HISTO-LOGIC®

Editor, Lee G. Luna, D. Lit., H.T. (ASCP) Technical Bulletin for Histotechnology Vol. VII, No. 3 - July, 1977
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GOLDEN FORCEPS AWARD WINNER

We are pleased to announce that Livia M. Molnar has been selected as the recipient of the Golden Forceps Award for 1977. Ms. Molnar, who is on staff at the University of Washington in Seattle, has been a long-time contributor to HISTO-LOGIC® with a total of eight articles appearing in past issues. She currently has additional articles in review which will appear in upcoming issues. Criteria for this award were clarity, practical applications and continued contributions.

Ms. Molnar is to be commended for her dedication to the field of histotechnology and the editor hopes others will be encouraged by her example. The Golden Forceps Award will be presented at the Symposium/Convention of the National Society for Histotechnology to be held in San Francisco, California, September 5-9, 1977. Reprints of her articles are available from Lab-Tek Products, Division Miles Laboratories, Inc., 30W475 North Aurora Road, Naperville, Illinois 60540.

Emanuele's PAS Modification for Cellular Elements

(See Results)

Peter V. Emanuele
Washington, D.C. 20306

Fixation: 10% buffered neutral formalin
Microtomy: Cut paraffin sections at 4 microns
Solutions:

Normal Hydrochloric Acid

Hydrochloric acid, concentrated (Sp gr 1.19)	83.5 ml
Distilled water	916.5 ml

Schiff's Solution

Dissolve 2.00 gm basic fuchsin in 400.0 ml hot distilled water. Bring to boiling point. Cool to 50° C. *Filter* and add 40.0 ml normal hydrochloric acid. Cool and add 2.0 gm anhydrous sodium bisulfite. Keep in the dark for 48 hours or until solution becomes straw color. Add 1 teaspoonful of activated carbon. Shake for 1 minute and filter through coarse paper. Collect the first 100 ml of the filtered solution and return to funnel. This eliminates residual carbon in container. *Store in refrigerator.*

1% Periodic Acid

Periodic acid	1.0 gm
Distilled water	100.0 ml

Mayer's Hematoxylin

Hematoxylin crystals	1.0 gm
Distilled water	1000.0 ml
1.0% sodium iodate	20.0 ml
Ammonium or potassium alum	50.0 gm
Citric acid	1.0 gm
Chloral hydrate	50.0 gm

Dissolve the alum in water, without heat; add and dissolve the hematoxylin in this solution. Then add the sodium iodate, citric acid, and the chloral hydrate. Shake after the addition of each chemical to insure each chemical is in complete solution. The final color of the stain is reddish violet. Stain keeps well for months.

Staining Procedure:

1. Decerate and hydrate to distilled water.
2. Oxidize sections in 1.0% periodic acid solution for 15 minutes.
3. Rinse slides in 5 changes of distilled water.
4. Place slides in Schiff's reagent for 25 minutes.
5. Wash slides in running warm tap water for 10 minutes.
6. Rinse slides in 5 changes of distilled water.
7. Stain sections in Mayer's hematoxylin for 15 minutes.
8. Wash slides for 15 minutes in running tap water. Warm water preferred.
9. Dehydrate in 95% alcohol, absolute alcohol, and clear in xylene, two changes each.
10. Mount coverglass with resinous media.

Results:

Intracellular and extracellular proteins that contain a glycoprotein component may be PAS positive. Collagen is PAS positive and serves as a positive control when it stains deep red. Lymphocytes and plasma cells with intranuclear PAS positive aggregates (Dutcher bodies) are observed in malignant lymphomas such as Waldenstroms macroglobulinemia and are associated with monoclonal proteinopathies. Intracytoplasmic PAS positive plasma cells (Mott cells, Russell bodies) may be observed in various inflammatory disorders. PAS staining of immunoglobulins is related to the quantity of glycoprotein: IgA and IgM immunoglobulins are usually strongly PAS positive, whereas IgG is usually weakly PAS positive or negative.

Did You Know

An Editorial

Hematoxylin is one of the few remaining natural dyes. It comes from Logwood,[1] the heart wood of the Campeachy Tree (Haematoxylon campechianum).[2]

Columbus may have been the first person to take Logwood to Europe. However, even he was uncertain of what he found.

It probably was Hernandez de Cordoba who first learned of the properties of Logwood. He visited the area of Campeche in 1517. The Spanish town of Campeche, founded in 1540, was named after the tree. Campeche made large profits on the export of Logwood until competition from Progreso (another Mexican city) reduced the profits.

In England in 1581, the use of Logwood was prohibited for the dyeing of cloth by "the Act of the Twenty-third of Queen Elizabeth." The act called the dyeing of cloth by Logwood extract a "practice false and deceitful," and it proscribed that "all Logwood found was to be burned."

In 1608 the Lord Mayor of London called the town's dyers together to investigate the use of Logwood. He found that although "they disclaimed the use of Logwood, the contrary was true." In spite of the unfavorable legislation, the demand for Logwood continued.

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In 1664 Oldenburg stated that he had worked on the fixing (mordanting) of Logwood, but with little success. In 1715 Dr. Barhan introduced Logwood into Jamaica from Honduras. By 1814 the tree had become a nuisance, spreading into fields where it was difficult to root out. This property of precociousness was turned to good use by employing the tree as a hedge for cattle.

In 1810 Michel Eugene Chevreul isolated the molecule responsible for the dyeing property of Logwood.

The name hematoxylin comes from the Greek word haimatodec which means blood-like, and xylon which means wood. Blood-like wood describes the color of Logwood that has been exposed to the air. Freshly cut Logwood is yellowish (the crystals of hematoxylin are sometimes acicular and vary in color from light yellow to rusty purple).

Hematoxylin was used in the tanning industry as a black dye. Used by itself, it imparts a brownish tinge to the leather. But used with other dyes it gives a rich, full black color. It also acts as a filler by helping with the glazing and finishing of the leather. Hematoxylin has also been used in the ink and drug industries. It was used to give ink color until a more lasting dye was found.

At one time a decoction of Logwood was thought to be good for chronic diarrhea, some forms of atonic dyspepsia, as an injection for leucorrhea, and as an ointment for cancer and hospital gangrene.

Hematoxylin was first used as a biological dye by Thomas Andrew Knight in 1803. He used it to determine the direction in which a fluid flowed in a transected potato runner. Sixty years later in a different country, hematoxylin was used as an animal tissue stain.

The demand for tissue dye was first felt upon the invention of the microscope by Leewenhoek, who in 1714 reported his use of saffron. But not until the improved microscopes of the 1850's was there a widespread demand for tissue dyes.

Wilhelm Waldeyer used hematoxylin in 1863 in an effort to study axis-cylinders. He did not use a mordant, and for this reason, his results were poor. Bohmer knew that alum was used as a mordant with hematoxylin in the textile industry. When he used hematoxylin with alum in 1865 to study meningitis epidemica, he obtained good results. Since Bohmer's time, many histologists, botanists, pathologists and cytologists have used hematoxylin solutions with excellent results. The dye that did not work at first, has become one of the most widely used biological stains.

1. So called from being imported in logs.
2. The hard, brownish-red wood of a tropical tree native to Central America, Mexico and West Indies, used in dyeing.

NOTE: All information contained herein was obtained several years ago from the Library of Congress by the editor of HISTO-LOGIC.* No references were obtained at that time, which explains their omission in this article.

Techniques for Studying Prenatal Ossification in Silver Nitrate Immersed Specimens

1. PAS-Alcian Blue Method
2. Modified Mallory Method
3. Modified Hematoxylin and Eosin

Livia M. Molnar
Department of Orthodontics
University of Washington
Seattle, Washington 98195

The method for radiological study of fetal pig specimens, developed by Hodges,[1] has also been used to study human prenatal specimens.[2] In our laboratory we have used this method for craniofacial studies of human and *Macaca nemestrina* specimens. We find that the immersed specimens yield good radiography, but cannot be used for light microscopy studies and photography because the silver nitrate overlaps the tissue and leaves a heavy silver artifact. We have modified three methods which produce properly stained and cleared slides that can be used for histological studies and photography without the silver artifact.

For radiological studies, use the silver nitrate staining method of Hodges.

Application of Silver, Decalcification and Microtomy Procedure

Fixation: Any fixative can be used.

1. Immerse the gross specimen in 0.5% silver nitrate solution for 4-8 days.
2. X-ray specimen.[1,2]
3. Decalcify in Bankuthy's decalcification solution.
4. Embed with Molnar's double embedding method.[3]
5. Embed specimen and section at 5-7 micra.

Solutions: All solutions required for the three methods are provided below.

Bankuthy's Decalcification Solution

Distilled water	1010.0 ml
Formic acid	90.0 ml
Hydrochloric acid, concentrated	80.0 ml
Sodium citrate	10.0 gm

0.5% Silver Nitrate Solution

Silver nitrate	0.5 gm
Distilled water	100.0 ml

10% Ferric Chloride

Ferric chloride	10.0 gm
Distilled water	100.0 ml

10% Sodium Thiosulfate (Hypo)

Sodium thiosulfate	10.0 gm
Distilled water	100.0 ml

0.5% Alcian Blue Solution

Alcian blue	0.5 gm
3% glacial acetic acid	100.0 ml

0.5% Periodic Acid

Periodic acid	0.5 gm
Distilled water	100.0 ml

Lillie's Cold Schiff's Solution

Basic fuchsin	1.0 gm
0.15 N hydrochloric acid	100.0 ml
Sodium metabisulfite	1.9 gm

Shake solution on mechanical shaker for 2 hours. Solution should be clear and yellow to light brown in color at this stage. Then add 500 mg activated charcoal. Shake solution for 2 minutes. Filter solution into clean bottle and wash the residue with a little distilled water to restore the original 100 ml volume.

Reducing Solution (Stock)

Sodium bisulfite	10.4 gm
Distilled water	100.0 ml

Reducing Solution (Working)

Sodium bisulfite stock solution	5.0 ml
Distilled water	100.0 ml

Tissue-Talk

National Society For Histotechnology
Symposium/Convention

The Third Annual Symposium/Convention of the National Society for Histotechnology will be conducted at the St. Francis Hotel, San Francisco, California, September 5-9, 1977.

Registration application is attached. Registration forms may be photocopied if more than one individual from the same activity wishes to attend. Mail registration and check to: **Registrar, P.O. Box 36, Lanham, Maryland 20801.**

Also attached is a St. Francis Hotel reservation card. Please complete and mail directly to: **St. Francis, Union Square, San Francisco, CA 94119, Attn: Reservations Manager.**

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SEPTEMBER 3-9, 1977

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I will be attending National Society for Histotechnology
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Company _____

Address _____ City _____ State _____ Zip _____

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☐ Please hold room on a payment guaranteed basis. If the reservation is not honored on the day of arrival the room will be billed for one night and then the reservation will be cancelled.

Please reserve accommodations as checked below:

PER DAY	MAIN BUILDING Single	MAIN BUILDING Double	MAIN BUILDING Twin	TOWER Single	TOWER Double	TOWER Twin
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Medium	$40 ☐ $45 ☐	$52 ☐ $57 ☐	$52 ☐ $57 ☐	$55 ☐ $67 ☐	$70 ☐ $82 ☐	$70 ☐ $82 ☐
Deluxe	$55 ☐	$70 ☐	$70 ☐			
		Suites			Suites	
2 Rooms (Parlor and Bedroom)	$110 ☐	$135 ☐	$160 ☐ $185 ☐		$165 ☐ $185 ☐	
Specialty Suite		$400, $450, $600			$350, $400, $600	

PLEASE CONTACT ST. FRANCIS DIRECT FOR ANY CHANGES IN ACCOMMODATIONS

All sleeping room accommodations are subject to 6% city tax. If a room at the rate requested is unavailable, one at the nearest available rate will be reserved.

Reservation requests must be received 30 days prior to commencement of convention. Requests received after 30 day cut-off confirmed subject to availability.

FORM 33 12-76 HH

NATIONAL SOCIETY FOR HISTOTECHNOLOGY
SYMPOSIUM/CONVENTION
SEPTEMBER 3-9, 1977

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Please check functions you desire to attend:

*Scientific Sessions ____ $40.00 (Wed-Fri)

Banquet ____ $15.00 (Thursday Evening)

Exam Reviews (Monday)
1. Histotechnology ____
2. Chemistry ____
3. Histochemistry ____
4. Anatomy ____

***WORKSHOPS**

MONDAY
No. 1 ____ $20 (all day)
No. 2 ____ $20 (all day)
No. 3 ____ $20 (1/2 day PM)
No. 4 ____ $20 (1/2 day AM)
No. 5 ____ $20 (1/2 day PM)
No. 13 ____ $20 (1/2 day PM)

TUESDAY
No. 6 ____ $20 (all day)
No. 7 ____ $20 (all day)
No. 8 ____ $20 (all day)
No. 9 ____ $20 (1/2 day AM)
No. 10 ____ $20 (1/2 day PM)
No. 11 ____ $40† (all day)
No. 12 ____ $20 (1/2 day AM)
No. 14 ____ $20 (all day)
No. 15 ____ $20 (1/2 day PM)

***Note:** The prices listed above for attending workshops and scientific sessions apply to NSH members only. Non NSH members must add $5.00 for each workshop and $10.00 for the Scientific Sessions.

†High cost necessary due to expensive solutions and chemicals.

Check or money order **must accompany** registration!
Payable to: National Society for Histotechnology
Mail registration to: Registrar, P.O. Box 36, Lanham, Maryland 20801
Please Note: Reimbursement of registration fees will be made upon receipt of cancellation notification prior to August 29th.
No refunds will be made after this date.

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Workshops

Workshop Title	Director	Date	Time	Location
1 Introduction to Stain Mechanism	Jack Wenger	Monday, 9/5/77	9:30 AM - 4 PM	California West Room
2 Tissue Identification	Lee Luna Charles West Edna Prophet	Monday, 9/5/77	9:30 AM - 4 PM	Grand Ballroom
3 Cytology	Ruth Kenney	Monday, 9/5/77	1 - 4 PM	Georgian Room
4 Macrophoto and Microphoto for Histology Techs	Paul Kolsanoff Eugenia Kolsanoff Don Longenecker	Monday, 9/5/77	9:30 AM - NOON	California East Room
5 Safety Seminar for Histotechnology	Ken Cohen	Monday, 9/5/77	1 - 4 PM	California East Room
6 Animal Dissection, Anatomy & Tissue Identification (Limited to 50 registrants)	Lucille Rossi Jean Mohler	Tuesday, 9/6/77	9:30 AM - 4 PM	Elizabethan D Room
7 Special Stain Wet Workshop (subject to change): Acid Fast, Congo Red, Fontana, Hall, Dahl, Gomori Trichrome, Lawson's Elastic, Aldehyde Fuchsin for HBAg, Methyl Green Pyronin Y, Southgate's Mucicarmine	Erwin Haas Lee Luna	Tuesday, 9/6/77	9:30 AM - 4 PM	Grand Ballroom
8 Immunofluorescence Antibody Techniques	Charles Culling	Tuesday, 9/6/77	9:30 AM - 4 PM	Colonial Room
9 Automatic Knife Sharpening	Marilyn Augustine Lyn Richardson Maria Sugulas	Tuesday, 9/6/77	9:30 AM - NOON	Georgian Room
10 Cryostat	Kay Jenkins	Tuesday, 9/6/77	1 - 4 PM	Georgian Room
11 Immunoperoxidase Technique	Diane Miller	Tuesday, 9/6/77	9:30 AM - 4 PM	Elizabethan C Room
12 Certain Applications and Staining of Mineralized Thin Bone Sections Using the Goldner's & Gomori's Trichrome Stains (Limited to 30 registrants)	Tony Villanueva Michael Crouch	Tuesday, 9/6/77	9:30 AM - NOON	Elizabethan A Room
13 *Introductory Histotechnology	Erwin Haas	Monday, 9/5/77	1 - 4 PM	Elizabethan D Room
14 *Histochemistry	Jerry Coates	Tuesday, 9/6/77	9:30 AM - 4 PM	Elizabethan B Room
15 *Human Microscopic Anatomy	Freida Carson Tom Palmer	Tuesday, 9/6/77	1 - 4 PM	Elizabethan A Room

*These workshops are **highly recommended** for those individuals taking the Thomas Edison examinations.

NSH/Thomas Edison Exam Reviews

The following sessions are primarily for individuals taking the Thomas Edison examinations in preselected subjects.

Subject	Instructor	Date	Time	Location
Introductory Histotechnology	Lecture Review (Jules Elias)	Monday, 9/5/77	9:30 AM - NOON	Elizabethan D Room
Chemistry	Lecture Review (Jules Elias)	Monday, 9/5/77	1 - 4 PM	Elizabethan A Room
Histochemistry	Lecture Review (Robert Escoffery)	Monday, 9/5/77	9:30 AM - 4 PM	Elizabethan B Room
Human Microscopic Anatomy	Lecture Review (Freida Carson Tom Palmer)	Monday, 9/5/77	9:30 AM - 4 PM	Elizabethan C Room
Chemistry — workshop will consist of audio visual equipment for review by interested histotechnologists:		Monday, 9/5/77	9:30 AM - NOON	Elizabethan A Room
Exams — given at the same time. Two days are set for those who wish to take more than one exam during the week.		Wednesday, 9/7/77	7:00 - 9:00 AM	Elizabethan A & B Rooms
		Thursday, 9/8/77	7:00 - 9:00 AM	Elizabethan A & B Rooms

All examinees must be pre-registered before taking exams. If you would like to take an examination and have not registered, submit $5.00 for study guide to: National Society for Histotechnology, P.O. Box 36, Lanham, Maryland 20801.

Business Meeting Schedule and Evening Activities

Activity	Date	Time	Location
Executive Board Meeting	Saturday, 9/3/77	9 AM - 2 PM	Elizabethan A Room
Executive Board, Committee Chairpersons, President & Secretary House of Delegates, Journal Editor, Newsletter Editor, Thomas Edison Coordinator, NAACLS NSH Representative	Sunday, 9/4/77	9 AM - Noon	Elizabethan A Room
House of Delegates	Sunday, 9/4/77	1:30 - 5 PM 7 - 10 PM	Colonial Room
NSH Membership Meeting	Wednesday, 9/7/77	7 - 10 PM	Grand Ballroom
Thomas Edison Exams	Wednesday, 9/7/77 Thursday, 9/8/77	7 - 9 AM 7 - 9 AM	Elizabethan A & B Rooms Elizabethan A & B Rooms
Exhibits Open	Tuesday, 9/6/77 Wednesday, 9/7/77 Thursday, 9/8/77 Friday, 9/9/77	7 - 9 PM 9:30 - 4:30 PM 9:30 - 4:30 PM 9:30 - Noon	California Rooms
LAB-TEK Cocktail Hour	Thursday, 9/8/77	6:30 - 7:30 PM	Colonial Room
Banquet	Thursday, 9/8/77	7:30 PM	Grand Ballroom

SCIENTIFIC SESSIONS

WEDNESDAY, SEPTEMBER 7, 1977

A.M. SESSION:

Practical Aspects of Gynecological Histology and Pathology	*Donald M. McKay, M.D.*
Kidney Biopsy Procedures	*Sharon Van de Velde*
Some Aspects of Histotechnology in Toxicology	*Barbara Kirkhart*
Histological Cell Block Preparations	*Richard Slocum*
Tissue Culture Techniques — How and Why	*Jean Mannagh*

P.M. SESSION:

Theory and Technique of Peroxidase and/or FITC Labeling of Tissue Sections	*Sharon Van de Velde*
FDA's "Good Laboratory Practice" Guidelines — Their Impact on a Histology Laboratory	*Jean Mohler*
H & E Staining — The Practical Aspects	*Edna Prophet*
How to Win at Embedding and Influence Tissue	*David L. West, Ph.D.*
The Jones Stain for Kidney Specimens	*Doris Jones*
The Von Kossa Affair	*Cel Rutledge*

THURSDAY, SEPTEMBER 8, 1977

A.M. SESSION:

Pigments, a Key to Their Identification	*Michael Johnson*
Reliability and Multiple Uses of Strong Silver Nitrate	*George Cole*
Why Differentiate Routine Hematoxylin	*Charles Culling*
Criteria for Grading HT Certification Microscopic Slides	*William B. Kingsley, M.D.*

P.M. SESSION:

The HT Certification Practical Examination	*William B. Kingsley, M.D.*
Lymph Node Preparations	*(To be announced)*
Quality Control	*Richard Slocum*
Fact from Artifact in Dermal Pathology	*J. D. Conroy, D.V.M., Ph.D.*

FRIDAY, SEPTEMBER 9, 1977

A.M. SESSION:

Study of the Skin, Muscle, Cartilage and Bone with Polarized Light Microscopy	*John McNeal, M.D.*
Imprint Techniques in the Surgical Pathology Laboratory	*Judy Briscoe*
Whales, Fish and Other Monsters I have Known. Histopathology of the Occasional Exotic Specimen	*Michael Lagios, M.D.*
The JB-4 Microtome in a Modern Histology Laboratory	*Sharon Van de Velde*
The Effects of pH and Exposure Period of Aldehyde Fixatives	*David L. West, Ph.D.*

Molnar's Modified Mallory's Solution

Distilled water	600.0 ml
Phosphotungstic acid	6.0 gm
Orange G	3.0 gm
Aniline blue	3.0 gm
Acid fuchsin	3.0 gm

Molnar's Modified Harris Hematoxylin

Hematoxylin	5.0 gm
Alcohol, absolute	25.0 gm
Ammonium or potassium alum	25.0 gm
Distilled water	500.0 ml
Mercuric oxide (red)	2.5 gm

Prepare as in Harris' original. Dissolve the hematoxylin in the alcohol, the alum in the water by aid of heat. Remove from heat and slowly add the mercuric oxide. Reheat until solution becomes dark purple. Remove from flame immediately and plunge the vessel into a basin of cold water until cool. Add 10 ml glacial acetic acid to the solution to increase precision of nuclear stain. Filter. Stain is ready for use as soon as it cools. Store at room temperature; filter each time before use.

1% Alcoholic Eosin Solution

Eosin Y, water soluble	4.0 gm
Distilled water	80.0 ml
Dissolve and add alcohol, 95%	320.0 ml

0.25% (12N) Hydrochloric Acid

Hydrochloric acid	0.25 ml
Distilled water	100.0 ml

Saturated Sodium Bicarbonate Solution

Sodium bicarbonate	10.0 gm
Distilled water	100.0 ml

PAS-ALCIAN BLUE METHOD

At this stage, apply the method outlined in steps 1-5 titled "Application of Silver, Decalcification and Microtomy Procedure" printed at the beginning of this article.

Staining Procedure:

1. Decerate slides and hydrate to tap water.
2. Place slides in 2 changes of 10% ferric chloride for 5 minutes each.
3. Place slides in 10% sodium thiosulfate solutions for 5 minutes. (Change hypo solution every 20-30 slides.)
4. Wash slides in tap water for 10 minutes.
5. Place slides in 1% alcian blue solution for 15 minutes.
6. Wash slides in tap water for 2 minutes.
7. Oxidize slides in 0.5% periodic acid for 10 minutes.
8. Wash slides in tap water for 5 minutes.
9. Place slides in Lillie's cold Schiff's solution for 10 minutes.
10. Place slides in working reducing solution for 3 minutes.
11. Formaldehyde, concentrated, 1 dip.
12. Wash slides in tap water for 5 minutes.
13. Dehydrate slides in 95% absolute alcohol and clear in xylene, 3 changes each.
14. Mount coverslip with resinous media.

Results:

Exclusively acid substances (various connective tissue mucins): blue
Neutral polysaccharides (glycogen and Brunner gland mucin): magenta
Cartilage: light blue
Nuclei: deep blue
Cell body of fungi: dark red to purple
Mucoid capsules: blue
Bone (colored by PAS): dark pink
Cytoplasm: pink

This procedure resolves the problem of the silver nitrate artifact. It can be used for human and *M. nemestrina* prenatal craniofacial specimens as well as any other bone specimens. Molnar's new PAS method permits nuclei and cytoplasm to be studied without the preparation of different staining methods to achieve the same results.

MODIFIED MALLORY'S METHOD

At this stage, apply the method outlined in steps 1-5 titled "Application of Silver, Decalcification and Microtomy Procedure" printed at the beginning of this article.

Staining Procedure: (Use glass dish and slide racks.)

1. Decerate slides and hydrate to tap water.
2. Place slides in 2 changes of 10% ferric chloride solution for 5 minutes each.
3. Place slides in 10% sodium thiosulfate for 5 minutes. (Change hypo after every 20-30 slides.)
4. Wash slides in tap water for 10 minutes.
5. Stain slides in Molnar's modified Mallory's solution for 3-5 minutes.
6. Differentiate slides in water (stop when you have reached the required color).
7. Dehydrate in 95% absolute alcohol and clear in xylene, 3 changes each.

Results:

Nuclei: red
Collagen fibrils: blue
Ground substance of cartilage and mucin: varying shades of blue
Erythrocytes and mucin: yellow
Elastic fibrils: pale pink or unstained
Teeth and bones: orange to reddish

This procedure, like the previous, resolves the problem of the silver nitrate artifact.

MODIFIED HEMATOXYLIN AND EOSIN PROCEDURE

At this stage, apply the method outlined in steps 1-5 titled "Application of Silver, Decalcification and Microtomy Procedure" presented at the beginning of this article.

Staining Procedure: (Use glass dish and slide racks.)

1. Decerate slides and hydrate to tap water.
2. Place slides in 10% ferric chloride solution for 10 minutes.
3. Place slides in 10% sodium thiosulfate for 15 minutes, 2 changes. (Change the hypo solution after every 20-30 slides.)
4. Wash slides in tap water for 10 minutes.
5. Stain slides in Molnar's modified Harris' hematoxylin, 45 seconds.
6. Rinse slides in tap water.
7. Differentiate slides in 0.25% (12N) hydrochloric acid, 2 dips.
8. Rinse slides in tap water.
9. Blue slides in saturated aqueous sodium bicarbonate solution for a few seconds.
10. Rinse slides in tap water.
11. Counterstain slides in 1% alcoholic eosin solution for 6 minutes.
12. Dehydrate slides in 95% alcohol and absolute alcohol, a few dips in each solution.
13. Clear slides with xylene, 2 changes.
14. Mount coverslip with resinous media.

Results:

A well differentiated stain with blue nuclei and bright rose background is obtained. This method will resolve the silver nitrate artifact and can be used for human fetal and *M. nemestrina* prenatal craniofacial specimens.

References:

1. Hodges, P.C.: Ossification in the Fetal Pig. A Radiographic Study. *Anat. Rec.*, 116:315-326, 1953.
2. O'Rahilly, R. & Meyer, D.B.: Roentgenographic Investigation of the Human Skeleton During Early Fetal Life. *Am. J. Roentgenol.*, 76:445-468, 1956.
3. Molnar, L.M.: Double Embedding with Nitrocellulose and Paraffin. *Stain Tech.*, 49:5, pg. 311, 1974.

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HISTO-LOGIC®

Plan to attend
NSH Symposium / Convention
Sept. 5-9 in San Francisco

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Technically Speaking

Robert A. Clark, Technical Services
Lab-Tek Products, Naperville, IL 60540

During the sectioning process many problems can and do occur. They may be related to the type of tissue being sectioned, the method of embedding (orientation), improper fixation and dehydration, or poor paraffin impregnation during processing.

There are other factors which may be involved and these are mechanical in nature. One important factor concerns the Tissue-Tek® II Chuck Adapter. If the unit is not properly adjusted or needs to be rebuilt, it can cause poor sectioning, thick and thin sections, gouging, vibration, etc. To find out if your Tissue-Tek II Chuck Adapter needs repair, do the following:

1. Remove the blade from the Microtome Knife Holder.
2. Insert a Tissue-Tek Ring or Tissue-Tek II Cassette into the Chuck Adapter and close the locking lever.
3. With thumb and index finger, grasp the Ring or Cassette and try to move it while in the Chuck Adapter.

(Any movement, no matter how slight, indicates the need to rebuild the Chuck or make further adjustments as described in the directions for the Chuck Adapter.)

How do you go about rebuilding the Chuck Adapter?

Lab-Tek Products now markets a repair kit for *Tissue-Tek II Chuck Adapter*, product number 4178. This kit will enable you to rebuild your present Chuck Adapter with ease and at a substantially reduced cost.

Editor's Note:

It is a pleasure to introduce a new column to HISTO-LOGIC.® Technically Speaking will be a regular feature authored by Bob Clark, Manager of Technical Services in Lab-Tek's home office. His laboratory experience with the U.S. Army and work as a hospital Histology Supervisor makes Bob an ideal author for this column. Bob will appreciate your comments and suggestions. Please jot them on your letterhead stationery and mail them directly to him in Naperville.

To receive your own personal copy of HISTO-LOGIC, or to have an associate added to the mailing list, submit home address to: Lab-Tek Products, Division Miles Laboratories, Inc., 30W475 North Aurora Rd., Naperville, Illinois 60540.

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.