Histograph

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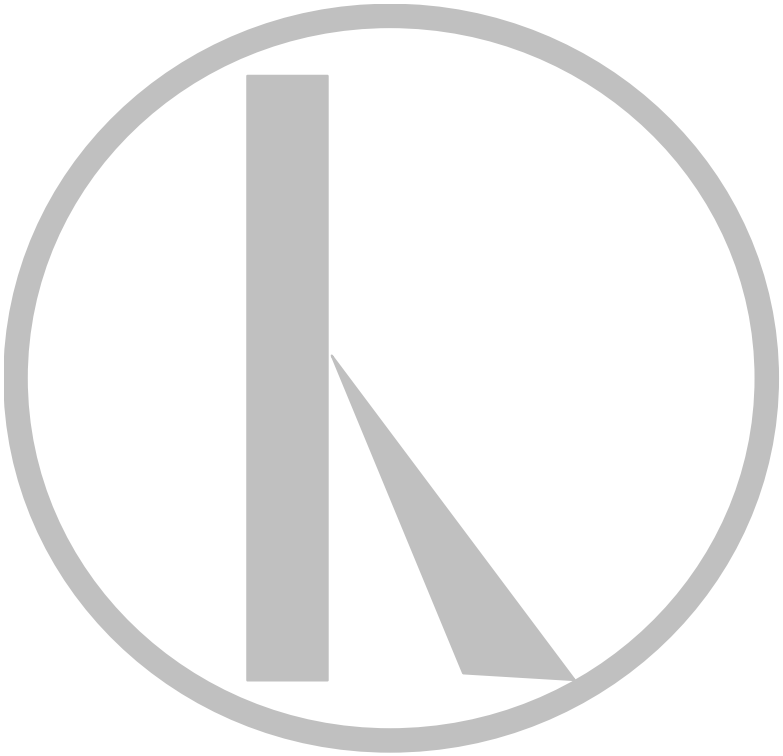
April 2013



Newsletter of the Histotechnology Society of NSW

Contents

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Editorial

We hope you had an enjoyable Christmas and New Year but unfortunately many of us have to get back into the grind of work, not like those lucky ones who have retired (you know who you are, bear with me as I have a jealous moment!). We are planning several workshops this year and we have our National meeting in Melbourne this month.

I had the pleasure of meeting our Chairperson's new twin grand-daughters and what a pair of petals they are. And what a proud Grandfather – I believe Trevor could run a marathon with the energy he is exuding!

This issue contains discussions on melanin and DAB, Rushton's Hyaline bodies and a safety article on Sorbents. The Fellowship of the Faculty of Science (RCPA) has released procedures for gaining a fellowship by research and a summary is included.

We hope you enjoy this issue and please provide feedback

Tony Henwood,
Editor
anthonyh@chw.edu.au

HISTOTECHNOLOGY SOCIETY OF NSW
POSTAL ADDRESS

PO BOX 871
SEVEN HILLS, NSW 1730

Chairman's Report

The next "National Conference" in Melbourne is now close, with details inside this Newsletter. If you have not already registered we encourage you to do so, it should be a good conference.

By now everybody will have well and truly recovered from their Christmas break. We had a great night at our Christmas function with Craig Peebles "The Write Guy" being our Guest speaker. Craig is a specialist in analysing hand writing and went through some case studies as well as the hand writing of some of our committee members. A most entertaining and interesting night.

We have three workshops planned this year with organisation well under way.

The first will be on "IHC trouble shooting" which is being organised in conjunction with Roche Diagnostics – Ventana on Saturday the 11th of May. A flyer is currently being prepared. If you are involved in Immunohistochemistry then keep this in mind.

We have commenced planning a practical "Microscopy" workshop, topic yet to be finalised, ideas would be welcome. This is being held in conjunction with the "Microscopy Society of Australia" with microscopy specialists from the MSA. Date to be finalised in July.

Tony Henwood has commenced organising a practical workshop on Frozen sectioning and immunofluorescence which is planned for September.

Planning is well underway for our next NSW conference. It will be held in Orange on Saturday and Sunday the 5th and 6th of April 2014. Our Secretary, Treasurer and myself recently did a venue inspection, it will be ideal for what we want. Keep a note of this in your diary; it will be a great weekend.

We are moving forward with our incorporation. The NSW Department of Fair Trading approved our name change to the "Histotechnology Society of NSW". We are currently working on a new Constitution as part of this process.

Another reminder that membership fees are now due.

Cheers,

Trevor Hinwood.
Chairperson.
Histotechnology Society of NSW.

Brown on Brown – Melanin and Immunohistochemistry

In the majority of laboratories, the most utilized chromogen in immunohistochemistry is diaminobenzidine (DAB). The presence of melanin pigments poses two fundamental problems. One is the direct physical masking effect on antigen-antibody interactions (1). Another is that due to its brown colour, sometimes it may be difficult to distinguish between DAB and melanin pigment. The main challenge lies in differentiating melanocytes from melanophages. Melanocytes often have fine cytoplasmic granules with a distinct nucleus, whereas melanophages have coarse melanin granules with obscured nuclei. In heavily pigmented epidermal lesions, melanin present in keratinocytes further obscures the recognition of melanocytes (2).

For the evaluation of such pigmented lesions, there are several options. Other chromogens, such Fast red or AEC result in red immunoperoxidase reaction product. Also, rather than using haematoxylin as the counterstain, techniques such as Giemsa or Azure B change melanin colour from brown to green. Finally, bleaching techniques eliminate melanin from the slides. However, these latter techniques may induce a change in the expression of several antigens. As an example, HMB-45 may become positive in macrophages.

Azure blue, when substituted for hematoxylin as a counterstain in immunostain preparation, has been used to help differentiate melanocytes from melanophages. Azure blue preferentially stains cytoplasmic melanin granules blue-green, whereas melanocytes are highlighted by brown DAB chromogen. Melanophages, which contain melanin and lack melanocytic determinants, appear clear with blue-green granules in the cytoplasm (2).

After the slides were bond washed for 4 min and rinsed in distilled water, they were stained with a mixture of the following solution: 100 mg of Azure blue (Sigma) in 4 ml of distilled water. Solution 2 was prepared as follows: 600 ml sodium acetate and 3.4 ml 0.1 M acetic acid were added to 27 ml distilled water. Both solutions 1 and 2 were combined and 5 ml of acetone was added. The slides were incubated for 60 min at room temperature, differentiated in 95% ethanol and dehydrated in several changes of absolute ethanol, followed by clearing in xylene with subsequent mounting (2).

However, the problem of physical masking remains because of the presence of excess melanin granules (1). Therefore, two main methods for bleaching out melanin pigments have emerged: treatment with potassium permanganate and oxalic acid ($KMnO_4$/oxalate) and treatment with dilute hydrogen peroxide (H_2O_2; 3% or 10% H_2O_2). Each method has advantages and disadvantages. The $KMnO_4$/oxalate method is faster as bleaching can be achieved within 1 hour and is easily incorporated in conventional daily immunostaining protocols. Nevertheless, this method reduces the antigenicity of some antigens used in immunohistochemical procedures. In contrast, the dilute H_2O_2 procedure does not reduce antigenicity when performed at room temperature, but requires 24 hours to bleach melanin pigments. Therefore, the dilute H_2O_2 treatment enables extensive application of antibodies, whereas $KMnO_4$/oxalate treatment has an effective but restricted range. To accelerate the bleaching process without interfering with the antigen-antibody reaction, a modified immunostaining method using warm 10% H_2O_2 for melanin bleaching has been reported (1).

Liu et al (1) found that, using 10% H_2O_2 in PBS contained in a glass coplin jar at 65^{o}C, the reaction time was shortened to 30 minutes, which maintained good cellular morphology and that immunostaining was unaffected.

Momose et al (3) found that irrespective of bleaching method, bleaching after antigen retrieval with microwaving or trypsinization was suitable, but bleaching prior to antigen retrieval frequently gave rise to marked tissue deterioration. They studied a range of antibodies and compared hydrogen peroxide to permanganate treatment:

	Hydrogen Peroxide	Potassium Permanganate
Reduced	CD4, CD31	MART1, CD1a, CD4, CD10, CD21, CD31, AE1AE3
Enhanced	CD21	
Unaffected	HMB45, MART1, S100, CD1a, CD3, CD5, CD8, CD10, CD20, CD68, CD34, D2-40, AE1AE3, Desmin, MiB1	HMB45, S100, CD3, CD5, CD8, CD20, CD68, CD34, D2-40, Desmin, MiB1

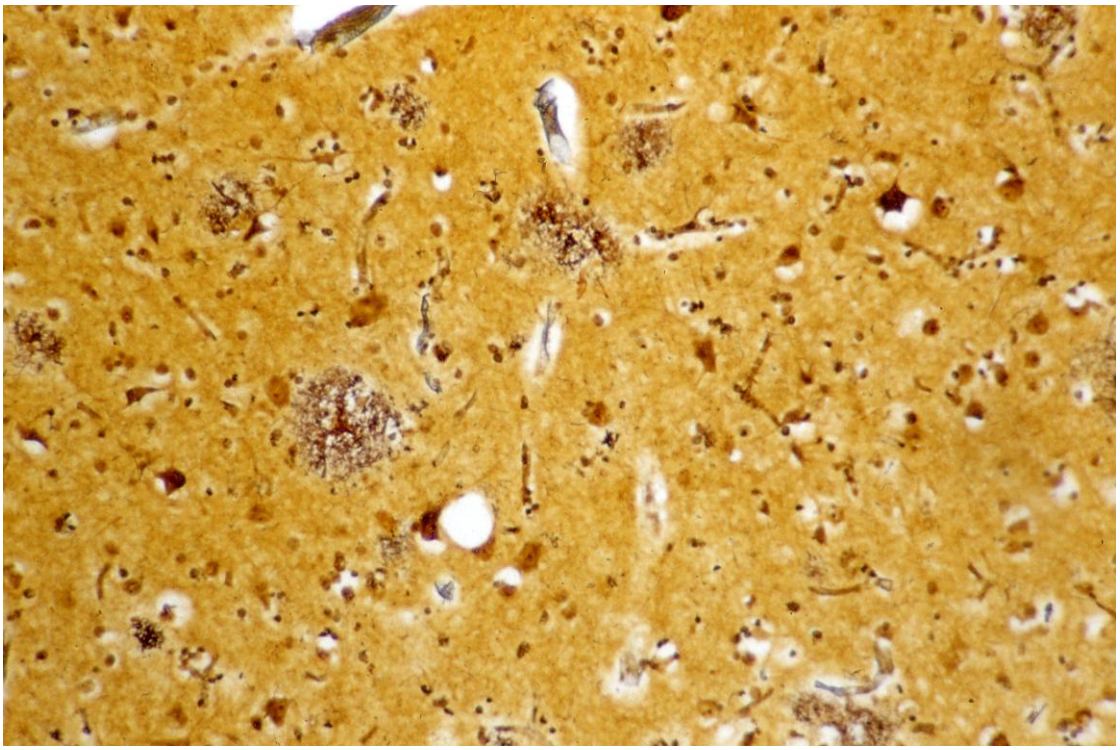
Fuchs et al (4) found that since DAB gave a diffuse or faintly granular dark brown reaction product, which is resistant to hydrogen peroxide then melanin could be removed post immunostaining. Melanin was bleached by incubating the sections for 18 hours at room temperature in 3.0% (v/v) hydrogen peroxide in 1.0% (w/v) disodium hydrogen

phosphate. With this procedure, the depigmentation was nearly complete in most specimens, and any residual melanin pigment could easily be differentiated from true positive immunoreaction due to its yellowish colour and coarse granularity.

References

1. Liu, et al (2012) "Melanin Bleaching With Dilute Hydrogen Peroxide: A Simple and Rapid Method" App Immunohistochem & Molec Morph
2. Hillesheim et al (2011) "An immunohistochemical comparison between MiTF and MART-1 with Azure blue counterstaining in the setting of solar lentigo and melanoma in situ" J Cut Pathol 38(7):565-569.
3. Momose et al (2011) "Re-evaluation of melanin bleaching using warm diluted hydrogen peroxide for histopathological analysis" Pathology International, 61(6), 345-350.
4. Fuchs et al (1992) "An immunohistochemical and prognostic analysis of cytokeratin expression in malignant uveal melanoma" Am J Path 141(1):169.

What Stain is this?



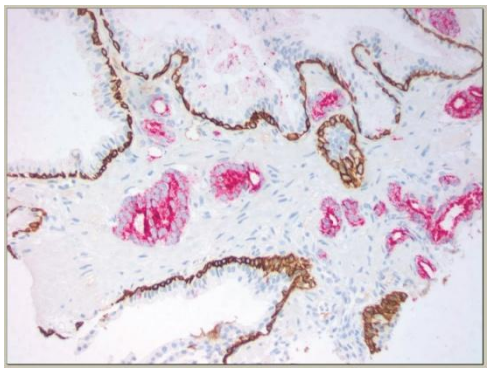
What Stain is this?

What is the tissue?

What is being demonstrated?

HISTOTECHNOLOGY SOCIETY OF NSW

IMMUNOHISTOCHEMISTRY -TROUBLESHOOTING



ONE DAY WORKSHOP

By Emma Jones Application Specialist Roche

An interactive session includes interpretation of stained slides under microscope and troubleshooting IHC common problems. Topics include pre and post analytical conditions, staining artifacts, IHC Controls, instruments/automation and other hidden external factors such as PH of water and many more.

When: 11th May Saturday 2013 Time: 10AM to 4PM

Where: Douglass Hanly Moir Pathology,
14 Giffnock Ave,
Macquarie Park 2113

Cost: $ 45.00

Morning tea, Lunch & Afternoon tea provided.
For any special food requirements see below for contact details.

Contact: **RSVP: 30th April 2013**

Bharathi Cheerala, Douglass Hanly Moir Pathology
Ph: 02 9855 5434 or 02 9855 6271 Email: bcheerala@dhm.com.au

Abstracts from the Literature

The Diagnostic Value of Fungal Fluorescence in Onychomycosis

Idriss, Munir H, Khalil, Ahmed, Elston, Dirk
Journal of Cutaneous Pathology. 40(4):385-390, April 2013.

Background: Fluorescence of pathogenic fungi has been previously shown when hematoxylin and eosin-stained sections are examined under a fluorescent microscope. We hypothesize that this phenomenon could aid in the evaluation of nail specimens for onychomycosis.
Methods: Forty-eight routinely stained nail sections of periodic acid-Schiff (PAS)-positive onychomycosis, along with 23 PAS-negative control specimens with a clinical diagnosis of onychomycosis, were studied under a fluorescent microscope to determine the clinical usefulness of this technique.
Results: In most cases, fluorescence of fungal organisms was noted. Fungi were identified by their tubular or annular shapes with fluorescence surrounding them. The sensitivity and specificity of the method were 96 and 90%, respectively. In some cases, it was difficult to identify the fungi because of the relative paucity of organisms, weak fluorescence and high background fluorescence of eosinophilic nail keratin.
Conclusions: We conclude that fluorescence microscopy can be used as a rapid screening tool for identification of fungi in nail specimens.

The Utility of Elastic Verhoeff-Van Gieson Staining in Dermatopathology

Kazlouskaya V, Malhotra S, Lambe J, Idriss MH, et al
Journal of Cutaneous Pathology. 40(2):211-225, February 2013.

Elastic fibres are important components of the skin and are responsible for skin elasticity. Genetic defects are well-known in numerous hereditary elastic tissue disorders and skin biopsies are often the first step in the evaluation of those disorders. Verhoeff-Van Gieson elastic staining is a simple method that is used for visualizing elastic fibres. With the development of modern immunohistochemical methods, the value of routine histochemical staining is sometimes underestimated. Histochemical stains are less expensive, easy to perform and help to resolve numerous diagnostic quandaries in dermatopathology. This article focuses on the value of elastic tissue staining in dermatopathology, with a focus on primary elastic tissue disorders, alopecia, inflammatory skin disorders and neoplastic proliferations.

High-throughput RNA sequencing of a formalin-fixed, paraffin-embedded autopsy lung tissue sample from the 1918 influenza pandemic

Xiao, Y.-L., Kash, J. C., Beres, S. B., Sheng, Z.-M., Musser, J. M. and Taubenberger, J. K.
J. Pathol., 229: 535–545, 2013

Most biopsy and autopsy tissues are formalin-fixed and paraffin-embedded (FFPE), but this process leads to RNA degradation that limits gene expression analysis. The RNA genome of the 1918 pandemic influenza virus was previously determined in a 9-year effort by overlapping RT-PCR from post-mortem samples. Here, the full genome of the 1918 virus at 3000× coverage was determined in one high-throughput sequencing run of a library derived from total RNA of a 1918 FFPE sample after duplex-specific nuclease treatments. Bacterial sequences associated with secondary bacterial pneumonias were also detected. Host transcripts were well represented in the library. Compared to a 2009 pandemic influenza virus FFPE post-mortem library, the 1918 sample showed significant enrichment for host defence and cell death response genes, concordant with prior animal studies. This methodological approach should assist in the analysis of FFPE tissue samples isolated over the past century from a variety of diseases.

NATIONAL HISTOLOGY CONFERENCE 2013

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https://www.nationalhistologyconference.com.au/

National Histology Conference Registrations will close 1 week prior to the Conference – 19th of April, 2013. Please ensure to register before this date so you don't miss out!

Check those ABS on the Sorbents

Safety Corner

Sorbents, what "toilet paper" you ask?

In the old days, we made our own Schiff's reagent. Oh how we remember boiling our basic fuchsin solution, cooling to 50^{o}C and then adding hydrochloric acid and sodium bisulphite. Walk into an old Histo lab, find the Bunsen (or gas outlet), look upwards and admire the bright red artwork on the ceiling. I must add that there is a safer cold Schiff method available or be real lazy, like I am, and buy it commercially.

But I digress. When the Schiff's solution is made it usually looks yellow. The yellow colour of Schiff's reagent is due to a contaminant. During synthesis of dyes of the fuchsin group Phosphine, a yellow acridine dye, is formed. It can easily be removed by treatment of Schiff's reagent with charcoal (1). CHARCOAL IS A SORBENT!!

Sorbents are chemicals or materials that can capture liquids or gases. Sorbents can be Adsorbents or Absorbents. Adsorbents adhere substances over the surface of the adsorbing material, e.g. activated carbon. Absorbents incorporate a substance throughout the body of the absorbing material, e.g. polypropylene which absorbs oil. A desiccant is a specific type of sorbent used to reduce humidity or moisture. They are hygroscopic meaning they have the ability to attract and hold water molecules (2).

Activated Carbon

Activated carbon can be manufactured from carbonaceous material, including coal (bituminous, subbituminous, and lignite), peat, wood, or nutshells (e.g., coconut). The manufacturing process consists of two phases, carbonization and activation. The carbonization process includes drying and then heating to separate by-products, including tars and other hydrocarbons from the raw material, as well as to drive off any gases generated. The process is completed by heating the material over 400°C (750°F) in an oxygen-free atmosphere that cannot support combustion. The carbonized particles are then "activated" by exposing them to an oxidizing agent, usually steam or carbon dioxide at high temperature. This agent burns off the pore blocking structures created during the carbonization phase and so, they develop a porous, three-dimensional graphite lattice structure. The size of the pores developed during activation is a function of the time that they spend in this stage. Longer exposure times result in larger pore sizes (3).

Diatomaceous Earth

Diatomaceous earth is a naturally occurring, soft, siliceous sedimentary rock that is easily crumbled into a fine white to off-white powder. It has a particle size ranging from less than 3 micrometre to more than 1 millimeter, but typically 10 to 200 micrometres. This powder has an abrasive feel, similar to pumice powder, and is very light as a result of its high porosity. The typical chemical composition of oven-dried diatomaceous earth is 80 to 90% silica, with 2 to 4% alumina (attributed mostly to clay minerals) and 0.5 to 2% iron oxide. It is an excellent absorbent for most laboratory spills,

especially organic liquids. It is often used as Kitty Litter (4).

Here are some other types of absorbents and adsorbents:

- Activated alumina is an adsorbent made of aluminum oxide (Al_2O_3). It is used as a desiccant for drying gases and air and as a fluoride filter for drinking water.
- Calcium oxide is a slow but strong and high capacity desiccant also known as quicklime. It is caustic and expands as it adsorbs and does so over several days.
- Clay or clay silicates are natural mineral absorbents and adsorbents that are used as spill cleaning agents, sealants, and packing materials because they are inexpensive, inert, and have a quick capture rate.
- Silica gel or silicon dioxide is a common desiccant used in food preservation, humidity control, and various medical devices. It has a higher water absorption capacity than clay silicates, it is very inert, and it can be regenerated through heating.
- Siliceous Volcanic Rock readily adsorbs solutions while remaining free flowing (anti-caking) and chemically resistant to microbiological degradation. This is quarried, crushed and screened into closely graded fractions having a specific range of particle size to suit various applications.
- Synthetic products and organic polymers (e.g. polypropylene, polyurethane). Synthetic sorbents are the most commonly used commercial sorbents in oil spill clean-up due to their oleophilic and hydrophobic properties (4).

References

1. *Meloan SN & Puchtler H (1986) "On the Structure and Chemistry of Leucofuchsin and Schiff s Reagent" J Histotechn 9(2):119-122.*
2. *http://www.globalspec.com/learnmore/materials_chemicals_adhesives/chemicals_raw_materials/desiccants_absorbents*
3. *http://en.wikipedia.org/wiki/Adsorption*
4. *Adebajo MO, Frost RL, Kloprogge JT, Carmody O, Kokot S (2003)" Porous Materials for Oil Spill Cleanup: A Review of Synthesis and Absorbing Properties" J Porous Materials 10(3):159-170.*

In Situ Hybridisation Workshop

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17th November 2012
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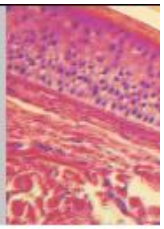

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UNSW Museum Course

RUSHTON'S HYALINE BODIES

The hyaline body (HB), a structure first reported in 1918 by Dewey (1), was described in detail by Rushton (2). Rushton's Hyaline Bodies (RuHBs) are hyaline concretions of linear, curved or circular shapes, measuring up to about 0.1 mm in length, and they are found in the lining epithelium of odontogenic cysts. They occur in between 4% and 10% of radicular cysts or dentigerous cysts, producing a positive result with aldehyde fuchsin and Prussian blue reaction for iron and with Orcein. They are eosinophilic and Gram-negative. They are negative with von Kossa stain for calcium and the periodic acid-Schiff reaction for mucopolysaccharides (2, 3).

RuHBs are stained by Orcein and aldehyde fuchsin, which do not stain either the epithelium or hair. These dyes are highly reactive to sulfonic acids. Keratin contains a high amount of cysteine, and the epithelium and hair, which are normally unstained, become reactive to these dyes after pre-oxidation (Shikatas Modified Orcein) (3).

Based on the results of histochemical examinations and electron microscopic observations, certain substances, such as keratins produced by the odontogenic epithelium, may play a role in HB formation. As histochemical reactions of HBs are similar to those of dental cuticles and different from those of epithelial keratins, particular secretory proteins other than keratins have also been considered. Alternatively, research has suggested that RuHBs might be derived from blood components, because HBs histochemically react to haemoglobin and iron stains. This hints at a haematogenous origin, although a histochemical reaction for haemoglobin is not specific. Electron microscopic examination suggested that HB was formed of degenerating erythrocytes, not of a keratinous product of epithelial cells. However, Browne et al. (1) disproved the possibility of both keratin and erythrocyte origin, based on the negative results of immunohistochemical staining (3).

Sakomoto et al (3) studied ten specimens with RuHBs obtained from 400 odontogenic cysts. HBs were stained by orcein and Congo red. Birefringence was faintly observed. Immunohistochemical examination revealed that HBs were positive for hair keratin and keratin 17. Hair keratin was concentrated in HBs, and cells with hair keratin expression were hardly seen, while cells with keratin 17 expression were observed near RuHBs. RuHBs were also positive for haemoglobin alpha chain. The presence of hair keratin in RuHBs was confirmed by Western blot analysis. The present study suggests that HBs are formed as a consequence of two independent events: unusual alteration of epithelial differentiation so as to provide hair keratin, and haemorrhage so as to provide erythrocytic substances.

Although the ectopic production of hair keratin appears more essential, their results reconcile the long-standing debate between two theories, the keratin theory versus the red blood theory, concluding that both substances are required for the genesis of RuHBs, and also suggesting that they might be novel non-pathological amyloidogenic proteins (3).

References:

1. *Dewy KW. (1918) "Cysts of the dental system" Dent Cos 60: 555.*
2. *Rushton MA. (1955) "Hyaline bodies in the epithelium of dental cysts" Proc R Soc Med 48: 407–9*
3. *Sakamoto K, Khanom R, Hamagaki M, Yamaguchi A (2012) "Ectopic production of hair keratin constitutes Rushton's hyaline bodies in association with hematogenous deposits" J Oral Pathol Med 41: 637–641*

FELLOWSHIP OF THE FACULTY OF SCIENCE RCPA UPDATE

On 10 December 2009, in an historic vote, the membership of the College agreed to the inception of The Faculty of Science under Article 57c of the Articles of Association. Applications for Founding Fellowship were accepted until December 2011 from among the ranks of distinguished scientists and pathologists who demonstrated a desire and commitment to promoting science in pathology through the College. Development of the pathway to Fellowship of the Faculty by examination is underway and will be available in the coming years.

Fellowship of the Faculty of Science (Research) RCPA by published works is a pathway now available to professionals who have outstanding research expertise in science in pathology and will make a vital contribution to the Faculty.

Fellowship of the Faculty of Science (Research) RCPA by published works

The application process requires an Informal Review in the first instance. The Informal Review will involve the applicant submitting their CV and list of publications for the Principal Examiner to assess as having potential to meet the standard expected for Fellowship by published works. Following a successful outcome of the Informal Review, the applicant may make a Formal Application, which includes two copies of their publications and their Sponsor's commentary. The relevant section of the By-law to Article 57c and the College Policy document "Fellowship of the Faculty of Science (Research) RCPA by published works" are available on the link below:

http://www.rcpa.edu.au/Careers/Training/FFOSC.htm

Tony Henwood MSc, BAppSc, GradDipSysAnalys, CT(ASC), FFSc(RCPA)
Laboratory Manager & Senior Scientist
Tel: 612 9845 3306

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    - complex skins and organs (breast, colon, prostate etc)
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- Folio generated from work completed – NPAAC requirement
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- Must have minimum 12 months experience in Cut Up assistance
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- Enquire now - maximum intake 12 only – 6 places already taken!
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Contact: Anne Prins 02 6125 4644 anne.prins@anu.edu.au
Penny Whippy 02 6174 5667 penelope.whippy@act.gov.au

Christmas 2012

HISTOTECHNOLOGY SOCIETY of NSW

ABN: 63 128 868 343
nswhistogroup@bigpond.com

Membership Application Form

PLEASE TICK: ☐ New Membership

☐ Renewal of Membership

☐ Change of details

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OCCUPATION_____POSITION_____

INSTITUTION_____DEPARTMENT_____

ADDRESS for CORRESPONDENCE: HOME OR BUSINESS? (Circle One).

STREET/P.O.BOX._____

SUBURB _____ POSTCODE._____

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E-MAIL ADDRESS: _____

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

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☐ $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)

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Or: I**nternet Banking**: BSB:**802 084**; Account number: **94099;**

Account name: **Histotechnology Group of NSW**.

Reference: **[Name – for member identification]**

SIGNATURE_____**DATE**_____

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