Greetings from the Region IX Director
Heather Nymeyer

The first two day Region IX Education Event can only be classified as a success with approximately 85 attendees and 17 vendors. The weekend started off with a vendor's meet and greet on the Friday evening and then it was followed on the Saturday by lectures. The Education Committee prepared a program of lectures that ranged from Basic Molecular Biology to Forensic case studies. I would like to extend my appreciation to the vendors that attended this meeting and to those that provided sponsorship for speakers, breaks and door prizes. I have included a complete list of vendors that were in attendance on page 4 of this newsletter. Congratulations and thanks to the Education Committee for all their hard work. I look forward to next year's meeting.

The NSH Symposium and Convention will be held this year in Denver, Colorado and by now members should have received the S/C schedule of workshops. If you are thinking of attending the S/C, register early, many of the workshops fill up quickly and you don't want to be disappointed. If you are attending, think about becoming an ambassador. The name “ambassador” may be a bit scary for anyone who has not volunteered; I can assure you that the job is not as bad as it sounds. While you are looking through the S/C calendar, remember to plan on attending the Region IX meeting to be held on Saturday. It will be at this meeting the Region IX awards will be presented and any changes to the Region IX bylaws will be voted on. According the Region IX bylaws, any proposed changes must be circulated to the membership within 30 days of the S/C. Therefore within the next few weeks any proposed changes will be sent to all the members. I would encourage you to visit the NSH website (www.nshrregionix.org) and submit your suggestions to me by the end of August (heather@nshrregionix.org).

It is hard to believe that almost 1 year has passed since I assumed the position as the Region IX Director. During this time, I have met some extremely interesting individuals and learned a lot about parliamentary procedures, bylaws and the financial aspects of an non-profit organization. I would encourage anyone, who would like to give themselves a challenge to become involved with a Region IX committee and start this exciting journey.

See you in Denver and have a great summer.
Education Update

It hardly seems that three months has past since the Education Day in Calgary. Summer seems to be flying by rather quickly.

As Heather mentioned, the Education Day in Calgary was a great success. The 1.5 day arrangement was favourably received by both attendees and the vendors-it gave more time for the attendees to meet with the vendors and find out what was new. So based on the feedback from all in attendance we will continue with the Friday evening get-together, in addition to the Saturday lectures.

We had a large turnout of people who came to listen to some fantastic speakers. The feedback we received from the attendees indicated that they loved all of the speakers, as well as the event as a whole-we must be doing something right. I would like to extend a very big thank you to Trish Johnson who literally stepped-in at the last moment to give Joanne Luider's talk after Joanne had to cancel the night before due to being very sick. Trish did an outstanding job and everyone commented on how well she did for a last minute substitution.

As usual there are problems which can arise at any point and we had a few, but we dealt with them the best we could. It can be hard arranging these events remotely, which is why we need to have locals involved in every step of the planning-without these people, the events would be much more difficult to organize. I would like to extend my heart felt thanks to Carol Leriger, Sandra Eyton-Jones, Tracey Lenek and Coral Toews, for all of their hard work in helping put this together. I would also like to give a big thanks to Carrie Giles, a Med Tech at SAIT who was wonderful in helping us get things organized on site on the Friday. Without her help things would not have gone as smoothly as they did.

Finally, I would like to extend my thanks to all of the vendors who supported us in this venture. We had a record number of vendors in attendance, and one who sent door prizes even though they could not make the event. Without the vendor support we would not be able to produce these events, so please show your appreciation throughout the year when you are dealing with these vendors. The vendors have given us a tremendous amount of feedback and we will address your concerns and suggestions in planning future events.

Speaking of future events, the next Region IX Education Day is heading further East, all the way to the Maritimes. It had been suggested a couple of years ago that we host one ‘down East’ so we are investigating the possibility. It was suggested originally to have it in Halifax, however, in Calgary Moncton was also proposed as a possible site. What I really need are people from both areas to volunteer to help organize this event-it can’t all be done from the opposite side of the country. We need to nail down a location and date asap, so please, contact me if you are willing to help.

Mark Elliott mark@nshregionix.org
Region IX Education Committee, Chair
NSH Region IX Education Day
June 8, 9th, 2007 Calgary, Alberta

Over 85 participants

5 great speakers

Watch for our next Education Day in 2008!

17 Vendors

Great Door Prizes
Region IX Committee Announcement
Ann Lynde appointed Region IX Secretary

At this year’s Region IX meeting in Denver, Colorado, Ann Lynde will assume the position of Region IX Secretary, replacing Linda Rollins. Linda, will be embarking on traveling and retirement over the next few months and will not be able to continue her role as secretary.

I would like to take this opportunity to thank Linda Rollins for serving as Region IX secretary for the last year and wish Linda well in her travels and retirement plans.

Heather Nymeyer
Region IX Director

NSH has Moved!

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Region IX would like to thank Surgipath Canada and Russell Myers, Ph.D. for allowing The Cutting Edge to reprint “The Basic Chemistry of Hematoxylin”.

**The Basic Chemistry of Hematoxylin**  Russell Myers, Ph.D.

Staining protocols utilizing hematoxylin are the most commonly used of the routine staining procedures performed in the histology laboratory. In addition, hematoxylin based protocols are among the oldest in the laboratory as some of the formulations of hematoxylin based solutions date back over one hundred years. The value of hematoxylin lies in the capacity of aluminum hematoxylin solutions to stain the chromatin of cell nuclei. Although the recent advances in cell biology and molecular pathology have resulted in significant improvements in the practice of diagnostic pathology, the changes in chromatin patterns together with alterations of nuclear size and shape that are revealed with hematoxylin stains remain key diagnostic markers in the evaluation of pathological changes. In spite of the importance and relevance of hematoxylin in diagnostic pathology, the chemistry of hematoxylin remains poorly understood. The following article is a review of the current state of understanding of the chemistry of aluminum based hematoxylin stains and the mechanism by which these stains act.
Oxidation and Formation of Hematoxylin

Hematoxylin is a natural product extracted from the heartwood of the legwood tree (Haematoxylon campechianum). Hematoxylin is relatively colorless and without further modifications has little or no value as a biological stain. The use of the word “hematoxylin” to describe a staining solution is somewhat misleading but continues to be used as a term of convenience. To produce a functional dye, hematoxylin is oxidized to hematein and subsequently is bound to one of several metal ions including aluminum (Al+++), iron (Fe+++), and chromium (Cr+++). A metallic ion bound to a dye that is involved in the binding of the dye to tissue is referred to as a mordant. The present article is concerned specifically with the chemistry of aluminum bound hematoxylin as a nuclear stain.

Conversion of hematoxylin to hematein may be accomplished by the action of a number of agents. Several older formulations such as Delafield’s and Ehrlich’s rely on atmospheric oxygen for oxidation. In these formulations, hematoxylin and aluminum salts are stored in loosely capped or cotton plugged bottles to facilitate oxidation. The process of bubbling air through hematoxylin solutions also has been used to speed up the oxidation process. Due to the extended period of time (4-10 weeks) necessary to “naturally ripen” or oxidize hematoxylin under these conditions, these procedures are rarely if ever used today. Currently most formulations incorporate a chemical oxidant such as sodium iodate that rapidly converts hematoxylin to hematein. Concentrations of sodium iodate typically are based upon the amount of hematoxylin and usually range from 0.10 to 0.20 grams of sodium iodate per gram of hematoxylin. Mercuric oxide was used as the oxidant of choice in Harris formulations for many years but is no longer used due to environmental issues concerning mercury. Today, mercuric oxide is replaced with sodium iodate in modified Harris formulations.

In contrast to hematoxylin which has been well characterized structurally, the molecular structure of hematein has been debated in recent years. The traditional structure of hematein which includes a quinoid ring is depicted in Figure 1. Regardless of the exact structure of hematein, we can assume that hematein is structurally similar to hematoxylin based upon NMR analysis and similarities in molecular weight. The change in structure results in the conversion of the relatively colorless hematoxylin to the redish/brown hematein. In addition, the oxidation to hematein is necessary in order to bind metal ions such as aluminum.

Dye Lake Formation

A number of different aluminum salts are used as a source of Al+++. These include aluminum ammonium sulfate [AlNH₄(SO₄)₂], aluminum sulfate [Al₂(SO₄)₃] and aluminum potassium sulfate [AlK(SO₄)₂]. Mayer’s and Harris formulations typically contain AlNH₄(SO₄)₂ or AlK(SO₄)₂ while Gill formulations contain Al₂(SO₄)₃. There is in fact probably little difference among the aluminum salts other than the amount of Al+++ that each is capable of contributing to the solution. AlNH₄(SO₄)₂ and AlK(SO₄)₂ have limited solubility in aqueous solutions and under most conditions do not completely ionize to produce free Al+++.
While it is generally accepted that hematein forms a complex or "lake" with Al\textsuperscript{+++}, the nature or structure of this complex has yet to be fully elucidated. The ratio of Al\textsuperscript{+++} to hematein molecules as well as the overall charge of the hematein Al\textsuperscript{+++} complex has been a subject of debate over the past 50 years. Most evidence, however, supports the concept that hematein-Al complexes are positively charged or "cationic" at most staining conditions.

In addition to the oxidants and aluminum salts described above, hematoxylin solutions often contain other additives or agents. Modifications to the solvent are made by the addition of polyhydroxy alcohols such as glycerin or more commonly ethylene glycol. Initially it was proposed that the polyhydroxy alcohols acted as stabilizers to reduce the over oxidation of hematein. In fact, however, these additives primarily act to increase the solubility of hematein and the hematein-Al complex within the solution. Although hematoxylin is relatively soluble in aqueous solution, hematein and hematein-Al are only sparingly soluble in water. Hematein and hematein-Al may precipitate and form a sediment or a metallic sheen on the surface of the solution. The inclusion of ethylene glycol in the solution reduces the precipitation or deposition of these products and thus increase the stability or shelf-life of the hematoxylin solution.

Various acids are sometimes added to hematoxylin solutions to reduce the pH. Acetic acid is added to Gill type hematoxylin while citric acid is found in Mayers. The addition of acids to hematoxylin solutions increases the selectivity of the stain for nuclei by reducing nonspecific background staining. The effects of reduced pH on staining is discussed in greater detail in the section "Mechanism(s) of Staining".

**Mechanism(s) of Staining**

A number of varied theories have been proposed to account for the staining of nuclei by hematein-Al complexes. Nuclear molecules that have been proposed to bind hematein-Al complexes include basic (positively charged) proteins such as histones as well as deoxyribonucleic acid (DNA). There is currently little evidence to support the suggestion that basic nuclear proteins bind hematein complexes. Evidence is more consistent with the theory that the hematein-Al complexes bind DNA. Evidence to support this theory include studies utilizing DNase enzymes. These studies have demonstrated that digestion of nuclear DNA prevents staining of all nuclei with hematein-Al solutions.

The attraction or binding of hematein-Al to DNA is likely due to the electrostatic attraction of the cationic hematein-Al complexes for the phosphate groups as these groups carry a negative charge at staining conditions. This theory is also consistent with the observation that other anionic sites such as those found in mucins and proteoglycans may create background staining by binding hematein-Al. In fact, background staining may be reduced by lowering the pH of the solution of hematein-Al solutions. A reduction of pH can be expected to protonate (addition of H\textsuperscript{+}) many of these sites such that they become neutral. The fact that these substances at a neutral charge no longer bind the positively charged hematein-Al complexes further substantiates the role of electrostatic forces as mediators of hematein-Al binding.

It has been suggested that simple electrostatic forces alone are not sufficient to account for the staining of nuclei with Al/hematein solutions. This theory is based upon the belief that the electrostatic forces mediate the initial attraction of the Al/hematein complexes for DNA but the electrostatic bonds are replaced with more stable covalent or coordinate covalent bonds. The greater stability of the covalent type bonds would account for the relative permanent nature of Al/hematein staining. There is, however, little direct evidence to support the role of covalent bond formation in the binding of hematein-Al complexes to DNA.

*Russell Myers, Ph.D., is Vice President of Immunohistochemistry at Surgipath Medical Industries, Inc.*
Final Call
NSH T-SHIRT CONTEST 2007

Each year NSH sponsors a Best T-shirt Design Award; Runner up for Best T-Shirt Design; Most number of attendees wearing a state or region T-shirt.

Design a T-shirt that Region IX members will proudly wear to the S/C in Denver, CO and you could win two bottles of BC Red and White wine. E-mail your designs by Sept 10, 2007 to:
Heather Nymeyer (heather@nshregionix.org) or
Rose Clarke (rose@nshregionix.org)

or if submitting designs by postal delivery pleases address them to
Heather Nymeyer
Region IX, Director
1176 Pleasant St
Kamloops, BC, V2C 3B9

The winning design will be printed in time for the S/C in Denver. We are hoping that the members attending the S/C will purchase these T-shirts to proudly represent our Region IX during the T-shirt competition. T-shirt costs will kept at a minimum and will be on sale at the Region IX meeting in October at the S/C.

Calgary Education Attendees
NSH Continuing Education Certificates

By now, all Calgary attendees should have received from the NSH a certificate indicating their CE Credits earned at this event. If you have not received this certificate, members can access their CEU credits by going to www.nsh.org and clicking on Members Only. You will be required to enter your password and the e-mail address on file at the NSH office.

NSH Region IX would like to thank Surgipath Canada for their continued sponsorship of The Cutting Edge Newsletter.
NSH S/ C Special Events

- **First Time Attendee Breakfast**  
  Sunday, October 28, 2007  
  7:00am - 8:00am  
  Adam's Mark Denver

- **National Society For Histotechnology Annual Awards Banquet**  
  Sunday, October 28, 2007  
  6:00pm  
  Adam's Mark Denver

- **NSH Official Meeting Schedule**  
  See Complete Schedule for Times & Dates  
  Committee meetings are open to all convention attendees. We invite you to become involved in the operation of the society and attend the meeting of your choice. The Board of Directors and House of Delegates will have an observation gallery for members to view their Society in action.

- **Poster Sessions**  
  Posters will be viewed Sunday through Tuesday (Oct 28-30)  
  Colorado Convention Center Hall

- **Annual T-Shirt Contest**  
  Tuesday, October 30, 2007  
  12:00pm  
  Exhibit Hall

- **Guess the Member Count**  
  Drawing Tuesday, October 30, 2007

- **Celebrate the Journal of Histotechnology Turning 30!**  
  All Week

- **Surgipath Medical Industries, Inc. Hospitality Event**  
  October 30, 2007  
  8pm - Midnight  
  Hyatt Regency Denver Capitol Ballroom
HI STOLOGY JUMBLE

Unscramble these four jumbled words to form new words. Then by using the letters that appear in the circles, complete the following phrase:

SHOEVEFRF

ONBUSI

GELATINC

TARESUAD

A SLOGAN THAT A HI STOLOGY DEPARTMENT CAN DISPLAY?

HI STOLOGY: _ _ _ _ _ _ _ _ _ _

Answer on page 11

THE CUTTING EDGE is the official newsletter of Region IX of the National Society for Histotechnology. It is distributed quarterly to 350 members in Canada.

The Objectives of THE CUTTING EDGE are to:

• Reflect both Canadian and North American articles pertinent to the practice of Histotechnology
• Publish articles that are beneficial to our discipline.
• Promote communication between Region IX members and members of the other NSH Regions
• Provide a medium for exchange of information among members.

Contributions are welcome. Please send your articles to:

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Production of The Cutting Edge is sponsored by Surgipath Canada Inc.

www.nshregionix.org

Newsletter Submissions
If you would like to publish an article, create a puzzle or announce a meeting event send your submissions to Heather or Mark.
MISSION STATEMENT
The National Society for Histotechnology is a non-profit organization, committed to the advancement of Histotechnology, its practitioners and quality standards of practice through leadership, education and advocacy.

HISTOLOGY JUMBLE SOLUTION

SHOEVERFRF = VERHOEFF’S
ONBUSI = BOUINS
GELATINCH = CHELATING
TARTESTUAD = SATURATED

A SLOGAN THAT A HISTOLOGY DEPARTMENT CAN DISPLAY?
HI STOLOGY: A CUT ABOVE THE REST!

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MEMBERSHIP APPLICATION
2007-2008 Membership Year

TYPE OF MEMBERSHIP:

☐ RENEWAL ☐ NEW MEMBER ☐ PROFESSIONAL ☐ STUDENT ☐ RETIRED ☐ INTERNATIONAL

Program Director/Supervisor Name (required for Student Status Only):

MEMBER NAME: __________________________

HOME ADDRESS:

Address: __________________________________________

City: ____________________________________________

Province/State: _______ Zip/Postal Code: __________

Country: ____________________________

HOME TELEPHONE: ____________________________

PERSONAL EMAIL: ____________________________

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Please Note: All NSH Correspondence will be sent to this address. In addition, this address is published in our online membership directory.

WORK ADDRESS:

Company: ______________________________________

Department: ____________________________

Title: ____________________________

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Country: ____________________________

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FAX: ____________________________

Work Email: ____________________________

Referred by NSH Member:

________________________________________

State Histology License Number (if applicable)

________________________________________

MEMBER DEMOGRAPHICS:

Date of Birth: _____/_____/______ Gender: ☐ Female ☐ Male

Year entered Profession: _________ I am a Manager/Supervisor: ☐ Yes ☐ No

CHECK ALL APPLICABLE BOXES:

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☐ RT (CSMLS) ☐ ART (CSMLS) ☐ MLT (CSMLS) ☐ NOT CERTIFIED ☐ OTHER

HIGHEST LEVEL OF EDUCATION:

☐ AA ☐ BA/BS ☐ MA/MS ☐ PHD ☐ MD ☐ DVM ☐ OTHER

I practice Histology in (choose one):

☐ University ☐ Hospital ☐ Private Lab ☐ Veterinary ☐ Marine ☐ Botany ☐ EM ☐ Research ☐ Industrial

PAYMENT: Remit fee with completed application in US funds to the NSH national office: 4201 Northview Drive, Suite 502, Bowie, MD 20716.

Circle Membership Dues: $60.00 (Professional/Intl) $30.00 (Student/Retired)

Circle Membership Pin ($30.00): Gold Gold/Blue No Thanks

Optional ADA Fund Contribution $__________ (Your donation to the ADA will aid in furthering the education of the physically challenged.)

Total Due $__________

☐ A check for the total amount due payable to “NSH” is included with this application.

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