Histograph

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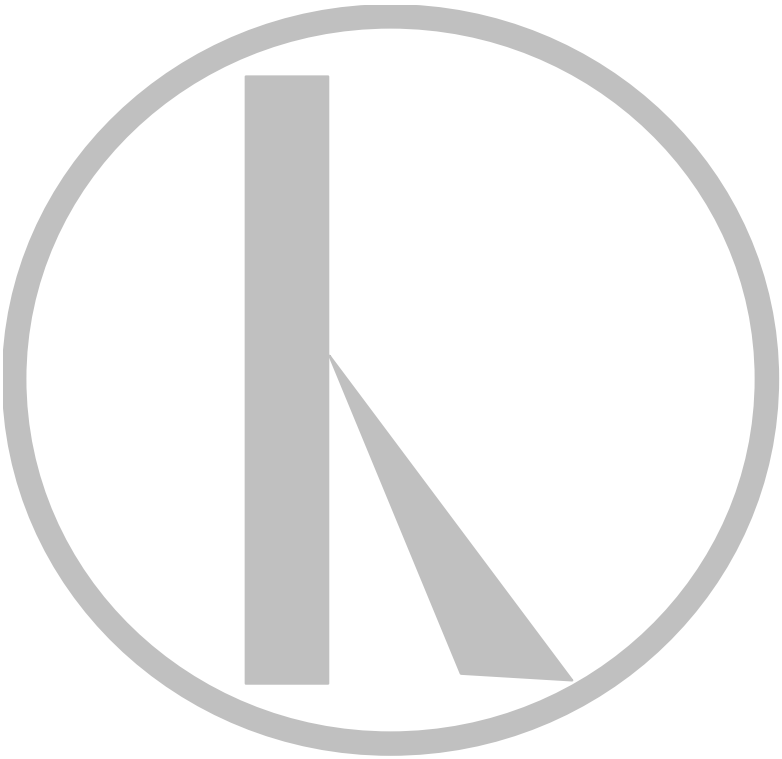
January 2008



Newsletter of the Histotechnology Group of NSW

Contents

Editorial

Firstly sorry about the lateness of this issue. It was meant to be a November/December 2007 issue but time got away from me. I will try better this year. Otherwise the committee will do unspeakable things to me. An H&E enema comes to mind!

Well, it was a great State meeting in Canberra. The organisers did a great job and I can't wait for the next Canberra Meeting. We are privileged to have summaries of some of the presentations given at this meeting in this edition of Histograph and more is to come in up-coming issues.

If you have any issues with the content of Histograph or have something to contribute, please contact me and we will organise it to your satisfaction.

Don't forget the Crossword. We did not receive a correct answer for the last issue Crossword, so I get to keep the bottle of wine! Remember, the first correct answer received by our chairman (trevor.hinwood@hdscientific.com.au) will receive a prize of a bottle of wine.

Tony Henwood,
Editor
anthonyh@chw.edu.au

Chairman's Report

Christmas has come and gone and many of us will hopefully have had a well earned break.

During the past year we have been able to present a number of well attended meetings focusing on different aspects of Histology. As well we had our Canberra Meeting which people are still talking about. We owe a great deal to several people in Canberra and Sydney who made the Conference the success it was. A good team effort.

Next year we will continue along the same lines and are already working on a joint meeting with the Brain and Mind Research Institute at Sydney University. This Institute has commenced important brain research which includes histology. Time frame around April 2008. We will keep you informed of developments.

We have also reinitiated a plan for a meeting in the Hunter Valley/Newcastle area during 2008.

The committee would like to wish you and your families a successful and healthy 2008. We look forward to seeing you in this year.

Trevor Hinwood,
Chairperson,
Histotechnology Group of NSW.

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Heparan Sulfate Mimetics: A New Class of Anti-Cancer Drugs

Chris Parish
Division of Immunology and Genetics
John Curtin School of Medical Research

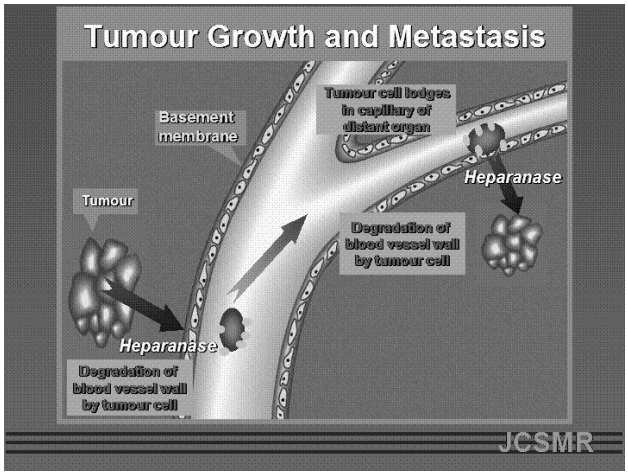
Heparan sulphate (HS) is a linear proteoglycan that has various functional roles:

- Structural – Extra-cellular Matrix (ECM), basement membranes (BM)
- Acts as a barrier to cell invasion (BM)
- Acts as an ECM depot for HS-binding growth factors/proteins
- Acts as a cell adhesion ligand - present on cell surfaces
- Allows the establishment of chemokine gradients in the ECM
- Acts as a co-receptor on cells for HS-binding growth factors

The endoglycosidase, heparanase, is a key regulator of heparan sulfate function. The important features of heparanase include:

- Aids cell invasion by degrading heparan sulfate in the ECM
- Heparanase also releases heparan sulfate binding growth factors from the ECM
- There is no alternative enzyme that can do the job!!

Heparanase is believed to be involved in tumour spread via the circulatory system since it can be produced by tumour cells. Heparanase dissolves the BM of blood vessels allowing entrance of tumour cells into the blood vessel and following lodgement of tumour cells in a distant capillary, heparanase produced by the tumour cells again dissolves the BM of the capillary allowing egress of the tumour cells. Heparanase is also believed to cause angiogenesis (new growth of blood vessels) preventing hypoxia of the tumour.



The focus of research at the Chris Parish group is to ascertain whether heparan sulfate mimetics can be synthesised that:

- Inhibit heparanase activity?
- Block action of heparan sulfate binding growth factors?
- Such mimetics have potential as anti-cancer drugs

Two such mimetics include phosphomannopentaose SO4 and phosphomannotetraose SO4, a mixture of these mimetics being termed PI-88.

PI-88 is a double-hit anti-cancer drug that:

- Inhibits tumour cell metastasis by blocking the endoglycosidase heparanase
- Inhibits tumour angiogenesis by blocking both heparanase and the action of HS-binding growth factors

The Chris Parish group found, using RIP-Tag Transgenic mice, that PI-88:

- Inhibits tumour growth at multiple stages of tumour progression
- Enhances apoptosis/inhibits cell proliferation within tumours

- Inhibits angiogenesis at all stages of tumour development
- Reduces VEGF-VEGFR complex formation on endothelial cells in tumours
- Inhibits tumour invasion into surrounding tissue and converts tumours to a benign phenotype

The data supports the view that PI-88 has multiple effects on tumour development.

The current clinical status of PI-88:

Phase I clinical trials using healthy volunteers and cancer patients have been completed. Apart from some anti-coagulative activity PI-88 is non-toxic.

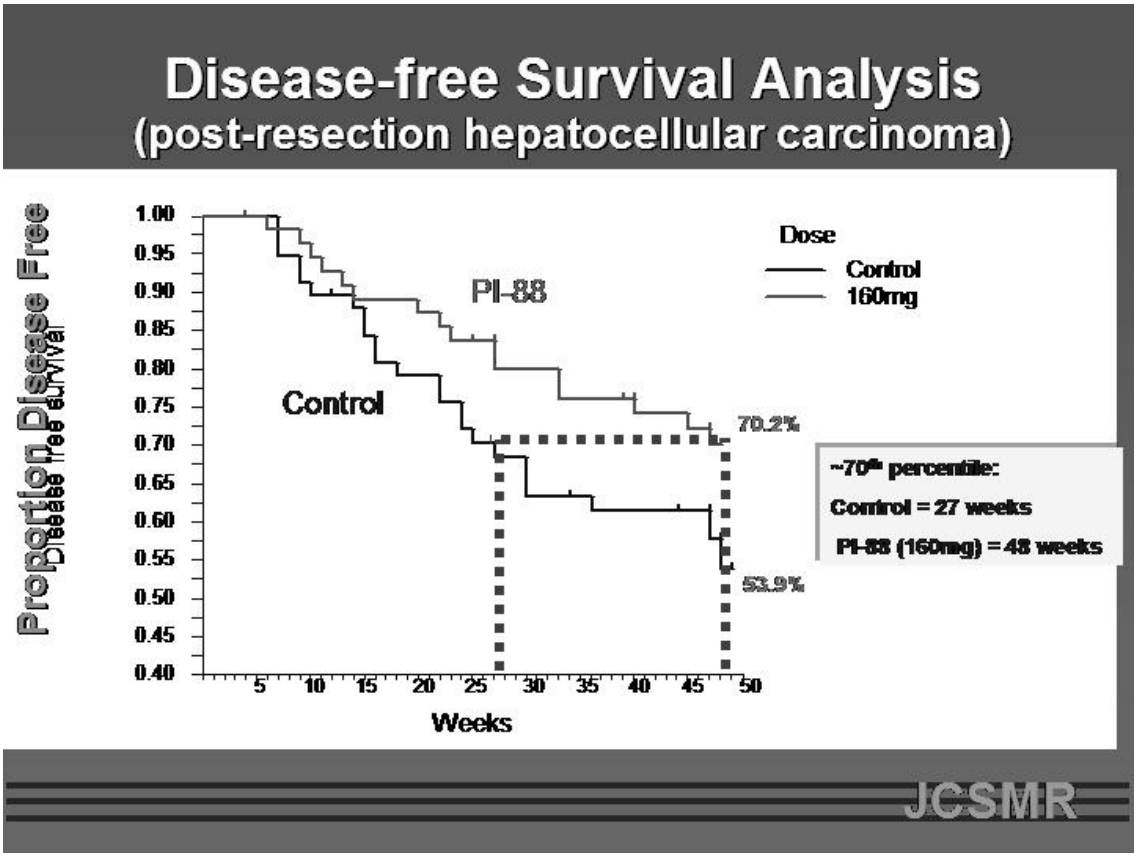
Phase II clinical trials in cancer patients are currently in progress

- melanoma (from 25-30% response rate - completed)
- melanoma + cytotoxic drug (in progress)
- non-small cell lung carcinoma + Taxol (completed)
- prostate cancer + Taxol (in progress)
- post-resection hepatocellular carcinoma (35-40% reduction in recurrence, ~80% delay in recurrence)

Phase III clinical trials in cancer patients are soon to commence including hepatocellular carcinoma (in 14 countries, 60-70 sites, 800 patients).

Conclusions:

- Low molecular weight heparan sulfate mimetics have considerable therapeutic potential as anti-cancer drugs, e.g., PI-88
- Heparan sulfate mimetics have negligible toxicity
- Second generation heparan sulfate mimetics are being developed with better pharmacokinetics and oral delivery



Fatal Enteritis Necroticans (Pigbel)

Lizhen Gui, M.D., Ph.D., Charu Subramony, M.D., Jonathan Fratkin, M.D., Michael D. Hughson, M.D.
Department of Pathology, University of Mississippi Medical Center, Jackson, Mississippi

Mod Pathol 2002;15(1):66–70

Enteritis necroticans is a life-threatening infectious disease caused by Clostridium perfringens, Type C, a β toxin–producing strain of clostridia. The disease, which is characterized by segmental necrosis of the proximal jejunum, is associated with a high mortality rate if not diagnosed early and treated with antibiotics or, if advanced, with antibiotics and surgical excision of necrotic bowel. It was first reported in Northern Germany after World War II among previously starved children and adults who ate large meals of meats and vegetables. At that time, the disease was called Darmbrand, meaning "burnt bowels". Because of the unique heat resistance of the spores, the causative organism was initially identified as C. perfringens, Type F, which was later found to be identical to C. perfringens, Type C. This disease was prevalent between 1944 and 1948, but as the nutritional status of the population improved, the condition disappeared in Europe,

In 1963, Murrell and Roth reported a similar disease in 17 patients from the Highlands of Papua New Guinea. The patients were mostly male children and young adults, ranging in age from 2 to 36 years, who presented with severe abdominal pain after ceremonial feasting on huge quantities of sweet potatoes and inadequately cooked pork contaminated with pig intestines. C. perfringens was isolated from the meat and from the stools of seven patients. C. perfringens, Type C was isolated from resected jejunal segments of affected patients, establishing the organism as the causative agent. This disease was called "pigbel," a Pidgin English term for abdominal pain after a pig feast.

Epidemiological studies indicated that this disease accounted for 10% of all deaths and for 50% of deaths in children between 6 and 10 years of age in the highlands of Papua New Guinea. A marked reduction in mortality and morbidity was obtained by treating the affected patients with Type C antiserum. When the children of New Guinea were vaccinated with a β toxoid, an 8-fold decrease in annual incidence of the disease was achieved.

The jejunum and ileum often show segmental necrosis. Microscopic examination shows Gram-positive club-shaped bacilli consistent with Clostridia coating a necrotic mucosa.

C. perfringens, a Gram-positive bacillus, can be classified into four subtypes on the basis of four major toxins produced by the organisms. Type A organisms produce α toxin; Type B, α, β, and ε toxins; Type C, α and β toxins; and Type D, α and ε toxins. Type A organisms cause gas gangrene and are a major cause of food poisoning throughout the world. Type C organisms cause enteritis necroticans.

Although Type C is widely distributed in the soil and in the stools of animals and man, enteritis necroticans is mainly confined to areas of the world in which there is severe protein malnutrition. Types B and D are not known to cause disease in humans. The gene for the α toxin is chromosomal and encodes a phospholipase that hydrolyses the membrane phospholipid of erythrocytes, endothelial cells, and muscle cells. The β toxin of C. perfringens, Type C, is carried on a plasmid and encodes a highly trypsin-sensitive protein that causes intestinal necrosis. The genes for the α and β toxins of C. perfringens, Type C, are designated *cpa* and *cpb*, respectively. The PCR amplification of *cpa* and *cpb* products with gene specific primers allows the identification of C. perfringens, Type C, and its discrimination from Type A.

A Stain to Try - Whippy's Elastic Stain

Penny Whippy, Senior Scientist, Histology, ACT Pathology

Principle

This connective tissue stain was specifically developed to produce a high contrast between the maroon elastic fibres and the green and yellow counterstain, with the inclusion of a nuclear stain for orientation. Orcein is an excellent dye for demonstrating elastic tissue, particularly very fine and delicate fibres such as those found in skin. Natural Orcein (obtained from lichens) has now been superseded by a synthetic product that gives stronger and more precise staining than the natural dye. The mechanism of elastic fibre staining with Orcein is not fully understood. It is thought to involve a combination of effects, with Van der Waal's forces and hydrogen bonding of the elastic fibres to phenolic groups of the dye molecule, being the two most likely.

Weigert's Haematoxylin is used to give a robust nuclear stain for orientation. Acidified Light Green is a nitromethylated triphenylmethane and at this concentration will stain collagen, but not elastic fibres. The small molecule of the picric acid in the Light green Picrate Solution stain the RBC's and also differentiates the Orcein and Weigert's to completion.

Fixation Most formalin-based fixatives are suitable tissue.

Controls Normal skin

Reagents

1% Orcein in 70% Ethanol with 1 % HCL - Corrosive

Orcein (synthetic) ……... 0.5 gm

70 % Ethanol ……..……..….. 50 ml

Hydrochloric Acid ……. 0.5 ml Add Orcein, 70% Ethanol and the stirring bar into a conical flask. Carefully add Hydrochloric Acid. Stir until dissolved. Filter into stock bottle.

70% Ethanol

Absolute Alcohol ……..70 ml

Distilled water ……..……30 ml Measure alcohol and water into a measuring cylinder. Store in a stock bottle.

Saturated Aqueous Picric Acid - Explosive
Commercial Product

Weigert's Haematoxylin

Commercial Product

Light Green Stock Solution
Light Green ……. 2 gm
Distilled water ……..…. 100 ml Glacial Acetic Acid ……..… 2 ml Place Light Green and distilled water into a conical flask and mix well.
Add Acetic acid and mix.
Filter and pour into stock bottle.

Light Green - Picrate (Whippy's) Solution- Explosive

Saturated aqueous picric acid …… 10 ml Light Green stock solution……..…… 5 drops
Pour Picric Acid into a conical flask.
Add Light Green solution, stir until dissolved.

Transfer into a stock bottle.

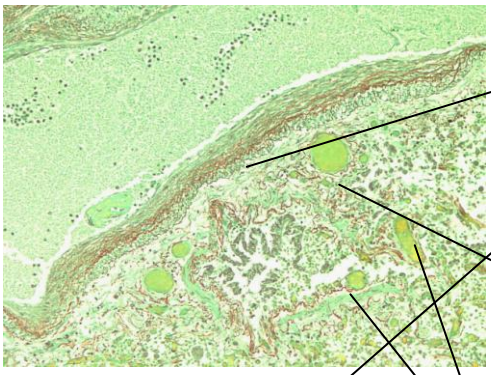
Procedure

1. Take sections down to 70% Alcohol.
2. Stain in Orcein Solution in a sealed coplin jar ……..…….. 30 mins
3. Rinse in running water. …… 30 secs
4. Stain in Weigert's Haematoxylin……..…… 5 mins
5. Rinse in running water…… 30 secs
6. Counter stain with Whippy's Solution.……..…… 2 mins
7. Rinse in running water…. ……..……..…… 10 secs
8. Dehydrate in absolute alcohol, clear and mount

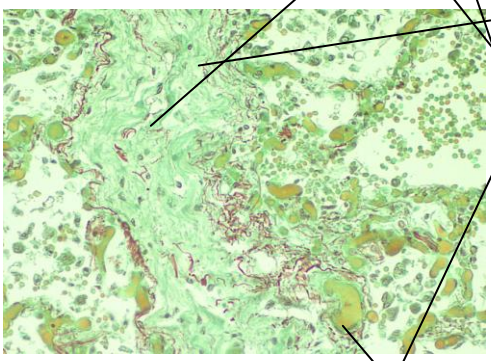
Results

Coarse elastic fibres	Maroon	Smooth Muscle	Green
Fine elastic fibres	Maroon	Red Blood Cells	Yellow
Nuclei	Black/Blue	Collagen	Lime Green

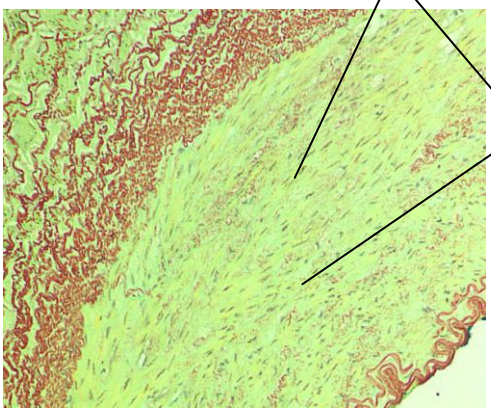
Whippy's Elastic Stain x 10 – Lung



Whippy's Elastic Stain x 10 – Lung



Whippy's Elastic Stain x 20 – Lung



Elastic Fibres

Collagen

Smooth Muscle

Red Blood Cells

Whippy's Elastic Stain x 20 – Blood Vessel

Method Limitations

Avoid 70% and 95% Alcohols when dehydrating – go to absolutes only.

Store Orcein solution in a sealed coplin jar to avoid precipitation of stain. Gurr/BDH Orcein has given consistent results and should be used in this method. Discard solution after one month.

Store at room temperature, on the shelf. To dispose of, discard after 1 month. Dilute with running water and flush to waste with a large volume of running water.

Light Green Picrate Solution may be stored, but a **freshly** prepared solution will give a sharper result.

Whippy, P. (2007) Development and Evaluation of a Rapid High Contrast Elastic and Collagen Stain. Annual Histotechnology Group of NSW Conference

Softening Toenails

At our recent State meeting there was some discussion on the optimum method for processing toe nails. These difficult tissues can cause some angst and consternation especially when they pop out of the wax block or refuse to be sectioned at 5µm!

- Thom Jensen has cut toe nail and even horse hoof using 10% ammonia water. After fixation, soak the specimen in the 10% ammonia water until it's flexible and then process it. Once processed, trim in ever-so-slightly so as not to chunk it and soak again if needed. You might want to add glue to the slide so the nail stays on during staining.
- Shelly Coker has used 10% hydrogen peroxide to soften nails. You simply trim into the nail, then soak the nail. Once softened, you can cut beautiful sections of nail. If the pieces are just too big to even trim, soak the pieces individually in peroxide, then embed and cut them.
- Lynn Burton has used Nair Hair removal solution for several years. Depending upon the thickness of the nail, it is left in overnight or even over the weekend. It cuts like a hot knife through butter after that.
- Rene Buesa places the nails in an all plastic cassette, and immerses it in 10% sodium hydroxide. Check the nail after 30 minutes and if soft enough wash them thoroughly before processing.

Solvent Recycler for Sale

One B/R Spinning Band Solvent Recycler for sale.
Model: 8400/M490
Excellent Condition
Includes:

Microprocessor controller
Recirculating water cooling unit
Spare Pot Flask (for ethanol recycling)
Manual

Price $15,000 ono

Contact Tony Henwood (02) 9845 3306
Histopathology Dept
The Children's Hospital at Westmead

Drug Development and Oncology: Links with Histology

A/Prof Desmond Yip, Medical Oncology Unit, Canberra Hospital.
ANU Medical School, Australian National University

The earliest treatment for cancer involved surgical removal of tumour masses. Surgery and radiation remove or kill the bulk of loco-regional cancer. However, small local tumor deposits and occult metastases may escape these treatments and result in subsequent systemic recurrence or relapse.

Cytotoxic chemotherapy agents work by a variety of mechanisms, but they share the property of affecting rapidly dividing cells more than they affect non-dividing or slowly replicating cells.

- Anti-metabolites interfere with DNA elongation
- Topoisomerase inhibitors and anthracyclines block a key enzyme in DNA replication
- Anthracyclines are also DNA intercalators and generate reactive oxygen intermediates that damage DNA
- Alkylating agents crosslink DNA
- Platinum-based compounds form DNA adducts
- Taxanes interfere with microtubule function

Hormonal therapies either modulate hormone receptors or inhibit enzymes necessary for hormone synthesis.

Targeted anti-tumour agents include biologic and small-molecule compounds that bind or block specific molecules important for tumor cell growth. These "magic bullets" have fewer side effects and dosing can be adjusted to the biological activity of the agent rather than to a maximum tolerated dose.

Targeted therapies have been classified into the "mabs" and the "nibs" which are the suffixes for the generic names of the drugs.

- Monoclonal antibodies (mabs) are proteins synthesized to bind onto specific tumour cell proteins or receptors present on the cell surface that are necessary for tumour growth and thereby neutralise their action.
- Small molecule inhibitors (nibs) block the activity of signaling pathways inside the cell to prevent tumour cell growth.

Monoclonal antibodies in cancer

Generic name	**Trade name (manufacturer)**	**Target**	**Clinical indication**
Rituximab	MabThera (Roche)	CD 20	Lymphoma
Trastuzumab	Herceptin (Roche)	Her2 receptor	Breast cancer
Bevacizumab	Avastin (Roche)	VEGF	Colorectal, lung, breast ca
Cetuximab	Erbitux (Merck)	EGF receptor	Colorectal cancer, head and neck SCC
Panitumumab	Vectibix (Amgen)	EGF receptor	Colorectal cancer

Small molecule signal transduction inhibitors

Generic name	Trade name (manufacturer)	Target	Clinical indication
Imatinib	Glivec (Novartis)	c-KIT, bcr-abl	GIST, CML
Erlotinib	Iressa (Astra-Zeneca)	EGFR	NSCLC
Gefitinib	Tarceva (Roche)	Her 1, EGFR	NSCLC, pancreas ca
Sunitinib	Sutent (Pfizer)	PDGFR,KIT,VEGFR,PDGFR,RET,CSF-1R	Renal cell cancer, GIST
Sorafenib	Nexavar (Bayer)	Raf kinase	Renal cell cancer

Members of the ErbB receptor family play integral roles in normal cell growth and survival[1, 2]. They have also been associated with negative outcomes and decreased survival rates in patients with a variety of tumor types, making ErbB family members attractive candidates for therapeutic intervention[3, 4]. In vitro studies have demonstrated that blockade of the ErbB-1 pathway can induce growth arrest, while inhibiting signaling through ErbB-2 leads to apoptosis in certain transformed cell lines[5, 6].

Ligand-mediated dimerization of the ErbB receptors and subsequent autophosphorylation or transphosphorylation leads to their association with a variety of cytoplasmic phosphotyrosine binding proteins. This results in the initiation of a phosphorylation cascade and activation of several downstream pathways involved in cell growth and survival, including the Ras/Raf/MAPK and PI3K/Akt pathways[7]. Stimulation of these pathways transmits a signal to the nucleus resulting in modification of gene transcription patterns that ultimately affects processes such as cell division, apoptosis, adhesion, migration, and/or differentiation.

Although the various dimer combinations activate overlapping downstream pathways, each receptor exhibits a unique phosphorylation pattern. The profile of the adaptor proteins that interact with each family member, and thus the quality and potency of the output signal, is distinct. For example, Erb-B-3 contains 6 binding sites for a PI3K subunit, leading to particularly potent activation of the Akt survival pathway through dimers containing this receptor[8]. In addition, the same receptor can also exhibit a different phosphorylation pattern and bind a unique subset of adaptor proteins dependent on ligand and dimerization partner. To illustrate, while EGF-activated ErbB1 homodimers recruit Shc and Grb2 and display rapid internalization, ErbB-1/ErbB-4 heterodimers activated by NRG-1 recruit Shc but not Grb2 and internalize more slowly[9]. This combinatorial diversity allows for exquisite control and fine tuning of signal transmission and cellular responses through the ErbB family of receptors.

Her2 alteration in breast cancer represents about 25% of these cases where its over-expression promotes invasion, survival and angiogenesis of cells. These cancers have a more aggressive clinical course with earlier relapses and relative hormone

insensitivity.
Immunohistochemistry is used to assess over-expression and in situ hybridisation is used to assess over-amplification.

There are multiple strategies that could potentially be used to block signaling through the ErbB receptors:

- Monoclonal antibodies (MoAbs) directed toward the extracellular domain of the receptor can be used to prevent interactions with ligands. This approach might also modulate signaling, dimerization, or receptor expression on the cell surface, as well as potentially triggering antibody-dependent cellular cytotoxicity or complement-mediated cytotoxicity.
- Small molecules directed toward the kinase domain can inhibit phosphorylation and activation of downstream signaling pathways.
- Receptor antagonists can be used to competitively block ligand binding.
- Ligands or receptor-specific antibodies can be conjugated to lethal toxins. Following binding to the receptor, the toxin is internalized and kills the tumor cells.
- Antisense oligonucleotides can be used to down-regulate the expression of ErbB receptors or ligands.
- Vaccines can be made to trigger the immune system to attack tumor cells over-expressing normal or mutant ErbB receptors.

While all of these strategies could potentially be used to inhibit ErbB receptors, so far MoAbs and small-molecule kinase inhibitors have been developed to the greatest extent in a clinical setting. Two commercial products are Trastuzumab (Herceptin), a monoclonal antibody to block ligand binding or receptor dimerization, and Lapatinib (Tykerb), a small-molecule kinase inhibitor.

Trastuzumab is a humanised monoclonal antibody targeting Her2. It is used in advanced breast cancer as a single agent or in combination with chemotherapy. Its main toxicity is reversible cardiotoxicity. Herceptin was approved by TGA in 2000 and reimbursed in 2001 in situations where Her2 IHC was 3+ or FISH positive.

There have been five randomised clinical trials in early breast cancer (14 079 women) (HERA, NSABP B31, NCCTG N9831, BCIRG 006, FinHER). Meta-analysis of these studies showed improvement in:

- Disease Free Survival by 40%
- Overall survival by 36%

The TGA approved Herceptin for ISH+ve early breast cancer in April 2006 and reimbursed in October 2006.

Lapatinib is a small molecule dual kinase inihibitor (EGFR and Her2). It is taken orally. It is used in trastuzumab resistant advanced breast cancer in combination with Xeloda. It has been found to cause a 51% reduction in disease progression. It was TGA approved in Jul 2007.

EGFR may play a critical role in regulating tumor cell growth, repair and survival, angiogenesis, invasion and metastasis. It is expressed in a significant percentage of human tumors and is correlated with poor prognosis, decreased survival, and/or increased metastasis

EGFR expression in solid tumors

Tumor Target	%
Colorectal cancer	72–89
Head and neck cancer	95–100
Lung cancer (NSCLC)	40–80
Breast cancer	14–91
Ovarian cancer	35–70
Renal cell cancer	50–90

Cetuximab (Erbitux) is an IgG1 monoclonal antibody targeting EGFR. Its binding blocks EGFR signaling and inhibits proliferation, angiogenesis and metastasis, and stimulates apoptosis. A registration study was done in EGFR ICH positive advanced colorectal cancer and demonstrated a benefit in combination with irinotecan in refractory patients. TGA approval was obtained in January 2005. It has been combined with radiotherapy in treatment of locally advanced head and neck cancer (TGA approved Jan 2007, reimbursed Sept 2007).

In advanced refractory colorectal cancer improved survival was seen in patients when cetuximab is given in combination with irinotecan chemotherapy or alone. There was no correlation of response with the degree of EGFR-IHC staining. Responses were also seen in patients who were EGFR IHC –ve. This has lead to an investigation of EGFR gene amplification and downstream mutations (KRAS, BRAF, MEK, AKT) to see whether these predict responses. So far the KRAS mutation seems to have a positive correlation.

The main side effect is an acneiform rash. The severity of the rash correlates with a positive clinical response to cetuximab in colorectal cancer.

Erlotinib (Tarceva), a small molecule tyrosine kinase inhibitor, is taken orally for the treatment of non-small cell lung cancer. The Phase III trial of Erlotinib in previously treated advanced non-small cell lung cancer has shown an improvement in symptoms and quality of life as well as survival in second or third line therapy of advanced non-small cell lung cancer compared to supportive care (6.7 vs 4.7 months). A side effect is an acneiform rash.

It was found that females, patients of Asian origin, non-smokers, and adenocarcinomas were more likely to respond. There was also a higher response rate in EGFR amplified or high polysomy. EGFR exon mutations were not correlated with response or survival.

Pharmacogenomics is the study of inherited gene variations that dictate drug response. It aims to predict whether a patient will respond to a drug. It uses techniques such as Single Nucleotide Polymorphism (SNP) screening and biomarkers to see if there are correlations with response. Most of the current clinical trials of new biological agents incorporate these biological substudies to determine predictors of response. This would hopefully bring on the era of

Personalised Medicine to allow selection of the most appropriate drug(s) out of the many available to use in an individual rather than going through a costly process of trying each drug in turn in a particular person.

The cost of bringing a new drug to market is $US110-500 million. Pharmaceutical companies must recoup the cost of research and development as well as make a profit before patent protection run outs. Targeted therapies are high cost drugs and examples of the cost of available biological agents in Australia is listed in the table below.

The Cost of Targeted Therapies

Drug	Per month ($A)
Herceptin	$4100
Tykerb	$3900
Erbitux	$8000
Tarceva	$3800

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Formalin or not formalin - that is the question

D Grehan and M McDermott
Our Lady's Hospital for Sick Children, Crumlin, Dublin 12, Ireland

We have all faced the dilemma. The laboratory receives a universal container in which a tissue sample is immersed in a clear liquid. The tissue is still pink and blood stained. So has the sample been placed in saline in error or is it in formalin and simply not yet fixed? In time honoured fashion, the laboratory technician or pathologist removes the lid of the container and gingerly inhales. Regrettably, by the time the characteristic odour of formalin is recognised, its noxious and irritant properties have already inflicted their damage upon the teary eyed investigator. It need no longer be this way!

An easily and rapidly applied technique can establish the presence or absence of formalin without placing the investigating staff in harm's way.

Place a few drops of reticulin solution in a beaker and add small drops of your test solution.
If the test solution is formalin, the reticulin solution will turn black. A similar effect can be produced by adding the test solution to Schiff's solution. In this case, adding drops of formalin will turn the combination a deep magenta colour. The addition of a test solution of saline (the most frequently encountered alternative) will produce no colour change to Schiff's solution and will turn reticulin solution white. Because all laboratories will have both reagents already prepared on their shelves, the test may be done in a matter of seconds.

Good old fashioned chemistry to the rescue!

J Clin Pathol (2001)54:734–735

Histotechnology Group of Queensland State Conference

Date 3-4th May 2008

Theme: Back to Basics

Venue Ramada Pelican Waters ★★★★★
Mahogany Drive
Pelican Waters
Caloundra (Sunshine Coast)

CONFERENCE DRAFT PROGRAMME

Confirmed Speakers

More Topics to be Added

Mr Bryan Llewellan	*Haematoxylin and Eosin in Detail*
Janette Thurley	*An Introduction to Fixation.*
Georgia Stamaratis	*Processing of Whole Eye Specimens*
Susan Bell	*Processing of Bone Marrow Specimens*
Chris Hagon	*Allopaecia*
John Pauli	*Cut-up by Technical Staff*
Leigh Owen	*Histology in the Solomon Islands*
Julian Richardson	*Histology in the 1800's*
Laura Arrowsmith	*How to setup and maintain your microscope*
Bryan Llewellan	*The Trichrome Stains in detail PT1*
Bryan Llewellan	*The Trichrome Stains in detail PT2*
St Vincents Hospital	*Basic MolecularTechniques*
Susan Campfield	*Neuropathology Case Studies*

HISTOTECHNOLOGY GROUP OF QUEENSLAND
STATE MEETING 3-4th MAY 2008

REGISTRATION FORM

Complete and Return

By Email
michael_doyle@snp.com.au

By Fax
07 3876 9306

By Mail
Michael Doyle
Sullivan and Nicolaides Pathology
134 Whitmore St
Taringa Q 4068

Please photocopy if more than one delegate attending

Delegate Details

Date:

Name:.....

Company/Institution:.....Email:.....

Company/Institution Address:.....

Suburb:.....State:.....Postcode:.....

Phone: ( )..... Mobile: ( )..... Fax: ( ).....

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(Includes registration, accommodation for Saturday night, Sunday breakfast, conference dinner, happy hour, all lunches, coffee breaks etc)

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	Twin Share	$	430*	$.....
Non-members	Single	$	520*	$.....
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❑ Conference Registration

(Includes registration, conference dinner, happy hour, all lunches, coffee breaks etc)

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❑ * Without conference dinner tick this box and deduct $100 from cost

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❑ **Extra Conference Dinner Tickets** $ 100 $.....

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Hospital told to return placenta to new mom

Associated Press
http://www.ctv.ca/servlet/ArticleNews/story/CTVNews/20070720/placenta_hospital_070720/20070720?hub=Health Updated: Fri. Jul. 20 2007 8:39 AM ET

LAS VEGAS — A woman has won a court fight to keep the placenta after her daughter's birth. She had planned to grind it up and ingest it as a way to fight postpartum depression, but now plans to bury it.

Clark County District Court Judge Susan Johnson granted a preliminary injunction Tuesday, ordering Sunrise Hospital and Medical Center in southern Nevada to return the placenta to Anne Swanson. Hospital officials said they will comply.

The hospital had refused to give the uterine lining to Swanson following the April 12 Caesarean birth of her daughter, with officials calling it contaminated biohazardous waste. The judge ordered the hospital not to destroy the placenta, which was frozen, and ordered that it be turned over to Swanson within two weeks.

Swanson, who was 30 when she gave birth, originally wanted to give her placenta to a friend to be dried, ground into a powder and packed into capsules. She said she now plans to dry, store and eventually bury the organ instead of eating it.

"I hope this brings about a better awareness about the benefits of placenta," she said, citing a theory that placental hormones can help control postpartum blues.

Amy Stevens, system vice president for Sunrise Health, which operates Sunrise Hospital, described the ruling as specific to Swanson. She said the hospital must comply with strict regulations in handling human biohazardous waste.

There is no Nevada law prohibiting hospitals from returning placentas to mothers. But several Las Vegas area hospitals told the Las Vegas Review-Journal the organ is usually destroyed unless a physician designates it for medical tests or a patient seeks it for specific religious or cultural reasons.

Joe Nocito [jnocito@satx.rr.com] has made two observations:

Firstly, the placenta was frozen and not placed in formalin. Every hospital I have worked in always placed the placenta in formalin (ok, some might have placed 15mls in the container, maybe 20).

The second thing I noticed was that Nevada has no law about returning specimens to the owner. I've worked at placed were we were being sued by a guy because he wanted his finger back, 18 months ago. Even though we sent him copies of the Texas law and MSDS on 10% NBF, he still found a lawyer to file suit. Also, I've had experiences where the patient has requested their specimen back because of religious reasons.

So here's my point. People who want their specimens back because of religious reasons do so up front. People who want to place their specimen on the coffee table for a conversational piece will request it back as an after thought. Like Billy Bob who wanted his finger back. "Hey Bubba, look what I took home from the hospital". "No you idiot, it's not chicken liver, it's my haemorrhoids". That's my story and I'm sticking to it.

Picture editing and PowerPoint tips

http://www.bibleplaces.com/tips.htm

1. IrfanView is an excellent freeware viewer program. Use it for naming and sorting pictures.
2. Microsoft Photo Editor comes with MS Office 2000 and includes a handy Autobalance feature. Sometimes the "auto" is not perfect, but often it is the quickest and easiest way to improve a picture's quality. There does not seem to be any similar feature that is as quick in Paint Shop Pro or Adobe Photoshop.
3. The pictures in the Pictorial Library are usually 1600x1200 resolution. That means they are too large for the typical screen or classroom projector (that doesn't mean they won't fit, it means that the program or the projector will not display all of the pixels). When building a PowerPoint for the classroom projector, resize the pictures to 1000x750 resolution. This reduces the file size (as sometimes my PowerPoints can be 40MB, even with this size reduction). And at 1000x750, you are still getting the full quality of an XGA projector.
4. When resizing pictures, it is essential to apply an "unsharp mask" after resizing. Resizing tends to blur pictures quite a bit and using "unsharp mask" brings the sharpness back.
5. If you are interested in building a PowerPoint file quickly with a series of photos in a certain directory, use the PhotoAlbum add-in available for PowerPoint 2000 from Microsoft's web site. This add-in has significant limitations, but for some purposes it can be a time-saver. The PhotoAlbum feature is included in PowerPoint 2002.
6. When adding pictures to a PowerPoint file, do not drag and drop or copy and paste from another source. If you do, PowerPoint interprets the picture as a tif file and the file size is greatly increased. Instead, add pictures by using the "Insert/Picture/From File" command on the PowerPoint menu.

Histograph Crossword

Last edition's answers

Across

2 Picric decal (6)

5 Lots of gloms (6)

6 Margin Marker (6)

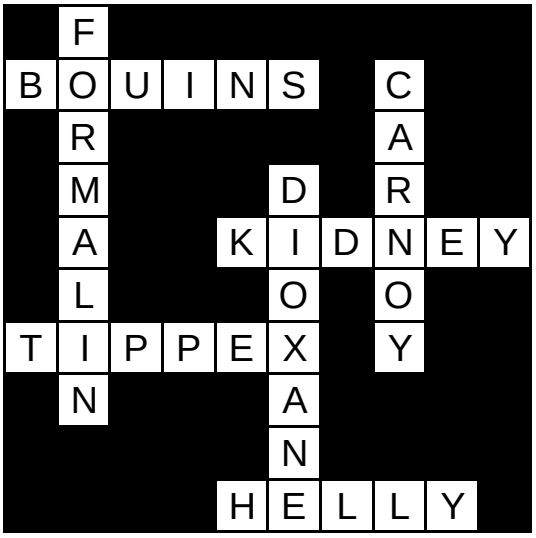
7 Hg No No (5)

Down

1 Common Fix (8)

3 Blast rbc (6)

4 Universal solvent (7)



This edition's Histo Crossword again carries the prize of a bottle of wine for the first correct answer received by our Chairman, Trevor Hinwood (trevor.hinwood@hdscientific.com.au).

Across

1 Silver and Alizarin (7)

3 Plasma bodies (7)

5 Pink NA (7)

8 Quincke pos (4)

9 Blue cholecyanin (7)

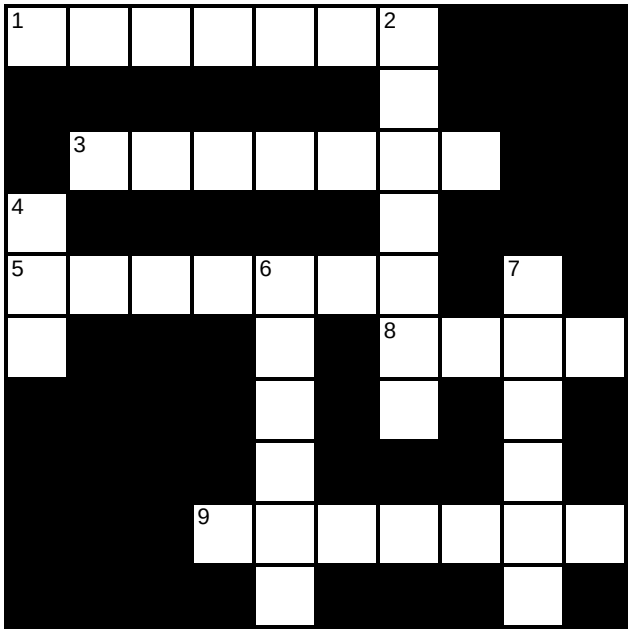
Down

2 Turnball (7)

4 Myelin blue (3)

6 Red and yellow (6)

7 Red Perls (6)



Canberra Surgical Cut Up Workshop

- *Held over four Saturdays in March, May, July, and Sept 2008*
- *Covers the 4 NCAAP guideline divisions*
- *Suitable for all staff, from Associate Diploma equivalency to Degree*
- *Must have minimum 12 months experience in Cat Up assistance*
- *Satisfactory completion of all 4 sessions = Certificate of Competency*
- *Held at Canberra Institute of Technology and run by Anne Prins and Penny Whippy*
- *Maximum intake 12 only so be quick!*

Contact:

Anne Prins 02 6125 4644
anne.prins@anu.edu.au

Penny Whippy 02 6244 2874
penelope.whippy@act.gov.au

Safety Corner - Potassium Permanganate

Potassium permanganate, commonly known as Condy's Crystals, is an oxidising agent and the crystalline form or concentrated solutions are corrosive. Solutions of greater than 0.02% strength may cause corrosive burns to the skin and mucous membranes (1).

The fatal oral dose is estimated to be about 10g. Death may occur up to one month from time of poisoning. Swelling and irritation of the tissues in the mouth and throat, nausea and vomiting may occur after swallowing solid permanganate or concentrated solutions. A high-pitched noisy breathing (stridor), slow pulse, shock and fall in blood pressure can occur. Liver and kidney damage may develop (2).

Middleton et al (4) have described a fatal case of haemorrhagic pancreatitis caused by severe potassium permanganate poisoning while Young et al (5) described a case of fatal acute hepato-renal failure following potassium permanganate ingestion.

Mahomedy et al (3) have described two cases of Methaemaglobinaemia caused by potassium permanganate poisoning following treatment dispensed by witch doctors. Justus & Gastmeier (6) have described a case of fatal potassium permanganate poisoning when it was mistaken for charcoal.

Potassium permanganate is regularly used in the management of suppurative eczema, and ulcers, and has previously been used as an abortifacient. Ingestion of dilute solutions can cause brown staining of the mouth and throat, sore throat, abdominal pains, vomiting and dysphagia. Concentrated solutions or dry crystals can cause swelling and bleeding of lips and tongue, pharyngeal oedema and swelling of the larynx, as well as gastrointestinal burns. Systemic effects do not usually manifest, due to poor absorbance, but can include tachycardia, hypotension, hallucinations, methaemaglobinaemia and cyanosis, metabolic acidosis, haemolysis, pancreatitis and coma. Some effects can be delayed up to 36 h post-ingestion including disseminated intravascular coagulation (DIC), cardiac failure and hepato-renal failure. It has been postulated that the damage from ingestion of potassium permanganate crystals is due to oxidative injury from free radicals generated by the absorbed permanganate ion. It was found that the clinical course following potassium permanganate poisoning closely resembled severe paracetamol overdose and early administration of N-acetylcysteine has been recommended (7).

References:

1. Temple & Smith (1998) International Programme on Chemical Safety Poisons Information Monograph 409 Chemical.
2. ProSciTech (2004) Potassium Permanganate MSDS.
3. Mahomedy et al (1975) Anaesthesia 30: 1903.
4. Middleton et al (1990) Postgraduate Medical Journal 66: 6578.
5. Young et al (1996) Human Experimental Toxicology 15: 25961.
6. Justus & Gastmeier (1967) Das Deutsche Gesundheitswesen 22: 8979.
7. Dhamrait (2003) Anaesthesia 58(6): 606

Beer is good for you - 50 Spanish nuns can't be wrong

Fifty Spanish nuns have tested the health benefits of beer in a scientific study financed by beer producers, the daily El Pais reported on Thursday.

"We have offered to do this service to humanity," said Sister Almerinda Alvarez, one of the nuns living in three Cistersian cloisters in the northern Leon province.

In the first phase of the experiment, the nuns ate normally without taking any alcohol. In the second phase, their diet was supplemented with half a litre of alcohol-free beer per day for 45 days. In the third phase, they took two hop capsules a day.

The moderate consumption of any kind of beer - dark or light, normal or alcohol-free - improves oxidative metabolism and lowers cholesterol levels, concluded the study by Valencia University and the Spanish Society of Dietetics and Nutrition Sciences.

As to the question why nuns were chosen to test the beer, Sister Alvarez said the researchers wanted "responsible people with an organised schedule and a balanced diet."

Printed from medicalsearch.com.au (7/6/07)
http://www.medicalsearch.com.au/news/viewrecord.asp?id=26338

Safran du Gatinais

Saffron, a culinary spice and colouring agent most familiarly used in paella, consists of the stigmas of the flowers of Crocus sativus. The spice has a very strong aroma and also contains a dye of some histologic interest. Because of its labour-intensive production saffron's extremely expensive.

Saffron is used histologically as a connective tissue stain, traditionally in one of the many techniques attributed to Dr. Masson, and in the Movat Pentachrome stain. It dyes collagen a yellow-orange colour that contrasts subtly with eosin.

Saffron has a Colour Index number (75100). The active colouring matter is called crocin, composed of crocetin and gentobiose.

To prepare the stain, the dye is extracted from the crude spice with ethanol. Because saffron is so expensive, the WHO tumour fascicles (in the 1960's) suggested extracting the dye into ethanol using a reflux condenser to achieve maximum yield. This alcohol extract has an obnoxious medicinal smell.

Safflower (Carthamus tinctorius) is a look-alike, sometimes called dyer's saffron or bastard saffron. It is odourless and contains a different dye, carthamin or carthamone, chemically unrelated to Saffron. It has not been used as a histologic stain. Safflower is sometimes referred to as saffron, and do not buy it for histologic use - remember it's odorless. (Safflower is grown commercially as an oil seed.)

Saffron was historically grown in France and Spain. It is still grown commercially in Spain, but most of it is grown in India. The traditional histologic designation “safran du Gâtinais” referred to the French product, which may no longer be available. (Saffron was grown in England centuries ago, hence the place name Saffron Walden.)

Bob Richmond
Samurai Pathologist, histoantiquarian and occasional blazoner wannabe
Knoxville

Histotechnology Group of NSW Membership Application

2007 - 2008

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