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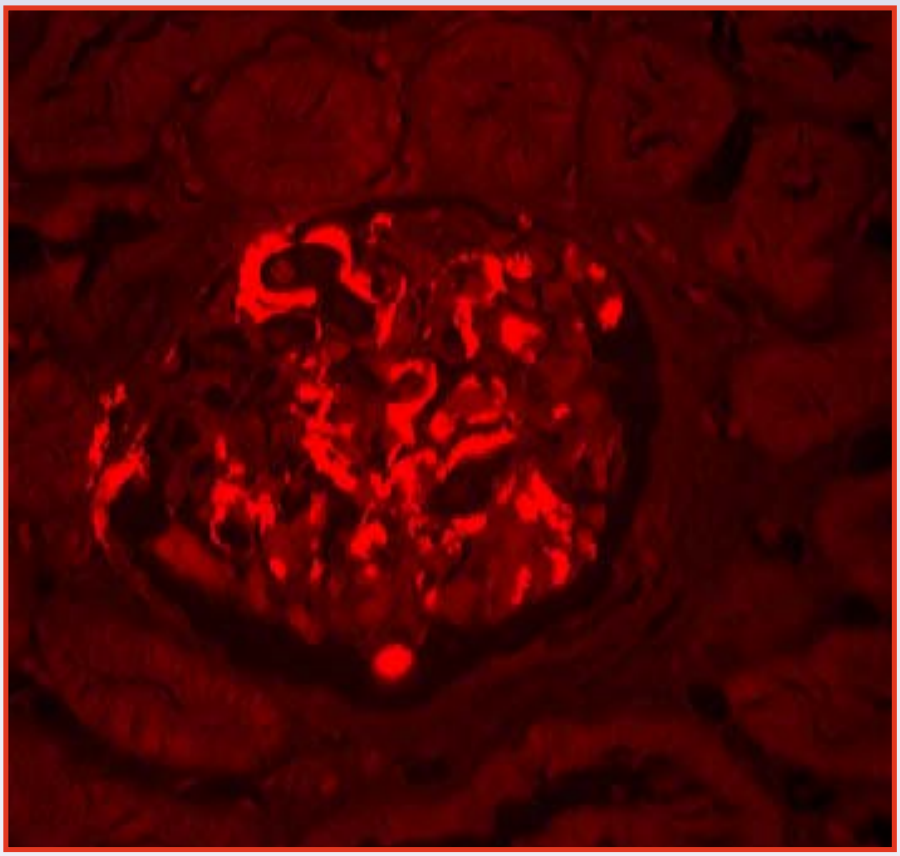

Fig. 1A. Congo Red stained 2-micron section of kidney viewed with fluorescence microscopy. 400×

A Novel Approach for the Demonstration of Amyloid in Thin (2 micron) Sections of Kidney

Rena Fail, HT (ASCP) and Sally Self, MD
Medical University of South Carolina
Charleston, SC 29425

Introduction

Staining for amyloid is commonly requested of clinical histology laboratories. The Congo Red method is considered to be the gold standard, especially when stained preparations are viewed with polarized light. Most references caution, however, that thick (8-10 microns) paraffin sections are required if one is to achieve the apple-green birefringence that is said to be characteristic of Congo Red-stained amyloid deposits. Failure to utilize thick paraffin sections may render small amyloid deposits indistinguishable from nonspecific background typically associated with binding of the dye to collagen and elastin fibers. Our laboratory utilizes a novel approach to achieve high contrast visualization of amyloid in thin paraffin sections of kidney when viewed with a fluorescence microscope.

Managing Editor, Gilles Lefebvre
Scientific Editor, Vinnie Della Speranza, MS, HTL (ASCP) HT, MT

Methods

Alkaline Congo Red Technique
(Putchtler, Sweat and Levine, 1962)[5]
The use of a staining solution fully saturated with sodium chloride increases the specificity of the results.

Fixation: 10% neutral buffered formalin

Sections: 2-micron paraffin sections

Solutions:

1. Mayer's Hematoxylin (Poly Scientific R&D Corp., Bayshore, NY)
2. Alcoholic Sodium Chloride (saturated)
   80% alcohol ... 500.0 ml
   Sodium chloride ... 5.0 gm
3. 1% Sodium Hydroxide
   Distilled water ... 100.0 ml
   Sodium hydroxide ... 1.0 gm
4. Alkaline Alcoholic Sodium Chloride (working)
   Alcoholic sodium chloride ... 50.0 ml
   1% sodium hydroxide ... 0.5 ml

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5. Stock Congo Red Solution (saturated)
   Alcoholic sodium chloride ..... 250.0 ml
   Congo Red ..... 0.5 gm
6. Working Congo Red Solution (prepare fresh)
   Stock Congo Red solution ..... 50.0 ml
   1% sodium hydroxide ..... 0.5 ml
   Filter: Use within 15 minutes

Stain Procedure:

1. Deparaffinize and hydrate to distilled water.
2. Stain nuclei with Mayer's hematoxylin for 10 minutes.
3. Blue in running water for 5 minutes.
4. Rinse in 3 changes of distilled water.
5. Treat with Alkaline alcoholic sodium chloride working solution for 20 minutes.
6. Stain in Congo Red working solution for 30 minutes for thin sections.
7. Dehydrate rapidly in 3 changes of 100% alcohol, clear in 3 changes of xylene, and mount in permount.

Results

Amyloid:

Light microscopy:	deep pink to red
With polarization:	apple-green birefringence
With fluorescence:	bright pink to red when viewed with 546 nm (green) illumination
Nuclei:	blue

Discussion

Amyloid is a homogeneous, highly refractile substance staining readily with Congo Red. Congo Red-stained amyloid appears pink to red by light microscopy, and apple-green with polarizing microscopy. This reaction is shared by all forms of amyloid and is believed to be due to the cross ß-pleated configuration of amyloid fibrils. This ß-pleated sheet configuration gives amyloid its polarscopic appearance when stained with Congo Red dye. Fine rigid non-branching fibrils, 7.5-10 nm in diameter of indeterminate length are seen in thin sections by electron microscopy. In kidneys, the fibrils are found within the mesangium and subendothelium, and occasionally within the subepithelial space. The amyloid fibrils may extend into the basement membrane, resulting in thickening and splitting of the structure. The adjacent epithelial foot processes are obliterated.[1]

The demonstration of amyloidosis in biopsies is important because some forms, notably those secondary to inflammatory processes, may be slowed or controlled with the administration of anti-inflammatory medications. Appropriate special stains are needed to demonstrate amyloid, particularly in end stage kidneys. Amyloidosis is difficult to distinguish from other late chronic glomerular diseases. Those most commonly used differentiating stains are the Congo Red, Vassar and Culling's Thioflavine T, and Highman's Methyl Violet.[2]

Nephrologists at our hospital began using the smaller 18- and 20-gauge needles to obtain kidney biopsies. Though less invasive and less painful for the patient, they posed a problem for our laboratory. Our technologists are required to obtain 20-23 consecutive slides with two serial sections of the kidney biopsy on each slide. These sections are routinely cut at 2 microns. Twelve to eighteen of these slides are stained with H&E, Masson's Trichrome, PAS, and Jones' Periodic Acid-Methenamine Silver (PAMS). Five extra unstained slides are retained for future studies to eliminate the need to recut the block. When amyloid staining becomes necessary, the need to obtain thicker recuts can often yield sections bearing little resemblance to the original sections.

Fig. 1B. Congo Red stained 2-micron section of kidney viewed with brightfield microscopy. 400×

Fig. 1C. Congo Red stained 2-micron section of kidney viewed with polarizing microscopy. 400×

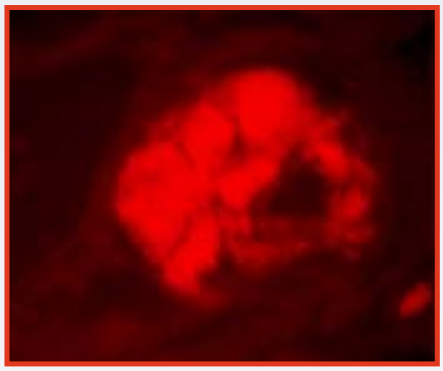

Fig. 2A. Congo Red stained 10-micron section of liver viewed with fluorescence microscopy. 400×

Congo Red (polarized), Thioflavine T, and the meta-chromatic stains such as Methyl Violet are methods we have used previously for staining amyloid. Metachromatic methods, by their very nature, are nonspecific. Tissue components other than amyloid, including fibrinoid, arteriolar hyaline, keratin, intestinal muciphages, Paneth's cells, zymogen granules, and jutaglomerular apparatus all stain with Thioflavine T.[3] As a result, Congo Red is the method of choice.

A few years ago, we had a slide that was stained with Thioflavine T which proved to be ambiguous. Congo Red was requested for comparison. In order to view a comparable section, it was necessary to perform the stain on a 2-micron section. Congo Red-stained amyloid appears red when viewed by light microscopy, and pale apple-green when polarized. On a 2-micron section, Congo Red viewed by either light microscopy or polarization is very pale, but does exhibit a pale green hue under polarization. In searching the literature we discovered that amyloid exhibits a faint blue-green autofluorescence. Following staining with Congo Red, amyloid exhibits a pink-red fluorescence when illuminated with 546 nm (green) wavelength light. This is the same channel (BP 546/12 excitation filter) on our fluorescent scope that we use to examine Auramine-Rhodamine stains. We further learned that thick sections can sometimes produce false positives. To avoid this, we use a fully saturated NaCl Congo Red solution which suppresses false positive staining of collagen and elastin.[4] Fluorescence of the 2-micron section appeared to be very sensitive and very specific. Amyloid fluoresced bright pink to red even on 2-micron sections (see Figs. 1A and 2A).

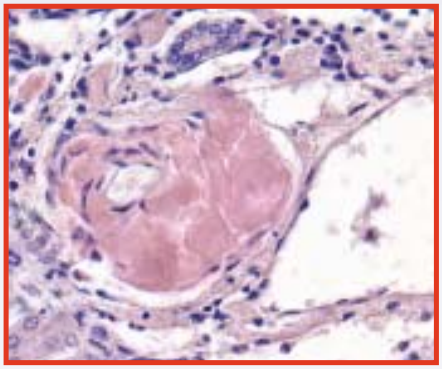

Fig. 2B. Congo Red stained 10-micron section of liver viewed with brightfield microscopy. 400×

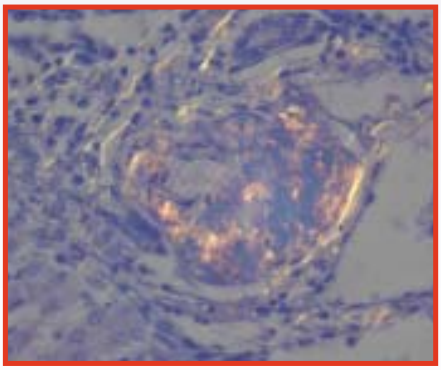

Fig. 2C. Congo Red stained 10-micron section of liver viewed with polarizing microscopy. 400×

Unlike Vassar and Culling's Thioflavine T or Highman's Methyl Violet, Congo Red-stained sections are permanent. They do not fade over time. Congo Red staining can still be seen years later with both light and fluorescent microscopy. Using 2-micron sections stained with Congo Red and viewed on the rhodamine channel is the easiest and most definitive way to diagnose amyloid. Fluorescence microscopy of Congo Red-stained sections can detect even minute deposits of amyloid.

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Artifact in Tissues Held in 70% Ethanol

M. Jane Chladny, HT(ASCP)
Laboratories of Veterinary Diagnostic Medicine
College of Veterinary Medicine
University of Illinois
2001 S. Lincoln Ave.
Urbana, IL 61802

Abstract

Artifactual vacuole formation in the white matter of rat central nervous system tissues exposed to 70% ethanol may resemble pathologic changes. Vacuolization in the fiber tracts of rat central nervous system tissue has been documented. An experiment was conducted to determine if similar vacuolization occurs in the white matter of other species. Samples of mouse, rat, cat, dog, sheep, cow, horse, and human brain, previously well fixed in 10% neutral buffered formalin, were held in 70% ethanol for 24 and 48 hours. A similar piece of brain from each species held in 10% formalin was used as a control. After routine processing, vacuolization artifact in the fiber tracts was observed in the tissues held in 70% ethanol in all species examined.

Introduction

In a research facility or histology laboratory where all tissues are well fixed prior to processing, an additional fixation step on the tissue processor is unnecessary. As practiced in some laboratories, a decision was made to substitute 70% ethanol for 10% neutral buffered formalin as the initial solution on the tissue processor. The removal of formalin from the processor was instituted not only for waste minimization, but also for the health and safety of laboratory personnel. This modification of the procedure produced well-processed tissues. For weekend processing, tissues were loaded into a processor on Friday afternoon, and held in 70% ethanol on delay mode until processing on Sunday evening.

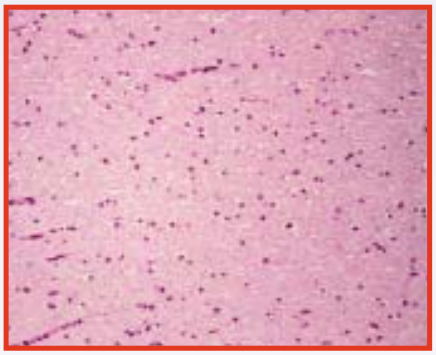

Fig. 1. Horse brain, formalin-fixed overnight processing. 100×

Following implementation of the modified processing schedule, a pathologist observed microscopic vacuolization in the white matter of rat brain sections used in a toxicology study. Because of inconsistencies in the appearance of vacuoles in different dose groups, it was questioned whether the vacuoles were lesions produced by

the test material or artifact. When the accompanying histology paperwork was examined, it was noted that the vacuoles appeared only in animals whose tissues were embedded on Mondays.

McLarrin reports that vacuolization may occur in the white matter of rat central nervous system tissues held in 70% ethanol longer than 48 hours.[1] Furthermore, Luna states that changes may be observed in rat central nervous system tissues in as little as 20 hours of exposure.[2] The observed vacuoles were processing artifacts, not lesions as first suspected. Formalin was returned to the first station on the processor to prevent the artifact in central nervous system (CNS) tissues.

The appearance of vacuoles in rat tissue prompted us to investigate the effect of exposure to 70% ethanol on brain tissue of other species.

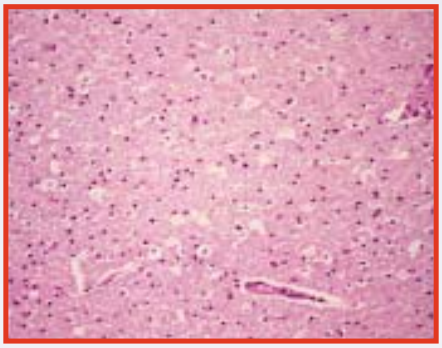

Fig. 2. Horse brain, formalin fixed, followed by 48 hrs in 70% alcohol. 100×

Materials and Methods
Samples of brain were collected from a mouse, rat, cat, dog, sheep, cow, horse, and human. Three pieces from the same area of the formalin-fixed brains were trimmed for evaluation. One piece was left in 10% neutral buffered formalin to serve as a control. Another piece was placed in 70% ethanol for 24 hours. The third piece was placed in 70% ethanol for 48 hours. Subsequent to exposure, all were processed using the following routine overnight processing schedule:

10% neutral buffered formalin	1 hr
10% neutral buffered formalin	1 hr
60% ethanol	1 hr
80% ethanol	1 hr
95% ethanol	1 hr
95% ethanol	1 hr
100% ethanol	1 hr
100% ethanol	1 hr
xylene substitute	1 hr
xylene substitute	1 hr
paraffin	15 min
paraffin	15 min
paraffin	15 min
paraffin	15 min

Processed tissues were embedded in paraffin, sectioned at 3 microns, and stained with hematoxylin and eosin.

Results
Microscopic examination showed some vacuolization in the fiber tracts of all species after 24 hours of exposure to 70% ethanol, although a marked increase in vacuolization and a spongy appearance was observed at 48 hours exposure. There was little change in the appearance of perivascular clearing in both the white and gray matter of the three treatment groups. Perivascular clearing is considered a common artifact, secondary to immersion fixation.

Discussion
The use of 70% alcohol is endorsed by many as an acceptable holding medium for fixed tissues. Vacuolization artifact can become problematic if 70% ethanol is used for CNS tissues awaiting processing. Although it is necessary to remove tissues from 10% formalin for immunohistochemistry to prevent masking of antigenicity, changes will occur in the white matter of CNS tissues held in 70% alcohol for more than 20 hours. This artifact was present not only in rat CNS fiber tracts, as previously reported, but it was also observed in the seven other species tested.

Acknowledgments
The author thanks Dr. E. J. Ehrhart for microscopic interpretation and photomicrography, and Rena Fail, Medical University of South Carolina, for human sample experimentation.

References
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2. Luna L. *Histopathologic Methods and Color Atlas of Special Stains and Tissue Artifacts*. Gaithersburg, Md: American Histolabs, Inc., Publications Division; 1992: 591.

A Breath of Fresh Air - Using Respirators in the Workplace

Maureen Doran HTL(ASCP)
Southern Illinois University
School of Medicine
Histology Center, 2055
Carbondale, IL 62901

Engineering controls are an employee's best form of protection from airborne contaminants. In environments where engineering controls are not feasible, respirators are used to protect workers. Where respirators are used, the Occupational Safety and Health Administration (OSHA) standard for respiratory protection must be followed. OSHA's recently revised respiratory protection standard, 29 CFR 1910.134, was published on January 8, 1998 and became effective April 8, 1998. The U.S. Department of Health and Human Services/NIOSH publication #99-143 recommends that a respiratory protection program contain the items described below.

Written Standard Operational Procedures (SOPs) describing the selection and use of respirators must be developed. Information and guidance needed for the proper selection, use, and care of these devices must be included. One person must administer the program and be responsible for implementing all aspects of the program. The program's administrator should have a

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background that provides sufficient knowledge to develop and oversee a respiratory protection program.

Respirator selection is based on the hazard to which the worker is exposed. A hazard assessment needs to be conducted to determine the type of respirator protection that is needed. The assessment should include a surveilance of work area conditions including exposure measurements/estimates. NIOSH has developed a new set of regulations for testing and certifying nonpowered, air-purifying, particulate-filter respirators. This certification provides for nine classes of particulate filters with three levels of efficiency. All of these new particulate respirators meet the CDC's performance criteria for protection against tuberculosis. The HEPA filters which were certified under 30 CFR11 are still acceptable for use in TB environments. More protective respirators may be needed for certain high risk procedures. OSHA does not allow the use of chemical cartridge respirators against substances with poor or unknown warning properties. In situations where the identity of the contaminant is unknown, or when the concentration of the contaminant is very high, a supplied-air respirator should be used.

The respirator user must be trained in the correct use of the respirator and be aware of its limitations. This training must include instructions for wearing, adjusting, and checking the seal of the respirator. A user seal check determines whether a respirator has been put on and adjusted to fit properly. This is done every time a respirator is worn.

All tight-fitting respirators must be fit tested before an employee is required to use respiratory protection. The standard specifies four accepted qualitative fit test protocols. Qualitative fit testing is acceptable as long as the contaminant concentration does not exceed 10 times the contaminant's permissible exposure limit. Fit tests are used to select the respirator that provides an adequate and comfortable fit. Fit testing is conducted at regular intervals, and also if there has been a change in the work environment or the employee's health.

Respirators must be cleaned and disinfected on a regular basis. If a replaceable-filter respirator is worn by more than one person, it must be cleaned and disinfected after each use. Respirators should be inspected during cleaning, and damaged or deteriorated parts must be replaced. Disposable-particulate respirators can be reused, but cannot be worn by more than one person. These respirators must be discarded if they are soiled, physically damaged, or the user experiences increased breathing resistance.

Respirators must be stored in a convenient, clean, sanitary location. They should be protected from dust, harmful chemicals, sunlight, moisture, and excessive heat or cold. Often replaceable-filter respirators are stored in resealable plastic bags, but this is not recommended for particulate filter-type respirators. It is important to insure that respirators are dry before storage.

Not all workers are capable of wearing a respirator. Certain facial features or injuries can prevent a proper face-to-respirator seal. Individuals with breathing or pulmonary difficulties can develop COPD from chronic labored breathing associated with their disease. Pulmonary function testing can determine an employee's ability to tolerate respirator use without suffering from restricted air flow. The standard requires that an employer obtain a written recommendation regarding the employee's ability to wear the respirator. Depending on each state's licensing agency, either a physician or other licensed health care professional is allowed to perform the medical evaluation required by the written respiratory program. The medical evaluation and/or exam is used to determine that a worker is physically able to do the work safely while wearing the respirator.

Wearing a respirator is a major inconvenience to most workers. Even the best respiratory program will fail if workers do not comply with the program. OSHA determined that respirators used without proper training and fit testing can increase the employee's potential for exposure. The employee must feel susceptible to the disease or the condition related to the hazard, according to Becker's Health Belief Model [1974]. The worker must believe that the benefits of wearing the respirator outweigh the inconvenience.

Sources of Additional Information

American National Standards Institute. Practices for Respiratory Protection, Z88.2, 1992.

American Industrial Hygiene Association Respiratory Protection Manual, AIHA 1993.

NCCLS. Clinical Laboratory Safety; Approved Guideline. NCCLS document GP17-A (ISBN 156238-300-0)1996.

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Histotechnology: The Next 100 Years

M. Lamar Jones, BS, HT(ASCP)

Introduction

As we move into the next millennium it is tempting to imagine where the discipline might be going. Before we can look ahead, it is only appropriate that we reflect over the last 100 years of histotechnology to appreciate how the discipline has changed in that time. About one-third into the

20th century we saw several trends that helped shape the histopathology laboratory, one of which was automated tissue processing. The late 1960s and early 1970s brought about some unique changes that impacted the work force up to the 1990s. The most dramatic advances took place within the last decade, setting the stage for histotechnology to move forward into the new millennium. Some of these advances include:

- the development of techniques to amplify and hybridize DNA and RNA probes to microbial and human genomes
- digital imaging of tissues and the advent of confocal microscopy
- the advent of the discipline of information technology
- the utilization of microchips to enhance the capability of automation in histology
- the wider availability of streamlined reagent systems that brought sophisticated technologies within reach of those with minimal training

Few would argue that the histopathology laboratory is probably the most manual and labor-intensive area of pathology, and as such, is an area ripe for automation. Only a few areas of histotechnology have had any type of automation thus far; notably processing, staining, and more recently, coverslipping. In recent years the skilled workforce has dwindled, driving the need for advances in instrumentation and technology. This reality combined with the exciting developments in computer technology have opened up the possibilities for further exciting changes. Because the field is rapidly changing, we need to be creative and flexible in order to keep pace and change with it.

What types of instrumentation will we see in the next several years? In the next 50 years? How will we cope with the changes brought about by technology? What will education for histotechnologists be like? How will we prepare for the changes that will reshape our histopathology laboratories? This article will offer a glimpse of how future generations of histotechnologists may practice their craft.

Automation

The histopathology laboratory continues today to be a labor-intensive area of diagnostic pathology. With budget restraints, personnel shortages, and quality issues, automation can offer solutions to many problems. Why will the emphasis on automation increase in the decades ahead?

In contrast to human labor, automation offers:

- consistent volume and quality of output
- rapid turnaround
- high speed and volume of output
- fewer possible errors
- constant availability
- consolidation of tests
- predictable cost

One type of automation consists of high end systems that move the specimen from the receiving area of the lab through the analyzing process, and then to be discarded or stored. Such systems are controlled by information systems that help to improve test ordering, as well as verification. Another type of automation is the modular system. This system will allow equipment to be operated either independently or together as work stations. It is ideal for small labs, can stand alone or form work stations, as other pieces of equipment can be added if needed. The mission for labs is to reduce manual processes, increase productivity, and achieve rapid turnaround times for procedures. And laboratory personnel can be cross-trained, which will increase flexibility and mobility. Today, consolidated work stations, robotics, and modular equipment are incorporated into the histopathology laboratory.

Grossing

The Gross Lab will be an integral part of both the surgical suite and the histopathology laboratory. In many hospitals, the histology lab is now located relatively close to the surgical suite. The histotechnologist may work very closely with the surgical team in taking and preparing the tissue specimen immediately after removal from the patient. The histotechnologist's training will include a greater emphasis on anatomy and surgical procedure. As enrollments in pathology residency programs decline, it may become increasingly necessary for histotechs to be capable of working alone.

The specimen will be placed onto the gross preparation area, described, and automatically digitally photographed. Magnetic resonance or x-ray technologies will also be utilized for appropriate specimens with all resulting images becoming part of the patient's permanent record. Imaging will occur by voice commands to the computer that controls the track system. A tissue processing cassette will be " stamped" out with a bar code that identifies that patient and specimen. The patient name, bar code and surgical number will be recorded digitally onto a tiny microchip that will travel along with, and become a permanent part of the specimen. Data on this processor chip will be permanent and can be accessed at any time with a special pen capable of being carried in a lab

coat pocket, which will decipher the code on the chip and then be played back in a human voice.

"Stamped" cassettes will move on a track system in front of the grossing area and the specimens will be placed in the cassettes. A divider will be placed robotically between specimens. Once the specimens are placed into the cassettes, they will be sealed robotically and taken to the processing chamber by the track system. The cassettes will be placed into the proper processing chamber, according to the size and type of tissue that is in the processing cassette. This will allow for the proper processing of each size of tissue. Care must be taken to place the specimen into the processing cassette exactly the way that it will be embedded, since this will be the last opportunity for human intervention before the sections are prepared and analyzed automatically.

Processing
Tissue processing will also have a different approach. The tissue processor unit will be built into the laboratory. In this manner, the reagents will be piped into the laboratory's processor. The reagents will be purchased and shipped in and connected to transport lines to the processor. The processor will be voice activated so there are no buttons to press, or any need to calculate the start, stop, and delay times. There will be a viewing screen to allow any specimen to be seen as magnified in the processing cycle in any reagent. This will also allow lipids, water, and other impurities to be seen as they are removed and replaced in the processing cycle. During processing, the density and specific gravity of the tissues and reagents will be recorded. The reagents will be pumped into the processing chamber and when completed, the used reagent will be pumped out directly to a disposal area. Technologists will no longer handle hazardous reagents. And the tissues may be embedded in some medium other than paraffin. This medium will be pumped into the processing chamber as the final reagent and solidified around the specimen. In a closed system such as this, processing will be completed in as little as an hour for routine surgicals, and biopsies in fifteen minutes or less.

Microtomy
Tissue specimens may no longer be sectioned with a microtome. Once the tissue specimens are processed and solidified, they will be tracked to an instrument called a **tissue analyzer.** Here the tissues will be sectioned with a laser or high pressure vibrating system. Once the section is obtained, it will be mounted onto a grid that will be placed into an imaging system after staining. Unlike paraffin, the synthetic embedding medium will permit any required staining method to occur without prior removal.

Staining
Up until the last several years, staining microscope slides in the histopathology laboratory has been primarily performed manually. Today it is not at all uncommon to have an automated H & E slide stainer even in the smallest histopathology laboratory. And for high-volume labs there are automated special stain and immunohistochemistry stainers available and in use. Staining tissue sections may likely be utilized for many more years and will remain a vital part of histotechnology. The H & E may still be utilized, but the real art and science of histo-technology will center around genetic code "staining." More of the immunohistochemistry and in situ hybridization will become the standard. The nice, artistically and scientifically prepared microscope slides may be replaced by computerized charts, graphs, and plates generated by the computer-driven image analyzer. Perhaps only infectious disease cases will actually have a stained glass slide prepared for documentation. Staining will be performed by robotics to include staining and coverslipping in one process all on a microchip-embedded microslide!

Microscopes
The microscope for the pathologist may very well be attached to a "lazy boy" or some type of command center pilot seat. Sections will be robotically transferred to the imaging system, which will not only provide visual images but will measure the density of stain molecules in the section.This will allow the computer to recreate three-dimensional images of microscopic structures, providing the pathologist with a capability for visualization of structural abnormalities never before imagined. Images will be projected onto a computer screen that covers one entire wall, providing a level of resolution and detail previously unheard of. Image quality will far exceed anything seen today as a result of enhancements to computer and electronic chips. This will be called a **"video-scope."**

The video-scope will either be remote controlled or voice activated. The system will automatically analyze and interpret the section images, offering the pathologist, by the sounds of a human voice, a run-down of the measured parameters and possible diagnoses. And should the pathologist need to see the "gross," or the "block," then the request would be made by simple voice command. After the block is sectioned by the tissue analyzer, it will be filed robotically. Upon request, the cassette can be retrieved robotically and viewed on the screen by the pathologist. For QC purposes, the bar code would be scanned to reveal the proper patient and surgical number.

Bar codes
Bar codes are already used in many facets of the clinical laboratory. Bar codes permit the tracking and verification of data such as that contained on:

- specimen labels
- reagent labels
- accession labels
- request labels
- microslide labels

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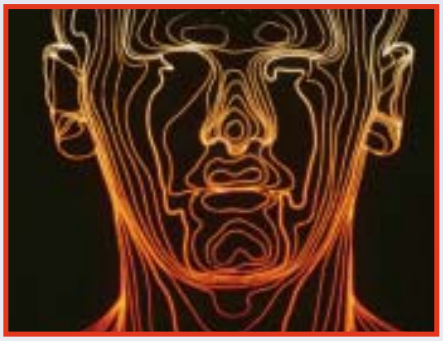
Within the next decade, we will see tissue cassettes embossed with bar codes.

There are tremendous advantages to using bar codes in the laboratory. They offer an efficient and error-free means of tracking that is inexpensive, and they provide a high degree of security. Some present-day immunostainers utilize bar codes to track patient slides, the stain that is needed for those slides, and the reagents needed to perform that stain.



Bar codes will revolutionize the histopathology laboratory, so much so that all patient and specimen tracking, as well as stain performance, will be bar code-driven.

Immunohistochemistry
Most immunohistochemical procedures are performed in the histopathology or special procedures laboratories. While many laboratories once relied upon manual methods to complete these stains, more recently instrumentation has been developed to permit automated staining to be done reliably and reproducibly.
I envision that a device will be developed which will radically change the way immunohistochemistry is performed. It may consist of a small electronic chip that may be read by a computer. A piece of fresh or fixed tissue will be placed directly onto the chip and viewed on a computer screen. Relevant data will be entered into the system and an antibody applied, making it possible for the result to be read directly. This may be the immuno stain and/or the image analysis of the future, called the **" immuno chip."**

Molecular Pathology
It has been only in the last few years that the word " molecular" has entered into the vocabulary of the histotechnologist. Already many of these tests are being performed in the histopathology laboratory. And we will see more of these types of tests performed by histotechnologists, perhaps in the next 10-15 years. Molecular techniques will become the gold standard. The practice of molecular pathology will change the medical field greatly, and it is off to a good start even now. We now see molecular pathology as a tool for diagnosing patients with infectious diseases and genetically transmitted diseases. Many histotechnologists are already performing DNA/RNA hybridization and apoptosis assays. For many of the infectious diseases and organisms, the histopathology laboratory may be the microbiology lab of tomorrow. Microslides properly prepared can be retrieved from the slide file for many years upon request, whereas micro culture plates only last a few days. Probes and antibodies will demonstrate microorganisms with a high degree of sensitivity and specificity.

Information Technology
The world of computerization has only entered the histopathology laboratory in the last several years. In most cases, histopathology was probably the last part of the laboratory to become computerized. But today, the computer is the " way of life." In the very near future, even as we speak, the complete testing process in pathology will include:

- electronic reporting
- electronic signature/verification
- full auto fax support
- auto-encoded reports containing CPT 4, snowmed, and ICD 9 codes
- code compliance with regulatory agencies
- image processing
- intranet and web pages for telepathology and consults
- voice recognition features
- direct third-party payer or managed care billing

The histopathology laboratory of the future will have more computerization than ever imagined. Histotechnologists will become quite skilled and knowledgeable with computers and information technology.

Education
Formal education for histotechnologists will be more necessary than ever before. As a result of the impact that histotechnologists will have on the health care industry, training will become more sophisticated.
A bachelor's degree plus at least a 2-year concentrated histopathology residency program will be required. Upon graduation, a Master of Science in Histopathology will be awarded. Licensure, as well as certification, will become the national standard for all technologists. The residency program will involve such training as actual case studies, and tissue and organ management. Histopathology will replace histotechnology, due to the increased sophistication of the technology that practitioners of the discipline will be required to employ. The technological changes of the discipline that I have alluded to will bring about a long desired elevation of professional stature and compensation.

It is unclear when changes of the sort that I have imagined may arrive. What is certain is that change will come. Technological innovations will bring opportunity to those who are ready to meet the challenge. Are we as histotechnologists going to be ready? Will we take the challenge? If not, we will be left behind, for technological change waits for no one. There is no question that the histotechnology discipline of the

future will require a level of knowledge, skill, and sophistication beyond anything previously imagined. Nevertheless, the proper handling of tissue specimens will still be done best in the hands of a well-trained, professional histotechnologist!

M. Lamar Jones is consultant at Histological Consultants, Inc., Alabaster, AL.

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All in a Day's Work !

M. Jane Chladny, HT(ASCP)
Laboratories of Veterinary Diagnostic Medicine
College of Veterinary Medicine
University of Illinois
2001 S. Lincoln Ave.
Urbana, IL 61802

All in a Day's Work! *is a salute to the efforts of histotechs everywhere, those unsung heroes who often go unrecognized for their contributions to the advancement of health and science. Please contact the editor if you would like to see your work featured in this column.*

The Laboratories of Diagnostic Medicine, part of the University of Illinois College of Veterinary Medicine, offer a variety of services which provide histotechnologists with daily challenges and continual learning. The primary responsibility of the histopathology laboratory is to provide diagnostic support to clinicians at the university's large and small animal clinics, veterinarians within the state of Illinois, and even some practitioners out of state. Specimens can range from a small lesion taken from a domestic animal, to necropsy tissues from a herd animal with a suspected infectious disease. It is sometimes necessary for owners to sacrifice one sick animal for disease diagnosis to prevent a widespread outbreak in the herd. Along with routine H&E slides, the laboratory performs up to twenty different special stains per day from a routine offering of about fifty stains. Additionally, immunohistochemical procedures are utilized for the diagnosis of tumors and infectious disease. Immunohistochemistry can be challenging in an animal lab. Because species cross reactivity is usually not known, experimentation with various antigen unmasking procedures, antibody dilutions, incubation times, and detection systems must be done. Those antibodies that do cross react are titered for optimum staining in each species requiring the immunohistochemical procedure. Currently, the laboratory offers nineteen infectious disease markers and thirty antibodies for tumor identification or differentiation.

Besides services for domestic and food animals, the university supports a zoo pathology program, which enables veterinary pathology residents to specialize in diseases of zoo animals.

Fig. 1. One of the many step sections through the dorsal plane of an entire frog.

Submissions from the Lincoln Park and Brookfield Zoos and Shedd Aquarium in Chicago, supply diversity in the species seen by the laboratory. The type of animal submitted for necropsy can cause the diagnostic workload to fluctuate a great deal. Tissues from a finch can be contained in a couple of blocks, or if a tiny pipe fish dies, the entire specimen is embedded in one block. However, if a beluga whale, or a horse exhibiting neurologic signs, is necropsied, nearly sixty cassettes may be submitted. Because pathologists and residents have come to expect only the highest quality slides, some tissues present an extraordinary challenge for the microtomist. Marine mammals and birds are unusually difficult to section. Many large, dense bones and keratinized specimens, such as hooves and nails, require pretreatment and special handling. Oversized specimens, such as large animal eyes, are embedded using lead Ls and placed on 3x2" slides at sectioning.

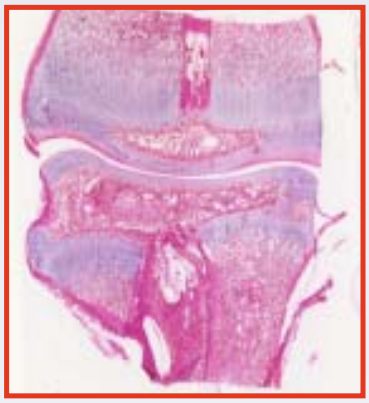

Fig. 2. Section of large bone including joint on 3x2" slide.

Slide preparation and staining for research projects is another important function of the histology laboratory at the College of Veterinary Medicine. In many cases, histotechnologists must assist an investigator, who may be unfamiliar with histologic procedures, to determine the methods most appropriate to complete his project. The laboratory has been involved in many interesting and unusual projects. As one might expect, some research done at the College of Veterinary Medicine is conducted for the betterment of animals. Annual trips to Africa supply the lab with tissues from wild animals found dead. Those tissues are examined in an effort to learn more about the

animals in their natural habitat, thereby aiding animals held in captivity in zoos. Also, the information obtained by the examination of these tissues is sent back to Africa to help wildlife management with health problems of their free range populations. One study examined samples of Alaskan seals and whales for possible metals toxicity.

Fig. 3. Embedding oversized specimen using lead Ls.

Aside from research to benefit animals, many of the projects involving the histology laboratory are for the advancement of knowledge to benefit humans. Studies have been done on cryptosporidium and rotovirus in an attempt to block effects of the agent which produces diarrhea and is fatal for many children in third world countries and underdeveloped areas of the United States. Animal models have been used to study amyotrophic lateral sclerosis (ALS) and multiple sclerosis (MS). In the MS project, investigators are working to identify the gene responsible for the disease. Once that information is obtained, further research may produce a method to block or alter the gene product. Another project is underway to target cells responsible for a brain tumor which affects children.

In addition to research to study diseases, animal models have been used in projects concerning human health and safety. Appetite and nutrition have been evaluated through varying zinc intake. The nutritional benefits of eating certain foods, such as broccoli, on disease prevention have been researched. An ongoing project studies the effects of ultrasound on animal and human health. Adverse changes in the lungs have been observed in several species. The results of this research project will be used to establish the safety of ultrasound use on humans.

Tasks are not limited to traditional slide preparations. Much like a hospital lab, the daily routine consists of H&Es, special stains, and immunohistochemical procedures; however, on occasion, glycol-methacrylate has been utilized, making embedding, sectioning, and staining a deviation from the norm. Plant tissues have been processed and special stains applied to sections of soybean roots for identification of fungus. The laboratory has even processed and cut industrial sludge. Another function of the lab includes preparation of slide sets used to support teaching of veterinary and veterinary pathology students. In most cases, 100 slides from a single block are prepared for the veterinary student teaching set.

Fig. 4. Histology laboratory staff (left to right): Melanie Cox, Dana Browning, Gigi Simon, Jane Chladny, and Karen Gebbink.

Histotechnologists at the University of Illinois find the best of both worlds: a diagnostic lab, as in a hospital, and a lab which supports research. Four of the five histotechnologists were previously employed by hospital laboratories. The diversity of tasks, species, and tissues found at the College of Veterinary Medicine provides a stimulating and challenging environment. The busy laboratory is staffed with excellent technologists who meet those challenges while gaining expertise in a wide range of histologic techniques.

Elastic Tissue Staining in Human Skin

Yufeng Yu, MD
Clifford M. Chapman, MS, HTL (ASCP), IQC
Pathology Services, Inc.
640 Memorial Drive
Cambridge, MA 02139

Abstract
There are several methods used in histology to visualize elastic tissue. Different methods stain thick elastic, and thinner elaunin and oxytalan fibers to varying degrees. We report on the effectiveness of these methods in staining elastic fibers in specimens of human skin.

Introduction
Elastic fibers are an integral and important component of skin. These fibers are composed of two major parts: a central core of elastin that is surrounded by microfibrils.[1,2] Thick elastic fibers present in the reticular dermis contain both parts. These thick elastic fibers connect to thinner elaunin fibers in the papillary dermis which, in turn, form a plexus with very thin oxytalan fibers that project perpendicularly toward the dermal-epidermal junction. These two types of thinner elastic fibers are composed mostly of microfibrils with little or no elastin.[3]

There are many different methods used in histology to stain elastic tissue. Different methods result in the various elastic fibers staining to different degrees. We report on the effectiveness of some of these methods in staining elastic fibers in specimens of human skin.

Methods
Specimen Preparation
Human skin specimens were fixed in 10% neutral buffered formalin for 24 hours, and then processed through a graded series of ethanol, cleared in xylene, and infiltrated in paraffin on a Sakura VIP tissue processor over a total processing time of 8 hours. Paraffin sections were cut on a rotary microtome at a

thickness of 5 microns, picked up on lysine-coated slides, and heated in an oven at 65°C for 45 minutes. After cooling, slides were deparaffinized in three changes of xylene for 5 minutes each, and hydrated to distilled water before staining.

Staining Procedures
Four different methods for staining elastic tissue were utilized: Verhoef Van Gieson,[4] Gomori's aldehyde fuchsin,[5] Weigert's resorcin,[6] and Miller stain.[7] Serial sections of formalin-fixed, paraffin-embedded human skin were prepared and numbered. Hematoxylin and eosin staining was used in addition to the above elastic tissue stains on adjacent sections from the same paraffin blocks.

Procedure: Miller stain for elastic tissue

0.5% Potassium Permanganate
Potassium Permanganate 0.5 g
Distilled water 100 ml

1.0% Oxalic Acid
Oxalic acid 1.0 g
Distilled water 100 ml

Miller Elastic Stain
Victoria Blue 4R(C.1 42563) 1 g
New fuchsin(C.1 42520) 1 g
Crystal violet(C.1 42555) 1 g

Dissolve in 200 ml of hot distilled water, then add in following order:
Resorcin 4 g
Dextrin 1 g
30% ferric chloride (fresh) 50 ml

Boil for 5 min then filter while hot. Transfer precipitate plus filter paper to original beaker and re-dissolve in 200 ml of 95% ethanol. Boil on a hot plate, or in a water bath for 15 to 20 min. Filter and make up to 200 ml with 95% ethanol. Finally, add 2 ml of concentrated hydrochloric acid.

Purchase Miller stain:
Rowley Biochemical (Danvers, MA) catalog # SO-709

Van Gieson stain
Acid fuchsin, 1% aqueous 2.5 ml
Picric acid, saturated aqueous 97.5 ml

Purchase Van Gieson stain:
Rowley Biochemical (Danvers, MA) catalog # SO-463

Method:
1. Deparaffinize and hydrate slides to distilled water.
2. Place into 0.5% potassium permanganate for 5 min.
3. Wash 7x with tap water.
4. Place into 1.0% oxalic acid for 3 min.
5. Wash 7x with tap water.
6. Rinse in 95% ethanol for 1 min.
7. Place into Miller Elastic Stain for 1-2 hours at room temperature.
8. Rinse 7x in tap water.
9. Rinse in 95% ethanol: 2 times 5 seconds each.
10. Rinse in water.
11. Place into Van Gieson's stain for 2 min.
12. Dehydrate *rapidly* through 95% ethanol - two changes 5 seconds each.
13. 100% ethanol - 2 times 2 min each.
14. Xylene - 3 times 3 min each.
15. Coverslip with Permount.

RESULTS:
elastic fibers blue/black
mast cells black
red blood cells green
connective tissue red

Procedure: Weigert's resorcin fuchsin elastic tissue stain.

Weigert's resorcin fuchsin
SD Alcohol, 3A 70% v/v
Hydrochloric acid 1% v/v
Resorcin-fuchsin 0.2% w/v
Distilled water 29% v/v

Purchase Weigert's resorcin stain:
Rowley Biochemical (Danvers, MA) catalog # F-370-1

Method:
1. Deparaffinize and hydrate to distilled water.
2. Immerse slides in the Weigert's resorcin fuchsin, and leave 6 hours to overnight at room temperature.
3. Remove slides from stain and wash in running tap water until all color comes out.
4. Dehydrate through alcohols and xylene.
5. Mount with Permount.

RESULTS:
elastic fibers light to dark purple

Results
While hematoxylin and eosin staining shows collagen in various shades of pink, specific elastic tissue fibers cannot be identified (Fig. 1). In contrast, the Verhoeff Van Gieson method shows thick black-stained elastic fibers in the dermis. However, the thinner elaunin fibers that run up and under the epidermis cannot be discerned (Fig. 2).

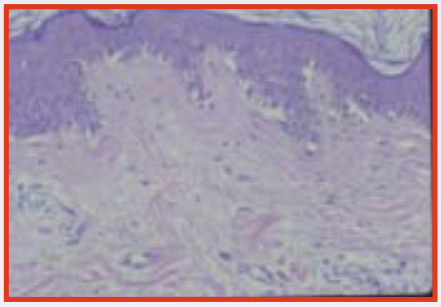

Fig.1. Hematoxylin and eosin. 780×

The Gomori aldehyde fuchsin method stains both the thick elastic fibers in the dermis, and the thinner elaunin fibers that approach the epidermis, a dark purple, which contrasts well with the light green counterstain. However, the thinnest oxytalan fibers are still difficult to identify (Fig. 3). Weigert's resorcin method results in dark purple-black staining of the finest oxytalan fibers that extend into the dermis. Additionally, thick elastic fibers and elaunin fibers are stained. While this staining is precise and delicate, there is virtually no counterstain (Fig. 4).

Miller's method stains all elastic fibers. Thick fibers in the dermis, thinner elaunin fibers leading from the dermis, and the thinnest oxytalan fibers extending into the epidermis are stained dark black. This staining contrasts well with the nuclear and connective tissue counterstains (Fig. 5).

Fig. 2. Verhoeff Van Gieson. 780×

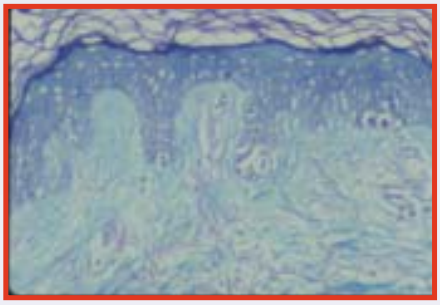

Fig. 3. Gomori aldehyde fuchsin. 780×

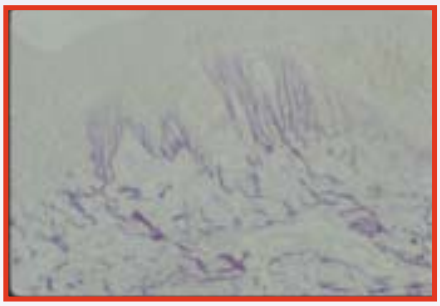

Fig. 4. Weigert's resorcin. 780×

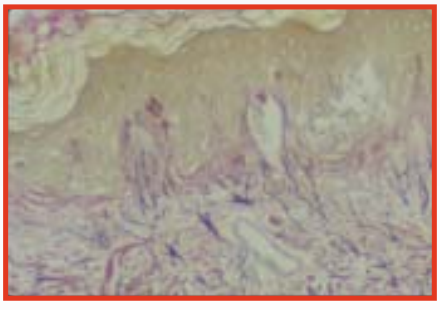

Fig. 5. Miller stain. 780×

Discussion

The major function of skin is to protect the body against exterior forces resulting from substances, chemicals, and microbes. While the epidermis is composed of tightly packed cells which secrete keratin proteins, the underlying dermis is made up of ground substance, collagen, and elastic fibers. The elastic fibers form the basis for the integrity and elasticity of the skin.[8]

Thick elastic fibers in the reticular dermis are composed of a core of elastin surrounded by microfibrils, and stain readily with all methods employed herein. However, the thinner elaunin and oxytalan fibers are not stained with the Verhoeff Van Gieson method. In addition, sections must be oxidized with potassium permanganate in both the Miller and Gomori methods in order for both elaunin and oxytalan fibers to be stained. This oxidation results in disulfide groups being formed, which cause the fibers to become basophilic and able to be stained by these methods.[3]

The finest oxytalan fibers are visualized by the Weigert's resorcin method. Under high power magnification these fibers can be seen extending to the basement membrane of the dermal-epidermal junction.

In the clinical histology laboratory, it is not surprising that the primary method of staining elastic tissue is the Verhoeff Van Gieson. Increased elastic tissue can manifest itself clinically as elastofibroma or pseudoxanoma elasticum. Conversely, decreased elastic tissue in skin may appear as macular atrophy or cutis laxa, which is a degenerative change in the elastic fibers.[8] Thus, the visualization of thick elastic fibers in the reticular dermis using the Verhoeff Van Gieson method is appropriate.

However, in a research setting, it is important to know exactly which fibers are being investigated. Using an inappropriate method could result in false negative results. Visualization of the thinner elaunin and oxytalan fibers requires the use of methods other than the Verhoeff Van Gieson. These methods must be evaluated for each use to determine if they will stain the elastic fiber type under investigation.

Acknowledgments

The authors wish to thank Ms. Karen Carlson and Pathology Services, Inc. for their assistance and support during the preparation of this manuscript.

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The History and Use of Hematoxylin

Maria G. Singhal
The Methodist Hospital
School of Histotechnology
Houston, Texas

Origin of Hematoxylin

Hematoxylin is a compound extracted from *Haematoxylon campechianum*, which is a derivative of the third largest family of plants - the legumes. The Aztecs used this compound to dye the fabric for their robes. The word "*Haematoxylon*" means "blood wood" and "*campechianum*" stands for the fact that both the trees and Mexican Indians who discovered the dye are native to the Bay of Campeche region. It was Hernando Cortez who first found that Indians used the thorny plants to make the violet and black dye. Spaniards introduced this dye to the European markets where it competed well with indigo, the original violet dye from India. The English also produced a similar dye

and called it "logwood," since it was shipped as unextracted logs. Haematoxylin was first produced by Cherrul in 1810, and Erdmann worked out the formula in 1842. It was discovered later that the actual staining occurred as a result of the oxidation product of Haematoxylin, called hematein.

Properties

In its pure form, Hematoxylin is a colorless powdery substance which has a tendency to crystallize into either tetragonal prisms containing three molecules of water, or rhombic crystals with one molecule of water. It is a very weak acidic dye with an isoeletric point around pH 6.5. It attains staining properties upon conversion to hematein and in the presence of various electrolytes. Oxidation of Hematoxylin occurs when hydrogen atoms are removed, forming hematein (see Fig. 1).

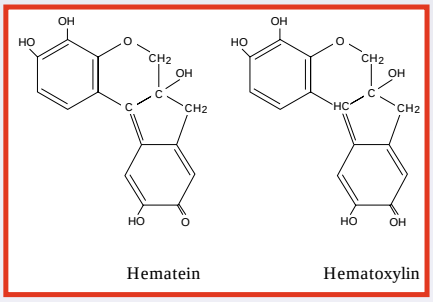

Fig. 1. Chemical Structures

Several oxidizing agents can be used to achieve oxidation of Hematoxylin, such as sodium iodate, potassium permanganate, or mercuric oxide. First exposure to atmospheric oxygen in the presence of ultraviolet light will cause formation of hematein, the latter being responsible for the color-related properties attributed to Hematoxylin. Pure Hematoxylin is soluble in water, ethanol, methyl cellosolve, and ethylene glycol.

Contaminants

The pure dye is difficult to obtain on a commercial scale. Commercial samples may be contaminated with wood from other parts of the tree, such as bark. These are insoluble and can be filtered out, but they decrease the effectiveness of the stain. Crystal contaminants may be due to soil from roots of the tree, or due to the attempts to stabilize the powder using sodium sulfite or antioxidants.

Uses in Histology

The first biologist who tried to use Hematoxylin was Waldeyer in 1863. However, he was not very successful in staining the axis cylinders of nervous tissue and, therefore, he gave up using Hematoxylin in favor of another dye. Two years later, Bohmer successfully employed Hematoxylin in combination with alum to develop his staining formula. In 1872, Merkel used Hematoxylin to stain muscles of animals in polarization studies. A great deal was accomplished in the field of Hematoxylin staining during the late 1800s. In 1879 (not fully confirmed), Kleinenberg proposed a formula for Hematoxylin containing alum and calcium chloride dissolved in 70% alcohol. Around this time, Delafield's Hematoxylin was also proposed in a formula which was furnished by Prudden in 1885, in response to an inquiry. Renaut and Ehrlich gave differing formulas for glycerinated alum-Hematoxylin in 1881 and 1886, respectively. However, the most important development in alum-Hematoxylin was made by Mayer in 1891. He emphasized the use of aluminum chloride as opposed to alum. During the 1880s, Bohmer, Heidenhain, Weigert, Apathy, and later, Platner developed methods introducing the use of chromium compounds with Hematoxylin. Later, Flesh and Pal proposed modifications to Weigert's formula. During the late 1880s and 1890s, researchers employed iron, molybdenum, vanadium, and salts of other metals. Benda, Heidenhain, and Weigert developed iron-Hematoxylin, differing in the use of iron salts and the method of application. Mallory worked with molybdenum, and Wolters worked with vanadium. These solutions produce different staining pictures due to the electrolytes and the mordanting action of the metal salts.

Common uses of Hematoxylin

Hematoxylin is one of the most valuable natural dyes used in nuclear staining today. Its primary use consists of staining histologic tissue samples for microscopic analysis. Two of the most important uses are: (1) staining in the Papanicolaou procedure for the diagnosis of cytopathology specimens, and (2) in combination with the contrast stain eosin, making the popular H&E stain for tissues in routine pathology. Its industrial use has declined over the years but it is still used to dye textiles, furs, and to prepare inks. It is used as a reagent for the detection and determination of various metals in fluids, extracts, and tissue.

Tissues that can be stained with Hematoxylin

Hematoxylin is extensively used to stain nucleic acids. Therefore, it demonstrates both nucleic DNA, as well as nucleic and cytoplasmic RNA. Various modifications have been used to stain a variety of substances, including mucins, mitochondria, muscle striations, myelin sheaths, and dentinal tubules.

Staining Procedure

Preparation of Harris's Hematoxylin:

Hematoxylin	5 g
Absolute ethyl alcohol	50 ml
Ammonium aluminum sulfate	100 g
Distilled water	1000 ml
Mercuric oxide	2.5 g

Dissolve Hematoxylin in alcohol. Completely dissolve the ammonium aluminum sulfate in water with heat. When both solutions are dissolved, mix the two solutions, and bring to a rapid boil. Remove from heat. Collect 50 ml of this solution. Label "without oxidizer," and put aside for empirical testing. To this solution,

add mercuric oxide slowly. Plunge flask into ice. Cool rapidly. The stain is ready for use as soon as a metallic sheen develops on the surface of the solution. Filter before use.

Empirical Testing

1. Odor:
   A good Hematoxylin stain should have a wine smell.
2. Color:
   Color should be a deep purple-red.
3. Tap Water Test:
   A few drops of good stain in tap water should turn bluish-black.
4. Filter Paper Test:
   A good stain, when placed on a piece of filter paper, should diffuse and produce a pattern with a maroon color ending in a dark purple.

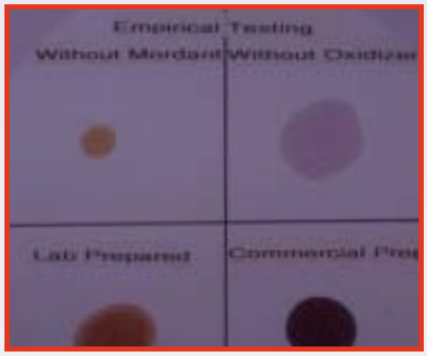

Fig. 2. Empirical test.

Progressive vs regressive staining

Progressive stains demonstrate particular elements and are selectively controlled by time and solution concentration. Staining continues with periodic viewing under the microscope until the desired results are observed. Regressive stains utilize the principle of rapid staining, only the tissue components are stained. The entire tissue is overstained and the excess solution is removed by differentiation until the desired stains are visible.

Differentiation

Differentiation is a process where excess Hematoxylin is gradually removed using a dilute acid rinse. Other reagents such as basic media, mordant, buffers, and oxidizers have been used as differentiating agents (bluing). Different alkaline solutions have been used to achieve bluing results: tap water, ammonia solutions, Scott's solutions, and lithium carbonate solutions. Sections must be replaced in an alkaline solution following the differentiation step.

Common causes of artifacts in staining due to the nature of Hematoxylin

Weakly stained section:

- Hematoxylin under-oxidized; Hematoxylin over-oxidized.
- Staining time too short.
- Over- or under-differentiation.

Nuclei not clear blue:

- Insufficient bluing.

Section stained too strongly:

- Insufficient differentiation.

Common alternatives to Hematoxylin

Brazilin
Celestine blue
Methylene blue
Toluidine blue
Thionine
Azure

Pros and cons of Hematoxylin use

Pros:

- The most widely used nuclear stain.
- Rapid, reproducible results in a wide range of tissues.
- Staining can be easily modified to suit individual needs.

Cons:

- Expensive.
- Results can vary if quality control is not maintained.
- Hematoxylin solutions easily oxidize.

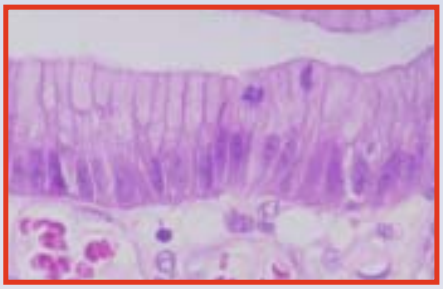

Fig. 3. Gastric mucosa, foveolar epithelium. Stained with Hematoxylin-eosin (H/E). 250× (oil immersion)

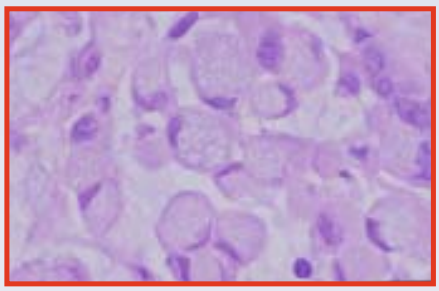

Fig. 4. Mucinousadenocarcinoma; signet ring cells. Stained with Hematoxylin-eosin (H/E). 250× (oil immersion)

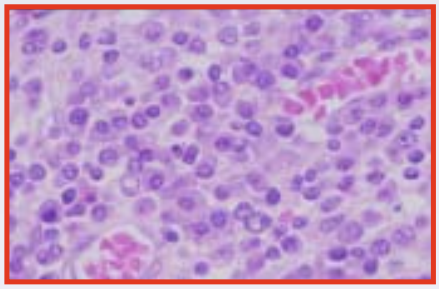

Fig. 5. Chronic inflammation; plasma cells. Stained with Hematoxylin-eosin (H/E). 250× (oil immersion)

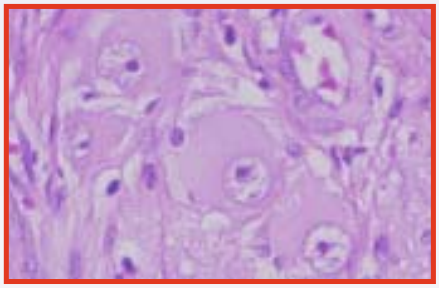

Fig. 6. Ganglioneurofibromatosis; ganglion cells. Stained with Hematoxylin-eosin (H/E). 250× (oil immersion)

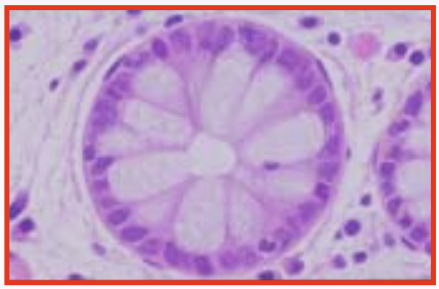

Fig. 7. Colon; normal crypt in cross-section. Stained with Hematoxylin-eosin (H/E). 250× (oil immersion)

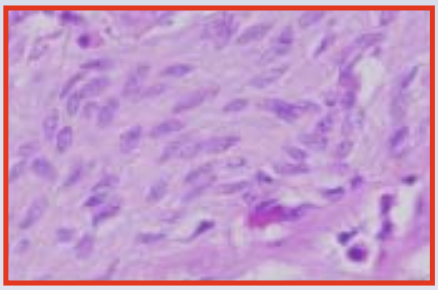

Fig. 8. Neurofibroma; nerve bundles. Stained with Hematoxylin-eosin (H/E). 250× (oil immersion)

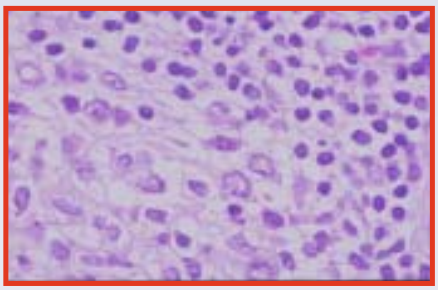

Fig. 9. Lymphoid follicle; lymphocytes. Stained with Hematoxylin-eosin (H/E). 250× (oil immersion)

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Memoirs of a Self-Made Histotech

(Part One)

Jeffrey S. Silverman
HT, HTL(ASCP) QIHC
Southampton Hospital
Southampton, NY

I've been privileged to have somehow found myself engaged in the honorable field of histotechnology, helping to diagnose disease in my fellow humans and their companion animal pets for the last quarter of the century. As a self-taught histotech who learned all he did through passion for histology, and luck in meeting lots of friendly employers and mentors, I've been asked to reflect back on the changes I've witnessed in my varied career.

I was the quintessential science nerd in school, dissecting frogs and pigs in grade school science fairs. Prior to that, I always took things apart, watches and clocks, old TVs and radios from friends and neighbors. One favorite neighbor had an auto repair business and he used to bring me carburetors, generators and such to dissect as well. My mother used to drag me to antique stores a lot when I was fourteen. On one such trip I found an antique copy of Animal Micrology by Michael F. Guyer (1906-1946 editions). I bought a well microtome from Edmund Scientific and practiced sectioning potatoes and cork. At grade school, I used to collect the science supply catalogs from the teachers and pore over the contents. My earliest memories of microscope slides were in junior high school, where there was a box of someone's ancient kidney and skin sections, probably prepared by some science teacher during his college histo course. After looking at them, I immediately set about dissolving off the coverslips and playing with whatever other stains were available in the school, using methods that I gleaned from my books.

Lo and behold, when I got to high school (class of 1971), I found that someone had purchased a rotary microtome that I was told no one could ever remember using. It was by an unknown Japanese manufacturer and even had a conveyor belt for the ribbons. It had a steel knife and a whet stone and strop! I made a paraffin processing set-up. We found an old asbestos-insulated incubator, put a 60 watt light bulb in it and made a paraffin oven. Somehow, the school, circa 1969-71, had a budget for this stuff, and in high school I pored over Fisher, Turtox, and Carolina Biological catalogs ordering paraffin, whatever fixings for Zenkers and Bouin's that weren't in the chemistry labs, an aluminum Boeckel slide drying plate, slides, coverslips, a few Peel-Away plastic tissue capsules, and dry stains like orange G, and aniline blue to concoct Masson Trichrome and Heidenhein's Azan stains (from the old books).

I managed to obtain some newer books that told of one-step dehydrant clearants like dioxane, so we bought some and that's what I used for processing. Toxic as hell, and I played with it with no PPEs and no hood during my high school years. I used xylene and cellosolve (ethylene glycol monoethyl ether) to decerate sections and stain. Next, at school, we euthanized a rat and later a guinea pig with ether in a big mayonnaise jar from the kitchen, dissected them, and used all the different fixatives on various organs. The processing and staining set-up was in my home in the basement because there was no room at the school. So I carried the tissues home on the bus in fixative, transferred the tissues from fixative by hand through dioxane, and infiltrated them in the bulb-heated paraffin oven. Embedding was from a pitcher in the oven into a unique Unimold from Lipshaw, a two-piece mold that made a block with a paraffin "stem" for the object clamp.

I did the microtomy at school in a prep room between two class-rooms. One day I slit open my finger and was too embarrassed to interrupt the class, so I applied pressure until the bell rang. I carried ribbons of sections home in my mother's nylon stocking boxes and mounted them later onto slides smeared with homemade Mayer's albumen after floating them on water placed onto slides I

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had sitting on the slide warmer! With Sly and the Family Stone and the latest Beatles 45 blasting on the Hi-Fi upstairs in the den, I prepared trichromes of guinea pig epididymis, kidney, and everything else, in my parent's basement. Damn near burned out the laundry sink with all the chemicals. The slides are still in use at my high school thirty years later.

Early in my senior year, the premed club visited the laboratories of our local county hospital, the Nassau County Medical Center. I told them of my experience and asked them about employment. I ended up volunteering every day after school and all that summer. I started out preparing five-gallon carboys of phosphate buffered formalin and decal solution for the gross rooms, cleaning cassettes, filing slides and blocks and other such scutwork. I embedded autopsies in checkerboard grids of aluminum squares. The surgicals went into Tissue-Tek embedding rings from rewashed steel cassettes, but I didn't yet do surgicals. I also dehydrated scores of vials of vaginal cell suspensions with amyl acetate for examination under the newly invented scanning electron microscope, to elucidate changes in the hormonal cycle and disease.

My mentor in histology, Dick Schroeder, knowing of my experience with things tinctorial, gave me the method for the Movat Pentachrome stain, told me that they wanted to use it for certain lesions and asked if I could shorten it from 18 hours to one or two. I did, by substituting 1 hour Bouin's mordant instead of a longer time in Zenkers's, and by using an acid Orcein Verhoeff to stain nuclei and elastica in one-half hour, replacing both the persnickety resorcin fuchsin elastic and Weigert hematoxylin steps of the original method. Heating some solutions also sped up the process. I published the result in ***Histo-Logic*** in 1972 under the late Lee Luna, who took me to task until I could argue that my modifications were either original or referenced. I was 19 years old. During my senior year I loved the lab so much, I paid classmates to drive me to the hospital during classes - I played hooky. That summer, I worked on larger surgicals, cleaned up the backlog of "posts," and learned special stains like PAS, retic, etc. It was a sad day when I had to leave to attend Columbia College 20 miles away in NYC. But I soon got a part-time job sectioning pig skin for the radiation research lab at NCMC. Man, a real job! There was also a ten-bed hospital on the east end that generated maybe 20 blocks a week, and I did those also for thirty bucks or so on the weekends. It wasn't long before my studies suffered and I was on academic probation for a year. I got a job through an employment agency for $150 a week at Saint Barnabas Hospital in the Bronx, commuting with my next door neighbor in the morning who dropped me off at the subway. I took the LIRR back home, all for $150 a week. But it was here that I really started to learn pathology.

St. Barnabas was a small, leisurely paced private hospital on a hill in the Bronx, formerly known as the Home for Incurables, surrounded by tenements on three sides and Little Italy (Arthur Avenue) on the other. Boy, the lunches we used to have on Arthur Avenue. For a small place, we saw some amazing cases. This was the first opportunity for me to regularly sit in on grossing, and it was for me tantamount to beginning a pathology residency that would last for twenty years. I seem to have a photographic memory for pathology and it wasn't long before I became proficient in identifying lesions, often resolving frozen section problems for the docs after having just read about "peritoneal gliomatosis secondary to ovarian teratoma." I voraciously read all of the journals and all of the textbooks in the office and began a slide collection.

I attended meetings of the NYC Pathologists Club slide seminars as a guest of my pathologist. In 1973, after attaining the necessary two years of training and experience necessary to sit for the HT exam of the ASCP, I took and passed the exam. Collecting slides really multiplied my accumulation of knowledge since I used to take our rare cases back to NCMC to share with Dr. Vincent Palladino and his residents and attendings. His active training program had numerous conferences and received countless donations of wet tissue, ranging from classics to rarities, from all of his former residents. I used to prepare, for free, sections of all of these donations and special conference cases, as well as his consults, to the relief of the overworked histolab upstairs, where I had first trained. I was their "pet histologist" and I didn't even work there. I used to race through traffic from the Bronx to East Meadow, Long Island to get there before the chief left for the day.

In this way, I amassed an amazing collection and saw many times what most practicing pathologists do during their service rotation, because this went on everyday. Through Dr. Palladino, I served variously as seminar histo-technologist, preparing 3000 slides of lymphomas one year, and projectionist for slide seminars of the Nassau/Suffolk Pathology Societies, working with such luminaries as Drs. Lauren Ackerman and Robert Scully. I gave informal slide seminars for the residents in the sign-out room of all the materials I brought or prepared for Dr. Palladino.

Look for Part 2 of Jeffrey Silverman's memoir in your next issue of ***Histo-Logic.***

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