Histograph

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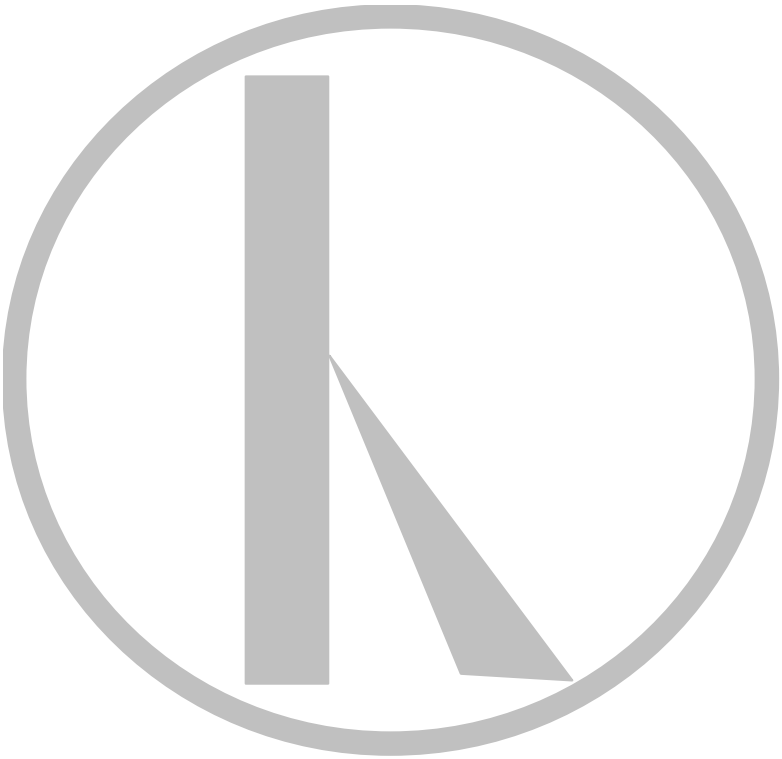

Newsletter of the Histotechnology Group of NSW

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Editorial

I hope you had a great time in Melbourne. Catastrophically, I was unable to attend since I was on-call for that weekend and being called in to work from Melbourne could be a trite inconvenient.

Some news around the traps, my 2IC, Andrew Gayagay, has been promoted to the senior scientist position at Prince of Wales Hospital. A great acquisition for POW - a terrible loss for the Children's Hospital. We wish you all the best.

This issue has some interesting articles on Harry Potter and Paul Ehrlich. At least the Spice Girls don't make an appearance in the abstracts, though Lassa, Rabies and yellow fever are in the top 40!

We hope you enjoy this issue and please, if you have something to say, please email or write to me.

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Chairman's Message

Congratulations to the Histotechnology Group of Victoria Committee for a successful National Meeting with over three hundred registered for Saturday and only a few less for Sunday.

The trade display was exceptional in both location and number of trade in attendance.

Saturday dinner night proved to be very enjoyable with plenty of food, drinks music and dancing.

The next National Meeting will be held in Queensland in October 2006, keep an eye out for the dates and location.

The Histotechnology Group of NSW is now well into planning the next state meeting at Mudgee on the 17th, 18th and 19th March 2006. We have several interesting speakers with two workshops planned. This is our 25th Anniversary year so we hope to have several surprises in store. Being St Patrick's Day on the Friday we have opted for this theme for the Saturday Night Dinner/Dance. Hope to see you there. Registration forms will become available shortly with the provisional programme.

The Christmas Lecture this year will be held at the North Ryde RSL on the 2nd of December 2005. It will be 7:00 for a 7:30 start, the guest speaker will be Stephen Fairy of Douglass Hanley Moir speaking on the topic of Renal Tumours. We need thirty people to make this a successful evening. Remember it's not just the lecture nor the food but often the camaraderie that makes the evening enjoyable.

Yours in Histotechnology

Bill Sinai

Paul Ehrlich – A History

I recently watched an old movie on Foxtel's TCM entitled: "Dr Ehrlich's Magic Bullet". It was made in 1940 and starred Edward G Robinson. The appearance of several characters well known to us in Histotechnology such as Robert Koch, W.H. Perkin and Ehrlich's cousin Carl Weigert had my immediate interest.

Paul Ehrlich was born on March 14, 1854 at Strehlen, in Upper Silesia, Germany. He was the son of Ismar Ehrlich and his wife Rosa Weigert, whose nephew was the great bacteriologist Karl Weigert. Ehrlich was educated at the Gymnasium at Breslau and subsequently at the Universities of Breslau, Strassburg, Freiburg-im-Breisgau and Leipzig. In 1878 he obtained his doctorate of medicine by means of a dissertation on the theory and practice of staining animal tissues. This work was the result of his great interest in the aniline dyes discovered by W. H. Perkin in 1853 (1). This thesis also included the discovery of mast cells. One of his many papers was: Ehrlich P. Hamatoxylinlösung. Z Wiss Micr (1886) 3:150. Can you pick the histotechnological relevance?

In 1878 he was appointed assistant to Professor Frerichs at the Berlin Medical Clinic, who gave him all the support to continue his work with these dyes and the staining of tissues with them. Ehrlich showed that all the dyes used could be classified as being basic, acid or neutral and his work on the staining of granules in blood cells laid the foundations of future work on haematology and the staining of tissues. In 1882 Ehrlich published his method of staining the tubercle bacillus that Koch had discovered and this method was the basis of the subsequent modifications introduced by Ziehl and Neelson, which are still used today. From it was also derived the Gram method of staining bacteria so much used by modern bacteriologists (1).

Paul Ehrlich received the 1908 Nobel Prize in Medicine for his scientific work in the field of immunity. A brush with a deadly disease at an early stage in his scientific career may have prompted Ehrlich's fascination with questions of immunity and the treatment of disease. Ehrlich had been afflicted by tuberculosis—a disease caused by an infection of bacteria that attack the lungs. His tuberculosis forced Ehrlich to spend two years in Egypt, seeking out a climate that would help his lungs to heal. Perhaps his knowledge that he carried within his lungs the tuberculosis bacteria led Ehrlich to develop his particular chemical approach to the treatment of disease. It was Ehrlich who envisioned the creation of "magic bullets," compounds that would have a specific attraction to disease-causing microorganisms. These magic bullets would seek out these organisms and destroy them, avoiding other organisms and having no harmful effects on the bodies of patients (2).

In his quest for the discovery of magic bullets—compounds with specific structures for combating specific diseases and that would leave all else alone—Ehrlich did not always fully realize his dream. He did find compounds effective in treating diseases like malaria and sleeping sickness, which are caused by protozoan parasites, but these were only partially effective. He came much closer to his ideal of the magic bullet when he found compounds of arsenic effective against the bacterium that causes syphilis. These compounds, named Salvorsan and Neosalvarsan, became accepted as the standard, effective treatment for syphilis. The success of these magic bullets earned Ehrlich enduring fame as one of the central figures in the establishment of chemotherapy (2).

References:

1. http://nobelprize.org/medicine/laureates/1908/ehrlich-bio.html
2. http://www.chemheritage.org/EducationalServices/pharm/chemo/readings/ehrlich.htm

Abstracts from the Literature

Diagnostic Utility of Immunohistochemistry in Morphologically Difficult Prostate Cancer: Review of Current Literature

M Varma & B Jasani

Histopathology (2005) 47 (1): 1

Immunohistochemistry is widely used to distinguish prostate cancer from benign mimics and to establish the prostatic origin of poorly differentiated carcinoma. We critically review the recent advances in prostate cancer immunohistochemistry, including the introduction of newer basal cell markers such as p63 and the discovery of the overexpression of -methylacyl coenzyme A racemase (AMACR) in prostate cancer. The description of newer urothelial markers to aid the distinction of prostate cancer from urothelial carcinoma is also presented together with refinements in the quality control of PSA and PSAP immunostaining. Although AMACR is a useful immunohistochemical marker for prostate cancer, it has significant limitations. These limitations are discussed and the need for interpreting AMACR immunoreactivity in the appropriate morphological context and in conjunction with basal call markers is emphasized. We also describe the utility of an immunohistochemical panel composed of PSA, PSAP and high molecular weight cytokeratin for distinguishing poorly differentiated prostate cancer from high-grade urothelial carcinoma. A morphological differential diagnosis based selection of immunohistochemical markers is highlighted as a novel approach in the diagnosis of prostate cancer in routine surgical pathology practice.

Transmission of Rabies Virus from an Organ Donor to Four Transplant Recipients

Arjun Srinivasan, Elizabeth C. Burton, Matthew J. Kuehnert, Charles Rupprecht, William L. Sutker, et al.

New England Journal of Medicine (2005) 352(11):1103-1111.

Background: In 2004, four recipients of kidneys, a liver, and an arterial segment from a common organ donor died of encephalitis of an unknown cause.

Methods: We reviewed the medical records of the organ donor and the recipients. Blood, cerebrospinal fluid, and tissues from the recipients were tested with a variety of assays and pathological stains for numerous causes of encephalitis. Samples from the recipients were also inoculated into mice.

Results: The organ donor had been healthy before having a subarachnoid hemorrhage that led to his death. Encephalitis developed in all four recipients within 30 days after transplantation and was accompanied by rapid neurologic deterioration characterized by agitated delirium, seizures, respiratory failure, and coma. They died an average of 13 days after the onset of neurologic symptoms. Mice inoculated with samples from the affected patients became ill seven to eight days later, and electron microscopy of central nervous system (CNS) tissue demonstrated rhabdovirus particles. Rabies-specific immunohistochemical and direct fluorescence antibody staining demonstrated rabies virus in multiple tissues from all recipients. Cytoplasmic inclusions consistent with Negri bodies were seen in CNS tissue from all recipients. Antibodies against rabies virus were present in three of the four recipients and the donor. The donor had told others of being bitten by a bat.

Conclusions: This report documenting the transmission of rabies virus from an organ donor to multiple recipients underscores the challenges of preventing and detecting transmission of unusual pathogens through transplantation.

Yellow Fever: The Recurring Plague

Oyewale Tomori,

Critical Reviews in Clinical Laboratory Sciences (2004) 41(4): 391-427

Despite the availability of a safe and efficacious vaccine, yellow fever (YF) remains a disease of significant public health importance, with an estimated 200,000 cases and 30,000 deaths annually. The disease is endemic in tropical regions of Africa and South America; nearly 90% of YF cases and deaths occur in Africa. It is a significant hazard to unvaccinated travellers to these endemic areas. Virus transmission occurs between humans, mosquitoes, and monkeys. The mosquito, the true reservoir of YF, is infected throughout its life, and can transmit the virus transovarially through infected eggs. Man and monkeys, on the other hand, play the role of temporary amplifiers of the virus available for mosquito infection. Recent increases in the density and distribution of the urban mosquito vector, Aedes aegypti, as well as the rise in air travel increase the risk of introduction and spread of yellow fever to North and Central America, the Caribbean, the Middle East, Asia, Australia, and Oceania. It is an acute infectious disease characterized by sudden onset with a two-phase development, separated by a short period of remission. The clinical spectrum of yellow fever varies from very mild, nonspecific, febrile illness to a fulminating, sometimes fatal disease with pathognomic features. In severe cases, jaundice, bleeding diathesis, with hepatorenal involvement are common. The case fatality rate of severe yellow fever is 50% or higher. The pathogenesis and pathophysiology of the disease are poorly understood and have not been the subject of modern clinical research. There is no specific treatment for YF, making the management of YF patients extremely problematic. YF is a zoonotic disease that cannot be eradicated, therefore instituting preventive vaccination through routine childhood vaccination in endemic countries, can significantly reduce the burden of the disease. The distinctive properties of lifelong immunity after a single dose of yellow fever vaccination are the basis of the new applications of yellow fever 17D virus as a vector for foreign genes, "the chimeric vaccine," and the promise of developing new vaccines against other viruses, and possibly against cancers

Do Prosthetic Particles Polarize? – Question to CAP

This following question was posed to the College of American Pathologists website (http://www.cap.org/apps/docs/cap_today/q_and_a/qa_1102.html). It was answered by Robert Novak from the Department of Pathology at the Children's Hospital Medical Centre of Akron in Ohio.

Q. Do prosthetic particles polarize when viewed with a polarizing microscope? I am trying to develop a procedure for staff to deal with physician requests to determine if there are particles from prosthetic devices in synovial fluid.

A. The presence of particles in synovial fluid that appear to be derived from the articular surface of prosthetic hips and knees has been studied, often in an effort to predict prosthesis wear and non-septic prosthesis failure. A correlation has been sought between the morphology and number of these particles and prosthesis problems using many techniques, including light microscopy with polarizing filters, light microscopy with oil red O staining, scanning electron microscopy, and various particle-counting methods. The latter two usually are performed after digestion of the synovial fluid with a strong base (NaOH or KOH) to remove organic materials before analysis. Because the question involves analysing synovial fluids in a clinical laboratory, the discussion

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will be limited to issues with light microscopic methods.

You must first consider the type of prosthesis you are dealing with to anticipate what type of particles you might encounter. In knee prostheses, the articular surface is usually a high-molecular-weight polyethylene, so polyethylene particles would be sought. In hip prostheses, metal-on-metal prostheses have been associated with the presence of metal fragments, whereas hip replacements with a polyethylene articular surface yield both metal and polyethylene particles. Metal particles cannot be polarized or stained and need an approach such as spectroscopy to be identified reliably. Thus, in the clinical laboratory, polyethylene particles are the most likely to be identified.

Polyethylene particles show birefringence with polarized light and stain with oil red O, though neither technique is obviously specific for polyethylene (1). This non-specificity of light microscopy is demonstrated in one study of particles in the synovial fluid of patients receiving hip prostheses, where about 50 percent of preoperative specimens were reported by the laboratory to have polyethylene particles, when the status of the patient was unknown to the laboratory (2). Furthermore, it has been demonstrated that polyethylene particles are present in synovial fluid in most patients with knee prostheses, if carefully sought, and that it is the number and size of the particles, not merely their presence, that correlate with prosthesis problems (3,4). Some have suggested it is the small globular particles (diameter, <5 μm) that are most significant, as these can be phagocytized by monocytes, resulting in the release of inflammatory cytokines, which lead to osteolysis and prosthesis failure. These particles are not as easily detected by light microscopic techniques as larger elongated forms, which are readily detected but may not be as clinically significant as the smaller particles.

In view of these considerations, merely finding apparent prosthesis fragments in synovial fluid may not be of particular value to your clinicians, and you may want to discuss with them alternative approaches to optimize the utility of the fluid analysis to their patients.

References:

1. Peterson C, Benjamin J, Szivek J, et al. Polyethylene particle morphology in synovial fluid of failed knee arthroplasty. Clin Orthop. 1999, 359:167-175.
2. Dorr L, Hilton K, Wan Z, et al. Modern metal on metal articulation for total hip replacements. Clin Orthop. 1996, 333: 108-117.
3. Bosco J, Benjamin J, Wallace D. Quantitative and qualitative analysis of polyethylene wear particles in synovial fluid of patients with total arthroplasty. Clin Orthop. 1994 ,309:11-19.
4. Calonius O, Saikko V. Analysis of polyethylene particles produced in different wear conditions in vitro. Clin Orthop. 2002, 399:219-230.

Harry Potter and the Recessive Allele

Recently Jeffrey Craig, Renee Dow and MaryAnne Aitken from the Royal Children's Hospital in Parkville, Victoria, had a letter to the editor published in Nature (Vol 436, 11 August 2005, page 776). They recommended the use of analogies as tools for introducing young people to scientific concepts. Taking their example from J. K. Rowling's stories about the young wizard Harry Potter, they suggest that wizarding is a monogenic trait, with the wizard allele (W) recessive to the muggle allele (M).

Wizards or witches can be of any race, and may be the offspring of a wizard and a witch, the offspring of two muggles ('muggle-born'), or of mixed ancestry ('half-blood'). Craig et al suggest that wizarding ability is inherited in a mendelian fashion, with the wizard allele (W) being recessive to the muggle allele (M). According to this hypothesis, all wizards and witches therefore have two copies of the wizard allele (WW).

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Harry's friends Ron Weasley and Neville Longbottom and his arch-enemy Draco Malfoy are 'pure-blood' wizards: WW with WW ancestors for generations back. Harry's friend Hermione is a powerful muggle-born witch (WW with WM parents). Their classmate Seamus is a half-blood wizard, the son of a witch and a muggle (WW with one WW and one WM parent). Harry (WW with WW parents) is not considered a pure-blood, as his mother was muggle-born.

There may even be examples of incomplete penetrance (Neville has poor wizarding skills) and possible mutations or questionable paternity: Filch, the caretaker, is a 'squib', someone born into a wizarding family but with no wizarding powers of their own.

They believe that, with the use of these examples, the concepts of mendelian genetics can be introduced to children as young as five, and then built on by gradually introducing specific terms such as 'gene' and 'allele', and relating these to chromosomes and DNA. At every stage, the children's familiarity with the Harry Potter characters can be used as a hook to engage them in discussing concepts of heredity and genetics.

Antony Dodd, Carlos Hotta and Michael Gardner from Cambridge, England, on the other hand, believe the assumption that wizarding has a genetic basis to be deterministic and unsupported by available evidence (Nature, 15 September 2005, Volume 437, page 318).

Following Craig and colleagues' analogy, Hermione, as a muggle-born witch, must have WM parents. However, as Rowling fans could point out, Hermione's parents were muggle dentists who lack any family history of wizarding. It's true, of course, that chance may not have thrown up a witch or wizard for many generations, or that any who did have magical powers may have kept them secret to avoid a witch hunt.

What about Neville's apparently poor wizarding skills? These cannot be explained by incomplete penetrance, as Craig and colleagues suggest. In incomplete penetrance, individuals either display the trait or not: they do not display an intermediate degree of the trait. Poor wizarding skills might be indicative of variable expressivity of an allele. However, both variable expressivity and incomplete penetrance are associated with dominant alleles. If the wizarding allele were dominant, rather than recessive as suggested, wizarding children such as Hermione could not be born to non-wizarding parents.

Dodd et al suggest that Neville's clumsiness may, perhaps, be an individual characteristic unrelated to his potential powers. However, they believe that it is not possible, from the evidence presented so far, to conclude that wizarding is a heritable trait.

Lassa Virus

Stephan Günther and Oliver Lenz

Critical Reviews in Clinical Laboratory Sciences 41(4): 339-390, 2004

Lassa virus is a RNA virus belonging to the family of Arenaviridae. It was discovered as the causative agent of a hemorrhagic fever—Lassa fever—about 30 years ago. Lassa fever is endemic in West Africa and is estimated to affect some 100,000 people annually. Great progress in the understanding of the life cycle of arenaviruses, including Lassa virus, has been made in recent years. New insights have been gained in the pathogenesis and molecular epidemiology of Lassa fever, and state-of-the-art technologies for diagnosing this life threatening disease have been developed. The intention of this review is to summarize in particular the recent literature on Lassa virus and Lassa fever. Several aspects ranging from basic research up to clinical practice and laboratory diagnosis are discussed and linked together.

2005 - 2006

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