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Newsletter of the Histotechnology Group of NSW

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Editorial

It is not long before our AGM and then the State meeting in Canberra. Mark both dates in your planner. We have been privileged to have heard two excellent presentations in the past few months. The first from Dr. Janice Brewer from Royal North Shore Hospital, gave an informative talk on Muscle Histochemistry. This month, Professor John Pollard from Sydney University presented Nerve biopsies. Even if most labs do not routinely process such specimens, it is important to understand the techniques and problems involved. You never know when you will get that querying phone call! A big thanks to Dako for their support.

Again I hope you find the bubble and squeak ("Bit of Everything") that makes up your journal of interest. If not email the editor and give him a serve. The crossword is a challenge, in fact a bottle of wine to the first correct answer received by the chairman (trevor.hinwood@hdscientific.com.au).

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Chairman's Report

Trevor Hinwood, Chairperson, Histology Group of NSW.

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Fixation Update

There has been a good deal of research into a replacement for formalin in the past few years. There has been a concerted search for a less toxic fixative. The following is a review of some of these developments.

Glyoxal Fixation

Glyoxal is a dialdehyde that behaves quite similarly to formaldehyde. Glyoxal containing fixatives are usually aqueous and buffered to pH 4 and contain a small amount of alcohol. It has been claimed that glyoxal is the most common replacement for formalin in North America (1). It is safer than formaldehyde since it essentially has no vapour pressure but skin and eye contact still needs to be avoided.

Richard Dapson et al (1) have published a review of glyoxal fixation and I highly recommend it as an exhaustive treatise on the subject. One pathologist (Rob Richmond) has expressed some concerns on Histonet. In response, Ada Feldman from ANATECH addressed some of these issues:

Interchangeability of glyoxal products.
Richmond: Prefer is described as a buffered solution of glyoxal with a pH of about 4. The formula is a trade secret. Competing glyoxal products probably also have trade-secret formulas. So can a lab change brands of buffered glyoxal without problems, or does it have to stay with a particular brand with its trade-secret buffering? - We've seen a similar problem with distilling aliphatic xylene substitutes: every one of them requires a separate distillation routine, at least on a spinning-band still. Will this acidic fixative be detrimental to later DNA or RNA extractions?
Feldman: The manufacturer of each fixative should be able to provide the information necessary to work with their fixative in conjunction with any other fixative that a lab may be using. Just as an example, Hollande's is not compatible with NBF and requires special attention. As for Prefer we can say it is compatible with the majority of other fixatives, glyoxal or not.

Limited time in the fixative. Richmond: Tissue can be left in neutral buffered formalin for quite a long time and still be stainable, but tissue stored in glyoxal becomes unstainable after about two weeks. Can glyoxal fixed tissue be transferred to 70% ethanol for more prolonged storage? - A very occasional surgical specimen requires additional blocks after a week - a bigger problem will be the pathologist who doesn't trim his autopsies promptly. (*Note: Dapson et al (1) did find that after two weeks, blocks lost their ability to stain with H&E and prolonged storage caused leaching of iron pigment. Silver stains for helicobacter were adversely affected).*
Feldman: There is a slight reduction in staining intensity after several weeks, but increasing staining time corrects this. So tissues are not rendered unstainable.

Transition period.
Richmond: A laboratory changing to glyoxal would have to keep IHC procedures for both fixatives working for some time. There would have to be some way to identify whether a block was fixed in formaldehyde or glyoxal.
Feldman: This is true any time you are switching fixatives or processing methods.

Eosinophilia. Richmond: - One ought to be able to distinguish eosinophils from neutrophils in tissue sections

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by nuclear morphology, without having to see granules. But quantitation of eosinophils - needed in an increasing number of GI biopsy situations - could be a problem. We might need an IHC for eosinophils in some of these settings.
Feldman: Your statements here are true.

Lysis of erythrocytes.
Richmond: Not much of a problem, since we're used to it with acid fixatives anyway.
Feldman: True again. It is often seen as an advantage because diagnostics cells are easier to see.

Breast cancer. Richmond: Elimination of nuclear bubble artefact in breast biopsy specimens may raise the apparent nuclear grade of tumours, and thus increase Nottingham (Elston-Ellis) scores.
Feldman: It would seem that the improved nuclear morphology would make marked nuclear variation easier to determine.

Prostate biopsies.
Richmond: I'd want to see some prostate biopsies fixed with glyoxal since with formaldehyde fixation, nucleoli are a strong criterion of malignancy, and if glyoxal fixation demonstrates nucleoli in benign ductal epithelium, this criterion is lost.
Feldman: Hope you can get a response from any of the glyoxal users with prostate biopsies. We would be interested in this answer also.

Ionic Fluids Fixation

Pernaka et al (2) from Poland have advocated the use of ionic fluids as replacements for formalin.

Ionic liquids (ILs) are a novel class of compounds, and are called solvents of the 21st century. They are molten organic salts, typically comprised of bulky cations and weakly coordinated anions. Some of the better studied ILs contain a heterocyclic cation based on a substituted imidazole or pyridine. They have advantageous chemical and physical properties, such as a negligible vapour pressure, low melting temperature (<100 °C), a broad liquid range, thermal, chemical and electrochemical stability, favourable solvation behaviour (many organic, organometallic and inorganic compounds can be dissolved in ILs) and have a large electrochemical window. They are also highly polar yet non-coordinating, non-flammable, easy to handle in a variety of standard experimental procedures and they are recyclable. This non-volatile nature means that ILs have been recognized as ‘green’ solvent alternatives to volatile organic solvents, and it decreases the risk of exposure and loss of solvent by evaporation. Careful selection of cation–anion pairs allows control of several chemical and physical properties of ILs. Moreover, it is possible to fine-tune their miscibility with water by changing the characteristics of side chains in the cation or the type of anion. In recent years, the number of possible cation and anion combinations has increased significantly. Pernaka et al (2) have categorized ILs, into room temperature ionic liquids (RTILs), protic ILs, task-specific ionic liquids (TSILs) and chiral ILs (2).

They found that 1-methyl-3-octyloxymethylimidazolium tetrafluoroborate (IL–[($C_8H_{17}OCH_2$)MIM][BF4]) exhibited tissue fixation properties similar to those of formalin and can be applied in both histological and immunohistochemical techniques. Tissue material fixed with [($C_8H_{17}OCH_2$)MIM][BF4] manifested a more intense staining than those fixed with formalin. With respect to the pattern, distribution and intensity of immunohistochemical staining, it was comparable in tissue material fixed with formalin or [($C_8H_{17}OCH_2$)MIM][BF4]. The time of fixation in formalin or IL had no effect on morphology.

Their study confirmed the suitability of 1-methyl-3-octyloxymethylimidazolium tetrafluoroborate as fixative in histopathological procedures, eliminating the necessity of using formalin.

HOPE Fixation

The HOPE (Hepes-Glutamic acid buffer mediated Organic solvent Protection Effect) technique comprises a new protection-solution with an organic buffer, with acetone as the only dehydrating agent, and pure paraffin of 52–54°C melting temperature, allowing for all pathologic routine investigations. In contrast to FFPE tissue, the HOPE-technique allows for the application of molecular methods, such as high molecular DNA and RNA isolation, which can be used for PCR and reverse transcription PCR (RT-PCR) (3).

There is some debate as to whether HOPE is an actual fixative. Essentially, HOPE-fixed sections show formalin-like morphology. However, the sections are somewhat difficult to handle because of their fragility. This is due to the absence of any type of protein cross-linking and the dynamic processes of immersion and outflow of the HOPE protection solution. HOPE-fixed sections provide an excellent preservation of proteins and antigenic structures for differential analysis by immunohistochemical and/or enzyme histochemical techniques. However, their most remarkable feature is the extremely low degradation of nucleic acids (DNA and RNA) combined with good results obtained by in situ hybridization techniques (4).

Honey Fixation

Recently Al-Maaini & Bryant (5) presented the use of honey as a substitute for formalin in fixation. Honey has been known for many years to be a successful antibacterial agent. They found that honey concentrations from 10-20% at room temperature gave comparable results to those tissues fixed in 10% formalin.

This paper caused a good deal of controversy on Histonet and some of the concerns were:

1. The honey used came from a source that would not be readily available to everybody, which means that tests with other types/sources of honey will be necessary;
2. There are no comparative methods, no statistical analysis of the results or documented comparisons with standard procedures, and
3. If to substantiate the results we have to judge by the photomicrographs, they are of a very poor quality and seem to reflect poor processing. That poor processing could be due to the processing protocol itself or to the honey fixation.
4. If honey collected from plants high in oestrogens would affect any type of oestrogen staining

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3. Goldmann et al (2004) “The HOPE-technique permits Northern blot and microarray analyses in paraffin-embedded tissues” Pathology - Research and Practice 200(7-8): 511-515
4. Olerta et al (2001) “HOPE Fixation: A Novel Fixing Method and Paraffin-embedding Technique for Human Soft Tissues” Pathology - Research and Practice 197(12): 823-826.
5. Al-Maaini & Bryant (2006) “The Effectiveness of Honey as a substitute for Formalin in the Histological Fixation of Tissue” Journal of Histotechnology 29(3): 173-9.

Human Hair Balls?

Hair Balls are usually found in the stomachs of cows and cats but did you know that can also occur in humans?

Every cat owner knows about hairballs--usually you "find" them in the middle of the night after you've stepped into a pile of something that looks and feels like anything but hair. Did you know that cats aren't the only ones to get hairballs? Humans and cud-chewing animals, such as cows, oxen, sheep, goats, llamas, deer, and antelopes get hairballs or other types of "bezoars" (pronounced BE zor). In fact, the Americans even commemorate National Hairball Awareness Day on April 27.

A bezoar is a mass of non-digestible matter that collects in the stomach (1-4). There are four types of bezoars:

- Trichobezoar: a mass of hair. One variant of trichobezoars is the "Rapunzel syndrome." This is a trichobezoar extending continuously from the stomach through the entire length of the small intestine. They are more hazardous than trichobezoars in that the twisted hairs develop a wire-like consistency. Peristalsis causes the tightly twisted hairs to be bowstringed tightly against the mesenteric wall of the intestine, and multiple perforations may develop due to pressure necrosis (3).
- Phytobezoar: a mass of undigested food particles usually from fruits and vegetables such as celery, pumpkins, grape skins, prunes and raisins. A special subset of phytobezoar is diospyrobezoar, an unusually hard bezoar formed in patients who ingest unripe or unpeeled persimmon fruits, and small seasonal outbreaks of diospyrobezoars have been reported during particularly bountiful harvests
- Lactobezoar: a mass of undigested milk, most commonly found in pre-term infants on highly concentrated formula
- Pharmacobezoar: a mass of medications such as extended release medicines or bulk-forming laxatives

"Bezoar" is a Persian word that means "protection from poison" because bezoars were believed to be a universal antidote against any poison. Bezoars from wild goats, antelopes, and other cud-chewing animals of Persia were introduced to Europe in the 11th century where they were popular in medicinal remedies until the eighteenth century. In China, ground-up cow bezoars have been used as medicine for over two thousand years, particularly to treat diseases of the mouth.

Bezoar stones were highly prized and could be worn as charms set in gold.

Human hairballs occur most often in children and young women who have mental disorders such as trichotillomania (chronic hair pulling) or pica (compulsive cravings to eat non-food items). Previous gastric surgery, poor mastication, overindulgence of foods with high fibre contents are

common factors predisposing to Bezoar formation. Bezoars may also be formed from almost anything else imaginable. Cases have been reported of bezoars due to peanuts, olive stones, shellac, tar, Gummi bears, sand, and toilet paper (3). A case of acute formed gastric bezoar has also been reported in a patient who attempted suicide by ingesting plaster.

Hairballs are difficult to diagnose, but some symptoms include cramps, loss of appetite, weight loss, and bad breath. They are rare in people with a normal digestive tract, but occur more often when people have pre-existing conditions such as diabetes, cystic fibrosis, renal failure, or a vagotomy or partial gastric resection. Most hairballs must be removed surgically.

Special endoscopic tools have been used to fragment very hard bezoars, bezotomes or bezotriptors, as well as lithotriptors and even lasers. Recently it has been reported, that gastric lavage with Coca Cola or direct injection of smaller amounts of Coca Cola directly into the bezoars is an effective, safe and cheap treatment.

References:

1. Kruse A (2006) downloaded from http://www.gastrosource.com on 4/10/06.
2. Erzurumlu K, et al (2005) Gastrointestinal bezoars: A retrospective analysis of 34 cases. World J Gastroenterol 11(12): 1813-1817.
3. http://www.ddc.musc.edu/ddc_pro/pro_development/case_studies/case040.htm.
4. Yasar, M., et al (2005) Kehr Sign with Gastric Bezoar: A Rare Case Report. The Internet Journal of Surgery. Volume 6 Number 2. http://www.ispub.com/ostia/index.php?xmlFilePath=journals/ijs/vol6n2/kehr.xml

Bomb squad safely detonates 90-year-old sample of acid in Wisconsin

COLUMBUS, Wisc. (AP) - It's not often that a bomb squad is asked to help clean up a pharmacy.

But experts say 57-grams of picric acid in the basement of a Wisconsin drug store packed the punch of nearly half a stick of dynamite. Store employees found the sample as they were cleaning out old chemicals. Store owner Nick Sharrow is relieved that the local bomb squad safely destroyed the sample, adding the substance is very similar to TNT.

Picric acid is not volatile as a liquid. But Sharrow says it can form highly explosive crystals as it ages. The sample discovered on the basement shelf dated back to around First World War. Picric acid was used as an antiseptic and in medical formulations to treat malaria, herpes and smallpox.

The drug store and apartments above were evacuated and the street closed to traffic during the removal.

The Canadian Press, Monday, April 16, 2007 *http://www.cbc.ca/cp/Oddities/070416/K041602AU.html*

HISTOTECHNOLOGY GROUP of NSW

ABN: 63-128-868-343

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P.O. Box 496
GUILDFORD NSW 2161
27 April, 2007

The Histotechnology Group of NSW invites you to attend its next weekend seminar which is to be held at Rydges Hotel, Canberra on 21st – 23rd September, 2007. This seminar is an excellent opportunity to meet many fellow histotechnologists not only from NSW but also interstate and find out the answers to all those problems you're having.

Please find enclosed a registration form. **Registration** includes all meals except Saturday night dinner which will be a special Floriade celebration. Please return your registration with payment by cheque or money order made payable to the "Histotechnology Group of NSW" to the Secretary by 1 September, 2007. You may also pay online, but please send your registration form by post to the above address or by fax: Attn Kathy Drummond 9855 5169

BSB: 802 084
Account no: 94099
Account name: Histotechnology Group of NSW

Please note: **accommodation** must be booked individually and confirmed by 7th September, 2007. Check into accommodation is after 1.00 pm and check out is before 10.00 am.

A copy of the provisional program is attached.

You will be able to participate in a cut up workshop (not a wet workshop and not hands on). Finding out what is expected and required by technicians who perform cut up and a review of case studies.

As usual there will be **competitions** and the **quiz** for which there will be prizes and the **"Hat Trophy"** for the best outfit on Saturday night as well as a **Lucky Door prize.** There are also prizes for the poster competition.

For further information please contact:

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We look forward to seeing you in September at Canberra.

Yours sincerely,

Kathy Drummond
(Secretary)

Histotechnology Group of NSW Annual Scientific Meeting

Canberra 21 – 23 September 2007 Provisional Program

Note for Friday workshop: You will need to register for the whole day

DAY	TIME	TITLE	SPEAKER
Friday	9.00-10.30	Scientific Cut up for Technicians Reviewing the UK system and the South Australian course	Tony Woods
	10.30-14.45	Morning Tea	
	14.45-12.30	Scientific Cut up for Technicians. Protocols, How, When, Where, Why	Grant Taggart
	12.30-13.30	Lunch	
	13.30-15.00	Scientific Cut up for Technicians Review of morning sessions including questions.	Tony Woods/ Grant Taggart
	15.00-15.15	Afternoon Tea	
	18.00	Cocktail Party	Trade Display Opening
Saturday	8.30-9.00	Registration	
	9.00-9.15	Opening	Sat Morning Chair
	9.15-9.45	Abstracts of Posters	Sat Morning Chair
	9.45-10.00	CSI – Canberra	TV stars
	10.00-10.20	Proffered Papers – 2 x 10 mins	Sat Morning Chair
	10.20-10.45	Trade Talks	
	10.45-11.10	Morning Tea	
	11.10-11.30	Forensic Dentistry	Dr David Griffiths
	11.30-11.50	Difficult stains – bring your problem stains to us!	Panel Discussion
	11.50-12.10	Histology education	Panel Discussion
	12.10-13.20	Lunch	
	13.20-13.50	IPX and Flow on Bone Marrow Trephines	Dr Dipti Talaukar
	13.50-14.10	Botanical Histology	Andrew Thornhill
	14.10-14.30	Sarcomas	Dr Chris Hemmings
	14.30-14.50	Alveolar Capillary Dysplasia	Rhonda Henley-Smith
	14.50-15.20	Trade Talks	
	15.20-15.45	Afternoon Tea	
	15.45-16.05	Entomological Histology	Eric Hines CSIRO
	16.05-16.40	Drug Development and Oncology links with Histology	Prof Chris Parish With Dr Yip
	17.30	Happy Hour	
	19.00	Dinner “What’s at the bottom of your Garden”	
Sunday	9.00-9.30	Registration	
	9.30-9.35	Opening	Sun Morning Chair
	9.35-9.55	Tough Love – Preparing bone and other tough tissues	Penny Whippy
	9.55-10.15	Trade Talks	
	10.15-10.35	WT1 as a complementary marker of melanoma	Bharathi Cheerala
	10.35-11.00	Morning Tea	Sun Morning Chair
	11.00-11.30	Quiz	Sun Morning Chair
	11.30-12.00	Trade Talks	
	12.00-12.20	Why we need recuts, etc.	Dr Genevieve Bennett
	12.20-12.40	Forensic Histology	Elizabeth Brooks
	12.40-13.00	Quiz and Poster results. Close and parting words	Trevor Hinwood
	13.00	Lunch	

Immunohistochemistry - The Overlooked

How Does Antigen Retrieval Work?

The introduction of antigen retrieval has enabled immunohistochemistry to become an integral component of morphologic diagnosis, routinely employed in cancer diagnosis, and for the identification of therapeutic and prognostic markers. The mechanism of antigen retrieval, as reviewed by Leong and Leong (1) however, remains speculative with the key to our understanding embedded in the actions of formaldehyde on proteins. One commonly held concept is that heat primarily breaks down protein cross-linkages that occur with aldehyde fixation, thus "unmasking" protein epitopes of interest. Enzymatic pretreatment is also thought to have a similar action whereas such "breakages" are the result of extremely rapid molecular movement induced by microwaves and ultrasound. The formation of rigid cage-like calcium complexes during formaldehyde fixation is another suggested mechanism of antigen masking requiring chelating agents for reversal. A more recent suggestion for the antigen retrieval phenomenon has evoked the Mannich reaction, which occurs with the cross-linking of some proteins. Such cross-linkages can be hydrolysed by heat or alkalis so that the process of antigen retrieval may be the simple removal of such cross-linked proteins that are sterically interfering with the binding of antibodies to linear protein epitopes in the tissue section. We are clearly not yet in possession of all the answers to the problem.

Electrostatic Forces

Thomas Boenisch (2) has demonstrated that electrostatic charges, i.e., net negative on antigens and net positive on antibodies, play an important part in immune reactions and therefore should be given greater attention.

Boenisch (2) and his team at DAKO stained 15 antigens both with and without the use of AR at the diluent pH of 6.0 and 8.6, respectively, and with increasing concentrations of cations. Without the use of AR, they found that antigens reactive with monoclonal antibodies of subtype IgG1 stained most intensely at the slightly acidic pH, whereas antigens reacting with monoclonal antibodies of subtypes IgG2a and IgG3 stained strongest at the alkaline pH of 8.6. When they did use AR, the IgG2a antibodies also stained strongest at pH 6, whereas optimum staining with a subclass IgG3 antibody remained at pH 8.6. They believe that this reversal of optimal staining from pH 8.6 to 6 with antibodies of subclass IgG2a is an indication of the antigen's recovery of lost negative charges during AR.

Varying the concentration of Na^+ ions (in the form of NaCl) can markedly influence staining intensities at all antibody concentrations. Larsson (2) referred to the presence of inorganic cations as forming a ''shield'' around the negatively charged antigens, thereby reducing or obstructing the attraction of the positively charged antibodies. (Boenisch also adds that adding an identical concentration of K^+ ions had an even stronger detrimental influence, but it is not known why.) It is also noteworthy to mention that phosphate buffered saline, a widely used antibody diluent, had one of the most pronounced negative effects on staining intensities. This makes sense because this diluent not only contains Na^+ ions from NaC^+ but also the monosodium (NaH_2^+) and disodium (Na_2H^+) cations of the phosphate buffer. Increasing additions of NaCl to Tris buffers also had an increasingly suppressive

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effect on staining intensities (2).

Albumin – foe & friend

Mittelbronn et al (3) studied the effect that albumin had on immunohistochemistry. They found that the most important effect concerning background staining reduction could be significantly attributed to the omission of albumin which usually is recommended as a reducer of background stainings.

However, in contrast to this negative effect, albumin could also increase specific staining intensity. Mittelbronn et al (3) recommend the careful use of albumin in immunohistochemistry because of the dichotomous effects mentioned above. Furthermore, their results imply that in case of a good specific staining pattern, the use of albumin in immunohistochemical solutions merely exerts significant negative background staining effects.

Japanese Antigen Retrieval

Namimatsu et al (4) from Tokyo have developed an antigen retrieval method based on the use of citraconic anhydride (a reversible protein cross-linking agent).

In general, formalin-fixed tissues demonstrated specific immunostainings comparable to that in fresh frozen tissues and significantly more enhanced than after conventional antigen retrieval methods. In particular, even difficult to-detect antigens such as CD4, cyclin D1, granzyme β, bcl-6, CD25, and lambda chain revealed distinct immunostainings. Different classes of antigens such as cellular markers and receptors, as well as cytoplasmic and nuclear proteins, consistently produced enhanced reactions.

References:

1. Leong and Leong (2007) Advances in Anatomic Pathology. 14(2):129-131.
2. Boenisch (2006) J Histochem Cytochem 54:961–964
3. Mittelbronn et al (2006) Appl. Immunohist Molec Morph 14(4):441-444.
4. Namimatsu et al (2005) J Histochem Cytochem 53:3–11.

Qualities Most Appreciated In a Boss or Supervisor

Recently readers of ADVANCE for Medical Laboratory Professionals (May 23, 2007) were asked to list those qualities they most appreciate in a boss or supervisor. How do we measure-up?

Honesty, integrity, ability to communicate clearly, ability to listen and give constructive criticism, and ability to delegate and trust that I can do the job
The ability to follow through is the quality I admire the most in a supervisor. The old saying that goes "Say what you mean and mean what you say" also extends to "If you say you are going to do something, then do it." Accountability goes a long way in establishing healthy working relationships and trust.
Flexibility. I work in a small lab and that is all I have ever known. As a 25-year old MLT, I would like to see more supervisors willing to work with young lab professionals to further their educations.
Open, level, honesty—to be treated with the respect that my years in the profession should award
Fairness to all—that he/she treats all individuals fairly and equally, be they a superior, doctor. or employee
Skills—that he/she is able (and willing) to do anything that needs to be done in the lab. Do not ask me to do something that you cannot or will not do yourself.
Ability to listen intently, delegate effectively, communicate and refrain from micro management (a manager who has to have a finger in everyone's pie).
He or she should be a problem solver, both technically and employee related; open-minded; confident; a great communicator; honest with integrity; able to bring new instrumentation in-house; encouraging; a team player; and encourage all educational opportunities for staff.

Whipping Disease

(Sorry I meant Whipple's Disease)

Whipple's disease is a rare bacterial disease that can be fatal if not treated. Unfortunately the clinical symptoms are often confusing and beset with "red herrings". Arthritis or arthralgia may be the only presenting symptom, predating other manifestations by years. Diarrhoea, weight loss and abdominal pain often occur sign-posting a possible area of investigation (1, 2).

The first case of what is now termed Whipple's disease was reported by Allchin and Hebb in 1895. In 1907 George Hoyt Whipple, the first American Nobel Laureate in physiology, described a 36 year old medical missionary with transient polyarthritis, fever, tiredness, and weight loss who then developed diarrhoea. Whipple attributed the illness to intestinal lipodystrophy caused by abnormal fat metabolism. The work of Paulley in England established the critical relation between antibiotic treatment and disease remission, and in 1961 "bacillary bodies" highly suggestive of an infectious agent were described (1).

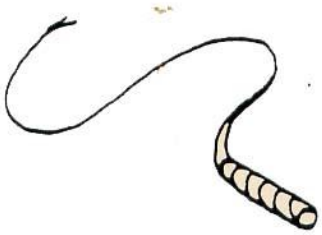
Wilson's group elaborated on the infective agent of Whipple's disease, using nucleotide sequencing and amplification by reverse transcriptase polymerase chain reaction (PCR) of bacterial 16S ribosomal RNA from the small intestinal biopsy of a patient with Whipple's disease. They identified a previously unknown organism related genetically to *Rhodococcus*, *Streptomyces*, *Arthrobacter*, and more weakly to *Mycobacter* species. Owing to the lack of similarity to any known genus and based on DNA sequencing and phylogenetic relations and its distinct morphology, the bacillus was assigned by Relman et al (3) to a new genus and species and termed *Tropheryma whippelii*, from the Greek words *trophe* (nourishment) and *eryma* (barrier), and George Whipple's surname. The organism is Gram positive, periodic acid Schiff (PAS) positive, and diastase resistant (1).

The characteristic histopathological features of Whipple's disease are found most often in the small intestine. These are variable villous atrophy and distension of the normal villous architecture by an infiltrate of foamy macrophages with a coarsely granular cytoplasm, which stain a brilliant magenta colour with PAS. These pathognomonic PAS positive macrophages may also be present in the peripheral and mesenteric lymph nodes and various other organs. The characteristic cytoplasmic granules are clumps of *Tropheryma whippelii* and their degradation products. Another feature is rounded empty spaces scattered through the lamina propria that contain neutral fat, described as intestinal lipodystrophy by Whipple in 1907. The histological differential diagnoses include *histoplasmosis* and *Mycobacterium avium-intercellulare complex* (1). The bacteria are ZN and Methenamine silver negative (2).

Confirmation of the diagnosis requires electron-microscopical evidence of the characteristic rod-shaped bacteria with trilamellar membrane or a polymerase chain reaction (PCR) assay showing the presence of *Tropheryma whipplei* (2).

The life saving value of antibiotic treatment in Whipple's disease was established in 1952. The current recommended treatment for Whipple's disease is long term trimethoprim-

sulphamethoxazole, as it crosses the blood-brain barrier. Folate supplementation is advisable, because trimethoprim-sulphamethoxazole may cause folate malabsorption. Dramatic symptomatic relief of gastrointestinal and musculoskeletal symptoms may occur in days. Histological improvement of the small bowel mucosa takes place rapidly (1).



References:

1. Ratnaike (2000) Postgrad Med J. 76:760-766.
2. Jones et al (2007) NEJM 356:68-74.
3. Relman et al (1992) N Engl J Med 327:293-301[Abstract].

What is happening this Year

13 June	Nerve biopsies
11 July	Prostate antibodies; AGM
21-23 September	Canberra conference
November	Christmas dinner and trivia night

Histograph Crossword

Across

2 Picric decal (6)

5 Lots of gloms (6)

6 Margin Marker (6)

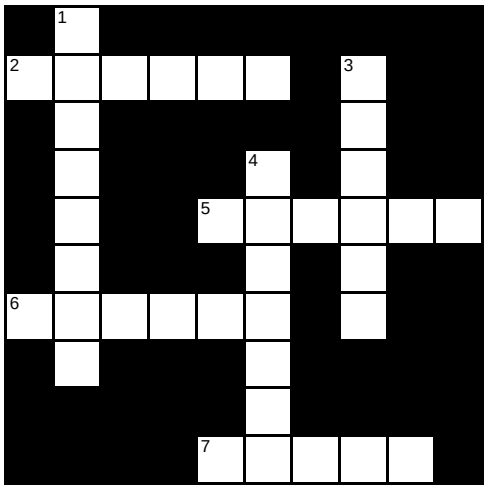
7 Hg No No (5)

Down

1 Common Fix (8)

3 Blast rbc (6)

4 Universal solvent (7)



Abstracts

Actual Practices in Nephropathology: A Survey and Comparison with Best Practices.

Pullman, James M. MD, PhD; Ferrario, Franco MD; Nast, Cynthia C. MD

Advances in Anatomic Pathology. 14(2):132-140, March 2007.

Nephropathology is a specialized field requiring routine tissue evaluation by immunofluorescence (IF), electron microscopy (EM), and light microscopy, and has published standards of best practice. Actual practices are less well documented. We therefore evaluated actual practices in nephropathology and their divergence from best practices. One hundred and twenty Renal Pathology Society members were given questionnaires regarding tissue handling, processing, and staining. Appropriate statistics for each question were calculated from results compiled into Microsoft Excel. Responses from 75 members showed that most received 16 or 18 gauge core biopsies, examined 9 slides for native kidneys, 8 slides for transplant kidneys, and for both biopsy types used hematoxylin and eosin, periodic acid-Schiff, trichrome, and silver stains. For native kidney biopsies, most collected for IF and EM if tissue was adequate, while clinical input could influence the rest. Almost all performed IF on adequate samples, with a minimum of 8 antibodies, including both light chains, those from Europe sometimes without proof of adequacy. Half performed EM unconditionally, the remainder based on specimen adequacy or clinical input. For transplant kidney biopsies, most collected tissue for IF and EM only with specific clinical indications, performed C4d IF on frozen tissue if available, but few used the native kidney IF panel. Very few performed EM unconditionally, but most would if given specific indications. We conclude that actual nephropathology practices within the Renal Pathology Society are geographically uniform and similar to published best practices, with divergence in performing IF and EM on the basis of specimen adequacy and clinical input, particularly in transplant biopsies.

Can Melan-A Replace S-100 and HMB-45 in the Evaluation of Sentinel Lymph Nodes From Patients With Malignant Melanoma?

Kucher, Cynthia MD; Zhang, Paul J. MD; Acs, Geza MD, PhD; Roberts, Shelley MS; Xu, Xiaowei MD, PhD

Applied Immunohistochemistry & Molecular Morphology. 14(3):324-327, September 2006.

The sentinel lymph node (SLN) biopsy has become an increasingly important procedure used in the primary staging of malignant melanoma. However, micrometastases in a lymph node can be easily missed on routine H&E-stained sections. Therefore, S-100 and HMB-45 IHC stains are standardly performed on grossly negative SLNs for detection of metastatic melanoma. Each of these IHC markers, however, is not ideal. The authors investigated whether the newer IHC marker Melan-A would improve the detection of metastatic melanoma in SLN biopsies. Forty lymph nodes previously diagnosed with metastatic melanoma were retrospectively evaluated for S-100, HMB-45, and Melan-A expression. In addition, 42 SLN biopsies for metastatic melanoma detection were prospectively collected and evaluated for S-100, HMB-45, and Melan-A expression. All lymph nodes with metastatic melanoma from the retrospective study demonstrated S-100 reactivity. Five of the lymph nodes with metastatic melanoma from the retrospective study failed to express either HMB-45 or Melan-A, all of which displayed a desmoplastic morphology. One of the metastases positive for S-100 and HMB-45 failed to show reactivity with Melan-A (3%). The prospective study found 10 lymph nodes from 42 cases to be positive for metastatic melanoma, which were positive for S-100 (100%). Nine of the involved lymph nodes were positive for HMB-45(90%), and nine were positive for Melan-A (90%). Melan-A, although very specific, cannot replace the use of S-100 and HMB-45 for the detection of metastatic melanoma in SLNs. It can, however, substitute for HMB-45 with equally good results.

Draft Information Bulletin for SAFE USE OF FORMALDEHYDE IN NSW HOSPITALS

Please find below a draft Information Bulletin SAFE USE OF FORMALDEHYDE IN NSW HOSPITAL MORGUES (MORTUARIES) AND PATHOLOGY LABORATORIES for review. The compliance of workplace controls for formaldehyde in hospital morgues (mortuaries) and pathology laboratories is recommended to be reviewed to reflect NICNAS recommendations. The bulletin includes a link to the full NICNAS assessment report on formaldehyde.

Information Bulletin

SAFE USE OF FORMALDEHYDE IN NSW HOSPITAL MORGUES (MORTUARIES) AND PATHOLOGY LABORATORIES

The purpose of this Information Bulletin is to advise the NSW public health system about the occupational health and safety and facility design implications for hospital morgues (mortuaries) and pathology laboratories arising from the National Industrial Chemicals Notification and Assessment Scheme (NICNAS) review of formaldehyde.

NICNAS reviewed formaldehyde under its "Priority Existing Chemical Program" in 2005-06. Occupational exposure to formaldehyde is predominantly by inhalation. The NICNAS formaldehyde assessment report concluded that the adverse health effects from exposure to formaldehyde include sensory irritation, skin sensitisation and carcinogenicity.

NICNAS has recommended that, based on the hazard assessment, formaldehyde classification for carcinogenicity be changed from Category 3 to **Category 2 "*May cause cancer by inhalation*"**. NICNAS is also recommending new exposure guidelines to the Office of the Australian Safety and Compensation Council (OASCC) that would reduce the allowable exposure to formaldehyde in the workplace. It is recommended that the standard be 0.3 ppm (0.36mg/m^3) 8h Time-Weighted Average (TWA) and 0.6 ppm (0.72 mg/m^3) Short Term Exposure Limit (STEL).

Recommendations 6 to 8 of the NICNAS Assessment Report contain specific training and workplace controls for morgues (mortuaries) and pathology laboratories.

The following workplace controls to reduce exposure of formaldehyde in morgues (mortuaries) and pathology laboratories are recommended by NICNAS:

- Use of local exhaust ventilation at each specimen station;
- Relocate specimen vats to areas with isolated ventilation or use local exhaust ventilation over the vats;
- Avoid the need for dilution of concentrated formalin products by purchasing diluted formalin products;
- Ensure effective ventilation, especially in areas where formaldehyde levels may be high, such as exhaust ventilation in storage areas, and down draught arrangements at dissection areas;

The compliance of workplace controls for formaldehyde in hospital morgues (mortuaries) and pathology laboratories is recommended to be reviewed to reflect NICNAS recommendations.

The full NICNAS assessment report on formaldehyde is available on: http://www.nicnas.gov.au/Publications/CAR/PEC/PEC28.asp

NICNAS is preparing a Safety Information Sheet specifically for safe us of formalin in forensic/hospital morgues (mortuaries) and pathology laboratories. A Safety Information Sheet will be available at end of April 2007, please check NICNAS website: http://www.nicnas.gov.au

Histotechnology Group of NSW Membership Application

2006 - 2007

I wish to become a member of the Histotechnology Group of N.S.W. and enclose

PLEASE TICK

- ☐ $38.50 for annual subscription of $35.00 and $3.50 GST.
- ☐ $16.50 for student subscription of $15.00 and $1.50 GST
  (Full-time or working toward first qualification)
- ☐ $82.50 for company subscription of $75.00 and $7.50 GST
  (2 representatives, one of whom must be a NSW representative)
- ☐ RENEWALS ☐ ANY CHANGES TO PREVIOUS DETAILS.

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