy dispersive spectrometry and its increasing availability to pathologists, it has become desirable to seek other methods which will not contaminate the tissue specimens with extraneous elements such as silicon and at the same time minimize crystal artifact. We have employed graphite as the quenching agent combined with liquid nitrogen and find it provides rapid freezing while minimizing the development of ice crystal artifacts, particularly in the superficial layers, permitting cryoultramicrotomy of the tissue for subsequent electron microscopy and energy dispersive microanalysis. Because of its low Z number, carbon does not interfere with spectral analysis of other elements of biologic interest. This technique is safe and relatively inexpensive, and eliminates the need for hazardous quenching agents. While we have not performed cytochemistry on our tissue, it should be equal to tissue prepared by the talcum powder method.

Materials:
Graphite powder; liquid nitrogen; cryostat chucks or silver pins for cryoultramicrotomy; Dewar flask (1 liter capacity); petri dish; small beaker.

Preparation:
Place a small beaker of graphite powder into a petri dish containing liquid nitrogen. Add liquid nitrogen to the graphite, stirring until a paste-like consistency is formed.

Method:
For energy dispersive microanalysis or other cryoultramicrotomy, the specimen should be approximately 0.5 to 2.0 millimeters. The tissue is rapidly excised and folded over a small silver cryostat pin. Rapidly plunge the pin into the graphite-liquid nitrogen mixture and leave until frozen. Keep the pin frozen in liquid nitrogen until cryoultramicrotomy is performed. After sectioning, the frozen sections are freeze dried over a period of 3 hours under vacuum and then can be analyzed with energy dispersive spectroscopy or with transmission electron microscopy.

References:
1. Moline, S.W., and Glenner, G.G.: Ultrarapid Tissue Freezing in Liquid Nitrogen. J. Histochem. Cytochem., 12:777-783, 1964.
2. Noble, L., and Challa, V.: Muscle Quenching with Liquid Nitrogen and Talcum Powder. Histo-Logic, 11:164-165, 1981.

A Tissue-Tek* Block Maker for Aspirated Bone Marrow and Small Hand Processed Specimens

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Despite the greatly increased use of trephine biopsies for the hematological/histological examination of bone marrow, many hematologists still carry out the aspiration procedure in parallel and examine sections of the aspirated marrow specimen.[1]

There is strong evidence that uncoagulated marrow samples provide the best histological information and, therefore, they require hand processing as small particles.[2] It is also occasionally necessary to hand process other minute specimens when there is a danger of losing them in an automatic tissue processor.

Traditionally many laboratories have processed and embedded their bone marrow aspirates according to the method of Cappell, Hutchison and Harvey-Smith.[3] With the advent of the Tissue-Tek System of embedding,[4] these flat-bottomed test-tube prepared blocks became difficult to handle as they did not conform to the Tissue-Tek System for section cutting and filing.

The equipment described in this paper was designed to overcome these problems. Marrow particles and other minute specimens are manually processed in 50 x 19 mm flat-bottomed specimen tubes (Figure 1). Tubes are available from Gallenkamp Griffin, Cat. No. T.UL-460-050J.

FIGURE 1

FIGURE 2

FIGURE 3

*Trademark of Lab-Tek Division, Miles Laboratories, Inc.

The solidified wax block which measures 17 mm in diameter is removed from the tube and cut to approximately 8 mm in length. This is pressed, specimen end first, into the hole in the metal block maker; an embedding cassette is placed on top and filled with hot wax. When completely solidified on the Tissue-Tek cooling platform, the block is easily pushed out of the block maker and is ready for sectioning.

The block maker (Figure 2) can be made from a solid block of aluminum, or similar easily worked metal, having an overall size of approximately 70 x 40 x 15 mm.

A centrally placed 17 mm diameter hole is drilled through the full thickness of the metal plate. Using the center of this hole as reference, a 40 x 30 x 3 mm depression is machined around it; this depression just accommodates a Tissue-Tek Cassette and acts as a shoulder for the accurate positioning of the cassette on the marrow-containing wax cylinder. A second deeper depression, 34 x 24 x 3 mm, is also machined out around the hole. This provides a supporting matrix of wax to firmly join cassette and specimen when the cassette is filled with molten wax.

The finished block produced by this technique can then be sectioned and filed in the usual way (Figure 3).

Note:
Not specifically mentioned in the article, but which would be fairly obvious to those working in the field, is the very important fact that the block produced is very accurately orientated for microtomy. This is particularly important when dealing with bone marrow aspirates, as when finally embedded the actual heavier marrow particles go to the very bottom of the block, with lighter blood proteins remaining above. This being so, precise orientation of the block allows for first face sections of the block being obtained.

References:

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Post-Decalcification Treatment of Bone Specimen

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The traditional procedure of washing bone specimen in running tap water overnight is unnecessary, and must be treated with reservation. Washing bone specimen overnight in water to remove the acid can produce "swelling" or expulsion of osteocytes and dislodgement of cells adjacent to trabecular surfaces. This can be circumvented by removing the bone specimen from the decalcifying fluid and placing it in two changes of 100 ml saturated lithium carbonate for a minimum of 1 hour each change. The specimen is then rinsed in several changes of distilled water with occasional manual agitation, checking after each 10-minute rinse for acidity with litmus paper.

Note:
Some specimens require three changes of distilled water, while others may require more, depending on the size of bone specimen. When acid is no longer present in the distilled water, the bone specimen can be processed in the conventional manner.

Caution:
Decalcifiers containing hydrochloric acid — When a bone specimen is fixed in formalin, and has to be decalcified in a solution containing hydrochloric acid, it is strongly recommended that the specimen must be placed first in 95% alcohol containing 5 drops of ammonium hydroxide for 1 hour, then washed approximately 2 hours in running water, before placing it in decalcifying solution. If the bone specimen is not deformalinized and washed for the proper time period, fumes of bis(chloromethyl)ether (BCME), a carcinogen, result.[1,2,3]

References:

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3. Federal Register, Department of Labor, Occupational Safety and Health Administration, 30:3757, Jan. 29, 1974.

Histopathology Control Slides Available

Histopathology control slides are now available for purchase through the Histopathology Staining Control Slide Program, initiated in 1979 by the American Registry of Pathology.

The following control slides are available at $75 per box of 25 slides (payable to the American Registry of Pathology): *M. Tuberculosis, M. Leprae, Gram Negative Bacteria, Gram Positive Bacteria, Fungus, Amyloid, Spirochetes, Cryptococcus, Copper, Hepatitis B Surface Antigen, and Legionella Pneumophila.*

Each box of 25 slides contains one stained slide and 24 unstained slides. A copy of the recommended staining procedure is included with each box of slides. The slides are not sold separately. Shipping costs will be paid by the ARP within the United States and Canada. For shipments to other countries, an additional $7.50 will be required for airmail shipment.

Address inquiries to: Histopathology Control Slide Program; American Registry of Pathology; Armed Forces Institute of Pathology; Washington, D.C. 20306. Phone: (202) 576-2043 or (202) 576-2978.

NSH Symposium/Convention

Plan to participate in the 8th Annual Symposium/Convention of the National Society for Histotechnology. We will convene at the Sheraton-Boston Hotel, September 13-17, 1982, for five days of informative workshops, scientific sessions and seminars. Expand your learning and your circle of professional associates. Send your registration form to us today!

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Liquid Embroidery to Identify Tissue Sections on Glass Slides for Peroxidase-Antiperoxidase

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Liquid embroidery can be used to mark the location of tissue sections, to make a well to keep reagents confined (thus cutting down on the amount of reagent), and to divide a section in two so that one side can be used for a control and the other side for the test. Liquid embroidery can be purchased at any handycraft store.

Method:

1. Apply an even line of liquid embroidery where desired.
2. Let air dry at room temperature.
3. Proceed with entire peroxidase-antiperoxidase method, except go very rapidly through xylenes at end, because prolonged time in xylene will make liquid embroidery dissolve and run.
4. Coverslip very carefully, using more mounting media than usual. Do not press down on coverslip.
5. Let slides air dry.

Note:
This method is not original and can be used for any glass slide methodology.

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The editor wishes to solicit information, questions, and articles relating to histotechnology. Submit these to: Lee G. Luna, Editor, Histo-Logic, P.O. Box 36, Lanham, Maryland 20706. Articles, photographs, etc., will not be returned unless requested in writing when they are submitted.