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Technical Bulletin for Histotechnology • Scientific Editor, Lee G. Luna, D. Lit. (Hon.) H.T. (ASCP) • Managing Editor, Brent Riley
Vol. XVIII, No. 4, Sept/Oct 1988

Methods for Staining Campylobacter Pyloridis

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In recent months our histology laboratory has received numerous requests to perform special procedures on gastric biopsies to aid in the identification of campylobacter pyloridis, a bacteria associated with active chronic gastritis. A better understanding of the organism is needed to assist the histotechnologist in the procedures necessary to identify this organism.

In 1983, J.A. Warren posed the question of unidentified curved bacilli on gastric epithelium in active cases of chronic gastritis (*Lancet* 1: 1273-1275, 1983). He observed a small, curved and S-shaped bacilli in numerous gastric biopsy specimens that were stained with the Warthin-Starry silver method. In 1984, D.M. Jones, et al, reported that a campylobacterlike organism had been isolated from patients with gastritis (*J Clin Path*, 37: 1002-1006, 1984). Using various special stains, including the Gram stain, PAS, Giemsa, and Warthin-Starry, he was able to demonstrate the organism histologically in 79% of the cases of gastritis, whether active or inactive, and by culture in 90% of the cases. The organisms were located on the surface of the mucosa in the layer of mucin, and in the gastric pits, and correlated strongly with detectable antibody and histological gastritis. Goodwin, et al, further classified the organism as campylobacter pyloridis, a true spiral bacteria possessing large amounts of urease enzyme (*J Clin Path*, 39: 353-365, 1986). (True spiral bacteria are distinguished from spirochetes by the absence of endoflagella and periplasmic fibers). The organism was shown to be strongly associated with active chronic gastritis, especially when polymorphonuclear leukocytes are present.

The histotechnologist has a choice of several special staining procedures that will adequately demonstrate the organism in gastric biopsy sections. Control material for the procedure can usually be obtained by reviewing past cases of gastric resections for chronic gastritis or peptic ulcer. Since the organism has been demonstrated in numerous studies in a majority of the patients with chronic gastritis and associated ulcers, the chances of obtaining good control material is high. In addition, sections from gastric biopsies that have demonstrated the organisms can be used. However, biopsy blocks are usually very limited in the number of control slides that can be obtained. Although campylobacter can be demonstrated with the H&E under close examination, they are best demonstrated with the following special stains.

Modified Steiner Technique for Spirochetes

Fixation
10% buffered neutral formalin

Microtomy
Cut paraffin sections at 4 micrometers

Solutions

1% Uranyl Nitrate

Uranyl nitrate	1.0	gm
Distilled water	100.0	ml

1% Silver Nitrate

Silver nitrate crystals	1.0	gm
Distilled water	100.0	ml

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2.5% Gum Mastic

Gum mastic	2.5	gm
Absolute alcohol	100.0	ml

0.04% Silver Nitrate

Silver nitrate crystals	0.04	gm
Distilled water	100.0	ml

2% Hydroquinone

Hydroquinone crystals	1.0	gm
Distilled water	50.0	ml

Reducing Solution

(prepare just before use)

2.5% gum mastic, alcoholic	20.0	ml
2% hydroquinone, aqueous	50.0	ml
Absolute alcohol	10.0	ml

Combine the gum mastic solution and the hydroquinone solution. Add the absolute alcohol and mix well. Filter through Whatman #4 filter paper. Immediately before placing slides in solution, add 5 ml of 0.04% silver nitrate.

Staining Procedure

1. Deparaffinize and hydrate slides to distilled water.
2. Place in 1% uranyl nitrate, preheated to 60°C, for 15 minutes.
3. Rinse thoroughly in distilled water.
4. Place in 1% silver nitrate for 1 hour at 60°C.
5. Rinse in 2 changes of distilled water.
6. Dehydrate in 2 changes of 95% and 2 changes of 100% alcohol.
7. 2.5% gum mastic for 5 minutes.
8. Allow sections to air dry for 1 minute.
9. Rinse in 2 changes of distilled water.
10. Place sections in reducing solution that has been preheated to 45°C. Allow sections to develop for 15-20 minutes, occasionally agitating sections.
11. Rinse in distilled water to stop reduction.
12. Dehydrate, clear, and mount in a synthetic medium.

Results

Spirochetes, fungi, and most bacteria (Fig. 1)	dark brown to black
Background	light yellow to brown

Reference

Garvey, W., Faith, A., and Bigelow, F.: Modified Steiner for the Demonstration of Spirochetes. *J Histotechnology* 8: 15-17, 1985.

Gimenez Technique for Campylobacter

Fixation

10% buffered neutral formalin.

Microtomy

Cut paraffin sections at 3-5 micrometers.

Solutions

Stock Carbol Fuchsin

Basic fuchsin	1.0	gm
95% alcohol	10.0	ml
4% phenol, aqueous	25.0	ml
Distilled water	65.0	ml

Stock solution stable for several months.

0.1 M Phosphate Buffer, pH 7.5

0.1M sodium phosphate monobasic	7.0	ml
0.1M sodium phosphate dibasic	31.0	ml

Working Carbol Fuchsin

Stock carbol fuchsin	15.2	ml
0.1M phosphate buffer, pH 7.5	38.0	ml

A precipitate will form immediately. Mix well and filter. Filter again immediately before use. Working solution is stable for 48 hours.

0.8% Malachite Green

Malachite green	0.8	gm
Distilled water	100.0	ml

Staining Procedure

1. Deparaffinize and hydrate slides to distilled water.
2. Working carbol fuchsin solution for 2-5 minutes.
3. Wash thoroughly in tap water.
4. 0.8% malachite green for 15-20 seconds.
5. Wash thoroughly in tap water.
6. Repeat steps 4 and 5 until sections become blue-green.
7. Blot and allow sections to air dry.
8. Clear in xylene and mount in synthetic medium.

Results

Rickettsiae and bacteria (Fig. 2)	bright-red
Nuclei	blue-green
Background	light-green

Reference

McMullen, L., Walker, M.M., Bain, L.A., Karim, Q.N., and Baron J.H.: Histological Identification of Campylobacter Using Gimenez Technique in Gastric Antral Mucosa. *J Clin Path* 40: 464-465, 1987.

Modified One-Hour Giemsa Stain

Fixation

10% buffered neutral formalin.

Microtomy

Cut paraffin sections at 4-5 micrometers.

Solutions

Azure II Eosin

Azure II eosin (Giemsa)	1.3	gm
Glycerin	80.0	ml

Incubate at 56°-60°C for 2 hours. Mix and cool.
Add:

Methanol	170.0	ml
Acetone	290.0	ml

May-Grunwald Stain Solution

May-Grunwald stain (Jenner)	0.15	gm
Methanol	290.0	ml
Acetone	290.0	ml

Stock Giemsa Solution

Combine Azure II eosin and May-Grunwald solutions. This stock solution can be used immediately but improves with aging.

Acetic Water Solution

Glacial acetic acid	0.01	gm
Distilled water	1000.0	ml

Working Giemsa Solution

Giemsa stock solution	10.0	ml
Acetic water	50.0	ml

Staining Procedure

1. Deparaffinize and hydrate slides to distilled water.
2. Immerse sections in working Giemsa solution for 1 hour.
3. Dehydrate sections in three changes of absolute alcohol.
4. Clear in xylene and mount in synthetic medium.

Results

Bacteria	deep blue
Nuclei	blue
Mast cell granules	purple
Cytoplasm	pink

Reference

Luna, L.: "Wet Workshop for Special Stains" Birmingham, Alabama, Nov. 2 1984. NSH-CEU Workshop #8471.

The size of the organism in the stained section is dependent upon the stain employed. Although they can be visualized at 400X, the organisms are best distinguished at 1000X under oil immersion. The silver impregnation and the organism may appear fat or stumpy (Figs. 1 and 2). The Giemsa stain, on the other hand, stains the inner portion of the organism, making the organism appear thinner and therefore it is more difficult to distinguish in the stained section (Fig. 3).

Campylobacter will be studied with great intensity until the exact relationship of the organism with the host is determined. The histotechnologist will play an important role in aiding pathologists and researchers in discovering this relationship.

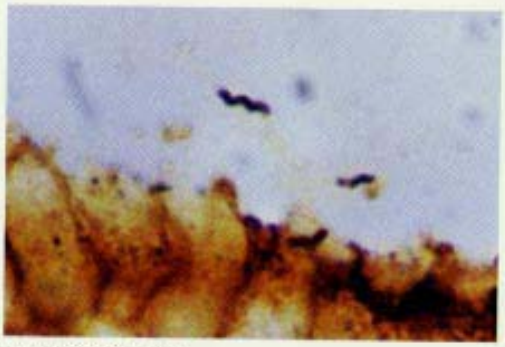

Figure 1: Modified Steiner Stain (1000X oil)

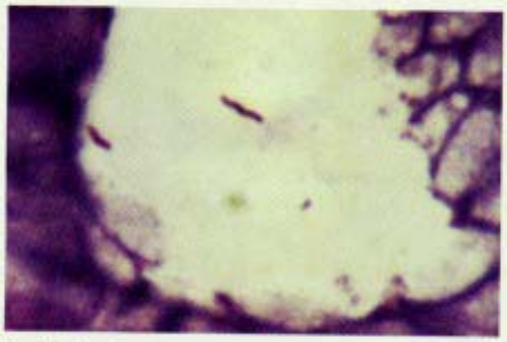

Figure 2: Gimenez Procedure (1000X oil)

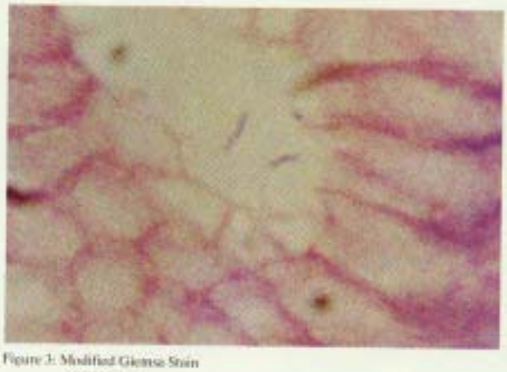

Figure 3: Modified Giemsa Stain (1000X oil)

References

1. Warren, J.A.: Unidentified Curved Bacilli on Gastric Epithelium in Active Chronic Gastritis. *Lancet* 1: 1273-1275, 1983.
2. Jones, D.M., Lessells, A.M., and Eldridge, J.: Campylobacter-Like Organisms on the Gastric Mucosa: Culture, Histological, and Serological Studies. *J. Clin Path.* 37: 1002-1006, 1984.
3. Goodwin, C.S., Armstrong, J.A., and Marshall, B.J.: Campylobacter Pyloridis, Gastritis, and Peptic Ulceration. *J Clin Path*, 39: 353-365, 1986.
4. Garvey, W., Fathi, A., and Bigelow, F.: Modified Steiner for the Demonstration of Spirochetes. *J HistotechnoI*, 8: 15-17, 1985.
5. McMullen, L., Walker, M.M., Bain, L.A., Karim, Q.N., and Baron, J.H.: Histological Identification of Campylobacter Using Gimenez Technique in Gastric Antral Mucosa. *J Clin Path.* 40: 964-645, 1987.

NSH Symposium/Convention There's Plenty to Do

If you are fortunate enough to be one of the many histotechnologists who will attend the NSH Symposium/Convention in Louisville, Kentucky, then you have plenty to look forward to. The 14th Annual Symposium/Convention will offer six days of nonstop activities. And if you need a break from the planned events, the city of Louisville can provide an impressive schedule of diversions.

The theme of this year's Symposium/Convention is "NSH Racing Forward." It will offer 41 different workshops to help satisfy your desire for new information about the histotechnology profession. In addition, two days of clinical and veterinary scientific sessions will be available. Top that off with ongoing technical and scientific exhibits, and you have all the right ingredients to become a better-informed histotechnologist.

Hospitality functions, sponsored by various exhibitors, will also be held throughout the week. This year, Miles will host a M*A*S*H theme party, because by Tuesday evening, October 11, you'll be ready for some R&R. There will be a gathering at Rosie's canteen to judge who looks most like their favorite M*A*S*H character. That means you'll have to put on your best fatigues. You'll also be serenaded by all the hits on the hit parade—to add a touch of home. Get on the incoming list.

You also won't want to miss the annual awards banquet, which is scheduled for Thursday, October 13. After a delicious dinner, several of your fellow histotechnologists will be honored for their outstanding achievements during the past year.

Then there is Louisville—and the beautiful surrounding area of Northeastern Kentucky. Louisville is known as the "Gateway to the South." And when you experience its leisurely Southern hospitality, you'll know why. In Louisville, you'll discover horses, fine architecture, friendly people, and delicious food. It's famous for baseball bats, tobacco, and Kentucky bourbon. It's also the home of bluegrass music and the most prestigious horse race in the world, the Kentucky Derby.

The convention hotel, the Galt House East, is located right downtown, overlooking the Ohio River. Within easy walking distance, culture and entertainment abound. The city has its own theater, ballet, and opera companies. And the Louisville Orchestra is well known as an interpreter of contemporary music. If you have time, take in a performance at Actors Theatre of Louisville or the beautiful Kentucky Center for the Arts. If folk music is more your style, there is almost always a bluegrass festival nearby.

Sightseeing is a major attraction in and around Louisville. A drive through bluegrass country will reveal impressive horse ranches in the midst of beautiful rolling hills. Historic Main Street and beautiful Victorian mansions provide a charming look into Louisville's past. Horse-racing fans can visit Churchill Downs Racetrack and Museum, home of the Kentucky Derby. And just outside the Galt House East, you can board the Belle of Louisville, a stern-wheeler excursion boat, for a trip down the Ohio River.

Several planned tours have been scheduled for Saturday, October 8, preceding the convention. You should register in advance for these tours, which include Lexington, Keeneland Race Course, and Highlights of Louisville.

No matter what you like to do, there'll be something of interest in Louisville. And compared to most other big cities, you'll find very reasonable prices. But then, Louisville is a very reasonable city—a perfect choice for the NSH 14th Annual Symposium/Convention.

Do You Know?

Lee G. Luna
American HistoLabs, Inc.
Gaithersburg, Maryland 20879

That ten percent formalin neutralized with calcium carbonate and filtered becomes acid within three hours after the exposure of tissue to the fixative.

That pseudocalcification artifacts are observed when calcium acetate is employed as a neutralizing agent for 10% formalin. The calcium deposits are difficult to distinguish from those

(continued on p. 30)

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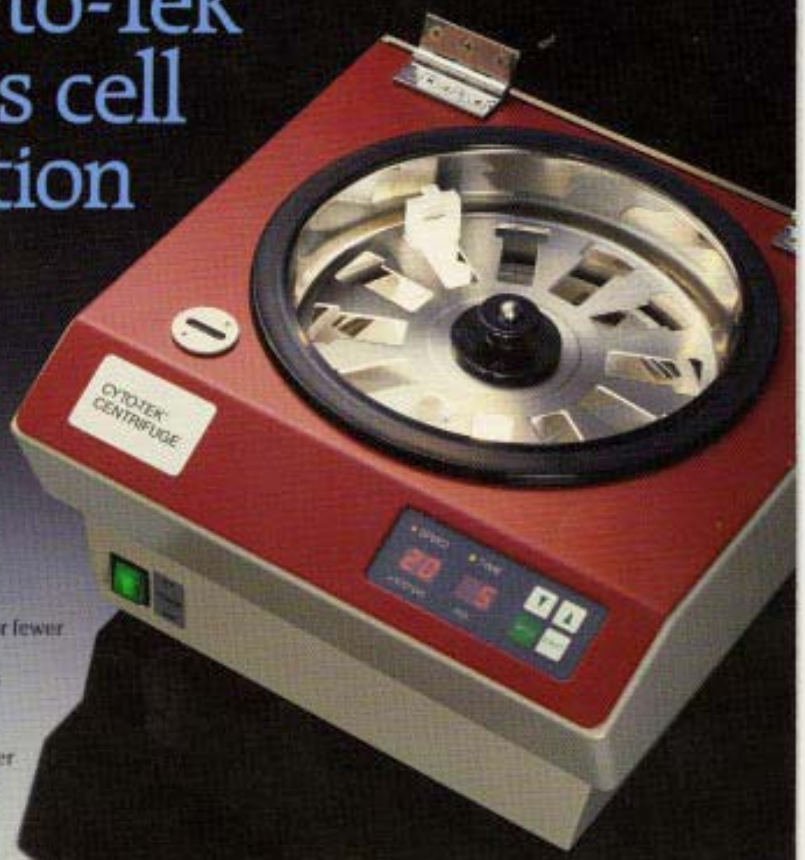

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produced during in vivo calcification. Hence, pseudocalcification can be mistaken for true calcification. Staining properties with the various calcium stains are identical to true calcification. Pseudocalcium can be distinguished from true calcification by the use of a mucosaccharide staining technique (alcian blue or Mowry's colloidal iron). True calcification produces staining immediately adjacent to the formation of calcium, while there is an absence of staining in specimens containing pseudocalcium.

That the pH of formol-calcium (Baker solution) is constant if no tissue is added to the solution. It changes from 9 to 6.4 if a large amount of tissue is added and to 7.3 with a smaller amount. Formalin with a phosphate buffer maintains a constant pH at all times.

That most tissue shrinkage is not caused by the formalin fixation, but by the subsequent dehydration, clearing and impregnation of tissue.

That staining reactions and tissue elements are altered and others obliterated by the deleterious and insidious effects of unbuffered formalin fixative. Formalin is oxidized to formic acid, and the following ruinous effects are produced: (a) The so-called formalin pigment will frequently react during the staining procedure to mask or, in some cases, simulate microorganisms, pigments, or other elements suggesting disease. Formalin pigment has a notorious reputation for reducing silver in procedures for staining fungi (Grocott's methenamine-silver), melanin (Fontana-Masson), spirochetes (Warthin-Starry), and reticulum. (b) Unbuffered formalin gradually destroys nuclear basophilia if tissue is exposed longer than three weeks. (c) Unbuffered formalin dissolves copper, iron, and calcium during the process of fixation. (d) It alters the staining of some cell granules. (e) It will also inhibit the aldehydefuchsin reaction for demonstrating Paget's cells.

That Zenker-formol, which causes a great deal of shrinkage, is widely used because of its excellent cytoplasmic preservation.

That tissues overexposed to Zenker's fixative will not exhibit the usual hematoxylin staining characteristics. Overexposure produces a monochromatic staining appearance. Chromatin material cannot be differentiated and, in general, nuclei are not distinct. Restoration of staining properties can be accomplished somewhat by placing the slides in a 10% aqueous solution of sodium bicarbonate for 3 hours followed by routine H&E staining.

That Zenker's fixative will often crystallize erythrocytes. Microscopically, the RBCs will exhibit a transparent or a light translucent appearance. These erythrocytes will possess excellent polarization properties.

That 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 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 at that time was 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.

That Bouin's fixative has an adverse effect on hyaluronic acid and it cannot be demonstrated by staining. It is not known if other mucosubstances are adversely affected. Buffered neutral formalin does not produce any gross alterations affecting the staining properties of hyaluronic acid.

That when fixatives containing acetic acid are used, the addition of an equimolar amount of sodium chloride or other similar salt will serve to protect erythrocytes and soluble cell granules from lysis during fixation.

That Flemming's solution without acetic acid is the best fixative for muscle from the standpoint of shrinkage.

That Regaud's solution gives a satisfactory fixation from the cytological standpoint. Unfortunately Regaud's solution has serious faults; it is unstable and begins to decompose within a few hours. The rate of penetration is slow and only very small tissue specimens can be fixed. It has the tendency to harden tissues excessively. Notwithstanding such drawbacks it can be used successfully for the study of mitochondria, which it preserves well.

The process of most types of fixation is complicated by the fact that fixing agents need time to reach the different tissue structures. Most workers conclude that fixation of the external specimen layers interferes with the penetration of the fixative's ingredients.

That tissues exhibiting marked shrinkage during fixation tend to show less change under subsequent dehydration, clearing and paraffin impregnation.

That fixing agents such as potassium dichromate increase the weight of tissue specimens during fixation as much as 15% over a period of 5 days. The increase in weight is in direct proportion to the dilution of the salt and freshness of the tissue.

That in tissue fixation the first toxic substance to reach the tissue cell is apt to determine the fixation image.

Reference:

Luna, L.G., Histological Procedures and Special Stains: *A Practical Guide*. Center for Histotechnology Training, Gaithersburg, Maryland. Available Spring 1989.

NSH Home Office Serves The Entire Histology Community

You might expect the home office of a major, national professional society to be a busy place, but you probably wouldn't expect that two people alone could handle the daily hustle and bustle of such an office. The NSH home office, in Lanham, Maryland, is not only operated by two individuals, but it's done effectively and efficiently.

Roberta Mosedale, Executive Secretary, and Sylvia Palmer, Office Assistant, currently handle all the day-to-day activities of the NSH. That's a heavy load when you consider that every facet of activity within the Society is channeled through the office. In her efforts to perform the necessary tasks, Roberta must wear many different hats.

First, she must be a reference librarian. Many requests for information come in every day, either by mail or phone. Sometimes a majority of the day is spent on the phone. If Roberta can't answer the question, she must know where to go to get the necessary information. People call or write for information concerning education, meetings, safety, journals, membership, and a host of other topics.

The office must process an average of 400 pieces of mail every week, including inquiries, requests, checks, bills, membership requests, journal subscription requests, address changes, and financial information. This mail often doubles around national convention time or membership renewal time. But regardless of how much there is, it must all be reviewed, filed, and/or answered.

The NSH office must also serve as the depository for all pertinent files. Correspondence to and from all the officers and chairpersons is also copied and filed at the office. This allows Roberta to keep a handle on what is going on and what progress the Society is making. She estimates that about 2,000 copies are made every month at the office.

All financial receipts are channeled through the NSH office. There, they are recorded, documented, and the necessary paperwork processed to the responsible committee and/or officers. Roberta must also process membership renewals, journal subscriptions, convention registrations, CEU credits, and all other Society business. She is responsible for maintaining and updating membership and journal subscription lists.

In performing her duties, Roberta must also monitor the activities of every committee. She also prepares materials for the national convention, including the printing of some materials and booklets. And she prepares and disseminates public relations information and helps set up the NSH teleconferences.

Roberta Mosedale and Sylvia Palmer completing data entry at NSH Office

The NSH National Convention/Symposium encompasses a large part of the home office responsibilities. In addition to handling registrations, exhibits, materials, and all connected correspondence, the office plans for the convention far in advance. They are currently working on the site selection for the 1993 convention.

"I like the variety best," Roberta explained. "There is no aspect of this job that could be considered dull. There's no routine. Every day is different."

It's a lot to handle. But then, Roberta Mosedale is no stranger to the NSH. She has been its Executive Secretary since the Society's inception in 1973. She first became involved with the NSH by working with the Annual Symposium on Histopathologic Technique, conducted by the Armed Forces Institute of Pathology (AFIP).

She began by working as a secretary in the Chief Dental & Oral Pathology Division of the AFIP, then moved into a position with the U.S. Army Surgeon General's Office. After resigning to spend more time with her family, she was asked to type correspondence for the AFIP at home. This eventually evolved into working at home for the NSH. In fact, she was instrumental in obtaining Society incorporation by the State of Virginia.

The original NSH home office was located in an extra bedroom in Roberta's house. It consisted of a small typing table and a telephone. As the NSH grew, an addition was added to Roberta's home to house the NSH office. Eventually, continued growth made it necessary to move the office to its current location.

The responsibilities of operating a national society home office can at times seem overwhelming, but with the efficiency and administrative skills of Roberta Mosedale, and the help of Sylvia Palmer, the NSH office runs like a well-oiled machine.

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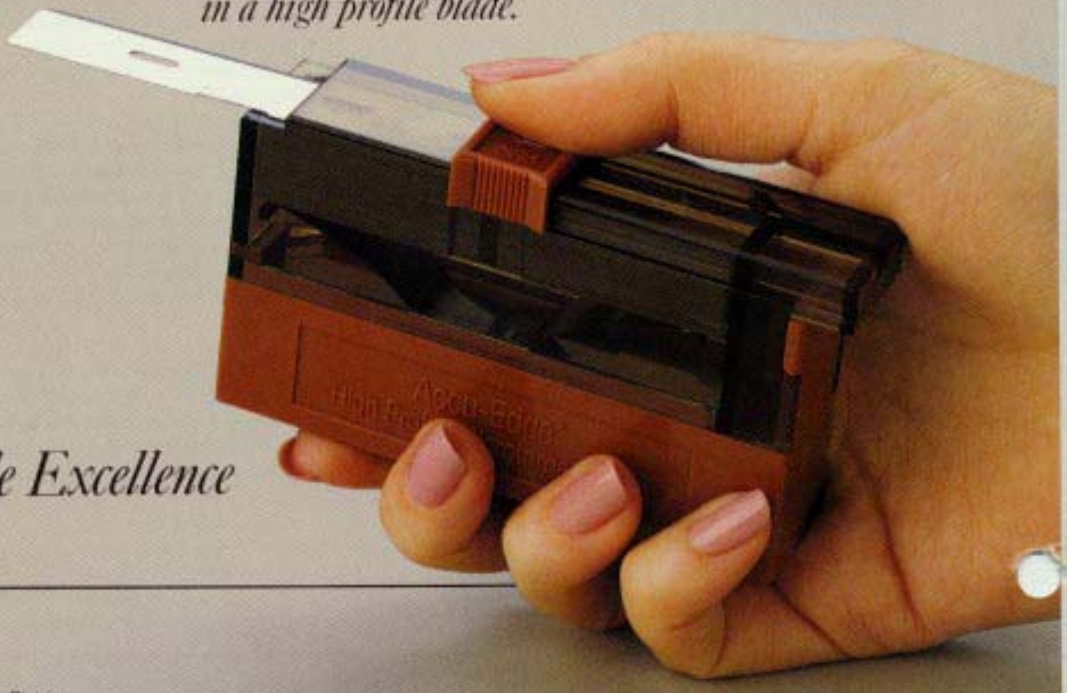

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Arland E. Olson Continues to Serve After 34 Years

Arland E. Olson was hired in 1954 to work in the Utah State University Histology Laboratory. He had Bachelor and Master of Science degrees in zoology, but he had no formal training in histotechnology. In fact, his only training consisted of two weeks while working alongside the person he replaced. After that, he was on his own. But he went on to a distinguished career as a histotechnologist.

Arland, a lifelong resident of northern Utah, recently semi-retired from his position as Research Associate in Veterinary Science at Utah State. In that first position, he taught himself the things he needed to know. Then in 1958, he had an opportunity to spend two weeks studying in an unstructured program at the Armed Forces Institute of Pathology (AFIP). While there, Arland had the opportunity to meet with a number of individuals—Lee Luna, Bud Cunningham, Peter Emanuele, Barbara Spillan, Dr. Lent Johnson, and Dr. Frank Johnson. He also spent time learning techniques in the Medical Illustration and Museum Gross Specimen Preparation divisions.

In addition to his primary work in histologic techniques, Arland was soon involved in medical photography, veterinary radiology, medical museum specimen preparation, large animal experiment supervision, and field survey and collection. One of his projects involved studying the effects of the very high fluoride content in the geothermal waters of Yellowstone National Park on the teeth and bones of bison, elk, and deer. Since then, he has devoted a great deal of time to multidisciplinary team studies of chronic fluoride toxicosis in domestic and wild animals.

He has also conducted studies involving both induced and inherited diseases of the musculoskeletal system, synovitis and sternal bursitis or "breast blisters" in turkeys, and the coccidiosis life cycle in calves. Arland has also coauthored nearly 60 articles, most of which were published in referred journals.

Arland began attending the AFIP Symposium on Histologic Technique in 1972, the year before the NSH was organized. The AFIP Symposium evolved into the NSH Symposium/Convention. He has only missed one symposium/convention since, and that was when he had coronary bypass surgery.

Arland joined the NSH as soon as it was formed and has been active ever since. He was a member of the Public Relations Committee from 1975 to 1978 and again from 1982 to 1986. He served as the first NSH Director from Region VII. He has also served on the Bylaws, Judicial, Budget and Finance, Nominations and Elections, and Credentials Committees. For his many contributions to the NSH, he received the Convention Award in 1983.

Arland supports the NSH in every way possible. He believes that the educational opportunities through the NSH-sponsored meetings and in the *Journal of Histotechnology* are extremely valuable to the histotechnology profession. He also believes in personal interaction among histotechnologists as an effective method of promoting and improving the profession. And he is impressed by the commercial support for the NSH because the resulting awards and educational opportunities encourage NSH members to work harder and earn recognition.

Today, Arland is still working on projects involving the effects of zinc on macular degeneration, an inherited benign bone tumor condition found in humans and horses. He is also continuing his research on chronic fluoride toxicosis.

After nearly 35 years in histology, and even in semiretirement, Arland Olson continues to grow as a histotechnologist. And he continues to contribute significantly to the betterment of the profession.

Evaluation of Commercial Hematoxylin for H&E Staining

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Since more laboratories are purchasing hematoxylin solutions, it seemed advisable to study the effectiveness of these solutions. The validity of the quality of these hematoxylin staining solutions could only be determined by the establishment of controls. It was decided that excellent controls would be examples of hematoxylin and eosin (H&E) stained slides from laboratories throughout the country. To this end thirty skin cases and thirty liver biopsies were randomly chosen to serve as the controls. H&E stained slides submitted with these cases were microscopically reviewed and photographed for the record. It was interesting but also disturbing to see that slides from most of these cases were not stained properly (Fig. 1), and in some cases the staining was useless (Fig. 2). Nevertheless these slides served as the control for this study.

Specimens Selected for Study

The following tissue specimens were selected for the reasons outlined below:

Brain specimen that contained *cryptococcus neoformans.* The capsular structure of this fungi was used to determine the density and tinctorial quality of the hematoxylins studied. It is important that structures such as these be made evident to the microscopist by the staining of hematoxylin.

Liver specimen that contained a large number of plasma cells but were poorly fixed in formalin. This specimen was selected to see how well the hematoxylins stained the nuclear chromatin present in plasma cells after inadequate to poor fixation.

Kidney specimen containing lead poisoning inclusion bodies, but also was improperly formalin fixed. This specimen was selected since kidney contains a wide diversity of cells from those exhibiting dense chromatin (in the glomerular tifts) to those exhibiting a dusting of chromatin material (cuboidal cell of the collecting tubules). Also, to see how well the lead poisoning inclusion bodies stained with these hematoxylins.

Tonsil specimen that was well fixed in Carnoys solution was selected because of the high concentration of plasma cells with their characteristics, varied size chromatin and "cart-wheel" appearance of the nuclear chromatin.

Hematoxylins Used for Study

The following hematoxylins were used for the study. Two things should be pointed out: 1) Gill's hematoxylin 1, 2, and 3 from one source only was obtained and used in the study. Although other sources were available, this was done since the authors assumed that all Gill 1, 2, and 3 formulations were identical and to include all of those available in the study would have been redundant. 2) All hematoxylins available in manufacturers/distributors catalogs at the time the study was initiated were used for the study. A solution of Mayer's hematoxylin made up in our laboratory was used to compare staining reactions. It should also be noted that the names of the hematoxylins provided below are as they appeared on the manufacturer's bottle.

1. Hematoxylin solution Gill #1
2. Hematoxylin solution Gill #2
3. Hematoxylin solution Gill #3
4. Hematoxylin solution Harris' formulation
5. Hematoxylin solution Harris alum hematoxylin
6. Hematoxylin solution Harris-type staining
7. Richard-Allan Hematoxylin
8. Mayer's alum hematoxylin formulation (control)
9. Acid Hematoxylin solution*

Note: All solutions, with the exception of the Richard-Allan hematoxylin and acid hematoxylin solution, contained an expiration date.

*Information suggests this hematoxylin is not used for H&E staining. It is used for differentiation of fetal and adult hemoglobins (HGB "F") versus HGB "A")

Staining Schedules

As noted below, some hematoxylins did not contain staining instructions. In those instances we used our discretion and provided an acceptable staining schedule that was based to some degree on the information obtained from the bottle. An eosin/phloxine counterstain was used to complete the H&E staining procedure.

Hematoxylin Solution and Staining Times
1. Gill's—No. 1
Progressive.
Used 2-minute staining time.
2. Gill's—No. 2
Progressive.
Used 15-minute staining time—1% Acid-Alcohol, 3 dips.
3. Gill's—No. 3
Progressive.
Used 15-minute staining time—1% Acid-Alcohol, 3 dips.
4. Hematoxylin Solution Harris Formula
Regressive. No staining instructions provided.
Used 15-minute staining time—1% Acid-Alcohol, 3 dips.
5. Hematoxylin Harris Alum Hematoxylin
Regressive. No staining instructions provided. Used 15-minute staining time—1% Acid-Alcohol, 3 dips.
6. Hematoxylin Solution Harris-Type
Regressive. No staining instructions provided.
Used 15-minute staining time—1% Acid-Alcohol, 3 dips.

7. Richard-Allan Hematoxylin
Regressive. No staining instructions provided.
Used 15-minute staining time—1% Acid-Alcohol, 3 dips.
8. Mayer's Alum Hematoxylin
Used 15-minute staining time.
Used 15-minute water wash for blueing.
9. Acid Hematoxylin (solution of Mayer's)
Progressive. No staining instructions provided.
Used 15-minute staining time.
15-minute running water wash for blueing.

Staining Results

The staining results of all hematoxylins used were determined by microscopic observation by the two authors. In addition a third experienced microscopist reviewed the slides and essentially reached the same conclusions as the authors. Staining quality was defined as:

a. Good results: Sharp nuclear chromatin staining of all specimens plus good staining of the capsules of the cryptococcus neoformans (brain); plasma cell after poor fixative (liver); lead poisoning inclusion bodies and other cellular elements (kidney); and nuclear chromatin of all cells in the tonsil specimen.

b. Acceptable results: Entities were seen although not very good.

c. Poor results: Entities were difficult to see and interpret.

Figure 2 provides an overview of all staining reactions on specific specimens and tissue entities. It should be pointed out that although Fig. 1 illustrates poor staining of some entities with some hematoxylins, these results no doubt were affected by poor fixation.

Conclusions

This study defined several interesting things, but hopefully also helpful. The most surprising finding was the poor H&E staining of most of the 60 skin specimens and liver biopsies reviewed. Approximately 80% of these specimens were poorly stained. Fifteen percent were adequately stained and only 5% were stained at the very good level.

All hematoxylins with one exception (acid hematoxylin solution) stained nuclear chromatin and other designated entities well with the staining times employed.

All hematoxylin solutions stained well after storage at room temperature for 6 months.

It is concluded that commercial hematoxylin formulations produce good H&E staining cellular entities in tissue sections.

It is further concluded that most H&E staining deficiencies seen on stained slides are not due to the hematoxylin used, but to the quality of fixation and the way the hematoxylin solutions were used. This finding strongly suggests that many of us are not using hematoxylin staining solutions (homemade or purchased) properly.

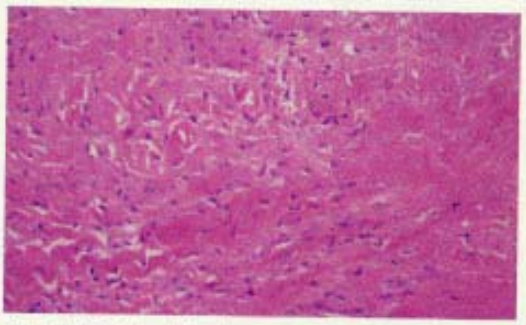

Figure 1. H&E stained section that shows poor (light) hematoxylin staining and over staining with the eosin. X 72.

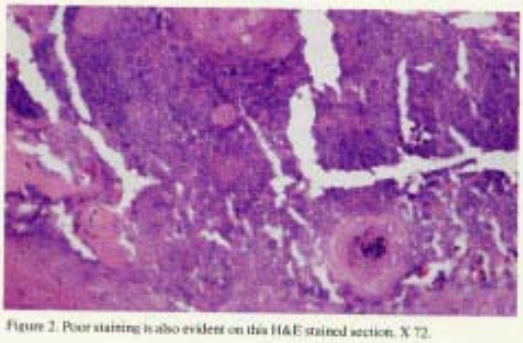

Figure 2. Poor staining is also evident on this H&E stained section. X 72.

Commercial Hematoxylin Stains Used	Tissue Staining Quality			
	Plasma Cells (Tonsil)	Lead Poisoning (Kidney)	RNA-DNA (Liver)	Crypto. Neoform. (Brain)
1. GILL—1	G	P	G	G
2. GILL—2	G	P	G	A
3. GILL—3	G	P	A	A
4. Harris Formulation	A	A	G	G
5. Harris Alum	G	G	G	G
6. Harris-Type Staining	A	A		
7. Richard-Allan	G	G	G	G
8. Mayer's (Regular)	G	A	G	G
9. Acid Hematoxylin Sol. of Mayer's	P	P	P	P

KEY: G=Good A=Acceptable P=Poor

FIGURE 3. Demonstrates the quality of staining of the different tissues and entities used for the study. It should be remembered that some staining results were influenced by poor fixation (see specimen selected for study).

New Histologic Page Numbering System

Beginning with the July/August issue of *Histo-Logic*, a new page numbering system was initiated. With the new combined format, we began renumbering the pages from page 1. Each issue will continue with the system of successive numbering.

Tenth Annual "Wet" Workshop Planned

The Tenth Annual Practical Stain-Technology "Wet" Workshop and Seminar has been scheduled for March 12-17, 1989. The program will be presented by Lee G. Luna and the Center for Histotechnology Training. The extensive workshop and seminar will provide registrants with an opportunity to perform 25 special stains, two microwave stains, and two immunochemical staining procedures, demonstrating more than 45 pathologic entities.

Some of the entities that will be stained are: Gram-positive *(Staphylococcus aurius)* and Gram-negative *(Pseudomonas)* bacteria; hepatitis B surface antigen; inclusion bodies; muscle; insulin; all pathogenic fungi; calcium; glycosaminoglycans (mucosubstances); myelin sheath; nerve fibers; mast cells; lepra and tubercle bacilli; nucleic acids—RNA and DNA; amyloid; silver reactive cell granules from the neuroendocrine system; copper; reticulum and other connective tissues; fibrin; spirochetes; Legionnaires' disease bacilli; cat scratch fever; and S-100 protein. Also, the proper tinctorial qualities of a good hematoxylin and eosin (H&E) will be discussed in great detail.

In addition to the practical special staining aspects, lectures will be presented daily on Chemistry of Staining; Silver Reactions; Fixation; Effects of Processing on Microtomy and Staining; Staining Mechanism; Tissue Artifacts; Cryotomy; Tissue Identification for Histotechnologists; Microtomy, Preferred Controls; Introduction to Immunochemistry Staining; Decalcification and other subjects directly related to the identification of technical problems and the production of high quality microscopic slides.

National Society for Histotechnology Continuing Education Units will be awarded. Each participant will receive 2.3 CEUs. If you are interested in this workshop/seminar, you should register early as participation is limited to 60. For more information, contact the Registrar, Center for Histotechnology Training, P.O. Box 736, Olney, Maryland 20832, (301) 330-1200.

To receive your own copy of *Histo-Logic*® or to have someone added to the mailing list, submit home address to: Miles Inc., Diagnostics Division, P.O. Box 70, Elkhart, Indiana 46515.

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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