# HISTO-LOGIC®

No reader should utilize materials and/or undertake procedures discussed in HISTO-LOGIC articles unless the reader, by reason of education, training and experience, has a complete understanding of the chemical and physical properties of all materials to be utilized and the function of each material by which any procedure is accomplished.

Editor, Lee G. Luna, D. Lit., H.T. (ASCP) | Technical Bulletin for Histotechnology Published: January, April, July, October | Vol. XI, No. 1 - January, 1981

## Stain Technology Workshop

This one-week practical, educational and innovative program will be presented March 8-13, 1981, in Silver Spring, Maryland, by Lee G. Luna. The course will be presented in three parts: (1) daily one-hour lectures on Histochemistry of Staining; (2) daily one-hour lectures on Tissue Identification which are correlated to special stains; and (3) participants will perform 20 special stains, demonstrating 25 pathologic entities. All slides, solutions, glassware, equipment and procedures will be provided. For information, contact: Registrar; P.O. Box 2453; Rockville, MD 20852; (301) 468-6552.

### PLEASE NOTE

Dates for next NSH Symposium/Convention at the Little America Hotel, Salt Lake City, Utah, have been changed to:

**NOVEMBER 16-20, 1981**

## Impregnation Techniques in the Preparation of Frozen Sections from Formalin-Fixed Tissue

**Leonard Noble**
**North Carolina Baptist Hospitals, Inc.**
**Winston-Salem, North Carolina 27103**

Preparing frozen sections from formalin-fixed tissue is usually performed by washing the fixed specimen in running tap water for several hours, followed by gently blotting the excess water from the specimen and subsequent freezing in a cryostat. This allows frozen sections to be obtained, but shattering of the section usually occurs and, therefore, detail is damaged. By gently warming the frozen block with one's finger, a fair section can sometimes be obtained, but at the expense of valuable time and, occasionally, lost temper.

The following are offered as alternatives to the previously mentioned techniques:

**Gum Acacia (Arabic) Sucrose Impregnation Technique[1]**
**Solutions:**

**Gum Acacia-Sucrose**

| | |
|---|---|
| Sucrose | 30.0 gm |
| Gum acacia (arabic) | 1.0 gm |
| Distilled water | 100.0 ml |
| Thymol | 10.0 mg |

Mix sucrose and gum acacia before adding the distilled water. Heat gently with constant stirring to facilitate dissolving. Refrigerate solution.

**Procedure:**

1. Wash formalin-fixed tissue in running tap water for several hours.
2. Place in gum acacia-sucrose solution overnight (vacuum will accelerate impregnation).
3. Remove tissue from solution and allow to drain for a short period.
4. Place tissue on cryostat chuck in usual manner, freeze and section.
5. Place frozen sections on gelatin or albumin coated slides and allow to dry for several minutes before staining.

**OCT* Impregnation Technique**

**Solutions:**

**OCT-Water**

| | |
|---|---|
| OCT | 50.0 ml |
| Distilled water | 50.0 ml |

Mix thoroughly and refrigerate.

**Procedure:**

1. Wash formalin-fixed tissue in running tap water for several hours.
2. Place in OCT-water solution overnight (vacuum can speed up impregnation).
3. Remove tissue from solution and allow to drain for short period.
4. Place tissue on cryostat chuck in usual manner, freeze and section.
5. Place frozen sections on gelatin or albumin-coated slides and allow to dry for several minutes before staining.

*OCT (Optimum Cutting Temperature) compound is a product of Lab-Tek Division, Naperville, Illinois.

**Reference:**

1. Holt, S. J.: *Experimental Cell Research*, Suppl. 7, p. 8, 1959.

## Strict Serial Sectioning with Particular Reference to Bone and Dense Fibrous Tissue

**J. R. Goodwin**
**Mater Misericordiae Public Hospitals**
**South Brisbane, 4101 Q'LD, Australia**

Long unbroken ribbons (collected in a teflon-coated collection device), containing 300 to 400 sections 5 microns thick, can be produced from decalcified bone or dense fibrous tissue by soaking the exposed surface of the embedded material in the following mixture: 5 parts of glycerine, 5 parts of ethanol and 1 part of Teepol.*

**Introduction:**

In a search of textbooks on histological technique, it becomes apparent that nowhere is a method described for obtaining serial sections in long unbroken ribbons. The production of such ribbons would be of the utmost value for the reconstruction of embryos and for detecting areas of interest whether they be malignant cells, anatomical configurations, localization of endogenous or exogenous materials, identifying areas of autoradiographic labelling, etc.

Lillie[1] mentions cutting convenient lengths as does Ann

*Teepol is a detergent sold by Shell; however, any liquid detergent will suffice.

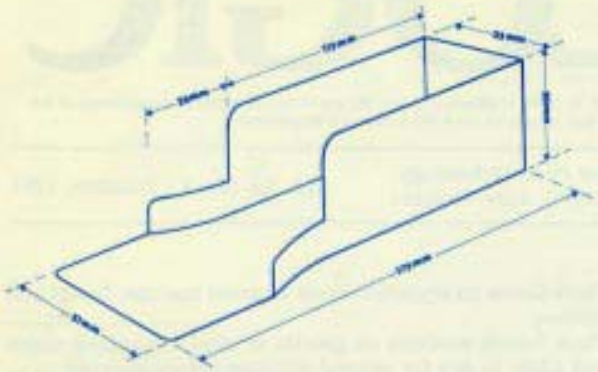

**FIGURE 1:**
Ribbon collection device.

Preece.[5] Culling[2] recommends ribbons 10 to 12 inches in length, while Drury and Wallington[4] maintain that the sectioning technique is little different from that in routine work, as does McManus and Mowry[7] who say that the procedure is the same as for the individual sections.

The difficulty in maintaining a strict sequence if sectioning is halted a number of times to deal with ribbons of moderate length is obvious. Cutting has to be commenced again, and even if the operator is aware that the first section cut on recommencement will be thicker due to expansion of the block, and the microtome advance mechanism is retarded, it is quite unlikely that the first section cut will be exactly the selected thickness. It will almost always be less than the selected thickness and is in any case subject to damage as it is the section that must be grasped to support the ribbon formed by succeeding sections.

Strict serial sectioning of bone or dense fibrous tissue is especially difficult, and even bone that has been completely decalcified will not ribbon well. Care in the selection of a processing schedule to minimize shrinkage and hardening may help, but the production of long (300 to 400 sections) unbroken ribbons is usually still not possible.

**FIGURE 2:**
Device inserted under the clamped knife mount and sectioning commenced.

**FIGURE 3:**
Sections numbered in desired intervals with a felt-tip pen and cut from the ribbon with light pressure.

Various means of softening the tissue once it has been embedded and cast in a paraffin block have been proposed. Lendrum[5] soaked the exposed face of the block in 9 parts of glycerine to 1 part of aniline oil. Daniel[3] used 9 parts of 70% alcohol to 1 part of glycerine or B.D.H. "Mollifex." Drury and Wallington[4] recommend soaking the face of the block in cold water with the addition of 0.2% Teepol, or any other wetting agent. (Badly processed fibrous tissue and muscle will benefit from this treatment.) Baker[1] recommended soaking in a mixture of 9 parts of 60% alcohol to 1 part of glycerine. Culling[2] recommends "Mollifex," while Preece[5] uses 1/2 teaspoon of any dry detergent to 100 ml of tap water leaving 45 minutes to 1 1/2 hours, and if not effective after 3 hours, soaks in plain water overnight.

**Methods:**
When presented with the problem of sectioning dog's teeth in situ within the jaw, the author had to find a means of serially sectioning this dense and difficult-to-section material, in most cases in its entirety, to locate areas of interest. By using some of the softening methods described it was possible to obtain 10 or 12 sections before the block needed resoaking. It became apparent that if usable lengths of ribbon were to be obtained, the blocks would have to soak for much more than overnight or even a few days.

A mixture containing 5 parts of glycerine, 5 parts of ethanol and 1 part of Teepol was found to cause little swelling of the tissue and enabled sections to be cut from this dense material with about the same results as embedded lung. After soaking for 4 weeks it was possible to obtain a ribbon of 400 sections at 5 micron thickness. Further soaking would possibly enable longer ribbons to be cut but this amount suited our purposes.

A means to handle such a long ribbon was needed and a device to fit a Spencer 820 series microtome† was constructed from light gauge galvanized sheet metal and coated with teflon (Fig. 1). The microtome knife was also coated with teflon. The device was inserted under the clamped knife mount and sectioning commenced (Fig 2). The microtome fronted the operator and as the sections were cut they slid down the knife and into the receiving compartment, where the ribbon commenced to fold and concertina. A small hair brush held in the left hand is used to assist this process and if necessary to gently push the ribbon down the slope. Long ribbons were obtained from material embedded in Paraplast. We have had no experience with other paraffin mixtures using the described technique. The device is removed from the microtome and taken to a clean area of sufficient length to receive the ribbon.

The last section off the knife is gripped by forceps and the first 10 to 20 cm of ribbon is laid on the bench. Thereafter, any convenient object (swab stick, small brush handle) is in-

†The "device" will work with other microtomes. The important aspect is that the front tongue of the device be able to fit under the knife when clamped on the microtome.

serted under the ribbon and another length pulled from the collection device, the device moved along the bench and the ribbon laid down. This is repeated until all the ribbon is laid out. Sections can then be numbered in desired intervals with a fine felt-tip pen, and cut from the ribbon with light pressure using a knife or scalpel (Fig. 3). This procedure has worked well for the author.

**References:**


1. Baker, J.R.: J. Roy. Micr. Soc., 61:75, 1942.
2. Culling, C. F. A.: *Handbook of Histopathological Techniques*, Second Edition, Butterworths, London.
3. Daniel, R.J.: Distribution of Glycogen in Developing Semen, J. Exper. Biol., 24:123, 1947.
4. Drury and Wallington: *Carleton's Histological Techniques*, Fourth Edition, New York, 1967.
5. Lendrum, A.C.: On the Cutting of Tough and Hard Tissues Embedded in Paraffin. Stain Tech., 19:143, 1944.
6. Lillie, R.D.: *Histopathologic Technic and Practical Histochemistry*, McGraw Hill, New York, 1965.
7. McManus, J.F.A., and Mowry, R.W.: *Staining Methods*, Harper & Row, New York, 1960.
8. Preece, A.: *A Manual for Histologic Technicians*, Second Edition., Little, Brown and Co., Boston, 1965.


## Negative Controls — Useful Idea


**Mack Alexander**
**Saint Joseph Hospital**
**Omaha, Nebraska 68131**


Histology laboratory regulating agencies require positive and negative controls for special stains. A neat and handy answer that has found favor with our pathologists is to place both controls on the same slide.

For the negative control we use brain tissue (the head of the caudate nucleus); this tissue is less reactive to a large number of special stains. Other forms of tissue are used for neuro controls. We section, and keep on hand, several hundred negative control slides.

When positive controls are needed, they are cut and picked up on the lower portion of the negative slide, marked, fixed, and held until needed. The obvious advantage to this technique is that both controls are treated identically in every step of each stain and a second slide has been eliminated.

## Reply to Editorial


**Graham Dann, A.R.T.**
**Medical Laboratory Technology**
**Lambton College**
**Sarnia, Ontario N7T 7K4**


*Ed. Note:* This information is in response to an editorial, "Solution to Problems in Staining Techniques," which appeared in **Histo-Logic**, Vol. IX, No. 2, April 1979.

On re-reading the April 1979 edition of **Histo-Logic**, I feel that the conclusion does not seem to completely answer the problem of the pH of the two acid fuchsin solutions, in the article "Solution to Problems in Staining Techniques."

The conclusion reached was that "the pH is dependent upon dye content" and referred to two solutions of acid fuchsin of 75% and 58% dye content. It seems to me that the pH difference of the two solutions was more likely due to the 42% non-dye content present in that particular dye sample. The dyes were from different manufacturers, so it is quite likely that the filler material would be different.

That the filler material may have been responsible for the pH difference could account for the very small change in pH of the 75% dye content solution, and the marked change in the 58% dye content solution, with the addition of acetic acid to both solutions, neutralizing any basic salts present in the 58% content solution.

## Other Gems for Histotechnology

This is the last of a four-part article listing various educational aids applicable to the field of Histotechnology. Part one — "Text Books," two — "Journals and Publications" and three — "Visual and Audio Aides," appeared in the April, July and October 1980 issues of **Histo-Logic** respectively.

**Histology — Self Assessment**
by Barbara Hrapchak
A. Histology Manual (with answer key)
B. Histology Examination only.
American Society for Medical Technology
5555 West Loop South, Suite 200
Bellaire, TX 77401

**Knife Sharpening Made Easy**
by Richardson, Judge, Sugulas
American Society for Medical Technology
5555 West Loop South, Suite 200
Bellaire, TX 77401

**Histology Teach and Test Flash Cards**
by Ann Preece
Ann Preece
Pathology Medical Group
P. O. Box 1989
La Jolla, CA 92037

**Toads — Technique, Observation & Discussion of Stains**
by Barbara Hrapchak
"Toads" is a tutorial presentation and review of special histologic stains divided into modules according to the type of tissue components being stained.
For more information on Toads or other programs, please contact:
CAI User Services
The Ohio State University
Division of Computing Services for Medical Education & Research
076 Health Sciences Library
376 West Tenth Avenue
Columbus, OH 43210
(614) 422-6192

**Immunohistochemistry Workshop Syllabus**
by Sharon Van de Velde

**Glycol Methacrylate Embedding & Staining**
by Sharon Van de Velde
Sharon Van de Velde
Special Anatomic Pathology
Cedars-Sinai Medical Center
Box 48750
Los Angeles, CA 90048

**Histologic Techniques in Electron Microscopy**
by Freida Carson, Ph.D.
American Society for Medical Technology
5555 West Loop South, Suite 200
Bellaire, TX 77401

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Vol. X
January - October 1980



## SUBJECT INDEX

Vol. X
January - October 1980

A Technical Bulletin for Histotechnology

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Addendum to previously published educational training aids:

**Text Books & Publications:**

1. Lillie, R. D.: *H. J. Conn's Biological Stains*, 9th Ed. (1977). Williams & Wilkins Co., 428 E. Preston St., Baltimore, MD 21202.
2. Clark, G.: *Staining Procedures Used by the Biological Stain Commission*, 3rd Ed. (1973). Williams & Wilkins Co.
3. Brimmer, F. M.: *Histological Methods and Terminology* (In Dictionary Form). The Mosaic Press, P.O. Box 41502, Tucson, AZ 85717.

## NSH Symposium/Convention
## Salt Lake City, Utah
## November 16-20, 1981

## Tentative Program

Following is a brief listing of planned workshops and lectures for the 1981 NSH Symposium/Convention in Salt Lake City. This represents a tentative program but gives a good indication of what will be presented. Exact titles with faculty and abstracts will be included in the April edition of **Histo-Logic** along with registration information.

**Workshops:**
Introduction to Stain Mechanism
Basic Chemistry
How to Write a Scientific Paper
Fluorescent Microscopy
Tissue Identification
Motivational Dynamics for Supervisors
The Muscle Biopsy — Start to Finish
Laboratory Calculations
Introduction to Immunofluorescence
Diagnosis of Leukemia by Special Stain and Enzyme Studies
Plastics
Immunoperoxidase
Photomicrography
Cryotomy
Laboratory Safety
Instrumentation
Time Management
Special Stain
If the Student Hasn't Learned, the Instructor Hasn't Taught

**Scientific Session Lectures:**
Lymphoma
Forensic Medicine
Liver Biopsy
Immunoperoxidase
Oncology
Histology/Cytology
Artificial Organs
Chemistry of Stains
ERA? PRA?
Skin Biopsy Technique
What Price Professionalism?
Sports of Histochemistry
Bone Marrow Technique
Bacterial Stains Demonstrating Gram Negative Rods — Legionnaires
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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 20801. Articles, photographs, etc., will not be returned unless requested in writing when they are submitted.

*Lab-Tek and the art of histology*

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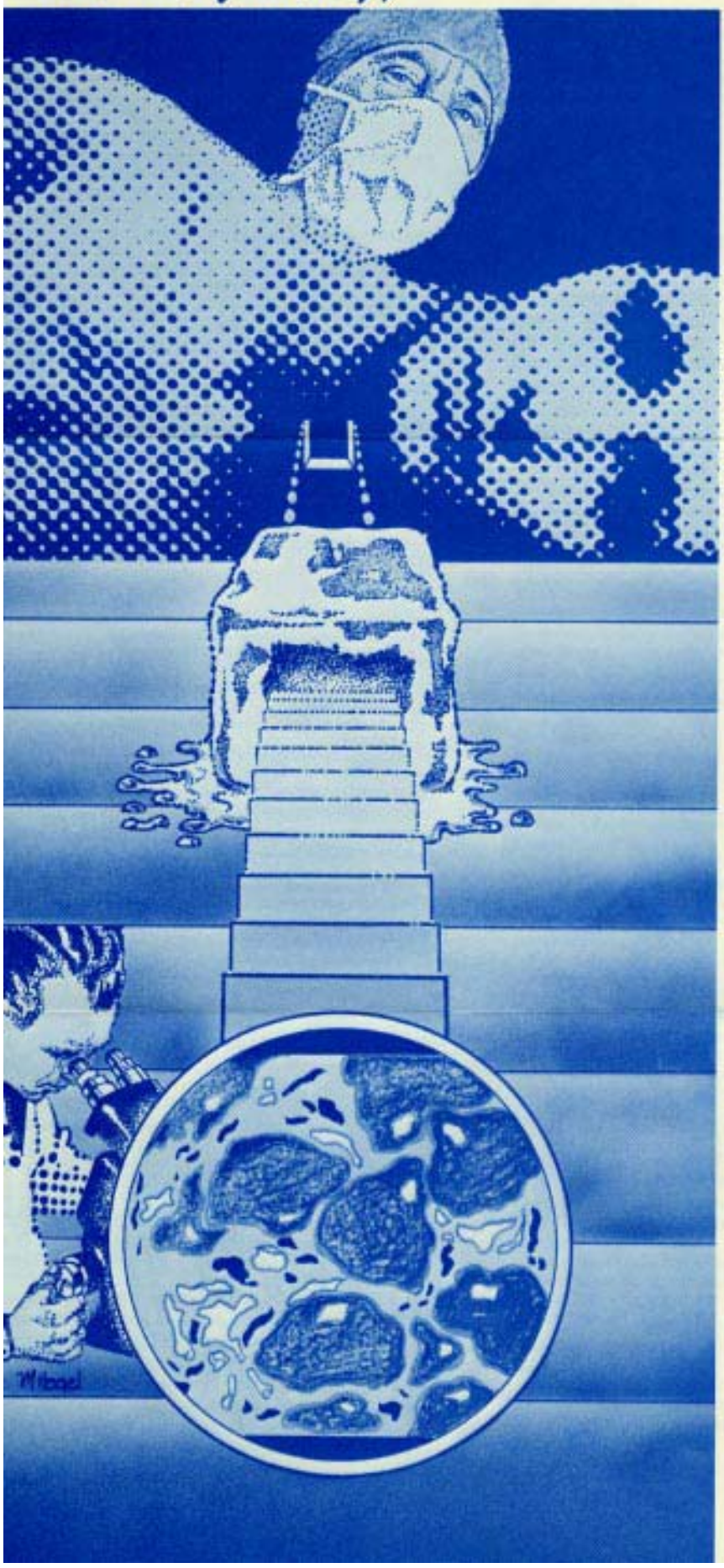

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