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Connective Tissue Staining in Glycol Methacrylate Sections

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A previous report by Koski et al[2] concluded that the Masson trichrome method and modifications do not stain collagen in sections of glycol methacrylate (GMA) as well as in paraffin sections. These authors noted that when aniline blue binds to the section, the plastic matrix is also stained. The differentiation that removes the aniline blue dye from the plastic also removes it from the collagen in varying degrees. To solve this collagen staining problem in GMA, these workers proposed the following alternatives: 1) to block hydroxyl groups on molecules other than 4-hydroxyproline and 5-hydroxylysine; 2) to find a method to etch or remove the plastic matrix; or 3) to entirely change the chemistry of the reaction.

Since Koski et al (1983) suggested these alternatives, several modified methods of the Masson trichrome stain have been reported for staining GMA sections[3,4,6].

A recent procedure was described by Philip L. Sannes for staining connective tissue (collagen) in GMA sections with picro-Sirius supra blue stain.[5] With slight modifications in Sannes' procedure, using Sirius supra blue GL (CI23160), a dye closely related to the Sirius supra blue (CI34215) used by Sannes, I found that collagen stained intensely bright blue. However, the counterstain of 0.2% Biebrich scarlet in 1% acetic acid faded after sections were mounted with Accu-Mount 60 (SP-Tissue Tek).

The method of Sannes for collagen staining offers an alternative to the Masson trichrome method for the staining of collagen in GMA sections. This article describes a modification of Sannes' procedure using Nuclear fast red (Kernechtrot—Chroma M139 P) as the counterstain, which obviates the need for hematoxylin and does not fade after sections are mounted.

Picric Acid Solution[1]

Add 6.0 gm of picric acid crystals to 200.0 ml of distilled water. While stirring with a magnetic stir bar, heat over a water bath until dissolved. Cool and store at room temperature.

Sirius Supra Blue GL Picric Acid Solution*

Place 1.0 gm of Sirius supra blue GL (CI23160) in a volumetric beaker and add picric acid solution up to a volume of 100.0 ml. Stir until dissolved.

*Sirius supra blue (CI34215) may be substituted.

Nuclear Fast Red (Kernechtrot) Solution

Nuclear fast red (chroma M139 P) . . . . . 0.10 gm
5% aluminum sulfate solution (5.0 gm in 100.0 ml distilled water). Boil aluminum sulfate solution; remove from heat and add the Nuclear fast red. While stirring, dissolve with the aid of heat (10 minutes). Cool, filter,

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and add a few crystals of thymol to retard bacterial growth.

Staining Procedure

1. Place sections in Sirius supra blue-picric acid solution for 40 minutes at 55°C. Remove to room temperature. Allow to stand 10 minutes.
2. Rinse slides in distilled water, 2 changes, 15 dips each.
3. Place slides in Nuclear fast red for 5 minutes.
4. Rinse slides in distilled water, 2 changes, 10 dips each.
5. Absolute ethanol, 2 changes, 5 dips each.
6. Absolute ethanol/xylene (1:1 ratio), 5 dips.
7. Xylene 2 changes, 5 dips each.
8. Xylene to mount.
9. Mount coverglass with synthetic mounting medium.

Results

Collagen	bright blue
Nuclei	purple
Smooth muscle	lavender
Erythrocytes	rose red

Remarks

Unlike modifications of the Masson trichrome method which apply aniline blue stain last to stain collagen, Sannes reverses the application of the collagen stain. Picro-Sirius supra blue stain is applied first, does not stain the plastic matrix and requires no differentiation. These advantages are not present in modifications of the Masson trichrome method. An increased concentration of Sirius supra blue enhances the intensity of the blue in the collagen while Nuclear fast red differentially stains nuclei, smooth muscle and erythrocytes. The dehydration and clearing steps are similar to paraffin techniques. Furthermore, fading does not occur in the stained sections after they are mounted.

The Sannes' method offers a new, reliable stain for collagen and a new approach in staining collagen in GMA sections. The method of Sannes and its modifications will require a new definition of the mechanism on how these staining reactions occur. When picro-Sirius supra blue stain is used as a first stain specific for collagen, the foremost questions are whether the hydroxyl groups on molecules other than 4-hydroxyproline and 5-hydroxylysine have been blocked or whether the chemistry of collagen staining has been changed.

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Methods for Staining Charcot-Leyden Crystals[1]

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Introduction

Charcot-Leyden crystals derive their name from the two German physicians, Jean Martin Charcot and Ernst Victor von Leyden who first described them. These crystalline structures, which are protein in nature, are found wherever eosinophilic leukocytes are undergoing fragmentation. The development of the crystal represents an uncommon fundamental intracellular process, with rapid protein crystal formation, that has no parallel in any other cell of the human body.[2]

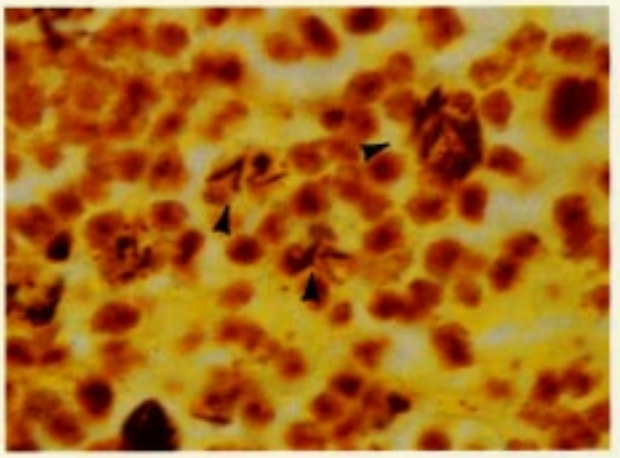

Figure 1: Charcot-Leyden crystals (arrows) can be seen in this photograph. Modified Brown and Brenn. X1000.

Charcot-Leyden crystals have been described in the tissue of patients with eosinophilic granuloma of bone, in the sputum of patients with bronchial asthma, in the stools of patients with amebic dysentery, in ulcerative diseases of the colon and a variety of other diseases.[3]

The crystals are often found in all stages of development in the cytoplasm of the cell (Figs. 1 and 2). There is no evidence that Charcot-Leyden crystals are found in the nucleus. The crystals are hexagonal or elongated dipyramidal shapes, depending on the plane in which they are sectioned. Some crystals exhibit coalescence of eosinophilic granules from which three or more sides of a crystal emerge. Some large crystals incorporate the granules into the crystal proper, while still other crystals appear perfect in shape without evidence of included granules.

Staining Characteristics

Charcot-Leyden crystals do not stain with sudan black although eosinophilic granules do. The crystals are negatively birefringent, insoluble in lipid solvents and do not stain with fat soluble dyes. The general staining properties of formalin-fixed Charcot-Leyden crystals are as follows:

1. Regaud's iron hematoxylin ..... black
2. Basic fuchsin (0.5% in 50% alcohol for 1-2 minutes) ..... magenta
3. Gallocyanin-chromalum (pH 2.5) ..... blue
4. Toluidine blue (pH 7.0) ..... dark blue
5. Acid fuchsin (0.5% in 50% ethyl alcohol for 1-2 minutes) ..... red

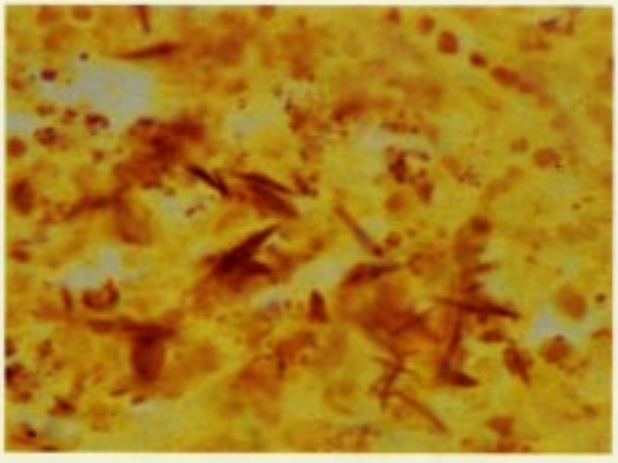

Figure 2: Charcot-Leyden crystal in different stages of development can be seen throughout the photograph. The crystals are stained pinkish red and vary in size. X1000.

6. Ponceau 2R (0.1% aqueous solution at pH 1.3) ..... orange-pink
7. Periodic acid schiff (PAS) ..... magenta
8. Trypan blue (0.1% aqueous solution at pH 0.1) ..... light blue

Histochemical studies of Charcot-Leyden crystals were conducted by Dawe and Williams.[4] This excellent study contains information on a wide variety of tests performed on these crystals.

Following are two procedures which I have used successfully for the demonstration of these crystals.

Modified Brown and Brenn for Charcot-Leyden Crystals[1]

Fixation
10% buffered neutral formalin

Processing
Paraffin embedded specimens

Microtomy
Cut sections at 4-6 micrometers

Solutions

1% Crystal Violet Solution

Crystal violet	1.0 gm
Distilled water	100.0 ml

5% Sodium Bicarbonate Solution

Sodium bicarbonate	5.0 gm
Distilled water	100.0 ml

Gram's Iodine Solution

Iodine	1.0 gm
Potassium iodide	2.0 gm
Distilled water	300.0 ml

Basic Fuchsin Solution (Stock)

Basic fuchsin, to saturation	approx 0.25 gm
Distilled water	100.0 ml

Basic Fuchsin Solution (Working)

Basic fuchsin (stock)	0.1 ml
Distilled water	100.0 ml

Picric Acid-Acetone Solution

Picric acid	0.1 gm
Acetone	100.0 ml

Ether-Acetone Solution

Ether	50.0 ml
Acetone	50.0 ml

Staining Procedure

1. Deparaffinize sections through two changes of xylene, absolute and 95% alcohols to distilled water.

Remove mercury precipitates with iodine and hypo solutions if necessary.

2. Place slides on staining rack. Pour on approximately 1.0 ml (or 20 drops) of 1% crystal violet solution, and 5 drops of 5% sodium bicarbonate solution for 1 minute. Agitate gently. If preferred, the two solutions may be mixed just before use.
3. Wash in water to remove excess stain.
4. Flood slides with Gram's iodine solution for 1 minute.
5. Rinse in water and blot with filter paper to complete dryness.
6. Decolorize with ether acetone solution dropped on slides until wash is clear.
7. Stain with basic fuchsin (working) solution for 1 minute.
8. Dip slide in water; blot gently, but not completely dry.
9. Dip slide in acetone to start reaction.
10. Differentiate immediately with 0.1% picric acid-acetone solution until sections are yellowish pink.
11. Rinse quickly in acetone and clear tissue in a mixture of equal parts of acetone and xylene.
12. Mount coverglass with resinous media.

Results

Charcot-Leyden crystals	red
Gram-positive bacteria	blue
Gram-negative bacteria	pink
Nuclei	red
Other tissue elements	yellow

Luna's Method for Charcot-Leyden Crystals[1]

Fixation

10% buffered neutral formalin

Processing

Paraffin embedded specimens

Microtomy

Cut sections at 6 micrometers

Solutions

Hematoxylin Solution (Stock)

Hematoxylin	1.0 gm
100% ethyl alcohol	100.0 ml

Ferric Chloride Solution (Stock)

Ferric chloride, 29% aqueous	4.0 ml
Distilled water	95.0 ml
Hydrochloric acid, concentrated	1.0 ml

Hematoxylin Solution (Working)

Hematoxylin solution (stock)	50.0 ml
Ferric chloride solution (stock)	50.0 ml

Biebrich Scarlet-Acid Fuchsin Solution

Biebrich scarlet, 1% aqueous	80.0 ml
Acid fuchsin, 1% aqueous	20.0 ml
Glacial acetic acid	2.0 ml

Acid Alcohol Solution

95% ethyl alcohol	100.0 ml
Hydrochloric acid, concentrated	1.0 ml

Biebrich Scarlet-Hematoxylin Staining Solution

Biebrich scarlet-acid fuchsin solution	6.0 ml
Hematoxylin working solution	45.0 ml

Lithium Carbonate Solution

Lithium carbonate	0.5 gm
Distilled water	100.0 ml

Staining Procedure

1. Deparaffinize sections and run to water.
2. Place slides in Biebrich scarlet-hematoxylin staining solution for 5 minutes.
3. Dip slides in distilled water to remove excess hematoxylin.
4. Differentiate slides in acid alcohol solution by dipping slide 5 times or until desired nucleic staining results are achieved.
5. Rinse slides in distilled water to remove acid alcohol.
6. Dip slides in lithium carbonate solution until section turns blue, usually five dips.
7. Wash sections in running tap water for 1 minute.
8. Dehydrate in 95% alcohol, absolute alcohol, and clear in xylene, 2 changes each.
9. Mount coverglass with resinous media.

Results

Charcot-Leyden crystals	red
Eosinophilic granules	reddish orange
Erythrocytes	reddish orange
Nuclear elements	blue

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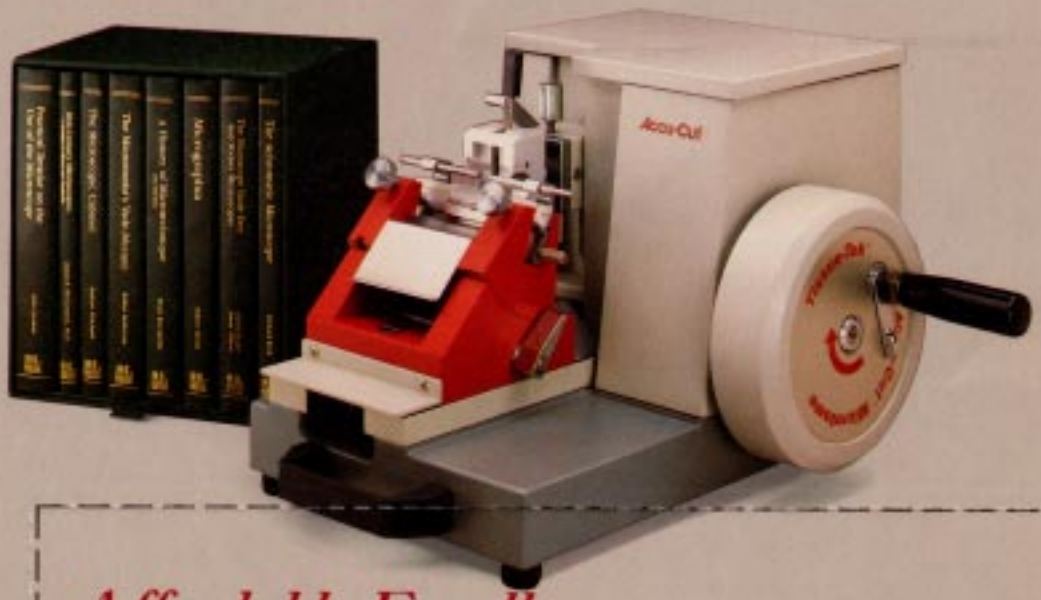

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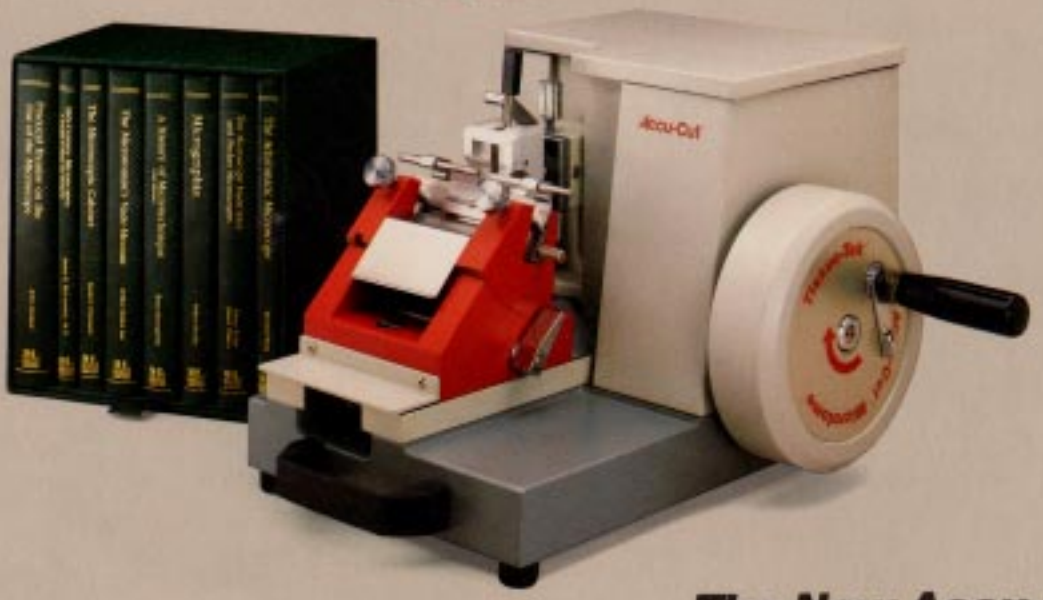

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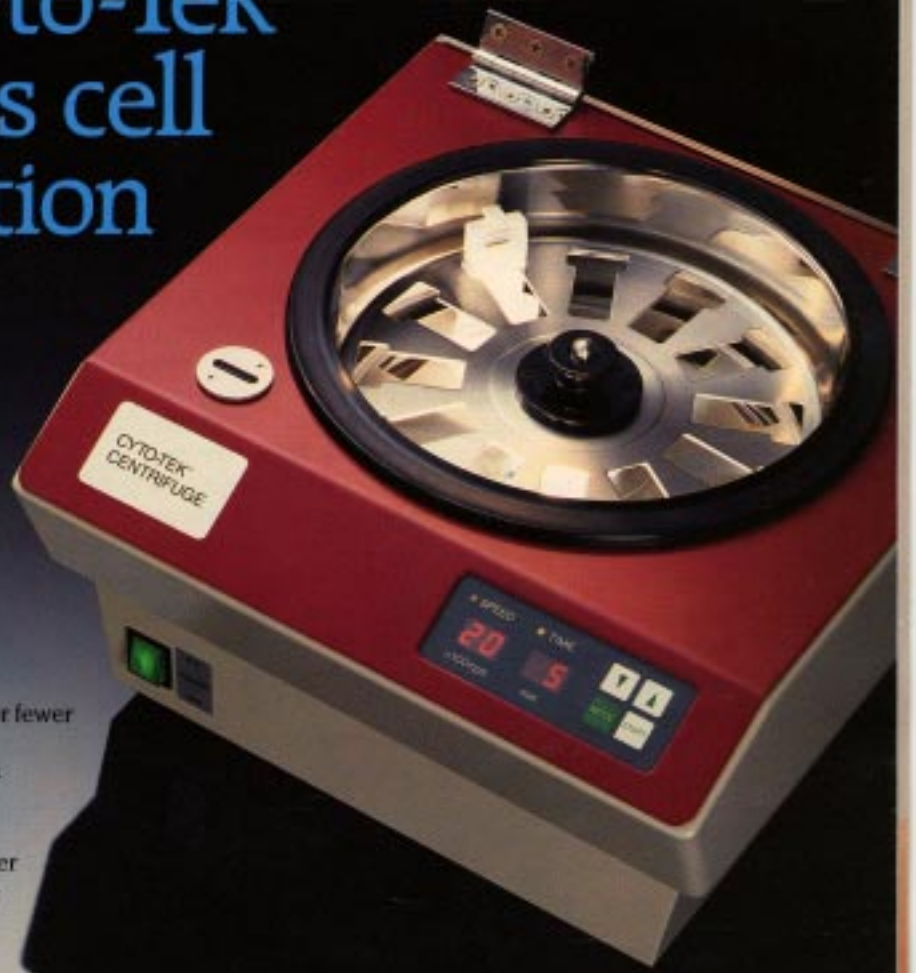

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State Society Newsletters Perform Critical Function

If anyone ever tries to tell you that histotechnology is not a creative profession, just show them some of these state society newsletter names: "Paraffinalia," "Micro-Tome," "The Slice of Life," "In Focus," "The Nucleus," "The Bevel," and "Tissue Paper." It's nice to see that even a serious profession like histotechnology can also be fun.

But these fun, creative names don't overshadow the fact that the publications they represent are serious and very professionally written and produced. At least 32 state societies are currently publishing their own newsletters. And in some states, even local chapters have their own newsletters.

Education and communication are probably the two most important functions of any professional organization. So it's no surprise that newsletters are an important part of most state societies. "The newsletter is often the only source of information exchanged within the state, especially in the rural areas," explained Lynn Montgomery, editor of *The Network*, which is published in Louisiana.

State newsletters have circulations ranging from about 50 to more than 400. Some have at least one special issue that is mailed to a much larger list than their normal circulation. This is usually the issue that covers their state convention.

Most of the state newsletters are published quarterly, although some have a difficult time maintaining a consistent publication schedule. Editors, reporters, and others who submit articles are all volunteers who also hold down full-time jobs as histotechnologists. "Unfortunately," said one editor, "we have to work around a lot of different personal schedules." Some editors are inundated with unsolicited articles. Then they have to decide what to print and what to hold for possible use in a future edition. Most, however, have to assign or ask for articles.

In Wisconsin, the state is divided into four districts. Each district has a co-editor who is responsible for submitting at least one article per issue on any histology-related topic. The Michigan newsletter, *The Mikro-Graf*, uses a different theme for each issue. Peggy Wenk, the editor, asks someone who has special experience in that area to write the feature article. She then adds references, quotes, and word games to support the topic.

Content of the newsletters is similar from state to state. Many publish the minutes from board meetings and other state meetings. Some have regular columns written by the editors or the state society officers.

The president of the Illinois society keeps a record of all the questions she is asked by members. She then writes a regular column to answer the questions. Her theory is that, if one person has a question, there are probably several more people who have the same question.

Job opportunities are also published in most of the newsletters. Other common types of articles include calendars of events; national, chapter, and state society news; new member profiles; book reviews; educational articles; health and safety information; and articles about new technology or new techniques. The Colorado newsletter, *Paraffinalia*, publishes feature stories about members and sometimes even sales reps from various suppliers. Some editors include articles reprinted from other state and national newsletters or journals.

The state newsletters are typically sent to society members at no charge. Sometimes they are also sent to nonmembers as a recruiting tool. Many of the state editors also have agreements with other states to exchange newsletters.

While the topics covered are similar in most, the look of the newsletters varies considerably. Some editors have bigger budgets because they have more members or because they accept advertising. This allows them to use professional typesetting and printing services. Some of the newsletters are even two color. But most are one color composed on a typewriter or word processor, and quick-printed.

No matter how the state newsletters are put together, they all play a significant role in communicating with and educating histotechnologists throughout the country. Those who devote their time and effort to publishing these newsletters deserve a great deal of credit.

Here is a list of state newsletters and their editors:

Alabama—"Histo-Info"
Dodie Madden
Birmingham, Ala

California—"Micro-Tome"
Linda McGlothen
Rancho Cordova, Calif

Colorado—"Paraffinalia"
Cindy Sweeney
Colorado Springs, Colo

Connecticut—"Paraffin Press"
Bonnie Yanosy
Trumbull, Conn

Florida—"Histotech"
Penny Ferre
St. Petersburg, Fla

Georgia—"Micro Time"
Mary Knight
Tallapoosa, Ga

Illinois—"The Micron"
Belinda Griffin
Springfield, Ill

Iowa—"ISH Newsletter"
David Cavanaugh
Ellsworth, Iowa

Kentucky—"The Slice of Life"
M. Lamar Jones
Lexington, Ky

Louisiana—"The Network"
Lynn Montgomery
Baton Rouge, La

Maine—"Tissue Talk"
Claudette Heath
Bangor, Me

Maryland—"The Maryland Micron"
John Murdock
Baltimore, Md

Massachusetts—"MASH"
Norma King
Boston, Mass

Michigan—"Mikro-Graf"
Peggy Wenk
Pontiac, Mich

Minnesota—"Histogram"
Andy Valls
Bloomington, Minn

Missouri—"The Bevel"
Cherie McCurdy
St. Louis, Mo

Nebraska—"Tissue Tribune"
Louanne Gruidel
Omaha, Neb

New Jersey—"NJSH Newsletter"
Alice Esposito
Waterford Works, NJ

New York—"On Stage"
Sandra Sendor
Hamburg, NY

North Carolina—"The Tarheel HistoNews"
Barbara Kirkhart
Chapel Hill, NC

Ohio—"Micro-Gram"
Liz Swihart
Toledo, Ohio

Oklahoma—"The Histo Informer"
Patty Eneff
Oklahoma City, Okla

Pennsylvania—"The Forum"
Shirley Given
Boothwyn, Pa

Rhode Island—"Tissue Paper"
Becky Bradley
Providence, RI

South Carolina—"Slice for Life"
Mequita Praet
Irmo, SC

Tennessee—"Microtome Express"
Grace Hanrahan
Memphis, Tenn

Texas—"Histo-Tex"
William W. Webb
Houston, Tex

Utah—"The Cassette"
Becky McLain
Salt Lake City, Utah

Virginia—"The Histo-Scope"
Francess Freund
Richmond, Va

Washington—"Washington State Histology Newsletter"
Cherie Dellwo
Spokane, Wash

West Virginia—"The Nucleus"
Pat Turner
Morgantown, WVa

Wisconsin—"In Focus"
Mary Ann Fabisiak
Stevens Point, Wis

National Society for Histotechnology Symposium Convention Demonstrates All-Around Commitment

Most of those who attended the recent NSH Symposium/Convention left with mixed emotions. It's always a relief to come to the end of a week of hard work and nonstop activity. But it's also disappointing to say good-bye to friends you might not see again for another year. Everyone will miss the fun and camaraderie that has become a hallmark of the annual event.

But perhaps the feeling that will dominate the hearts and minds of all those who participated in this year's program is one of pride—pride resulting from the strong commitment evident throughout the week. This commitment was generated by the society and its members, as well as by the histotechnology profession and the industry that serves it. All are committed to the advancement of the profession, as well as to education, service and, of course, friendships.

More than one thousand people attended the fourteenth annual symposium/convention held October 9-14 in Louisville. And they all left with a renewed commitment to the histotechnology profession.

The commitment to education was especially strong at the symposium/convention. Workshops were well attended and well presented, covering everything from basic chemistry and math to "The Why's and Why Not's of the Do's and Do Not's in Immunohistochemistry." Forty-one workshops were held in all from Sunday through Tuesday.

Clinical and veterinary scientific sessions began on Wednesday and continued through Thursday. Dr. J. B. McCormick kicked off the sessions with the Professor C. F. A. Culling Memorial Lecture. His talk, "Three Centuries of Histotechnology," began by explaining the 17th-century philosophy of Robert Hooke. Dr. McCormick then used a collection of prepared images to conduct a tour of the significant events in the world of histotechnology in the 17th, 18th, and 19th centuries. Other scientific sessions covered topics from new biopsy techniques to advances in cancer research.

Histotechnologists were also involved in preparing educational material. Technical exhibits were displayed Friday morning demonstrating a variety of specialized techniques used by various technologists. This gave registrants an opportunity to learn more about what other technologists are doing in their laboratories and research centers.

Dr. and Mrs. McCormick share a toast with friends.

A Miles representative gives product information to two delegates.

The poster session was another way histotechnologists could present their research or explain their techniques. This was the first year for the posters, which were on display Tuesday through Thursday.

Scientific exhibits from a number of manufacturers and suppliers were also well attended. Histotechnologists got a good look at the latest in laboratory equipment, supplies, and services. The Diagnostics Division of Miles Inc., introduced the new Accu-Cut® Microtome, an extremely accurate, well-balanced instrument with a precision knife advancing system. They also displayed the Tissue-Tek® VIP™ System, Tissue Embedding Con-

sole System, and Microtome/Cryostat, along with the Cyto-Tek® Centrifuge, Accu-Edge® Knives and Blades, and all of their Cassette accessories.

The Miles exhibit provided Pocket Pal™ Planners, and held drawings twice a day for a set of The Science Heritage Limited Series on the History of Microscopy and Microscopical Techniques.

Delegates can't "camouflage" the fun at the NSH M•A•S•H party.

Clinking glasses and a bubbling fountain welcome delegates to the Miles champagne reception.

This symposium/convention demonstrated a commitment to more than just the serious professional matters. There was also plenty of time and effort devoted to the lighter side of histotechnology. On Tuesday night, Miles Inc., sponsored a M•A•S•H party that attracted virtually everyone at the convention, including a few Klinger clones, Hot Lips look-alikes, replica Radars, twin Trappers, Sherman Potter impersonators, and Henry Blake fakes. Some even won prizes for their costumes.

The party featured plenty to eat and drink and a variety of good music for dancing. Everyone had a sMASHing

of good music for dancing. Everyone had a sMASHing good time. Photos taken at this year's party will be on display at the Miles exhibit during the 1989 NSH Symposium/Convention.

Miles also continued to sponsor the pre-banquet champagne reception featuring hors d'oeuvres, and a string quartet. Champagne was served in "keepsake" glasses.

The traditional awards banquet was held on Thursday. Awards were presented for outstanding service to the histotechnology profession. Among the awards were four presented by Miles for authorship of journal or newsletter articles. The Golden Forceps Award was presented to Cheryl Crowder, HTL (ASCP), from the School of Veterinary Medicine at Louisiana State University. Her article, "Helpful Hints for Microwave Oven Use: More Than Just Staining," was published in *Histo-Logic*. The Diamond Cover Award recipient was Lawrence Kass, MD, from Cleveland Metropolitan General Hospital. His article, titled "Basic Blue 41: A New Panoptic Stain for Blood and Bone Marrow Cells," was judged the best work published in the *Journal of Histotechnology*.

Two new awards were also presented by Miles this year: The Diamond Cover Editor's Award and the Diamond Cover Merit Award. The Editor's award went to Charles Churukian of the University of Rochester Medical Center. The purpose of this award is to honor and identify those individuals who contribute constant support, articles, and advice to the *Journal of Histotechnology*.

The Merit Award was presented jointly to James Weidmann, BSCT, HT (ASCP) and Matthew Freund, Ph.D., from the University of Medicine and Dentistry of New Jersey. The two recipients co-authored an article for the *Journal of Histotechnology* titled "Localization of Prostate Cells in Human Semen: A Comparison of the Burstone Technique and the Prostatic-Specific Antigen Staining Method."

Recipients of the Miles awards each received a set of The Science Heritage book collection.

Because of the strong commitment demonstrated at this year's symposium/convention, future events should be bigger and better than ever. Plans are already well underway for the 1989 NSH Symposium/Convention. The fifteenth annual event will be held in Las Vegas. Don't miss it!

A Useful Method for Demonstrating Bone Canaliculi

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American HistoLabs, Inc.
Gaithersburg, Maryland 20879

The following Giemsa procedure can be used successfully for demonstrating a variety of entities, including bone canaliculi and cement lines. Bone canaliculi are demonstrated especially well as seen in Figs. 1 and 2 (arrows). Bone specimen which have been exposed to formalin fixation for extended periods of time (2 months or longer) may not demonstrate the canaliculi well. As with other Giemsa type stains, this procedure demonstrates other entities (see Figs. 3-5) including cartilage. This procedure is not useful for differentiating cell types which are normally demonstrated with other Giemsa-Wright procedures.

Solutions

Giemsa Solution (Stock)

Giemsa powder	1.0 gm
Glycerin, A.C.S.	66.0 ml
Alcohol, Methanol, A.C.S.	66.0 ml

Mix glycerin and Giemsa powder. Place in a 60°C oven for two hours. Let cool, and add the methanol alcohol.

Buffer Solution pH 6.0

Potassium phosphate, monobasic	5.0 gm
Sodium phosphate, dibasic	1.0 gm
Distilled water	1000.0 ml

Giemsa Solution (Working)

Giemsa solution (stock)	1.0 ml
Buffer solution pH 6.0	50.0 ml

Alcoholic-Ammonia Solution

Methanol, 60%	95.0 ml
Ammonium hydroxide, concentrated	5.0 ml

Staining Procedure

1. Deparaffinize slides in xylene, and run to water.
2. Place slides in alcoholic-ammonia solution for 5 minutes.
3. Stain slides in working Giemsa solution for 45 minutes.
4. Rinse slides briefly in distilled water.
5. Dehydrate rapidly in 95% alcohol, absolute alcohol and clear in xylene, two changes each.
6. Mount coverglass with resinous media.

Results

Bone background	bluish purple
Canaliculi	reddish purple
Cement lines	blue
Cartilage	reddish purple

Reference

1. Luna, L.G.: Histological Procedures and Special Stains: A Practical Guide. This book is scheduled for release early spring 1989.

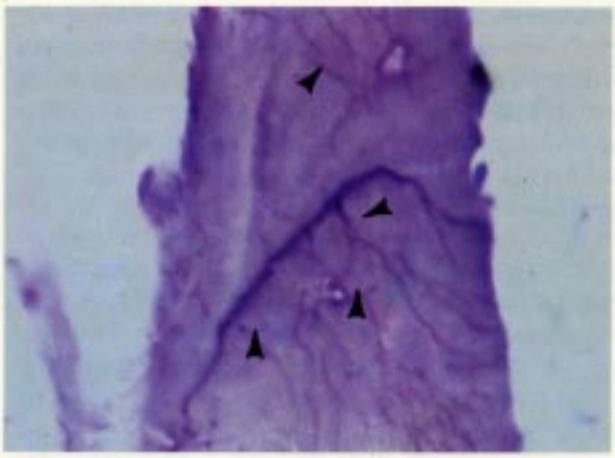

Figure 1: Bone canaliculi (arrow) can be seen radiating from a cement line (middle) and osteocytic space top (right). X1000.

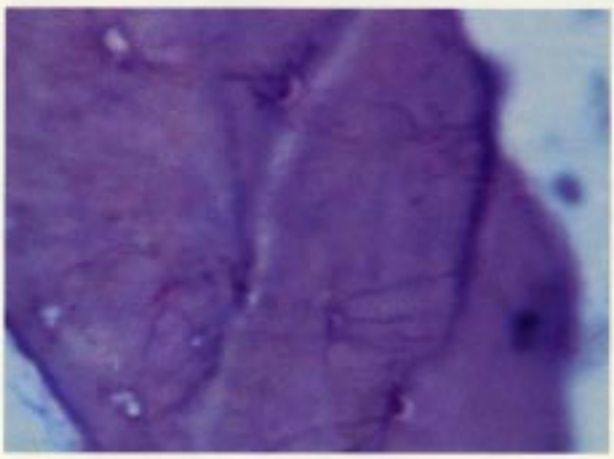

Figure 2: Canaliculi can be seen radiating from osteocytes. X1000.

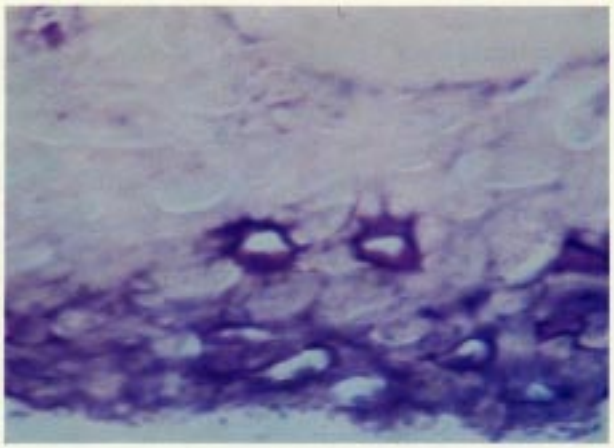

Figure 3: Glycoprotein-rich osteocyte walls can be seen staining with the modified Giemsa. X1000.

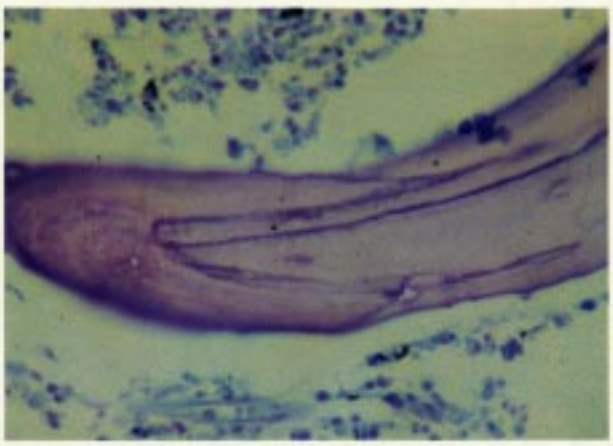

Figure 4: Bone spicule demonstrating glycoprotein-rich cement lines. X400.

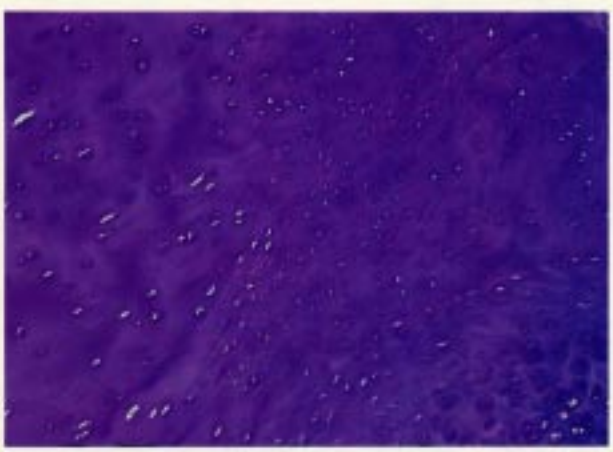

Figure 5: Ground substance in cartilage stained with the modified Giemsa procedure. X1000.

Biological Stain Commission Meets*

The general meeting of the Biological Stain Commission was held June 9-10 in Arlington, Virginia. The annual meeting was conducted by Frederick Kasten, President of the Commission, and a number of important subjects were discussed.

E. A. Schenk, Secretary, presented a report of the activities of the Assay Laboratory for 1987. A total of 129 dye samples were received for certification; 125 were found acceptable and 4 were rejected because of low dye content and/or poor staining performance. There were 104 dye samples received for "recheck." Currently 58 dyes are on the certified list, and a number are awaiting completion of assay protocols in anticipation of joining the list of certified dyes.

The Secretary reported that the commission has 59 members. Dr. Richard Horobon of Sheffield, U.K., has been appointed representative of the Royal Microscopical Society. Robert Mowry, Nominating Chairman, placed the names of Dr. David Penney, University of Rochester Medical Center, and Professor Dietrich Wittekind, University of Freiburg, Germany, in nomination for trustee. Also placed in nomination for a continuing three-year period as trustees were the names of Frederick H. Kasten, Arthur LaVelle, and Robert W. Mowry. All were unanimously approved.

Recognition was given Dr. Elmer Stotz, Trustee and Treasurer of the Commission, for more than 30 years of service. Dr. Stotz passed away in 1987. Dr. Henry Schneider, who is retired from his longtime trustee position, was also recognized for 30 years of service.

Several topics were discussed in the President's Forum:

1. European Committee for Clinical Laboratory Standards (ECCLS)—Professor Wittekind reported on problems and procedures in Europe associated with the standardization of dyes and stains. He cited the complexity of the problem since each country feels it should have its own representative. Currently, the main effort is directed toward obtaining dyes in pure form, which can then be used as reference standards

*Excerpted from the annual meeting report prepared by the commission's secretary, Eric A. Schenk, MD.

to check the quality of manufactured and marketed products.

2. Standardization of Immunoreagents—Horobin, Mowry, and others stressed the need to have some standardization of antibodies and other immunoreagents. Jules Elias discussed future plans in terms of priority: a) the preparation of a document for publication which details the findings and recommendations of his committee; b) obtaining funds for research which will provide data essential to the development of assay standards (e.g., the effect of various fixatives on immunostaining); c) identifying laboratories with expertise where product testing might be performed.
3. Azure B: Schenk presented a report on the now commercially available pure azure B. After considerable discussion of this product, it was suggested that pure azure B may be more suitable for calibration than for staining.
4. New Fuchsin: As published by C. Churukian and E. A. Schenk, this appears to be an excellent dye in acid-fast staining procedures for mycobacteria. Because it can be manufactured effectively free of less-methylated homologues and is currently sold in this form, it was proposed that new fuchsin be certified as such.
5. Embedding Media: A number of problems related to interactions between various types of plastic embedding media and stains were discussed.
6. Hematoxylin: Wittekind proposed standardization of hematein, the oxidation product of hematoxylin and active dye. Richard Dapson suggested that since hematein has no shelf life, it would be more useful as a calibration standard than as a commercial dye.
7. Fluorochromes: Horobin discussed a number of fluorochrome dyes currently used as tracers in neurobiology and problems associated with their use. It was agreed that these dyes, as well as fluorochromes used in flow cytometry, need to be studied and standardized.
8. Alcian Blue: Staining properties of Alcian blue 8GX and Astra-blue were discussed, as were some of the determining factors required for Astra-blue, before certification can be considered.
9. FITC: Korytko questioned specified dating of fluorescein isothiocyanate. Horobin felt that the basis may have been related to the propensity of FITC to take up water. It was agreed that, unless a compelling reason was uncovered for dating, this requirement could be eliminated.
10. Aniline Blue: A vendor requested aniline blue be relabeled, since it is actually methyl blue. However, the commission suggested that since neither dye is available in pure form and the active staining component has yet to be identified, aniline blue should continue to be certified according to the existing protocol.

The Board of Trustees meeting was held on June 10. During the meeting, the publication committee reported that a revised edition of *Biological Stains* has top priority. Testing protocols will be updated, and possibly printed as a separate publication. A revised edition of *Staining Procedures* will also be published.

New members appointed to the commission were Dr. David Wilbur, University of Connecticut, representative for the American Society of Clinical Pathologists and Dr. Anthony diSaintAgnese, University of Rochester Medical Center.

Some new committee members were also appointed:

Nominating Committee	**Publications Committee**
Lee Luna, Chair	Frederick Kasten, Chair
Robert Mowry	G. Stephen Nettleton
David Penney	Dietrich Wittekind
Eric Schenk	

There are currently 14 scientific societies with representation in the Commission. Plans are to expand this list to include cell biologists, the neural sciences, cytotechnologists, and hematology technologists.

Work will begin on writing protocol for dyes currently under consideration for inclusion in the certification program. These include acridine orange, Coomasie blue, and trypan blue.

The report on immunoreagents was discussed, with a proposal made that an ad hoc trustee committee be appointed to formulate and advise how this matter of standardization might be pursued by the Commission.

The 1989 meeting of the Biological Stain Commission will be held June 8-9 in Rochester, New York.

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1988-1989

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Do You Know?

- That the compounding of 5-P-dimethylamine benzylidene Rhodamine (working solution) used for staining copper can be simplified. (The word Rhodamine will be used throughout this article in place of 5-P-dimethylamine benzylidene Rhodamine.) It has been the practice to shake the Rhodamine stock solution to create a colloidal suspension to achieve proper staining of copper. **The modification suggested below eliminates the need to shake the stock solution prior to preparation of the working solution.** It is my opinion that the modified solution provides more consistent staining of copper.

Note: The new modification method of compounding Rhodamine was submitted by Jack B. Wenger. The remaining information is provided by Lee G. Luna. The staining procedure for demonstrating copper is included to assist those who are not familiar with the procedure or do not have ready access to references.

Okamoto and Utamura's Method for Copper

Fixation
10% buffered neutral formalin

Processing
Paraffin embedded specimens

Microtomy
Cut sections at 6 micrometers

Solutions

Rhodamine Solution (Stock)

5-P-dimethylamine benzylidene Rhodamine*	0.2 gm
Absolute alcohol	100.0 ml

Making Up Solution—**Old Way:**

Rhodamine Solution (Working)

Rhodamine solution (stock)	6.0 gm
Distilled water	94.0 ml

Note: Shake stock solution before measuring and mixing solution.

Making Up Solution – **New Way:**

Rhodamine Solution (Working)

Sodium acetate trihydrate	2.0 gm
Distilled water	100.0 ml
Formalin, concentrated (37%)	0.3 ml

ADD:

Rhodamine solution (stock)	6.0 ml

The Rhodamine stock solution should not be shaken before adding to the above solution. Formalin is used as a preservative.

Mayer's Hematoxylin

Preparation instructions can be found in most books on histopathological techniques.

0.5% Sodium Borax Solution

Sodium borax	0.5 gm
Distilled water	100.0 ml

Staining Procedure

1. Decerate slides and hydrate to distilled water.
2. Incubate in Rhodamine working solution at 37°C for 18 hours.
3. Wash well in several changes of distilled water.
4. Stain in Mayer's hematoxylin for 10 minutes.
5. Rinse with distilled water.
6. Quickly rinse in 0.5% sodium borax.
7. Rinse well with distilled water.
8. Dehydrate in 95% alcohol, 2 changes, 1 minute each.
9. Two changes in absolute alcohol, 1 minute each.
10. Clear in three changes xylene.
11. Mount coverglass with resinous media.

Results

Copper	bright red
Nuclei	light blue

Reference

Lindquist, R.R., *Arch of Path* 87: No. 4, pp 370-379, 1969.

*Catalogue #2748 Eastman Organic Chemicals Distillation Products Industries, Rochester, New York.

(Submitted by: Jack B. Wenger, Histopathology Laboratories, AFIP, Washington, DC 20306 and Lee G. Luna, American HistoLabs, Inc., Gaithersburg, MD 20879)

- That basic fuchsin is not a pure dye. It can be a mixture of pararosaniline, rosaniline, magenta II and new fuchsin or may only contain rosaniline and magenta II.
- That pararosaniline is the unmethylated form of basic fuchsin.
- That pararosaniline can be substituted for basic fuchsin in all staining methods which require the use of basic fuchsin.
- That pararosaniline must be used to prepare Gomori's aldehyde fuchsin.
- That pararosaniline is marketed as a certified pure dye.
- That new fuchsin is also marketed as a pure dye but is not certified.
- That new fuchsin may be substituted for basic fuchsin in the Brown-Brenn and similar bacterial stains.
- That Schiff's reagent prepared with basic fuchsin that contains new fuchsin will be difficult to decolorize. It will require additional treatment with activated charcoal and even then may not become water clear or light straw color.

(Submitted by: Charles J. Churukian, Special Stains Laboratories, Department of Pathology and Laboratory Medicine, The University of Rochester Medical Center, Rochester, NY 14642)

- That the compounding of Fontana Silver solution can be simplified considerably. The silver staining results with this modified solution are identical to those seen when this solution is prepared in the conventional way. Silver reactive products are well delineated and background staining is similar to Fontana staining using the conventional method of preparing Fontana solution.

Modified Solution:

1. Make 1000 ml of 10% silver nitrate in distilled water. (This becomes the stock silver solution, which can be kept for many months.)
2. Place 65.0 ml of concentrated ammonium hydroxide in a clean glass container (stoppered flask).
3. Place a teflon-coated magnetic stir bar in the container with the 65.0 ml of ammonium hydroxide.
4. Turn the magnetic stirrer to full speed.
5. Slowly add 10% silver nitrate to the ammonium hydroxide solution until solution just becomes opalescent (a translucent brown). This solution color remains until used.
6. Allow solution to sit overnight at room temperature in the stoppered flask before using.

Note: I have found this modification to simplify and speed up the preparation of Fontana Silver solution. It is especially useful since Fontana Silver solution is used extensively and frequently in many laboratories for both the Fontana-Masson method for silver reactive groups and Manuel's reticulum. The remaining stock 10% silver nitrate solution can be stored in the refrigerator until needed for subsequent procedures requiring Fontana silver nitrate solution.

(Submitted by: Jack B. Wenger, Histopathology Laboratories, AFIP, Washington, DC 20306)

Questions in Search of an Answer*

The following staining result has puzzled me for some time. Therefore, I would appreciate having information regarding what causes the problem and/or how to prevent it.

In performing various stains for mucosubstances such as Mowry's Alcian Blue and Colloidal Iron, *slides treated with hyaluronidase and/or sialidase stain darker overall than those not treated with these enzymes.* This, as we all know, is contrary to expected results since the enzymes are used to remove hyaluronic and sialic acid from tissue sections. In a discussion with Lee G. Luna, he suggested that enzyme contamination was the most likely culprit. He suggested that bacterial or fungal organisms may produce mucosubstances which may be deposited on the slide during the enzyme treatment. This newly deposited mucosubstance then reacts with the staining solutions. Since this is only a possible answer, I am still anxious to know if someone has a specific answer to this staining anomaly.

Jennie Achstetter
Histopathology Laboratories
AFIP, Washington, DC 20306

*Replies to Questions in Search of an Answer should be sent to Lee G. Luna, Scientific Editor, *Histo-Logic*, P.O. Box 36, Lanham, MD 20706.

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

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