Histograph

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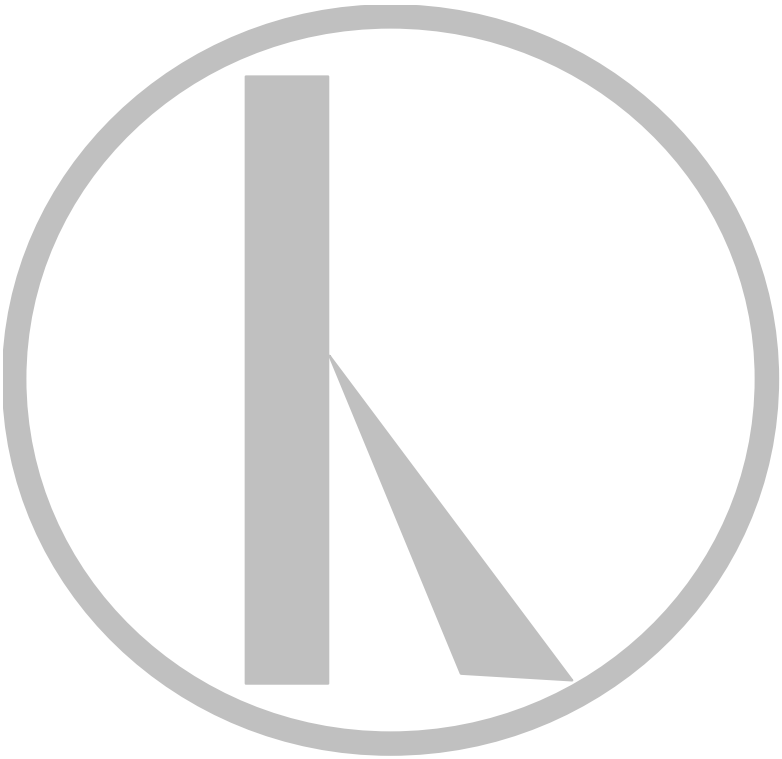

Newsletter of the Histotechnology Group of NSW

Contents

Editorial

We all hope you had a good Christmas holiday and that this year is comfortably challenging, rewarding and safe. Only one month to go before our National Meeting in Adelaide and this should be the years highlight.

Just a reminder to consider sending articles for publication in Histograph: original research, reviews, professional comment on current issues facing histotechnologists. Everything is welcome.

This current print run is surprisingly smaller than usual, since only financial members will be receiving Histograph. So if your colleagues wonder why they have not received this issue, ask them if they have paid their dues. If they have then ask them to contact our secretary so it can be put right pronto.

Tony Henwood,
Editor
anthonyh@chw.edu.au

Chairman's Report

The 4th National Histology Conference in Adelaide is fast approaching and we encourage our members to participate. There is still time to register for what will be another great meeting of Histology minds. More information can be found on their Website www.nhc.org.au

Our new website is now operational and details can be found in a separate report in this newsletter. There will be further development work which Margaret James will continue to be involved in. Details are www.histonsw.org.au

It was good to see so many people in attendance at our Christmas meeting (separate report in this magazine). Thank you to Leica Microsystems for their sponsorship of the evening and Dr Richard Jaworski for his informative presentation on Astronomy. A great night was had by all.

In March, Dr Warick Delprado of DHM Pathology presented a "Prostate Update". The actual presentation was "The why, what and what the …….". Again a good attendance of over 40 people.

Warwick emphasised the importance of the patient - there is a lot of technical work involved in the preparation of both prostate core biopsies and radical prostatectomies. He showed us the two methods of taking core biopsies. He also showed the importance of identification of margins on radical prostate specimens. The "What the" component involved other parts of the human anatomy which appeared with the prostate such as rectal mucosa, amyloid around a blood vessel and fragments of a hip replacement which was disintegrating.

Remember NATA Accreditation requires personnel who work in laboratories to attend Continuing Education sessions. All meetings sponsored by the Histotechnology Group of NSW count towards these requirements.

Our next meeting is on the 22nd of April. Our guest speakers are Dr Jim Lawson (UNSW) and Noel Whittaker (Molecular Biologist). Topic is "Is breast cancer sexually transmitted (by HPV)? More information on our Website. All welcome.

If you have colleagues who have not received this Histograph it will mean they are non financial and will need to pay this year's membership fee as soon as possible. Non financial members will not receive future Histographs.

We would welcome any ideas you have on topics or guest speakers for meetings. We can be contacted through the Website or committee members.

Trevor Hinwood,
Chairperson,
Histotechnology Group of NSW.

Safety Zone Prion Diseases

Transmissible spongiform encephalopathies (TSEs), also known as prion (Proteinaceous Infectious particles) diseases, are fatal degenerative brain diseases that occur in humans and certain animal species. They are characterized by microscopic vacuoles and the deposition of amyloid (prion) protein in the grey matter of the brain. All forms of TSE are experimentally transmissible (1, 2).

Human TSEs occur in sporadic, familial, and acquired forms. The most common form, sporadic Creutzfeldt-Jakob disease (CJD), has a worldwide death rate of about 1 case per million people each year, and typically affects people between 55 and 75 years of age. The disease usually begins with a progressive mental deterioration that soon becomes associated with progressive unsteadiness and clumsiness, visual deterioration, muscle twitching (myoclonus), a variety of other neurological symptoms and signs, and is often associated with a characteristic periodic electroencephalogram. The patient is usually mute and immobile in the terminal stages and in most cases, death occurs within a few months of onset of symptoms. TSEs are invariably fatal and there is no proven treatment or prophylaxis (1).

Similar neurodegenerative diseases also occur naturally in some animal species (scrapie in sheep and goats, chronic wasting disease in deer and elk), or as a result of exposure of susceptible species to infected animal tissues (transmissible mink encephalopathy, bovine spongiform encephalopathy, and spongiform encephalopathy in domestic cats and a variety of captive zoo animals) (1).

TSE agents exhibit an unusual resistance to conventional chemical and physical decontamination methods. They are not adequately inactivated by most common disinfectants, or by most tissue fixatives, and some infectivity may persist under standard hospital or healthcare facility autoclaving conditions (e.g. 121°C for 15 minutes). They are also extremely resistant to high doses of ionizing and ultra-violet irradiation and some residual activity has been shown to survive for long periods in the environment (1).

Precautions for working with high and low infectivity tissues from patients with known or suspected TSEs

1. Whenever possible and where available, specimens should be examined in a laboratory or centre accustomed to handling high and low infectivity tissues; in particular, high infectivity tissue specimens should be examined by experienced personnel in a TSE laboratory.
2. Samples should be labelled ‘Biohazard’.
3. Single-use protective clothing is preferred as follows:
   - liquid repellent gowns over plastic apron;
   - gloves (cut-resistant gloves are preferred for brain cutting);
   - mask;
   - visor or goggles.
4. Use disposable equipment wherever possible.
5. All disposable instruments that have been in contact with high infectivity tissues should be clearly identified and disposed of by incineration.
6. Use disposable non-permeable material to prevent contamination of the work surface. This covering and all washings, waste material and protective clothing should be destroyed and disposed of by incineration.

Distribution of infectivity in the human body

Infectivity Category	**Tissues, Secretions, and Excretions**	
High Infectivity	Brain	
	Spinal cord	
	Eye	
Low Infectivity	CSF	
	Kidney	
	Liver	
	Lung	
	Lymph nodes/spleen	
	Placenta	
No Detectable Infectivity	Adipose tissue	Tears
	Adrenal gland	Nasal mucous
	Gingival tissue	Saliva
	Heart muscle	Sweat
	Intestine	Serous exudate
	Peripheral nerve	Milk
	Prostate	Semen
	Skeletal muscle	Urine
	Testis	Faeces
	Thyroid gland	Blood

7. Fixatives and waste fluids must be decontaminated using sodium hydroxide adsorbed onto materials such as kitty litter and disposed of by incineration as a hazardous material.
8. Laboratories handling large numbers of samples are advised to adopt more stringent measures because of the possibility of increased residual contamination, e.g. restricted access laboratory facilities, the use of 'dedicated' microtomes and processing labware, decontamination of all wastes before transport out of the facility for incineration.

It is important to note that formalin and glutaraldehyde-fixed TSE tissue retains infectivity for long periods, if not indefinitely. As a result, they should be handled with the same precautions as fresh material and be considered infectious throughout the entire procedure of fixation, embedding, sectioning, staining, and mounting on slides, until or unless treated with formic acid. Treatment with formic acid reduces infectivity to negligible levels. Although exact procedures may vary, formic acid treatment consists of placing small pieces of fixed tissue, no more than 4 to 5 mm thick, in 50 to 100 ml of 95% formic acid for an hour, and then transferring them to fresh formalin for another two days before further processing. The entire procedure is conducted using continuous, gentle agitation.

Slides made from sections which have been treated with formic acid can be considered non-infectious. Slides made from sections that have not been treated with formic acid may also be handled without specific precautions, once the cover

slip is sealed to the slide and chemically disinfected to ensure external sterility, but should be labelled as a hazardous material.

Reference

1. WHO Infection Control Guidelines for Transmissible Spongiform Encephalopathies Report of a WHO consultation Geneva, Switzerland, 23-26 March 1999 http://www.who.int/csr/resources/publications/bse/whocdscsraph2003.pdf
2. Belay, E.D., (1999) "Transmissible Spongiform Encephalopathies in Humans" Annu. Rev. Microbiol. 53:283–314

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Pineal Gland the Internal Eye

Buried deep within the centre of the brain, the pineal gland has a structure similar to our lateral eyes.

For thousands of years, the pineal gland was recognized as the human body's connection to deeper realms of thought—a window into other dimensions. While this notion has faded with the passing of time, science has begun to focus its efforts toward understanding the secret functions of the "hidden eye."

The pineal gland performs a host of important bodily functions, such as sexual development, metabolism, and the production of melatonin. Yet scientists have found features present in the pineal gland that elude a simple explanation. Because of the unique structure of this organ, scientists have concluded that it must have once served some now latent functions. Modern medicine has revealed that this gland buried deep within the centre of the brain contains photoreceptor cells. Yet the predominant opinion is that these features merely describe latent abilities from an earlier age in our evolution.

According to science's evolutionary understanding of the pineal body, this organ once existed as a disordered system of nerve fibres located outside the surface of the skull. It specialized in capturing changes in light, providing its owner with more escape possibilities in the event of a predator's attack. This understanding sees the pineal gland performing functions similar to the eyes, the only difference being in its curious insistence on receding inside the skull.

A recent hypothesis proposed by David Klein, head of Neuroendocrinology at the National Institute of Child Health and Human Development (NICHD), suggests that primitive retinas had exercised the dual function of both capturing the image and producing melatonin. He believes that over time this latter function had migrated to the pineal gland, an emancipated organ, while the degeneration of the retina as a product of melatonin in mammals continues without coherent explanation.

Even though nowadays the pineal gland is recognized as being good for secreting endogens, it's certain that it still contains an important photosensorial capacity, a bodily process that is scientifically recognized.

Surprisingly, if both eyes were removed and the anatomical path from the frontal area of this gland was exposed to light, this organ could still respond to stimulus in a similar manner as the lateral eyes. This fact has some researchers considering whether the pineal gland is more than a degenerated eye. What if many of the still misunderstood processes of the brain reside in this small conical space?

According to Dr. Sérgio Felipe de Oliveira, Master of Science at the University of São Paulo's medical school and director of the Pineal Mind Clinic, an increase in pineal activity is intimately related with psychic activity such as visions or meditation.

Furthermore, besides the multiple endogenous functions of the pineal gland (control of the hypothalamus and biological rhythms, and protection from free radicals) it is also responsible for emitting N,N-dimethyltryptamine (DMT), known by some as the "spirit molecule." The liberation of this molecule is considered to be one of the most powerful hallucinogenic neurotransmitters known to man. It increases during sleep, in certain meditative

states, during near-death experiences, as well as with the ingestion of hallucinogenic plants.

Sceptics question the validity of these supposed episodes of heightened awareness into other dimensional planes, preferring instead to believe such experiences to be merely chemically induced phenomena limited to the brain. But they have trouble offering a reasonable explanation for the relationship of the liberation of DMT (and the consequent formation of images in the pineal) with near-death experiences.

Such is recognized by Dr. Rick Strassman, who has conducted exhaustive studies of the effects of DMT in humans. Research of this kind begins to approach the pineal gland as more than a vestigial eye relegated to producing hormones, but as an inborn window into other planes of existence.

This view of the pineal gland is not new. It represents the sixth chakra of ajna spoken of in the Vedic tradition, the window of Brahma as it is known in Hinduism, the Celestial Eye as the ancient Chinese call it, the Niwan Palace as it is known by Taoists, or the "Seat of the Soul" according to Descartes. Could this tiny cone hidden at the centre of the brain embody the potential to peer into realms that science is simply unable to grasp?

Vintiñi L (2008) http://en.epochtimes.com/n2/science-technology/celestial-eye-niwan-dmt-third-eye-3008.html

Histology Group of South Australia

Is Proud to Present The

REGISTRATION FORMS

NOW ONLINE

www.nhc.org.au

Workshop 1

Dr. Craig James

Surgical Grossing Of Skin Specimens

Workshop 2

Dr. John K C Chan

Immunohistochemistry - Technical And Interpretation Pitfalls

Keynote Speakers

Dr J K C Chan – Immunogenetics Of Tumours, Achieving New Heights By Immunohistochemistry

Dr J Robin Warren – How A Lifetime's Work With Helicobacter Pylori Led To A Nobel Prize In Medicine

Friday 8th May	10:00	Workshop 1
	13:00	Workshop 2
	18:30	Trade Opening with Cocktail Party
Saturday 9th May	09:00 - 17:00	Plenary Sessions
	18:30	Pre-Dinner Drinks
	19:30	Conference Dinner
Sunday 10th May	09:30	Plenary Sessions
	14:00	Finish With Late Lunch

ABCs of RUOs, IVDs, and ASRs

Those of us doing immunohistochemistry may have noted that our antibodies now carry more letters than a professor emeritus. What do they mean and what impact will they have on our testing? Some definitions:

RUO	Research use only
	Antibodies, nucleic acid sequences, etc., labelled 'Research Use Only' (RUO) purchased from commercial sources may be used in home brew tests if the laboratory has made reasonable effort for FDA-approved/cleared kits, and ASR class reagents. The results of that failed search should be documented by the laboratory director
IVD	In Vitro Diagnostic use
	a subset of medical devices which are "reagents, instruments, and systems intended for use in the diagnosis of disease or other conditions, including a determination of the state of health, in order to cure, mitigate, treat, or prevent disease or its sequelae. Such products are intended for use in the collection, preparation, and examination from the human body"
LTD	Laboratory Developed test
	"Homebrew tests" or "In-house Tests". FDA has stated that "clinical laboratories that develop [in-house] tests are acting as manufacturers of medical devices and are subject to FDA jurisdiction under the Act"
ASR	Analyte Specific Reagents
	ASR's are antibodies, both polyclonal and monoclonal, specific receptor proteins, ligands, nucleic acid sequences, and similar reagents which, through specific binding or chemical reaction with substances in a specimen, are intended for use in a diagnostic application for identification and quantification of an individual chemical substance or ligand in biological specimens
GPR	General Purpose Reagent
	a chemical reagent that has general laboratory application, that is used to collect, prepare, and examine specimens from the human body for diagnostic purposes, and that is not labelled or otherwise intended for a specific diagnostic application

The FDA has stated that "clinical laboratories that develop [in-house] tests are acting as manufacturers of medical devices and are subject to FDA jurisdiction under the Act". However, the FDA has generally exercised enforcement discretion over Laboratory Developed Tests (LDTs), and not actively regulated them. Instead, FDA decided to try to ensure the quality of the reagents used in LDTs. So, FDA created the ASR Rule.

Most of the antibodies being sold as ASR's today are those that are meant to be stand-alone predictive or prognostic tests (ER, PR, etc) and so require either FDA pre-market approval, requiring validation testing by the manufacturer, or are Class III infectious agent antibodies that require extensive proof they detect what they are supposed to detect.

Of importance to us is that many antibodies are being introduced to direct drug treatment (eg GD2, MGMT etc). One would hope that they meet the requirements of ASRs or IVDs.

The College of American Pathologists has established rules for its accredited labs on using RUO reagents. According to CAP's anatomic pathology checklist, RUOs "purchased from commercial sources may be used in laboratory-developed tests only if the laboratory has made a reasonable effort to search for IVD- or ASR-class reagents. The results of that failed search should be documented by the laboratory director."

Tim Morken (LabVision) has noted that apparently the CAP has recognized that some antibodies are not available through FDA registered vendors, so have allowed labs this as a way out of that problem. Tim believes that this is going to open a whole new can of worms because now labs are going to use antibodies that have been manufactured under no requirements at all - FDA registered labs must follow GMP (good manufacturing practices) and undergo FDA inspections. RUO-only vendors have none of these requirements. Tim comments that getting ASR's is a better bet than getting RUO's in this case. An RUO vendor does not have to test the antibody at all.

So in conclusion, did any of this make sense to you? I am still confused (maybe my normal state of mind). For further clarification I would recommend the references below.

References
1. Park, R., "CAP clarifies RUO policies" http://www.devicelink.com/ivdt/archive/08/03/004.html
2. FDA (2006) "Guidance for Industry and FDA Staff Commercially Distributed Analyte Specific Reagents (ASRs): Frequently Asked Questions" http://www.fda.gov/cdrh/oivd/guidance/1590.pdf

Mild Increase in Advertising Charges

After several years we have decided that a modest increase in advertising charges is warranted. The committee decided that the new prices be:

A4 PAGE - PRINTED COPIES SUPPLIED BY ADVERTISER
1 or 2 sides..... $75.00

A4 B&W PAGE - PRINTED IN THE JOURNAL
1 A4 page $100.00

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1 A4 page $300.00

As Thanksgiving approaches what do you think about a gall bladder in vinegar?

From Vinnie Della Speranza in the USA:

“I have a patient requesting her gall bladder be returned to her for religious reasons. The premise I've been given is so that, upon death, the patient may be stored with her body parts.

My facility has concerns about providing it to her in formalin (for obvious reasons) or alcohol. The patient admits this is a family practice with momma's appendix already being stored in the attic.

It can get a bit toasty warm here in the South so attic storage of a specimen in alcohol may not be prudent and I can't be absolutely certain it wouldn't burn the house down, another potential liability for my institution.

I'm tempted to give it to her in food grade vinegar, to avoid the potential liabilities from using anything that could be considered hazardous. Assuming that returning her gall bladder is a given, what do you think of using vinegar for this purpose?”

We had an issue with this concerning tonsils (the kids wanted them). We rinsed out the excess formalin after the final sign out by a pathologist (2 weeks), patted it dry and gave the sample to them in a clean container. This way it was fixed but they did not have the issue of formalin. I'm guessing that 2 weeks fixation should be sufficient for everything to be fixed. *Bernice Frederick HTL (ASCP)*

I was in this "pickle" once, and we washed the specimen and then just gave it to the patient clean and dry in a clean container. It was then up to the patient to figure out what to do with the specimen. I believe they had it frozen at the funeral home. Sorry Vinnie, could not pass up the pickle joke here. *Mike Pence*

One thing to keep in mind about Vinegar, however, is that fungus will still grow in it after a while. Kind of gross! *Judy Collins*

and nematodes, surprisingly known in the trade as vinegar worms; for people in the U.K., when you visit the chippie and pick up the vinegar bottle, the cloudiness you observe if you give it a good shake is/are nematodes, not to worry tho', consider it extra protein, something for nothing, not to be sniffed at in these credit-crunchy times!. *R.E. Edwards*

Once specimens arrive at our lab, they are ours. We do not give anything back to the patient. No legal liability that way. *Tom Podawiltz, HT (ASCP)*

Tom, I'd normally take this approach but if push came to shove I don't believe it would hold up in court. That may depend on the language in your surgical consent signed by the patient but that aside, the cost of responding to a patient's legal action would be much greater than the small effort it takes to render the specimen safer to turn over. *Vinnie Della Speranza*

We do release specimens to patients (with photo id required), but we have forms that they must read and sign (releasing us of any potential issues). On the form, we explain the hazards and how to store human tissue, glass slides, paraffin blocks, etc. We rinse all the fresh specimens to remove as much formalin as possible. We keep a copy attached to the report and send a copy with the patient or family member. *Jacqueline Farnsworth*

We will only release items to the funeral home, Find out if they already have arrangements with one and release it to them. Otherwise it won't get released. *Jessica Vacca*

And of course you editor had to say his two cents worth: You could use paraffin oil (also known as mineral oil). It is used successfully in museum techniques for the preservation of formalin fixed specimens. Rinse the formalin fixed specimen in water, place in ethanol (which will also bring back the colour), blot lightly and place in the oil. The oil has the added advantage that bile will not tend to leach out of the specimen. It also will not evaporate.

See: Henwood (2002)"Color preservation in pathology museum specimens" published in Biotech Histochem 2002 Jul; 77(4): 230.
Tony Henwood

Vinnie
You can also use glycerin.
Barry Rittman,

Tony,
I really like this idea of using mineral oil to transfer the specimen back to the patient. I used to work in a hospital and would get requests for tissues just as Vinne has described. Our hospital administration would instruct us on cases where the tissue had to be returned to the patient for religious burial reasons and we would seal the tissue in a bag using a kitchen sealer with no additional fixative present. In the future your legal department may want to draft up a tissue release form that would help eliminate any liabilities for mishandling of the tissue. *Christina Thurby*

I would think that embedding the whole thing in paraffin would be the best approach. They have to deal with this issue in the UK and they are required to give tissue back to anyone who wants it now. I visited a lab there where they were making little wooden coffin boxes to hold paraffin blocks. The patient should be charged for the tissue processing. *Patsy Ruegg, HT(ASCP)QIHC*

Use brine as a base for all pickling. Mix 6 cups distilled water, 3 cups white vinegar and 1/2 cup pickling salt in a large pot. Boil gently.
Histonet Alias

At a risk of inviting a series of puns/jokes, I do remember a Journal of Histotechnology article that discussed the use of honey as a fixative and a preservative. *Mark Frei MT(ASCP)*

You know, I really think that the vinegar is a great idea! I can't think of any reason off-hand why it would be hazardous, flammable, toxic, or bring on any liability. Even the isopropyl can be flammable, and if you told her to use it, you might be somewhat at fault (though I know a bit of a stretch). In any case, I think that I'll try to remember that in case I encounter any similar requests... *Joelle Weaver*

Does she already have a funeral home picked out? Perhaps she can ask the funeral home to "store" it for her, and then release it only to a funeral home. I would consult my legal department to see what they feel is appropriate, that way you are dotting all your t's and crossing your eyes >.< as well! *Susan M Weber HT(ASCP)*

I'd think that vésicule biliaire vinaigrette would be inclined to get the moldies. Of several not very satisfactory solutions, Tony Henwood's suggestion of mineral oil (paraffin oil) might be the

safest, though messy if it gets spilled.

What religion requires decades-long preservation of gallbladders? Highly observant Jews sometimes request return of tissues, but their requirement is that the tissue be buried in a Jewish cemetery - as soon as possible, not waiting for the rest of the patient to arrive. Do Muslims have any issues here? - I'm not aware of any Christian tradition that has any rules about this problem.

In my personal experience, the most common problem of this sort has been the patient who wants an amputated leg buried with him. Whenever I've dealt with this problem, a funeral director has bailed me out. As far as I know, there was no religious issue with the legs, just personal (or cultural) preference.

The most bizarre situation of this sort happened to me about ten years ago. A rural midwife had asked an OB-GYN to remove a retained placenta after a difficult delivery. The OB-GYN put the placenta in formalin and sent it to a pathology service some distance away. The midwife called the lab, and was furious to learn that the placenta had been put in formalin. It seems that (I hope you're not reading your e-mail with lunch) the midwife had her patients eat their babies' placentas.

I think the JCAHO or somebody banned returning gallstones to patients, a practice that used to be quite a nuisance for pathologists. *Bob Richmond*

Another option is to dehydrate the specimen (ethanol or acetone) and infiltrate with one of the resins (I have used GMA in the past) allow it to set and hand it over to the patient.

They can even mount it on a small wooden pedestal (The "Family Sports Trophy"??- Oh this is getting sad!) *Tony Henwood*

What religion requires decades-long preservation of gallbladders? Highly observant Jews sometimes request return of tissues, but their requirement is that the tissue be buried in a Jewish cemetery - as soon as possible, not waiting for the rest of the patient to arrive. Do Muslims have any issues here? - I'm not aware of any Christian tradition that has any rules about this problem. *Claire Ingles*

Patient is listed in our system as Presbyterian.

I'm guessing that patients know that if they cite religion as the basis for their request they are less likely to be denied.

I like the mineral oil and glycerin suggestions as they are probably the least problematic from a safety perspective. *Vinnie Della Speranza*

There are laws here in Texas that regulate all body parts as hazardous waste. If it isn't going to a funeral home the body part must go to a licensed hazardous waste handler.

Our legal department has indicated they don't feel that under Texas law there is an exception for releasing body parts even with a subpoena. We had to have that discussion once. *Ross M Stapf*

Amazing how different areas have different regs (or lack of). I thought I would attach our policy, seems to work and, drafted with the best intentions, hopefully prevents litigation and meets the needs of patients:

BIOPSY SPECIMENS: RETURNED TO PATIENTS PROCEDURE (c)

Background

At present, very few patients request the return of resected organs or other samples. When patients are specifically informed of what happens to such specimens, it is likely that such requests will increase in frequency, especially in relation to larger samples including whole organs, the disposal or retention of which may have emotional implications. Laboratories should have established protocols to

permit compliance with such requests while minimising any risk to the patient or others from infection or toxic chemicals. Procedure

1. Contact the Histopathology Department, ensure that patient details and patient representative (usually a parent) details (including contact number) are supplied.
2. Request is received and action approved by the Pathologist involved with the case.
3. Specimens are not usually released until one month after the report has been verified.
4. Histopathology staff will then liaise with the patient's representative and undertake the following procedure.

i. The specimen is washed in gentle running water for one hour (minimum).
ii. The biopsy is blotted dry and sealed in a biodegradable plastic bag
iii. The bag is labelled with "The Children's Hospital at Westmead", patient's name and the biopsy specimen laboratory accession number.
iv. The specimen is placed in a small esky or similar container.
v. A copy of the 10% formalin MSDS form is attached to the container.
vi. The details of the specimen transfer from Histopathology to the family representative are recorded on the release form (Attachment 1), a copy is given to the parents and the original is attached to the specimen report.
vii. Explanation is given to the parents regarding the possible hazardous nature of the specimen. That is:

- If the specimen is for burial, ensure a deep hole.
- Ensure that the specimen is kept away from children or pets.
- Stress that the danger does not necessarily only lie with the - possible infectious nature of the tissue but also the hazardous nature of the preservative.
- Remind the parents to be familiar with the MSDS form and if concerned contact the laboratory (phone number included on the MSDS). The above information is included on the Release Form

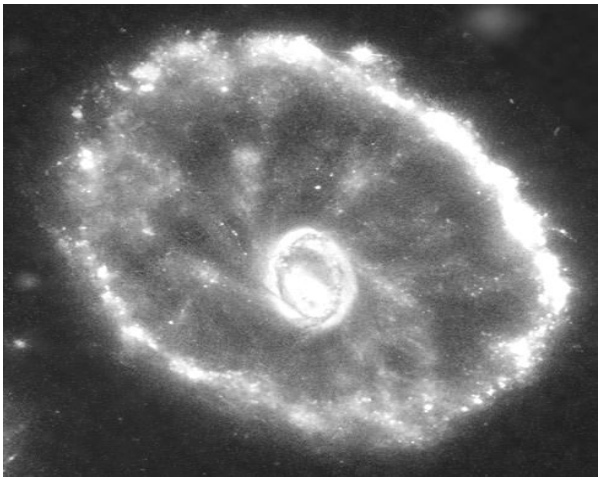
You might want to check with local regulations concerning burial. Here, and at other facilities that I've been a part of, we receive these requests regularly, usually associated with amputated limbs, however, we make it a policy to release no "soft tissue" to a patient, but instead we ask that they make arrangements with a funeral home (since this is supposed to be for purposes of burial, eventually) and it is only to the funeral home that we will release the part. What happens to the part after that, is between the patient and their funeral home representative, which is much more familiar with regulations and practices for these situations. This is the only way to complete way protect the hospital from liability of having a body part "stored in the attic".
Terri L. Braud, HT(ASCP)

References:

Royal College of Pathologists (2001) "Transitional guidelines to facilitate changes in procedures for handling 'surplus' and archival material from human biological samples" http://www.rcpath.org/index.php?PageID=208

Christmas Party with the Stars

Histotechnology group of NSW
Dec 5th 2008



I just want to thank the histotechnology group of NSW for the wonderful Christmas party you arranged in December 2008.

50 guests attended the party and enjoyed tasty food and drinks that were way too good. We all had such fun seeing everyone and really enjoyed ourselves. What a great way to kick off the holiday season!

Our appreciation and thanks must also go to Leica Micro Systems for sponsoring the event and for the gifts and other treats.

The trivia questions, one of the highlights of the night, were tricky, fun and enjoyable with many people sharing the prizes. Thanks to Liam and Julie for providing such great entertainment.

The keynote speaker for the event was Dr Richard Jaworski; histopathologist at DHM who also happens to be an amateur astronomer. Dr Jaworski took us on a sky tour to explore stars, planets, galaxies and other celestial bodies. His talk provided a great overview of the universe and space beyond earth's atmosphere and aroused curiosity in many of us. Thank you Richard for that splendid sky tour!

We look forward to more fun ahead!

Bharathi Cheerala

Douglass Hanly Moir Pathology

Serial coffee drinkers see Dead People

Medical search (http://www.medicalsearch.com.au/News/viewrecord.aspx?id=36360) has reported that serial coffee drinkers are more likely to have hallucinations or feel "the presence of dead people",

The UK-based study quizzed 200 students on their caffeine intake and found those with the highest consumption were also more prone to report seeing, or hearing, things that were not there. Those who consumed a daily equivalent of seven cups of instant coffee or more - high caffeine users - were three times more likely to have extra-sensory experiences than low users, who had less than one cup daily. The Durham University study took in all caffeine consumption including coffee but also tea, caffeinated energy drinks or chocolate bars and caffeine pills.

"This is a first step towards looking at the wider factors associated with hallucinations," said lead author, Simon Jones, a PhD student at the university's psychology department.

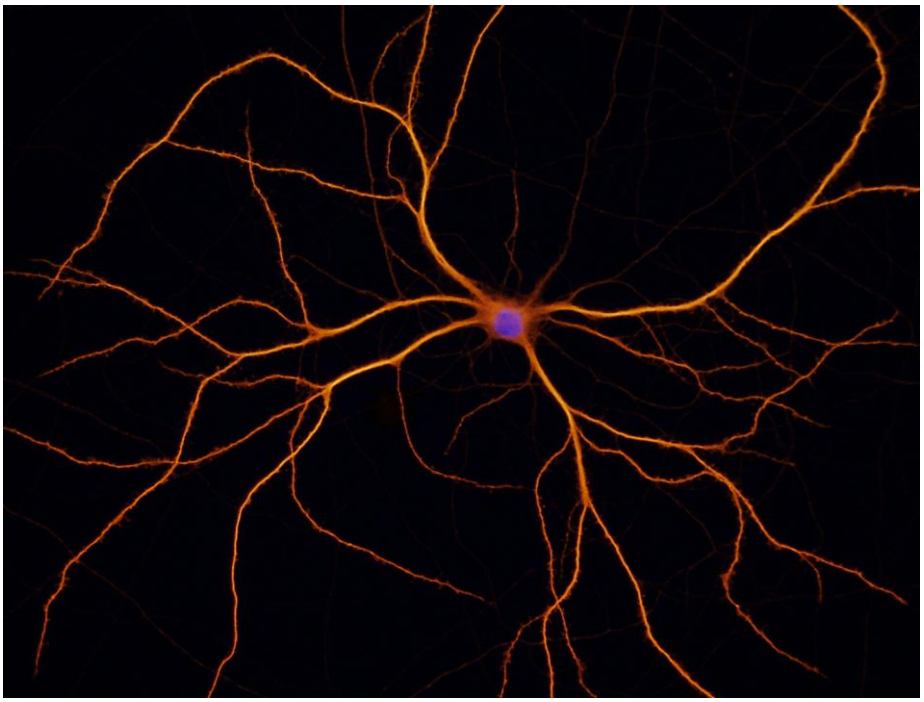
"Previous research has highlighted a number of important factors, such as childhood trauma, which may lead to clinically relevant hallucinations. Given the link between food and mood, and particularly between caffeine and the body's response to stress, it seems sensible to examine what a nutritional perspective may add."

When under stress, the body releases a stress hormone called cortisol. More of this stress hormone is released in response to stress when people have recently had caffeine. It is this extra boost of cortisol which may link caffeine intake with an increased tendency to hallucinate, say the scientists.

"However, one interpretation may be that those students who were more prone to hallucinations used caffeine to help cope with their experiences," said study co-author Dr Charles Fernyhough. "More work is needed to establish whether caffeine consumption, and nutrition in general, has an impact on those kinds of hallucination that cause distress."

People taking part in the study reported "seeing things that were not there, hearing voices, and sensing the presence of dead people". Jones said such hallucinations were not necessarily a sign of mental illness, and around three per cent of people regularly heard such voices.

Results of the study are published in the academic journal Personality and Individual Differences.



3rd A&PD Symposium

Hosted by: Alzheimer's & Parkinsons Disease Laboratory
Uncovering the mysteries of neurodegenerative disease

PATHOMECHANISMS IN NEURODEGENERATION

With support from Brain and Mind Australia, Olympus, Pathtech & Pfizer

Thursday, May 21, 2009 – Friday, May 22, 2009
at the Brain & Mind Research Institute
100 Mallett Street, Camperdown NSW

For Registration contact

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PROGRAM:

Thursday afternoon session (May 21st)

12:00 noon Coffee & registration
Session 1 chaired by Perry Bartlett
1:00 pm
Welcome, Jürgen Götz (BMRI)
1:05 pm Etienne Baulieu (Former President of the French Academy of Sciences, INSERM, Le Kremlin-Bicetre, France) Neurosteroids, microtubules and the aging brain
1:50 pm Peter Gunning (UNSW, Randwick) Principles of assembly of the actin cytoskeleton in neurons
2:15 pm James Vickers (Menzies Research Institute, Hobart, Tasmania) Axonopathy in Alzheimer's disease
2:40 pm MacDonald Christie (University of Sydney, BMRI) Assessing synaptic plasticity after nerve injury
3:05 pm Estelle Sontag (University of Newcastle) Importance of folate and PP2A methylation pathways in neurodegenerative disorders
3:30 pm Coffee break
Session 2 chaired by Barry Halliwell
4:00 pm Perry Bartlett (QBI, The University of Queensland, Brisbane) Is ageing dementia caused by diminished neurogenesis?
4:25 pm Carol Dobson (POWMRI, Randwick) Identification of a novel gene for frontotemporal lobar degeneration-motor neuron disease
4:50 pm John Hodges (POWMRI, Randwick) Frontotemporal dementia: towards more accurate in vivo diagnosis
5:15 pm Tony White (University of Melbourne) Modulating brain biometal availability as a protective strategy in neurodegeneration
5:40 pm Ralph Martins (Edith Cowan University) Blood biomarkers for Alzheimer’s disease
6:05 pm Drinks

Friday morning Session (May 22nd)

8:30 am Coffee & donuts
Session 3 chaired by Herbert Herzog
9:00 am Barry Halliwell (National University of Singapore) Oxidative damage in neurodegenerative disease
9:40 am Paul Lockhart (Murdoch Childrens Research Institute, Parkville) Investigating the role of parkin and PACRG in protein degradation pathways
10:05 am John Drago (Howard Florey Institute, Parkville) Insights from transgenic animal models of Parkinson-plus syndrome
10:30 am Malcolm Horne (Howard Florey Institute, Parkville) Secretion of α-Synuclein
10:55 am Glenda Halliday (POWMRI, Randwick) Upregulation of parkin in atypical Parkinson's disease
11:20 am Coffee break
Session 4 chaired by Etienne Baulieu
11:50 am Ann Turnley (University of Melbourne) Regulation of neurite growth and neurotrophin signalling by SOCS2
12:15 pm Andrew Hill (University of Melbourne) Cellular biology of prion disease
12:40 pm Simon Hawke (University of Sydney, BMRI) Gene therapeutic approaches for therapy of prion diseases
1:05 pm Herbert Herzog (Garvan Institute, Darlinghurst) Role of NPY in stem cell development
1:30 pm Lars Ittner (University of Sydney, BMRI) Truncated tau in transgenic mice
1:55 pm Thank you and invitation to lunch

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Astrocytes – Party Animals

The nervous system consists of two classes of cell, the neuron and glia. Although it is without doubt that neurons are essential for nervous system function, studies over the past decade are raising our awareness about the diversity of roles played by glial cells in nervous system function (1).

There are three categories of glia: Schwann cells and oligodendrocytes - the myelin-forming cells of the peripheral nervous system (PNS) and CNS, respectively, that wrap layers of myelin membrane around axons to insulate them for impulse conduction—and astrocytes, which are closely associated with neurons in the brain but do not form myelin. Astrocytes ensheath synaptic junctions, associate with nodes of Ranvier, and respond to disease and injury by clearing cellular debris, secreting trophic factors and forming scars. The name refers to their stellate morphology observed in histological preparations, but their morphology varies widely. Some astrocytes span the entire width of the brain radially from the hollow ventricles to the pial surface, providing scaffolding along which neurons migrate during fetal development. Others stretch from blood capillaries to neurons, transporting ions and other substances to sustain neurons and to regulate the extracellular environment. So intimate is the association between astrocytes and neurons that monitoring activity of these non-neuronal cells is a reliable surrogate for measuring neural activity. The marvellous imaging techniques that provide a window into brain function for both basic research and medical diagnosis actually rely on responses of astrocytes to the changing metabolic demands of neurons. The prevailing view, based on histology, has been that the stellate processes of astrocytes form a tightly intermingled web throughout the brain, but now it seems that these cells are much larger than previously thought, and the processes of adjacent astrocytes do not overlap extensively. Thus, parts of the brain (such as the hippocampus where memories are formed) are divided by astrocytes into separate compartments, each one the sole domain of an individual astrocyte. The functional significance of this structural organization is completely unknown (3).

In the CNS, oligodendroglia extend multiple processes to myelinate several axons at a time. In the PNS, a single highly versatile cell, the Schwann cell, performs all of the functions of CNS astrocytes and oligodendrocytes forming myelin, ensheathing synaptic junctions, and bundling small-diameter axons together (3).

Microglia make up a fourth category of non-neuronal cells in the brain. In contrast to macroglia, which derive embryologically from ectodermal precursors within the nervous system, microglia derive from bone marrow monocyte precursors. Like their counterparts in the hematopoietic system, microglia respond to injury or disease by engulfing cellular debris and triggering inflammatory responses. New findings suggest that microglia can respond to neural impulse activity, and thereby mediate neuroimmune interactions, for example in chronic pain conditions (3).

The discovery that chemical transmitters evoke Ca2+ elevations in cultured astrocytes sparked the imagination of a small group of neuroscientists who diverted their attention to the investigation of this class of glial cell. Although these cells play critical roles in supporting neuronal

function, astrocytic Ca2+ excitability and the consequent induced release of chemical transmitters (gliotransmitters), has led to an emerging new understanding of the functional roles played by these glial cells; it is now appreciated that astrocytes listen and talk to synapses and play roles in synaptic modulation and in mediating synaptic cross-talk (1).

By mopping up excess neurotrophic factor from neuronal synapses, astrocytes may finely tune synaptic transmission to affect processes such as learning and memory (2).

The major cellular events of learning and memory are long-term potentiation (LTP) and long-term depression (LTD), both of which affect neurons' ability to communicate with one another. Neurons that have undergone LTP display a stronger electrical response to the same level of a stimulus, whereas neurons that have gone through LTD display a weaker response. These changes are thought to result from modifications of the neuronal synapses, such as alterations in the density of postsynaptic receptors, or downstream signalling events (2).

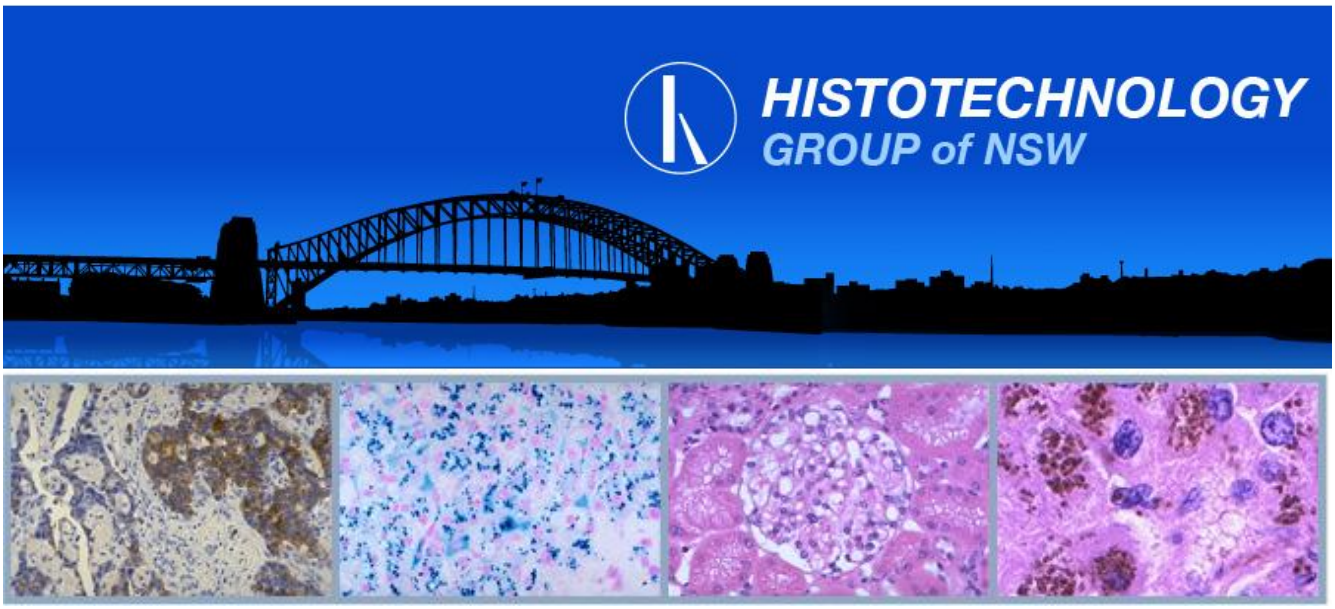
Secretion of the neurotrophic factor BDNF (brain-derived neurotrophic factor) has been implicated in long-term synaptic modification, and the function of BDNF on synaptic strength depends on its particular form: in its pro-BDNF form it is believed to promote LTD, and in its mature form it prompts LTP. Neurons were thought to secrete pro-BDNF, which then matured into BDNF in the synaptic space. However, a recent study suggests that only mature BDNF is secreted, pro-BDNF being processed intracellularly (2).

To get to the bottom of things, Bergami et al. investigated the fate of both forms after LTP induction in brain slices from the rat cortex. By fluorescent immunohistochemistry they showed that that neurons indeed secrete both mature and pro-BDNF, but that a large amount of the pro-BDNF is immediately taken up by astrocytes (2).

Astrocytes, previously thought to be unimportant in neuronal transmission, have recently been implicated in long-term modulation of neuronal synapses. For example, they release the neurotransmitter glutamate into the synapse prompting LTP. By specifically mopping up pro-BDNF, astrocytes seem to have another means to assist in LTP. However, while it's likely that most pro-BDNF gets degraded inside astrocytes, say the authors, some gets recycled and re-released, suggesting that astrocytes in fact fine-tune synaptic plasticity (2).

References

Haydon PG & Carmignoto G (2006) Physiol Rev 86:1009-1031
Bergami, M., et al. (2008) J. Cell Biol. 183(2): 213–221
Fields RD & Stevens-Graham B (2002) SCIENCE 298: 556-452



Web Site Report

Our new Website is operational. Take the time to have a look and pass the word on to your colleagues.

The Website has been constructed with a number of sections:

Home--- Home Page.
About Us--- History of the group.
Committee--- Details of the committee.
Scientific Meetings--- Details of forthcoming/proposed meetings.
Links--- Links to other State Groups/organisations and meetings.
News--- News items.
Contact us--- Method of enabling enquiries and comments to be forwarded to the committee.
Membership--- Information on becoming a member and membership form.

On the home page is a login for members to access our Histograph newsletter and other information. This is still under construction.

We would welcome feedback and comments on the present format and suggestions on items that could be added.

Trevor Hinwood.

Is RNA fixed with formalin?

Prior evidence indicates that under normal fixation conditions (room temperature to 37^{o}C) there is no reaction between native DNA and cross-linking fixatives. Higher temperatures are required to break hydrogen bonds, thereby unmasking purine and pyrimidine bases for reaction with the fixative (Hopwood 1985). Cross-linking fixatives do react with histones and other nuclear proteins.

In 1985 Solomon and Varshavsky stated that formaldehyde produces DNA-protein crosslinks both in vitro and in vivo but at the same time displays virtually no reactivity toward free double-stranded DNA.

But in recent years, this belief has been tested. The reaction between formaldehyde and nucleotide monomers has been shown to progress in two steps. The first step is addition of a formaldehyde group to a base in the form of N-methylol (N-CH_2OH). The second slow step is electrophilic attack of N-methylol on an amino base to form a methylene bridge between two amino groups (Masuda et al 1999).

Formalin reacts with human DNA through interaction with the hydrogen bonds, fixation and denaturation of DNA proteins, cross-linking between proteins and DNA, and methylation of the nucleic acid (Miething et al 2006).

Masuda and his co-workers (1999) studied alterations in RNA due to formalin fixation by measuring changes in molecular weights observed using Matrix-assisted Laser Desorption/Ionisation Time of Flight mass spectroscopy

Masuda et al (1999) have demonstrated these two reactions as molecular weight changes of oligo RNA and determined the extent of progression of these two steps. They found that the reactivity of the bases of RNA was in the order A, C >> G > U, which suggests that the tertiary amino group was the primary target for formalin fixation. Nearly 40% of adenines received mono-methylol addition after incubation in buffered formalin for 16 hours at 4^{o}C. They also showed that the majority of the methylol additions to oligo RNA were reversed by heating in Tris-EDTA buffer.

So what does this all mean? Well it seems that double stranded DNA has minimal reaction with formaldehyde but if denatured to single strands then a reaction has a high probability of occurring. RNA, being single stranded, has a high probability of reacting with formaldehyde.

References:

Hopwood (1985) Histochem J 17:389-442.

Masuda et al (1999) Nuc Ac Res 27(22):4436-4443.

Miething et al (2006) J Histochem Cytochem 54:371–374.

Solomon & Varshavsky (1985) Proc. Natl. Acad. Sci. USA 82:6470-6474

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2008 - 2009

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