

HISTO-LOGIC^{T.M.}

Lee G. Luna, D. Lit., H.T. (ASCP), Editor

A Technical Bulletin for Histotechnology

Vol. III, No. 3 - July, 1973

Announcement

We are pleased to announce that Mrs. Elaine S. Boyd, H.T. (ASCP), is the recipient of the Golden Forceps Award. Her paper, "An Evaluation of the Problems in Lymph Node Preparation," was selected from entries submitted to HISTO-LOGIC from July 1, 1971 through March 1, 1973.



Mrs. Boyd will be flown, all expenses paid, to the "Symposium on Histopathologic Techniques" which will be conducted October 1-5, 1973, in Silver Spring, Maryland. There she will be presented the Golden Forceps Award.

Criteria for selecting the winning article were originality, clarity, and scientific contribution.



An Evaluation of the Problems in Lymph Node Preparation

Elaine S. Boyd, HT (ASCP)
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Thousand Oaks, California 91360

Lymph Nodes, My Achilles' Heel

In view of the fact that all histotechnologists encounter extreme difficulties in processing lymph nodes, I would like to share with you some of my findings and techniques for improved lymph node preparation.

In order to obtain a diagnostic section of lymph node there are certain rules of processing and fixing which must be followed very closely. These will be enumerated in the order of the processing technique which has been found most helpful.

Blocking (Macro Sectioning) of the Lymph Node

The node should be cut (macro sectioned) immediately by the pathologist. Failure to cut the node immediately, or placing the entire node into a fixative will result in a poorly fixed central portion of the node. The intact capsule forms a protective shield around the lymphatic tissue, which delays the penetration of the fixative, sometimes for hours, producing varying degrees of central autolysis with poorly stained, ballooned cells, and a contrasting deeply stained peripheral zone.

Fixation

As in all tissues, fixation is of vital importance, and is a trouble

spot which is often overlooked. If formalin is used as a fixative, it must be buffered with sodium phosphate monobasic, and sodium phosphate dibasic (4 gm and 6.5 gm/1000 cc) respectively. Acid formalin (unbuffered) rapidly produces a dramatic alteration of staining, resulting in a deeply acidophilic character of the connective tissue, a lack of differential staining, and a ballooning of the nucleus, which may then appear vesicular. (Change the fixative often, or you may be putting your tissues into what is, essentially, distilled water.) It must be remembered that a fixative is exhausted after four baskets of tissue have passed through the solution. This is true of all fixatives. Zenker's is a better fixative than formalin for nodes, in my estimation. It gives a better preservation of the cytologic detail, as well as excellent staining quality.

It must be noted that the time of fixation is of great importance. The tissue should, for best results, remain in the fixative for a period not less than twenty-four hours. This is not only desired, but preferred. The handling of these specimens should never be rushed in any of the technical phases.

Processing

Beware of contamination!!!! (Carrying one solution into another.) This point cannot be stressed too strongly. Change the dehydrants each day. If you use isopropyl alcohol as a dehydrant, it must be followed by methanol, anhydrous, to completely remove the water from the sections.

Paraffin Infiltration

The temperature of the paraffin bath is vitally important. Paraffin heated above 60° C destroys the lymphoid tissue almost completely. The differential characteristics will also be altered, resulting in a muddy bluish-pink color rather than the sharp differentiation which is preferred.

Cutting Technique

A sharp knife is a must in cutting lymph nodes, so there will be no compression or tearing of the section. The knife must be fairly cold, and the paraffin block likewise should be cold. An ice cube is satisfactory for this step. The rate of speed of the wheel of the microtome should be approximately 15 revolutions per minute. The block should be cut no thicker than four microns so there is no layering of the cells.

Staining

Again, avoid contamination of the solutions. All solutions should be fresh, and the slides drained well between each bath. Also keep in mind that lymph nodes take up the nuclear stain more readily than does the average tissue, and the time in this solution should be almost cut in half, in order to obtain the proper differentiation of the nuclear chromatin and the nucleoli.

Conclusion

The preceding hints are a must if one expects to obtain a good section of lymph node each and every time. The procedure which has been found to be most satisfactory is as follows:

Fixation and Processing Schedule

1. Fix cut lymph node with Technicon FU-48 for 18-24 hours.

2. Alcohol, 80% for 1 hour.
3. Alcohol, 95% for 1 hour.
4. Alcohol, 100% for 1 hour. (Longer if necessary.)
5. Acetone, 2 changes for 30 minutes each.
6. Paraffin, 2 changes, 1 hour each.
7. Embed, and cut at 3-4 microns.

Staining Technique

1. Decerate and hydrate to 80% alcohol.
2. Place in iodine alcoholic solution for 2 minutes. (1% iodine in 70% alcohol.)
3. Place in sodium thiosulfate for 2 minutes. (5% aqueous)
4. Distilled water, 1 minute.
5. Stain in Harris' hematoxylin for 3 minutes. (Add 5 cc glacial acetic acid/95 cc hematoxylin prior to use.)
6. Rinse in tap water until excess dye ceases to run.
7. Decolorize in acid alcohol, 2 quick dips. (2% HCl in 70% alcohol)
8. Wash in running tap water for 10 minutes.
9. Place in distilled water for 1 minute.
10. Counterstain in Technicon eosin, 3-5 dips.
11. Dehydrate in 95% alcohol, absolute alcohol, and clear in xylene, 2 changes each.
12. Mount #1 coverslip with resinous media.

In processing tissues using different fixatives, Technicon FU-48 was found to be the most beneficial. It was my observation that tissues fixed in this solution presented little problems with section coming off slides. The slides stained very well, and were the ones most complimented and accepted by the pathologists.

So my Achilles' Heel, I hope I have conquered you! May you remain on the slide where you belong!!!

Acknowledgment

The author wishes to thank the following doctors for their valuable assistance in the preparation of this manuscript:

1. Dr. Lloyd Smith, Pathologist, Miller Clinical Laboratory, Modesto, California.
2. Dr. Robert J. Lukes, Professor of Pathology, University of California, Los Angeles, California.

References

1. Culling, C. F.: *Handbook of Histopathological Techniques, Aims and Effects of Fixation*, 2nd ed., Washington, D.C.: Butterworth, Inc., 1963, vol. 3, pp. 20-22.
2. Boyd, W.: *Surgical Pathology, The Lymphatic System*, Philadelphia: W. B. Saunders Co., vol. 27, pp. 568-569.
3. Ham, A. W.: *Histology, Hemopoietic Tissue*, J. B. Lippincott, 1969, vol. 15, pp. 308-318.
4. Lynch: *Medical Laboratory Technology, Care and Selection of Waxes*, 2nd ed., Philadelphia: W. B. Saunders Co., 1969, vol. 36, p. 1007.

Multiple Staining Procedures for the Islands of Langerhans

Ellen S. Slaughter, HT (ASCP)
and Robert E. Brown, M.D.
Washington, D.C. 20306

In our experience there is no single staining procedure that can identify satisfactorily and simultaneously the three types of islet cells in human pancreas. We recommend Gomori's aldehyde-fuchsin stain for beta cells, Gomori's chrome alum-hematoxylin-phloxine stain for alpha cells, and Hellerström and Hellman's modification of Davenport's silver impregnation

technique for alpha₁ (or delta) cells. The critical aspects for successful application of each procedure involve fixation, staining times, and proper differentiation, with use of a known positive control.

At present there are three known types of islet cells, and each one is associated with the production of a specific hormone:

1. Beta cells - associated with production of insulin.
2. Alpha₂ cells - associated with production of glucagon.
3. Alpha₁ (or delta) cells - associated with production of gastrin.

Because there are clinical states associated with the abnormal production of these hormones, one can readily appreciate the need for a reliable staining technique to identify and study each specific cell type. Unfortunately, in our experience there has been no single stain that satisfactorily identifies each of the aforementioned cell types. We have found it necessary to employ three separate islet cell stains in each case. These include:

1. Gomori's aldehyde-fuchsin stain for beta cells.^{2,6}
2. Gomori's chrome alum-hematoxylin-phloxine stain for alpha cells.^{3,6}
3. Hellerström and Hellman's modification of Davenport's silver-impregnation technique for alpha₁ or delta cells.^{1,4,5}

With this combination we can assess the following:

1. The approximate proportions of the different cell types.
2. Which cell types are proliferating, as in nesidioblastosis (neof ormation of islets).
3. Which cell types are showing cytopathologic features such as nuclear hypertrophy.
4. The state of granulation or degranulation. (This is particularly helpful in attempting to distinguish neoplastic from normal islets, because the former are usually but not always relatively degranulated.)

The purpose of this report is threefold:

1. To point out some of the practical applications of special stains for islet cells.
2. To present our particular modification of Gomori's aldehyde-fuchsin and chrome alum-hematoxylin-phloxine stains.
3. To review Hellerström and Hellman's modification of Davenport's silver-impregnation technique, pointing out the critical steps in each method.

Procedures

Modified Gomori's Aldehyde-Fuchsin Stain for Beta Cells

Fixation

Use 10% buffered neutral formalin and fix for at least 24 hours.

Microtomy

Cut paraffin sections at 6 micra.

Solutions

	Zenker's
Distilled water,	1000.0 ml
Mercuric chloride,	50.0 gm
Potassium dichromate,	25.0 gm
Sodium sulfate,	10.0 gm

Add 5.0 ml of glacial acetic acid to 95.0 ml of Zenker's solution before use. Discard after use.

	1% Alcoholic Iodine
Iodine,	1.0 gm
Alcohol, 80%,	100.0 ml

5% Sodium Thiosulfate (Hypo)	
Sodium thiosulfate	5.0 gm
Distilled water	100.0 ml

0.5% Aldehyde Fuchsin	
Basic fuchsin (CI No. 42510)	1.0 gm
Alcohol, 70%	200.0 ml
Hydrochloric acid, concentrated	2.0 ml
Paraldehyde	2.0 ml

Let stand at room temperature for five days or until stain is deep purple. Store in refrigerator. Filter before use. This is a stable solution. May be reused.

0.25% Metanil Yellow	
Metanil yellow (CI No. 13065)	0.25 gm
Distilled water	100.0 ml
Glacial acetic acid	0.25 gm

This is a stable solution. May be reused.

Staining Procedure

1. Decerate sections and hydrate to distilled water.
2. Mordant in Zenker's solution overnight.
3. Rinse in tap water for 3 off-and-on changes.
4. Remove mercury precipitate in alcoholic iodine for 5 minutes.
5. Remove iodine with sodium thiosulfate for 5 minutes.
6. Wash in running water for 5 minutes.
7. Rinse with 2 off-and-on 70% alcohol rinses.
8. Stain with aldehyde-fuchsin for 1 hour.
9. Rinse in 95% alcohol, 2 off-and-on changes.
10. Rinse well with 6 off-and-on tap water rinses.
11. Counterstain with metanil yellow for 3 minutes, (agitate).
12. Alcohol, 95%, 2 off-and-on changes.
13. Absolute alcohol, 2 off-and-on changes.
14. Xylene, 3 off-and-on changes.
15. Mount coverslip with resinous media.

Results

Beta cells Brownish purple

Remarks

The principal modifications of this stain are the mordanting with Zenker's solution and increasing the time in aldehyde-fuchsin. Because the time in aldehyde-fuchsin may vary, depending upon factors such as the age of the stains and individual technical variations, a control must be run with each case. The end point can be judged by using control beta cell staining as a guide.

Modified Gomori's Chrome Alum-Hematoxylin-Phloxine Stain for Alpha₂ Cells

Fixation

Use 10% buffered neutral formalin and fix for at least 24 hours.

Microtomy

Cut paraffin sections at 6 micra.

Solutions

Bouin's	
Picric acid, saturated aqueous solution	750.0 ml
Concentrated formaldehyde solution (37-40%)	250.0 ml
Glacial acetic acid	50.0 ml

This is a stable solution. May be reused.

Potassium Permanganate	
Potassium permanganate	1.5 gm
Distilled water	500.0 ml

Sodium Bisulfite	
Sodium bisulfite	5.0 gm
Distilled water	100.0 ml

Chromium-Alum-Hematoxylin

Solution A (Stock)	
Hematoxylin	1.0 gm
Distilled water	100.0 ml

Solution B (Stock)	
Chromium potassium sulfate	3.0 gm
Distilled water	100.0 ml

Working Solution	
Solution A (Stock)	100.0 ml
Solution B (Stock)	100.0 ml
Potassium iodate	0.2 gm

Mix well and boil until hematoxylin is a deep blue. Filter before each use. This is a stable solution and may be reused.

1% Acid Alcohol	
Hydrochloric acid, concentrated	1.0 ml
Alcohol, 95%	100.0 ml

0.5% Phloxine	
Phloxine (CI No. 11320)	0.5 gm
Distilled water	100.0 ml

This is a stable solution. May be reused. (Enhancement of phloxine staining may be achieved by making up the phloxine in a solution of distilled water that contains 0.001% CaCl₂ because there may not be enough endogenous Ca⁺⁺ in the tap water wash that precedes the phloxine step in the staining procedure.)

5% Phosphotungstic Acid	
Phosphotungstic acid	5.0 gm
Distilled water	100.0 ml

Staining Procedure

1. Decerate sections and hydrate to distilled water.
2. Mordant in Bouin's solution overnight at room temperature.
3. Rinse in distilled water using 3 off-and-on changes.
4. Place in potassium permanganate for 1 minute.
5. Place in sodium bisulfite until sections become clear.
6. Ten off-and-on tap water rinses.
7. Place in filtered chromium alum hematoxylin for 20 minutes.
8. Place in acid alcohol for 45 seconds.
9. Wash in warm running tap water until sections turn blue.
10. Place in phloxine for 20 minutes.
11. Rinse in 3 off-and-on changes of distilled water.
12. Place in phosphotungstic acid for 5 minutes.
13. Rinse in 80% alcohol for 10 quick dips.
14. Dehydrate through the alcohols quickly to 3 changes of xylene.
15. Mount coverslip with resinous media.

Results

Alpha₂ cells Red

The principal modification of this procedure is in the timing. It is important to run a control section of pancreas so that the proper end point can be attained.

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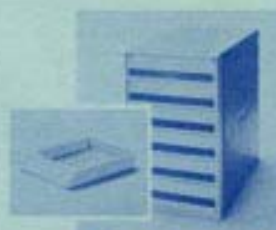


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Editor's Corner

Virginia Histology Workshop

The Virginia Society of Histology Technicians is presenting their fall histology workshop in Richmond on September 7-8, 1973. For information contact: Mrs. C. Durkowski, 9613 Krause Road, Chesterfield, Virginia 23832.

6th Annual Histology Conference

The Illinois Society for Histotechnologists presents its 6th Annual Conference, to be held at the Ramada Inn, Champaign, Illinois, August 23-25, 1973. There will be wet labs, histochemical techniques, and special presentations. For information contact: Mr. Kenneth H. Urban, 2141 N. Magnolia Avenue, Chicago, Illinois 60614.

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.

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

Symposium on Histopathologic Techniques

The Symposium on Histopathologic Techniques will be conducted at the Sheraton and Holiday Inns, Silver Spring, Maryland. The dates for this year's program are October 1-5, 1973. Listed below are the titles for the workshops, scientific sessions, and seminars being conducted. **PLEASE NOTE THE FOLLOWING SPECIAL INSTRUCTIONS:**

Registration application: The registration application is attached. *This will be the only announcement made concerning the symposium.* Registration for scientific sessions is limited to 600. We request that you file your registrations by our September 15 closing date. Late applications, however, will be accepted provided the quota has not been filled.

Registration forms may be xeroxed 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.

Accommodations: All accommodations for rooms must be made directly with the hotels. For your convenience, the addresses and toll-free phone numbers are provided below:

Sheraton Silver Spring
8727 Colesville Road
Silver Spring, Md. 20910
(800) 325-3535
(Symposium Headquarters
Hotel)

Holiday Inn
8777 Georgia Avenue
Silver Spring, Md. 20910
(800) 238-5400
(Approximately four
blocks from Sheraton)

In order to insure reduced rates, please indicate on your reservation that you are a registrant for the Histopathology Symposium.

Workshops

WORKSHOP NO. 1: MONDAY, OCTOBER 1, 1973

9:00 A.M. - 4:30 P.M., (Holiday Inn)

RESULTS ORIENTED MANAGEMENT FOR HISTOLOGY SUPERVISORS

Mr. Robert Rike

TRAINING WORKSHOP - A.M.

The morning session will focus on how, when and why people learn, development of a five-step training technique and several practical applications.

LEADERSHIP AWARENESS WORKSHOP - P.M.

The afternoon session is designed to focus on the qualities, skills and abilities needed for effective leadership; also, types and styles of management, and personal style identification.

WORKSHOP NO. 2: MONDAY, OCTOBER 1, 1973

9:00 A.M. - 4:30 P.M., (Sheraton)

INTRODUCTION TO TISSUE IDENTIFICATION

Mrs. Edna Prophet and Mr. Richard Verfuert

The purpose of this workshop is to acquaint the participant with basic tissue structures. The value of this knowledge in stain technology will be illustrated with the use of photomicrographs of special stains.

WORKSHOP NO. 3: TUESDAY, OCTOBER 2, 1973

9:00 A.M. - 4:30 P.M., (Holiday Inn)

SPECIAL STAIN SEMINAR/WORKSHOP

Mr. Bart Wenger

This seminar will deal with photomicrographic presentation of numerous special stains. Special emphasis will be placed on (1) preferred stains for demonstrating specific entities, (2) special requirement for successful results, (3) solution stability, (4) common problems and solutions in stain technology, and a question and answer period.

WORKSHOP NO. 4: TUESDAY, OCTOBER 2, 1973

9:00 A.M. - 4:30 P.M., (Sheraton)

ADVANCED CRYOTOMY

Mr. Frank Avallone

This workshop is designed for those desiring advanced information on cryostat technology. Included will be a problem-solving session. The workshop will be enhanced by several lectures dealing with various facets of cryostat technology. Registrants are encouraged to bring problem tissue specimens.

WORKSHOP NO. 5: TUESDAY, OCTOBER 2, 1973

9:00 A.M. - 4:30 P.M., (Sheraton)

MICROTOMY

Mr. H. K. Russell

The problems relative to poor versus good microtomy will be emphasized. The various steps in cutting which may be responsible for variable results will be discussed, to include the effects on tissue sectioning of the following: fixation, processing, embedding, microtomy, and tissue orientation. In-depth knife sharpening methods will be included.

WORKSHOP NO. 6: WEDNESDAY, OCTOBER 3, 1973

7:00 P.M. - 10:00 P.M., (Sheraton)

BASIC CRYOTOMY

Mr. Edward Cunningham

The purpose of this workshop is to provide the basics of cryostat technology, particularly for those technicians who wish to learn this facet of histology or those having difficulties with routine stat techniques. Participants will have an opportunity to use different cryostats and learn the pros and cons of each. "Stat" cryotomy and new H & E staining procedures will be performed.

WORKSHOP NO. 7: WEDNESDAY, OCTOBER 3, 1973

7:00 P.M. - 9:00 P.M., (Sheraton)

COMMUNICATIONS FOR THE MANAGER

Dr. James Young

Effective communication skills are a prerequisite for managers. Learn how you can become a better manager by improving and practicing communication skills. Prepare yourself to be more effective in your present position and to be qualified for a more responsible position.

Scientific Sessions

WEDNESDAY, OCTOBER 3, 1973:

THE SPECIFICITY OF HISTOCHEMICAL METHODS FOR DETECTION OF AMYLOID
Mr. Jules Elias

COMPARATIVE STUDIES OF STAINING TECHNIQUES FOR BACTERIA IN TISSUE SECTIONS AND PROTOZOA IN FECAL SPECIMENS
Mr. Richard Taylor

THE HISTOTECH'S ROLE IN FORENSIC PATHOLOGY
Miss Gerre Wells

COMPARISONS OF SELECTED SPECIAL STAINS
Mr. Jerry Coates

CHANGES IN THE pH OF TISSUE DURING PROCESSING
Mr. Dominic Europa

EXHIBITOR'S SEMINAR - 1:30 - 4:30 P.M.

This period provides the exhibitor with an opportunity to present a short lecture of their choice. They may discuss various items on exhibit, answer questions, present a short technical paper, present information relative to any facet of manufacture, distribution and/or problems relative to histotechnology service, etc. The planning committee feels this will be a very informative and worthwhile seminar.

THURSDAY, OCTOBER 4, 1973:

NEW HISTOLOGY AND HISTOTECHNOLOGY PROGRAMS LEADING TO A B.S. DEGREE
Mr. Rick Hasal

METHOD OF PREPARING, SECTIONING, STAINING AND APPLICATION OF UNDECALCIFIED SECTIONS OF BONE
Mr. A. Villanueva

POTHOLES ALONG THE ROAD THAT TISSUE TRAVELS FROM SURGICAL CLAMP TO MICROSCOPE
Mr. Richard Taylor

CYTOGENETICS: A ROLE FOR THE HISTOTECHNOLOGIST
Mr. Jules Elias

DEMONSTRATIONS OF FOREIGN MATERIAL IN LUNG
Dr. Russell Harley

METHODS FOR DISINFECTING CRYOSTATS
Mr. Dan Romieka

BASIC AUTORADIOGRAPHY TECHNIQUES
Mr. Kenneth Urban

KIDNEY BIOPSY - LIGHT AND ELECTRON MICROSCOPY TECHNOLOGY
Mr. Frank Avallone

VARIATIONS IN BONE DECALCIFICATION METHODS OR STAINING OSTEOID SEAMS IN UNDECALCIFIED, UN-EMBEDDED BONE SECTIONS
Mr. A. Villanueva

HISTOCHEMISTRY
Dr. Frank Johnson

THURSDAY EVENING:

6:30 to 7:30 P.M., complimentary cocktails sponsored by Lab-Tek Products, Naperville, Illinois.

Histopathology Symposium Banquet, 7:30 P.M.

FRIDAY, OCTOBER 5, 1973:

IDENTIFICATION OF TISSUE - A UNIQUE APPROACH
Mr. Alfred Tyler

(TO BE ANNOUNCED)
Mr. Donald Bodrug

FLUORESCENT TECHNIQUES AND APPLICATIONS
Mr. Ed Valeski

RESEARCH IN AND FOR HISTOLOGY
Ms. Betty Mayle

ROLE OF IMMUNOPATHOLOGY IN THE CLINICAL LABORATORY
Dr. Donald Tourville

REGISTRANT SEMINAR & PROBLEM-SOLVING CLINIC - 1:00 - 3:00 P.M.

The purpose of this seminar/clinic is to allow the symposium registrants to discuss hints or ideas on histologic technique. Each speaker will be allowed three minutes for their presentation and/or discussion of specific questions. Please plan to participate; you may be the one who provides the answer to someone's problem.

CLOSING REMARKS
Mr. Lee Luna

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5. Lomsky, R., Langr, F., Vortel, V.: Immunohistochemical Demonstration of Gastrin in Mammalian Islets of Langerhans. *Nature* 223:618-619, 1969.
6. Warren, S., LeCompte, P. M., Legg, M. A.: *The Pathology of Diabetes Mellitus*, 4th ed., 449-508, Lea and Febiger Publishers, Philadelphia, 1966.



Color Coding Rush Specimens

Donald L. Meyers
Chief Histotechnologist
Johns Hopkins Hospital
Baltimore, Maryland 21205

A color coding method has been devised in our laboratory to identify rush from semi-rush specimens.

The method utilizes Lab-Tek® Embedding Rings which are easily dyed with most dyes soluble in Toluol or Xylol. For red rings, use 1% Oil Red O in Toluol. For black rings, use magic marker ink in Toluol (50/50).

Dip rings in prepared dye solution for a few seconds (too long will dissolve rings) and place on paper towels to dry.

We use the black rings for rush biopsies and red for all other biopsies. This allows one to quickly identify rush biopsies while they are being cooled after embedding.



Let's Get Our Facts Straight

An Editorial

It is generally believed that glacial acetic acid should not be added to Zenker's fixative solution until just before use. This is no longer true. Glacial acetic acid can be added to the mixture and stored for 3-5 months without deleterious effects.

The omission of glacial acetic acid, until just before use, was necessary in 1894 when Zenker proposed this fixative mixture. At that time glacial acetic acid was then recovered from pyroligneous acid. Pyroligneous acid was obtained from the destructive distillation of wood, which was composed of nearly equal parts of methanol, acetone, and glacial acetic acid. Each of the major constituents commercially prepared in the past was considered contaminated with the other two. The contaminants contributed to a short shelf life. Today, modern chemistry ensures that the product will be pure and meet the purity requirements.



Freezing Artifact

An Editorial

Intra and extracellular ice crystals displace tissue, producing vacuoles and voids which remain after the ice thaws. Freezing prior to fixation for cryotomy, storage under refrigeration during fixation, freezing during cryotomy prior to fixation and paraffin processing, and exposure to low temperatures from the elements during transit, i.e. mailboxes and aircraft cargo bays, are the most common means of introducing these artifacts. **MORPHOLOGY:** Vacuoles and voids of various diameters will be observed throughout the tissue. Some nuclei exhibit ballooning and appear light gray in the H & E preparation. Collagen will polarize slightly more than normal. Epidermis and mucosa frequently separate from the underlying structures.

Hellerström and Hellman's Modification of Davenport's Silver-Impregnation Method for Alpha₁ or Delta Cells

Fixation

Use 10% buffered neutral formalin and fix for at least 24 hours.

Microtomy

Cut paraffin sections at 4 micra.

Solutions

0.1N Nitric Acid

Nitric acid (concentrated) 6.45 ml
Alcohol, 95% 993.55 ml

Pyrogalllic Acid

Pyrogalllic acid 5.0 gm
Alcohol, 95% 100.0 ml

Mix well and then add

Concentrated formaldehyde solution (37-40%) 5.0 ml

Mix well.

Silver Nitrate

Silver nitrate 10.0 gm
Distilled water 10.0 ml

Dissolve and add

0.1N nitric acid solution 90.0 ml

Mix well. Adjust this solution to pH 5 with concentrated (58%) ammonium hydroxide (need approximately 15 drops). Filter. Discard after use.

Staining Procedure

1. Decerate sections and hydrate to distilled water.
2. Place in Bouin's solution at 37° C for 2 hours.
3. Wash in running water for 1 hour.
4. Rinse once in distilled water.
5. Dehydrate through graded alcohols to 95% alcohol.
6. Place in filtered silver nitrate solution overnight in a 37° C oven in total darkness.
7. Immerse for 8 seconds in 95% alcohol.
8. Develop for 60 seconds in a solution of pyrogalllic acid. (Agitate)
9. Place through 3 changes in 95% alcohol for 1 minute each.
10. Alcohol, 95%, 2 off-and-on changes.
11. Absolute alcohol, 2 off-and-on changes.
12. Xylene, 3 off-and-on changes.
13. Mount coverslip with resinous media.

Results

Alpha₁ or delta cells Black

The critical steps in this procedure include: (1) Using acid-cleaned glassware; (2) avoiding use of metal staining apparatus; (3) adjusting the pH of the silver solution to *exactly* 5; (4) filtering the working silver nitrate solution to avoid precipitation; and (5) avoiding the use of mercurial fixation. A *control must also be run* simultaneously so that the proper end point can be reached.

References

1. Cavallero, C., Solcia, E., Sampietro, R.: Cytology of Islet Tumors and Hyperplasias Associated with the Zollinger-Ellison Syndrome. *Gut*, 8:172-177, 1967.
2. Gomori, G.: Aldehyde fuchsin: New Stain for Elastic Tissue. *Am. J. Clin. Path.*, 20:655-666, 1950.
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HOME ADDRESS _____
Street City State Zip

EMPLOYED BY _____

PLEASE CHECK FUNCTIONS YOU DESIRE TO ATTEND:

SCIENTIFIC SESSIONS _____ \$25.00 (Wed., Thur., Fri.)

BANQUET _____ \$10.00 (Thursday Evening)

WORKSHOPS

No. 1	_____	\$20.00	Quota 60	(Monday)
No. 2	_____	\$20.00	Unlimited	(Monday)
No. 3	_____	\$20.00	Unlimited	(Tuesday)
No. 4	_____	\$20.00	Unlimited	(Tuesday)
No. 5	_____	\$20.00	Unlimited	(Tuesday)
No. 6	_____	\$10.00	Quota 40	(Wed. Eve.)
No. 7	_____	No Fee	Unlimited	(Wed. Eve.)

CHECK OR MONEY ORDER MUST ACCOMPANY REGISTRATION APPLICATION. MAKE PAYABLE TO: V.S.H.T.
MAIL REGISTRATION TO: REGISTRAR, P.O. BOX 36, LANHAM, MARYLAND 20801.