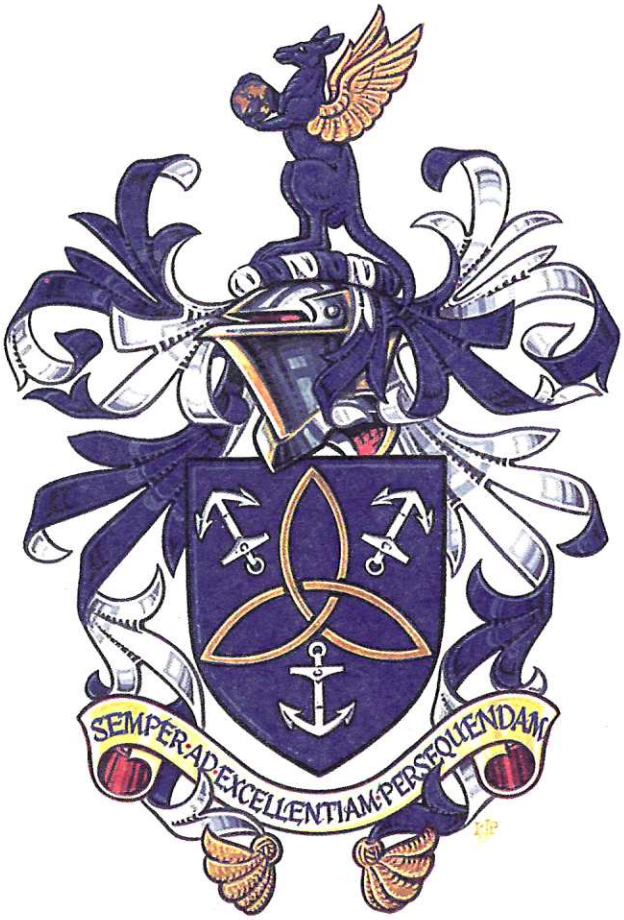
The Garnett Passe and Rodney Williams Memorial Foundation

Frontiers in Otorhinolaryngology 2002



July 31 - August 2, 2002
The Sheraton Noosa, Hastings Street,
Noosa Heads, Queensland
Australia

THE GARNETT PASSE AND RODNEY WILLIAMS MEMORIAL FOUNDATION

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The Garnett Passe and Rodney Williams Memorial Foundation
Frontiers in Otorhinolaryngology 2002
Programme

WEDNESDAY, 31 JULY 2002

5.30 – 6.30pm	Registration	The Noosa Ballroom
6.30 – 9.30pm	Welcome, Drinks and Buffet Dinner	Dr Peter Freeman (Chairman of Trustees)

THURSDAY, 1 AUGUST 2002

8.00 – 8.10am	Opening Remarks	Dr Dean Beaumont (Chairman of the Board)
Session 1	**HEAD AND NECK CANCER**	**Chair: Professor Douglas Tracy**
8.10 – 8.30am	Overview of squamous cell cancer of the head and neck	Professor William Coman
8.30 – 9.10am	Molecular progression of squamous cell cancer in the head and neck – new direction approaches	Professor David Sidransky
9.10 – 9.30am	E2F and the disruption of normal growth and differentiation control in squamous cell cancer formation	Dr Nicholas Saunders
9.30 – 9.50am	Perineural spread of cutaneous head and neck cancer SCC – clinical and research aspects	Dr Benedict Panizza
9.50 – 10.00am	Discussion	
10.00 – 10.30am	Morning Tea	
Session 2	**OTOLOGY**	**Chair: Professor John Funder**
10.30 – 11.10am	Mechanism and prevention of aminoglycoside - induced hearing loss	Dr Jochen Schacht
11.10 – 11.30am	Spontaneous recovery of hearing loss following ototoxicity	Associate Professor Stephen O'Leary
11.30 – 11.50am	Strategies for hair cell repair and replacement	Dr James Pickles
11.50 – 12.10pm	Avoiding ototoxicity in clinical practice – fluoroquinolones	Professor Marcus Atlas
12.10 – 12.30pm	Discussion	
12.30 – 1.30pm	Lunch	
Session 3	**RHINOLOGY**	**Chair: Dr Kevin Kane**
1.30 – 2.10pm	Interactions between common colds and allergic airway inflammation	Associate Professor Lennart Greiff
2.10 – 2.30pm	Chronic sinusitis and macrolide treatment	Associate Professor Anders Cervin
2.30 – 3.00pm	Diagnosis and therapy of olfactory and gustatory function	Dr Thomas Hummel
3.00 – 3.10pm	Discussion	
3.10 – 3.40pm	Afternoon Tea	

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3.10 – 3.40pm	Afternoon Tea	

Session 4	HEAD AND NECK CANCER	Chair: Professor William Coman
3.40 – 4.10pm	Clinical research in the management of neck metastasis in head and neck cancer	Professor Harold Pillsbury
4.10 – 4.50pm	Novel molecular and therapeutic approaches in head and neck cancer and lung cancer	Professor David Sidransky
4.50 – 5.10pm	*Invasion and metastatic markers in HNSCC*	*Professor Peter Parsons*
5.10 – 6.00pm	Head and Neck Cancer Forum: Problems and research studies (Moderator: Professor William Coman)	Professor David Sidransky Professor Harold Pillsbury Professor Ian Frazer Dr Nicholas Saunders

Friday, 2 August 2002

Session 5	RHINOLOGY	Chair: Professor Peter Wormald
8.00 – 8.40am	Nasal trigeminal function	Dr Thomas Hummel
8.40 – 9.00am	Olfaction in human disease	Professor Alan Mackay-Sim
9.00 – 9.20am	Olfactory ensheathing cells and spinal cord repair	Dr Francois Feron
9.20 – 9.40am	Furthering our understanding of the olfactory system	Professor Anne Cunningham
9.40 – 10.00am	Rhinomanometry in clinical practice	Dr Kevin Kane
10.00 – 10.30am	Morning Tea	
Session 6	**OTOLOGY**	**Chair: Dr Peter Freeman**
10.30 – 11.00am	Overview of vestibular function	Dr John Waterston
11.00 – 11.20am	The eyes are the window to the labyrinth	Dr Phillip Cremer
11.20 – 11.40am	New technology directions for cochlear implants	Professor Harold Pillsbury
11.40 – 12.10pm	Protective effects of cochlear implantation on the deafened auditory system	Associate Professor Robert Shepherd
12.10 – 12.30pm	Control of inner ear and brainstem processing by descending neural pathways	Professor Donald Robertson
12.30 – 1.30pm	Lunch	
Session 7	**OTOLOGY**	**Chair: Professor Marcus Atlas**
1.30 – 2.10pm	Oxidative stress in noise-induced hearing loss	Associate Professor Jochen Schacht
2.10 – 2.30pm	ATP receptors in the noise-damaged cochlea	Associate Professor Peter Thorne
2.30 – 3.30pm	Otology Forum: Hearing loss and dysfunction (Moderator: Professor Marcus Atlas)	Associate Professor Jochen Schacht Professor Donald Robertson Dr James Pickles Dr David Pohl
3.30 – 4.00pm	Afternoon Tea	
Session 8	**RHINOLOGY**	**Chair: Dr Dean Beaumont**
4.00 – 4.20pm	Mechanisms of rhinitis	Associate Professor Lennart Greiff
4.20 – 4.40pm	Healing of the nasal mucosa after endoscopic sinus surgery in the sheep animal model and the effects of different nasal packs on this process.	Professor Peter-John Wormald
4.40 – 5.40pm	Rhinology Forum: Problems and research studies (Moderator: Dr Dean Beaumont)	Associate Professor Lennart Greiff Dr Thomas Hummel Professor Peter-John Wormald Professor Alan Mackay-Sim
5.40 – 6.00pm	Closing Remarks	Dr Dean Beaumont
7.00pm	Depart for Conference Dinner	Guest speaker: Professor Ian Frazer

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Abstracts of Invited Speaker Presentations

OVERVIEW OF SQUAMOUS CELL CANCER OF THE HEAD AND NECK

William Coman
University of Queensland, Brisbane, Queensland

Squamous carcinomas affecting the head and neck generally have a better prognosis than lung cancer. However, head and neck tumours impact on major quality of life determinants such as taste, smell, vision, hearing, voice, swallowing and appearance.

The traditional methods of treatment using radiotherapy, chemotherapy and surgery all have significant disadvantages for the patients. In advanced tumours, all three treatment modalities may be needed.

Newer methods of treatments require research and appropriate clinical trials. Photosensitisers have been used in a limited way for photodynamic therapy, but not yet found general use. Immune therapy treatment has so far been disappointing.

Gene therapy offers greater hope in being able to selectively target malignant cells and arrest growth.

My knowledge of the natural history of head and neck squamous cell cancer in its development and spread is essential to the understanding for the need and methods of selectively identifying and killing cancer cells.

MOLECULAR PROGRESSION OF SQUAMOUS CELL CANCER IN THE HEAD AND NECK – NEW DIRECTION APPROACHES

David Sidranksy
Johns Hopkins School of Medicine , Baltimore, Maryland USA

Clonality is a fundamental characteristic of human cancer. One transformed cell gives rise to daughter cells, all of which exhibit the same genetic change that initially provided a growth advantage to the parent cell. The faithful transmission of these and other genetic changes in subsequent daughter cells has been well documented *in vitro* and *in vivo*. Correlation of these clonal and epigenetic genetic changes with histopathologic progression has led to the development of a molecular progression model for head and neck cancer (HNSCC). Promising nucleic acid markers involved in HNSCC progression include oncogene mutations, LOH, methylation, and mitochondrial mutations.

We have demonstrated that point mutations in critical oncogenes and tumor suppressor genes can be used in PCR detection of rare neoplastic cells in bodily fluids and surgical margins. A PCR-based plaque hybridization assay was employed to detect rare infiltrating cells in apparently normal histopathologic margins and lymph nodes following surgical resection in patients with head and neck squamous cell carcinoma (HNSCC). Many patients would have been upstaged using molecular techniques to augment morphologic assessment by light microscopy.

Microsatellite analysis can identify LOH or instability in tumors. We have detected microsatellite alterations in the saliva and plasma DNA of patients with HNSCC. In 21 patients, we found 6 with these genetic changes in the serum. 5 of these 6 patients have died and 3 developed metastatic disease Another emerging marker is based on the concept of promoter hypermethylation which results in absence of transcription and inactivation of tumor suppressor genes. We have also used promoter methylation to detect the presence of cancer cells in saliva and serum DNA from patients with head and neck cancer. Genetic and epigenetic alterations in serum appear to correlate with disease burden and a poor prognosis. Thus, evolving molecular markers will change the management of head and neck cancer patients in the near future.

E2F AND THE DISRUPTION OF NORMAL GROWTH AND DIFFERENTIATION CONTROL IN SQUAMOUS CELL CANCER FORMATION

Nicholas Saunders, on behalf of
Nicholas Saunders, Louise Knop, Alison Dahler and C. Fai Wong.
Epithelial Pathobiology Group, Centre for Immunology & Cancer Research,
University of Queensland, Brisbane, Queensland

The E2F transcription factor family are traditionally thought to be regulators of cell cycle traverse. As such, a contributory role for the E2F family in various human tumours was quickly established. Most evidence suggests that the dysregulation of E2F observed in human tumours is predominantly associated with defects in E2F regulation rather than specific mutations in E2F family members.

In recent years, we have been able to demonstrate that the E2F family are important regulators of keratinocyte proliferation and that they are severely dysregulated in squamous cell carcinomas of the skin and head and neck region. In particular, we have shown that squamous cell carcinoma cells are associated with defects in the transcriptional and post-transcriptional regulation of E2F1. More recently, we have shown that the dysregulated E2F activity observed in squamous cell carcinoma cells may also be associated with the active suppression of squamous differentiation in these tumour cells.

This has led us to propose that the dysregulation of E2F in squamous cell carcinomas not only promotes deregulated growth but suppresses the ability of the cancer cells to undergo terminal differentiation. This predicts that new treatments that inhibit E2F activity in head and neck cancer cells may serve to inhibit cancer cell growth and reinstate a normal terminal differentiation programme. This obviously presents an attractive therapeutic possibility. Data supporting this proposition will be presented.

PERINEURAL SPREAD OF CUTANEOUS HEAD AND NECK CANCER SCC – CLINICAL AND RESEARCH ASPECTS

Benedict Panizza
Princess Alexandra Hospital, Brisbane, Queensland

Two-thirds of all Australians will have at least one skin cancer removed. The overall incidence of perineural spread with cutaneous SCC is 3.7%. While small nerve involvement indicates a greater chance of local recurrence, large nerve spread carries with it a very poor prognosis for survival.

The understanding of perineural spread is poor. The literature is limited and treatment guidelines scarce. Whilst much effort is currently being given to the mechanisms of cancer spread, these are predominantly for the lymphatic and vascular pathways. It is probable the mechanisms involved with perineural spread are different and hence warrant separate investigation.

In order to investigate the phenomenon of perineural spread, the author has tried to establish a "bench to bedside" analysis with other medical and scientific teams. The purpose is not only to find interesting molecular facts but also to obtain findings that will guide others on a practical basis. To this end the following projects have or it is hoped will be begun.

Epidemiology: A retrospective analysis of all documented cases of head and neck cutaneous SCC with perineural spread. This will identify incidence, patterns of spread and regions of failure amongst others.

Histology: A histologic and possibly electron microscopic review of all large nerve specimens including the nerves ganglion has commenced with Sullivan and Nicolaides pathologists. This will identify barriers to spread and patterns of tumour migration.

Radiological: MR neurography using thin slices, surface coils and a high resolution matrix is currently being used with Queensland X-ray to identify more accurately the degree of tumour spread and to monitor patients post therapy.

A review of current treatment: Skull base techniques have been used to resect tumours en-bloc with their respective ganglions. This has allowed histological clearance and helps direct post-operative radiotherapy. Patient morbidity and survival rates will be monitored to see if the historical cure rates can be improved.

Molecular Biology

Immunohistochemistry: Research has begun with the Queensland Institute of Medical Research (QIMR) looking at various markers of tumour spread (such as neural cell adhesion molecules) on resected specimens. It is possible that a molecular marker be identified that could be used on all skin lesions specimens to identify those at risk of perineural spread with the implication of changing subsequent treatment.

Gene Chip Analysis: As central tumour spread is controlled with skull base techniques, the control of peripheral tumour spread is dependant on radiotherapy. Cutaneous failure is controlled by local resection. This has provided clean large tumour sample (cf perineural spread) which has been stored in the tumour bank. These specimens will be available for gene expression profiling to identify differences in gene expression and hence an understaning of perineural spread.

MECHANISM AND PREVENTION OF AMINO-GLYCOSIDE-INDUCED HEARING LOSS

Jochen Schacht
Kresge Hearing Research Institute and Department of Otolaryngology, The University of Michigan, Ann Arbor, Michigan, USA

Aminoglycosides (primarily gentamicin) are the most commonly used antibiotics today. The adverse effects of these drugs towards the kidney (nephrotoxicity) and the inner ear (ototoxicity) have largely limited aminoglycoside therapy in industrialized societies but they are still the drugs of choice in developing countries because of their efficacy combined with low cost. The emergence of tuberculosis also plays a significant role in their heavy use. This wide-spread application of aminoglycosides makes them the major cause of preventable hearing loss worldwide.

The ototoxic damage to the cochlea is primarily based on the destruction of the outer hair cells, leading to permanent hearing loss that begins at the high frequencies. Basic research has recently provided evidence that the destruction of hair cells is linked to oxidant stress caused by aminoglycosides. Aminoglycosides can chelate iron, and the resulting iron complex is redox-active, catalyzing the formation of free radicals. The initial damage caused by free radicals (e.g., lipid peroxidation) is followed by downstream events of gene activation that eventually lead to apoptotic or necrotic cell death. Free-radical formation as the underlying mechanism of ototoxicity has also received strong support by the fact that antioxidants attenuate both aminoglycoside-induced free radical formation *in vitro* and prevent ototoxicity in guinea pig and mice *in vivo*. This presentation will discuss the sequence of the molecular events triggered by aminoglycosides and delineate the activation of gene transcription for apoptotic and survival pathways. We will then report on recent clinical trials suggesting that antioxidant therapy is a viable approach to prevent the ototoxicity of aminoglycoside antibiotics in patients.

The work in Dr. Schacht's laboratory is support by research grant DC-03685 from the National Institutes of Health (NIH). We also thank The George and Christine Strumbos and The Kent and Carol Landsberg Foundation for their support.

SPONTANEOUS RECOVERY FROM SENSORINEURAL HEARING LOSS FOLLOWING OTOTOXICITY

Stephen O'Leary
Department of Otolaryngology, University of Melbourne,
The Royal Victorian Eye and Ear Hospital, Melbourne East

Background: Ototoxocity of the chemotherapeutics agent, cisplatin usually results in a permanent, sensorineural hearing loss but there are anecdotal reports of a spontaneous recovery of hearing following the ototoxicity[1-3]. Furthermore, some of the signs of cisplatin ototoxicity, such as tinnitus and the subjective impression of decreased hearing, recover spontaneously in approximately one-third of affected patients[2]. The experimental literature also has anecdotal reports of recovery of auditory sensitivity following cisplatin administration. This presentation describes experiments that investigated the mechanisms underlying the spontaneous recovery of hearing following cisplatin ototoxicity.

Methods: Albino guinea pigs were equipped with permanent round window electrodes. Clinical grade cisplatin solution was diluted with physiological saline to 0.1 mg/ml and injected intraperitoneally for several days. Before, during and after treatment with cisplatin we measured the compound action potential (CAP) and the cochlear microphonics (CM) in response to pure tone bursts (2 - 16 kHz) in awake, unanaesthetized animals. The endocochlear potential was recorded as a terminal experiment, and the cochleas prepared for light microscopy.

Results:

- With a cisplatin dose of 2 mg/kg/day, the time needed to induce a hearing loss of 40 dB at 8 kHz varied per animal from 5 to 11 days
- Hearing loss occurred suddenly, progressing from no loss to its maximum within 2 days
- In each animal the cochlear potentials gradually recovered in the first weeks after treatment
- At the lower frequencies, recovery was mostly complete. At the higher frequencies complete recovery was not seen within the present maximum observation period of 12 weeks
- Loss and recovery of CAP and CM occurred virtually simultaneously.
- The endocochlear potential decreased and later recovered in parallel with the loss and recovery of the CAP
- There was a permanent loss of hair cells in the basal regions of the cochlea.

Conclusions: These findings suggest a pivotal role for the endocochlear potential in the hearing loss from cisplatin ototoxicity, and its recovery, and a plausible mechanism for the hearing recovery sometimes reported in patients following cisplatin ototoxicity. The incomplete recovery of hearing at high frequencies was due to a permanent loss of hair cells in the basal region of the cochlea.

The pattern of hearing loss and the mechanism of the recovery described here may provide a model for understanding other types of sudden hearing loss associated with a spontaneous recovery of hearing, such as idiopathic sudden sensorineural hearing loss.

1. Aguilar-Markulis,NV, Beckley,S, Priore,R, and Mettlin,C: Auditory Toxicity Effects of Long-Term Cis-Dichlorodiammineplatinum II Therapy in Genitourinary Cancer Patients. Journal of Surgical Oncology 16:111-23, 1981.
2. Bokemeyer,C, Berger,CC, Hartmann,JT, Kollmannsberger,C, Schmoll,HJ, Kuczyk,MA, and Kanz,L: Analysis of Risk Factors for Cisplatin-Induced Ototoxicity in Patients With Testicular Cancer. Brit.J.Cancer 77:1355-62, 1998.
3. Laurell,G and Jungnelius,U: High-Dose Cisplatin Treatment: Hearing Loss and Plasma. Laryngoscope 100:724-734, 1990.

STRATEGIES FOR HAIR CELL REPAIR AND REPLACEMENT

James O. Pickles, Vision, Touch and Hearing Research Centre, School of Biomedical Sciences, University of Queensland, 4072 Qld. Australia.

Current work on hair cell repair and replacement will be reviewed. First, work showing enhanced formation of hair cells after genetic manipulation in the embryonic cochlea will be discussed, showing the roles of the different cellular signals that govern the formation of hair cells. Applications of the work to possible hair cell replacement after damage will be discussed. The roles of the possible cellular signals in maintenance of the normal hair cell state, and the repair of the cells after damage, will be reviewed, as will work on gene transfer into hair cells, using adenoviral and other vectors.

Supported by the Garnett Passe and Rodney Williams Memorial Foundation.

AVOIDING OTOTOXICITY IN CLINICAL PRACTICE – TOPICAL FLUOROQUINOLONES

Professor Marcus Atlas on behalf of
Marcus Atlas*, Peter O'Sullivan*, J Bowman~, Clay Gollege~
*Lions Ear and Hearing Institute
*Department of Otolaryngology and ~ Microbiology, Sir Charles Gairdner Hospital Perth

Topical antibiotics are the major drugs used in the management of chronic suppurative otitis media (CSOM) and otitis externa. Until recently, most of these ototopical agents contained aminoglycosides, with the potential to cause ototoxicity when absorbed through the round window. Despite these risks, these agents are still used widely in the middle ear because of the very real and major risks of complications due to CSOM. There is conflicting evidence as to the incidence of irreversible inner ear damage following the use of ototopical aminoglycosides.

Topical fluoroquinolones have recently proven to be an extremely useful and non-ototoxic agent in the treatment of both of these conditions as confirmed by both animal models and clinical trials. Concerns, however, have been raised by the Expert Group on Antimicrobial Resistance about the widespread use of topical fluoroquinolones possibly resulting in fluoroquinolone resistance.

Two principal mechanisms of resistance to flouroquinolones have been described:

- Alteration of the DNA gyrase which is the target site of the flouroquinolone.
- Diminished accumulation in the cell as a consequence of either decreased uptake or increased efflux.

Despite these theoretical concerns, there has been no convincing scientific evidence that this has in fact been the case in countries where topical fluoroquinolones have been widely used.

In a retrospective examination of ear swabs over a ten year period at the Sir Charles Gairdner Hospital, susceptibility to ciprofloxacin among *Pseudomonas aeruginosa* has remained remarkably constant at 84% for 1990 and 82% for 2000 respectively tested at an NCCLS susceptibility breakpoint of $\leq$ 1mg/l

The supposedly resistant strains are tested at an NCCLS breakpoint of $\geq$ 4 mg/l whereas topical use of ciprofloxacin results in far higher surface concentrations of the drug. The concentration in otorrhoea of another topical fluroquinolone, oflaxacin, found 30 min after administration of a 0.3% Otic Solution, have ranged from 388.8-2849.8ug/g. The upper limit is close to the concentration of the drug itself i.e.3000ug/ml. High concentrations have also been reported in middle ear mucosa after topical administration of the same solution but mucosal concentrations can vary widely and are thought to be dependant on many factors such as the site of biopsy and eustachian tube function.

As the breakpoint is calculated for systemically administered medication, very little is known about its relevance to topically administered ciprofloxacin. We have looked at strains from ear isolates that are resistant to ciprofloxacin and determined whether

the supposed resistance is low, intermediate or high level, and therefore examined if this resistance could be overcome by achievable ototopical concentrations. Isolates were screened by a combination of E-strip MIC testing to detect accurate low level resistance and serial agar dilutions susceptibility testing at concentrations of 25 mg/l, 50 mg/l, 100 mg/l, 250mg/l and 500mg/l. Results will be presented and discussed.

INTERACTIONS BETWEEN COMMON COLDS AND ALLERGIC AIRWAY INFLAMMATION

Lennart Greiff[1], Steven Myint[2], Carl Persson[3].
Departments of Otorhinolaryngology[1] and Clinical Pharmacology[3], University Hospital, Lund, Sweden. Department of Microbiology[2], Leicester University, Leicester, UK.

Interactions between common colds and chronic airway diseases are currently receiving great attention. The interest is focused on asthma since common colds have emerged as a major cause of asthma exacerbations. Accordingly, interactions between "eosinophilic inflammation", characterising asthma, and inflammation associated with rhinovirus-infections have emerged as prime study objects in disease models and at natural disease. We have, guided by experimental observations, developed the hypothesis that disease-mechanisms relevant to these interactions may be explored using the human nasal airway *in vivo* as a primary model. Specifically, conditions such as allergic rhinitis, as well as experimental measures such as rhinovirus-inoculations, allergen-challenges, and lavage-techniques, may be employed for this purpose. Importantly, such studies may be relevant also for chronic nasal conditions.

Allergic rhinitis and common colds are usually considered as markedly dissimilar conditions. Corroborating this notion, we have demonstrated that allergic rhinitis and common colds are characterised by different cytokine patterns. However, attention may now be given to essential similarities between the conditions: The production of chemokines/cytokines with eosinophil granulocyte activating properties, e.g., eotaxin and interleukin-8, is increased in allergic rhinitis as well as in common colds. Increased eosinophil activity is an established feature of allergic airway inflammation. However, we have demonstrated that increased mucosal levels of eosinophil products characterise also rhinovirus infections, and, importantly, that allergen exposure may produce abnormally great eosinophil responses in patients with allergic rhinitis suffering from common colds. Also, allergic inflammation and inflammation produced by common cold infections are characterised by plasma exudation, and allergen challenge produces abnormally great exudative responses in patients with allergic rhinitis suffering from common colds compared with non-infected subjects. Hence, it is conceivable that exudation of potent plasma proteins, produced by common cold infections, may contribute to and potentiate other inflammatory conditions.

In summary, allergic/eosinophilic airway inflammation and airway inflammation associated with common colds have important similarities. Furthermore, common colds may potentiate the inflammation that characterises allergic/eosinophilic airway diseases. The co-appearance of eosinophil active chemokines/cytokines and eosinophil products in allergic subjects suffering from common colds suggests a mechanism by which rhinovirus infections may potentiate these conditions. These observations further suggest that, e.g., eotaxin generation and actions should be considered as a potential target for therapeutic interventions.

CHRONIC SINUSITIS AND MACROLIDE TREATMENT

Anders Cervin on behalf of
Anders Cervin[1,2,3] MD, PhD, Alan Mackay-Sim [1] PhD, Ben Wallwork [1], MD
and William B Coman [3,4] MD

1 School of Biomolecular and Biomedical Science, Griffith University, Brisbane, Australia.
2. Department of Oto-Rhino-Laryngology, Head and Neck Surgery, Lund University Hospital, Lund, Sweden
3. Department of Oto-Rhino-Laryngology, Head and Neck Surgery, Princess Alexandra Hospital, Brisbane Australia.
4. The Garnett-Passe Professor of Oto-Rhino-Laryngology, Head and Neck Surgery, University of Queensland, Brisbane Australia

In the present study, the effect of long-term, low-dose clarithromycin treatment in a population suffering from persistent chronic sinusitis was studied. The specific aims being patient selection, clinical outcome, and impact on quality of life.

30 patients, 21 women, mean age was 46.3$\pm$ 10.4 years (mean $\pm$ s.d.), were treated with clarithromycin 250 mg x 1 daily for 12 weeks. Five patients failed to complete the treatment, of the remaining 25 patients, 14 (56%) responded subjectively to the treatment. Patients with a serum IgE above 240 ug/L were less likely to respond, $p<0.01$.

At the follow up at 12 weeks, Sacharine Transit Time was reduced from 27.7$\pm$5.1 min to 17.2$\pm$4.1 min, $p=0.07$. Endoscopic nasal examination scoring was significantly reduced, $p<0.05$. The scoring for Quality of life questions (related to the influence of the chronic sinusitis on work, recreational activities and sleep) were all significantly improved, $p<0.05$. In the symptom specific scoring, significant improvement was seen in sticky secretions and headache, $p<0.05$. The use of pain relievers was reduced, $p=0.07$.

The present study gives a rationale for choosing the patients more likely to respond to long-term, low-dose clarithromycin treatment and could provide the basis for future placebo controlled studies.

DIAGNOSIS AND THERAPY OF OLFACTORY AND GUSTATORY DYSFUNCTION

Thomas Hummel
Department of Otorhinolaryngology, University of Dresden, Dresden, Germany

The ability to smell gives us the means to sample chemicals that are in the air around us. It can alert us to dangers such as spoiled food and natural gas or it can delight us with the aromas of fresh bread and fine perfume. Thus, disorders of the chemical senses can affect our lives severely.

Olfactory loss may be caused by mechanical obstruction or inflammation of the olfactory epithelium due to allergic/non-allergic rhinitis, and chronic sinusitis w/o polyps. Treatment of olfactory loss related to sino-nasal disease is possible. Apart from surgical approaches and/or treatment with antibiotics, both systemic and topical steroids are effectively used in the therapy of olfactory loss related to sino-nasal disease. This situation is different in oflactory dysfucntion due to trauma or following viral infection so the upper respiratory tract (URTI). While several treatments have been tried there are only few appropriate studies on the pharmacological treatment of this disorder. Potential candidates for this therapy (e.g. alpha-lipoic acid) will be discussed. Last not least, because many chemosensory disorders are secondary to a wide variety of diseases, olfactory or gustatory complaints may help in the development of certain differential diagnosis, e.g., in Parkinson's disease. In turn, treatment of the underlying diseases may restore chemosensory function in these patients.

Future developments appear possible especially in light of the availability of standardised tools for the assessment of chemosensory dysfunction.

CLINICAL RESEARCH IN THE MANAGEMENT OF NECK METASTASIS IN HEAD AND NECK CANCER

Harold J. Pillsbury
Chairman, Department of Otolaryngology
University of North Carolina Medical Center, Chapel Hill, North Carolina

The presence of metastases in cervical lymph nodes is the most important determinant of prognosis and treatment in patients with head and neck squamous cell carcinoma (HNSCC). The cure rate for patients with pathologically positive lymph nodes is approximately one-half that of patients with uninvolved nodes. There are two main areas of research aimed at improving control rates of neck metastasis- better detection of metastasis in the clinically N0 neck and improved treatments for patients with cervical metastasis.

Currently, selective neck dissections are used to pathologically stage the N0 neck in high risk patients. Sentinel node biopsy is now routine in many centers for melanoma and breast carcinoma and is an active area of research in HNSCC. Several studies have suggested that the sentinel node technique is applicable to HNSCC and these results will be reviewed. The American College of Surgeons Oncology Group has recently opened a national sentinel node trial, and if the technique continues to be validated, it will likely become standard of care for the N0 neck. Routine pathology fails to detect 10% of metastases, and identification of the sentinel node allows for more thorough examination of only a few high-risk nodes. Several techniques are currently available for detecting micrometastasis and this is an active area of research.

External beam radiation is the standard post-operative treatment for patients with known cervical metastasis. Recent studies have shown better control with postoperative concurrent chemoradiation therapy using cisplatin-based regimens. However, these treatments are quite toxic, and new therapies are being explored. Intraoperative electron beam radiation delivers a boost to the surgical bed, allowing for decreased external beam dosing postoperatively and therefore fewer complications. In addition, re-irradiation of recurrent neck disease has also been shown to improve local control and to be fairly well tolerated. Several less toxic chemotherapuetic agents are currently in clinical trials, including epidermal growth factor receptor blocking agents and agents that either restore p53 function or take advantage of p53 mutation commonly found in tumor cells. Some of these studies have entered into phase III trials and these will be reviewed.

NOVEL MOLECULAR DIAGNOSTIC AND THERAPEUTIC APPROACHES IN HEAD AND NECK AND LUNG CANCER

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Our increased understanding of the key molecular events that drive head and neck cancer progression provides new targets for therapeutic intervention. Commonly activated oncogenes include cyclin D, EGFR receptor and p63, a member of the p53 family. Frequently inactivated tumor suppressor genes include p16, (a CDK inhibitor) and p53. In a subset of tumors, foreign HPV abrogates the genetic function of p53 and RB. All of these key genetic changes represent novel therapeutic targets that can be used to treat tumors more effectively.

Epidermal growth factor receptor (EGFR) is an attractive oncogenic target because it is found on the surface of most HNSCC cells. Targeted inactivation of EGFR using antibodies or tyrosine kinase inhibitors can be used to treat tumors with precision. Combination therapy has been effective in cisplatin resistant tumors. Adenoviruses with selective replication in p53 deficient cells have had success in local therapy. In patients with positive molecular margins, adjuvant treatment with such viruses may obliterate residual disease.

Finally, HPV positive tumors represent appropriate targets for immunotherapy. HSP/E7 vaccines hold promise in preclinical models for effective vaccination of HPV positive tumors. New trials are planned with particular attention to subtle disease response in addition to standard immunologic parameters. As more of targeted therapies are developed it is important to accurately identify the target and to be able to monitor the response to new agents. Many of the sensitive diagnostic strategies presented previously are being employed to assess response to treatment and to monitor the course of disease over time.

INVASION AND METASTATIC MARKERS IN HNSCC

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Background: Queensland has the highest skin cancer incidence in the world and a high incidence of head and neck cancers (HNSCC) with 333 new cases in 1997 (29% of the national total). New approaches and molecular markers are needed to improve the management of HNSCC. We have applied similar research technology established in our skin cancer work to study invasion and metastasis in HNSCC.

Aims: Utilising our HNSCC tumour bank, we have used cDNA microarray profiling to study molecular changes in the transition from normal mucosa to primary tumours and subsequently to metastases.

Methodology: Tumour tissues: Snap frozen and stored at –70°C. Normal mucosa, primary tumours and metastatic lesions were banked from the same patient whenever possible. RNA extraction: RNA extraction using RNeasy™, QIAGEN. Microarray chips: 19K OCI (Ontario Cancer Institute) and QIMR 5K chips. Reference RNA: RNA from a SCC cell line (Colo-16) used in each of the microarrays. Control: Normal oral mucosal. Data visualisation, mining and analysis: ImaGene™, BioDiscovery, Inc and GeneSpring™, Silicon Genetics. Statistical analysis: GeneSpring analysis software will be used for demonstrating significant differences in gene expression. For correlation with clinical data e.g. nodal status, tumour size, tumour grade, Mann-Whitney student t-test will be used and for disease free interval and overall survival Kaplan-Meier and SPSS survival analysis.

Results: This study is on going but preliminary results showed that about 25% of the top 30 genes up-regulated in tumours compared with normal mucosa were proteolytic enzymes; and another 25% were genes associated with cell motility. One third of the top 30 genes down-regulated in tumours were genes associated with the inflammatory response and the immune system. Many other changes were observed that require confirmation in a larger series, and validation by independent methods.

Discussion: The clinical significance of these results awaits correlation with clinical data. However, the genes that are highly up-regulated as in proteolytic enzymes – urokinase plasminogen activator, MMP-2, fibronectin 1 (10-100 fold)-have the potential to be serum diagnostic and prognostic markers, or determinants of treatment modality for HNSCC patients in the future.

NASAL TRIGEMINAL FUNCTION

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Intranasal trigeminal function is more and more understood as an integral part of human chemosensory perception. Sensations like burning, stinging, warmth, coolness, or itching are produced by almost all odorants so that they can be perceived by anosmics. Electrophysiological responses to trigeminal stimuli allow the specific assessment of trigeminally mediated information at different levels of processing including the periphery or the cortex. Information regarding the localization of these processes can be derived from magnetoencephalographic recordings or functional imaging data. When using these techniques in combination with psychophysical measures, it seems to be possible to specifically describe how and where the processing of irritation takes place, how it may interact with olfactory mediated sensations, and how it is modulated, e.g., by environmental influences or analgesic drugs.

OLFACTION IN HUMAN DISEASE

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In Australia, the clinical assessment of the sense of smell is often neglected or only qualitatively assessed. Often the sense of smell only becomes important in medico-legal cases. On the other hand, the inability to detect odours can have profound psychological and social effects on the patient and may affect nutrition. Olfactory loss is always accompanied by complaints of loss of "taste" because of the paramount contribution of olfaction to the perception of food flavour. Loss of gustation is much rarer and usually results from physical damage to the gustatory nerves (branches of cranial nerves VII, IX and X).

In otorhinolaryngology olfactory disturbances can arise from diseases of nasal obstruction, head trauma, and surgical intervention. Restoration of olfactory function is often achieved via surgical or drug treatment of the nasal obstruction or nasal airway disease, but rarely is normal olfaction a stated outcome of clinical practice. An adequate recognition of the problems associated with olfactory disabilities and disturbances is often the first stage in rehabilitation if not by cure then at least through counselling.

Quantitative olfactory tests have been fundamental to the development of a fuller understanding of olfactory function that has developed over the last 20 years. For example, it is now clear that normal ageing leads to gradual loss of olfactory function after 60 years of age, a loss exacerbated by smoking. Loss of olfactory function is now associated with many diseases including schizophrenia, Alzheimer's disease, and Parkinson's disease, as well as the more obvious nasal and paranasal sinus diseases and upper airway obstruction normally found in the ENT clinic. Thus, patients presenting with a poor sense of smell can alert the clinician to the possibility of neurological, as well as, otorhinological disease. Recent research, for example, indicates that a loss of the sense of smell is probably one of the earliest symptoms of Parkinson's disease.

There are two olfactory function tests available commercially which are used widely throughout the world. The most commonly used is the University of Pennsylvania Smell Identification Test (UPSIT), which is a 40 odour, multiple choice, odour identification test. The UPSIT presents the odours encapsulated in plastic strips and released by scratching or scraping. The other commercially available olfactory function test is the "Sniffin' Sticks", developed in Germany. This test presents odours in felt-tip pens. Three aspects of olfactory function are tested: identification, discrimination and threshold, making this test only partly reliant on odour familiarity and less reliant on odour memory. This test is widely used in European clinics but in Australia its use has not extended beyond the research laboratory.

With the aim of bringing these quantitative tests into the Australian ENT clinic we have developed normative values for Australian populations on both these commercially available tests. In our first study we developed a normative adjustment for the UPSIT (Mackay-Sim and Doty, 2001). More recently we have tested over 500

persons aged 10 - 90 to develop age-adjusted norms for the Sniffin' Sticks test. Using this test we have confirmed in the Australian population a severe loss of smell function in over 100 persons with Parkinson's Disease. This loss is evident in patients at the earliest ages of diagnosis.

OLFACTORY ENSHEATHING CELLS AND SPINAL CORD REPAIR

Francois Féron, on behalf of
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For many years spinal cord injury has been seen as clinically irreversible but recent animal experiments provide new hope. One of these advances has been the demonstration that an unusual cell, the olfactory ensheathing cell, can assist recovery after spinal cord injury. The olfactory ensheathing cell is a specialised glial cell with properties both of Schwann cells and astrocytes. It normally ensheaths the olfactory neurons as they course from the nasal olfactory epithelium to their termination in the brain, the olfactory bulb. There is increasing evidence that olfactory ensheathing cell transplants can re-myelinate damaged spinal cord axons, assist reentry of sensory axons after dorsal root section, assist growth of spinal cord axons through injury sites including complete spinal cord transection. The focus of our work on these cells has been to develop technologies to bring them quickly to clinical trial. To this end we have concentrated on proving the regenerative abilities of the olfactory ensheathing cells which are available from the olfactory mucosa via biopsy through the nose. We have demonstrated that transplantation of these nasally-derived olfactory ensheathing cells can assist reconnection of the fully transected spinal cord in the rat (1). Transplantation at the time of spinal cord transection (acute injury) resulted in significant recovery of locomotor behaviour as well as evidence for reconnection of some brainstem motor pathways. Recent experiments indicate that olfactory ensheathing cells can also assist recovery when transplanted 4 weeks after spinal cord transection (chronic injury, 2). These results advance the cause of spinal cord repair because they demonstrate that cells potentially available in humans have significant powers of assisting spinal cord injury. We have now developed methods for the culture and purification of olfactory ensheathing cells from small biopsies of human olfactory mucosa. Four weeks after biopsy pure populations of olfactory ensheathing cells can be generated in large enough numbers for transplantation.

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FURTHERING OUR UNDERSTANDING OF THE OLFACTORY SYSTEM

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Cell biological and molecular studies over the last 10 years have revealed many unique characteristics of the olfactory sensory system. The olfactory pathway has come into great prominence as a model system for studies of axonal guidance mechanisms. Better understanding of the processes of neurogenesis, neuronal turnover and growth factor use in this system will provide new therapeutic approaches to disorders of the sense of smell.

Our studies have aimed to identify the neuronal progenitor cell of the olfactory neuroepithelium (ON), and results to date show this to be a GBC-1 antigen positive 'stem' cell capable of forming neurospheres *in vitro*. These large progenitor cell colonies produce progeny of the neuronal lineage and have provided us with a remarkable *in vitro* model of the earliest stages of olfactory neurogenesis. Treatment of neurospheres with growth factors and subsequent molecular analysis by single-sphere RT-PCR has enabled us to follow for the first time the molecular responses of progenitor cells to different growth factors. Current studies comparing the characteristics of these cells to stem cells isolated from the neonatal forebrain subependymal zone suggest they may be more restricted in their developmental potential. Understanding their unique features and potential is of intense relevance to those investigating clinical disorders of olfaction, and also more broadly to the field of adult stem cell biology.

The intermediate filament protein, nestin, has been used as the classical marker for proliferating progenitor cells in the developing nervous system. Nestin is thought to be important in providing cell motility and fluidity of cell shape, processes critical during development. The ON is a region of the nervous system that uniquely supports robust, ongoing neurogenesis, so one might expect nestin to mark proliferating olfactory progenitors. Using immunohistochemistry, we examined nestin protein expression in the mature ON and found it to be restricted to the basal compartment with expression by the endfeet and inferior processes of the sustentacular cells, rather than the adjacent progenitor cells. This is fascinating, as during embryonic CNS development radial glial cells, which provide a scaffold upon which young neuroblasts migrate, express nestin. In the ON, sustentacular cells may play an analogous role providing a scaffolding for the migration of recently proliferated olfactory neurones. Nestin's function here may reflect the intense requirement for cell mobility and remodeling in the basal ON, and this may be a key, previously unrecognised, process in permitting neurogenesis. Recently, radial glia themselves have been reported to be a form of progenitor cell, generating both neurones and glia (Campbell & Gotz, 2002). This unexpected finding, in conjunction with our *in vitro* data, highlights a potential relationship between the olfactory sustentacular and neuronal lineages, which is an important focus of our ongoing studies.

Further experiments have defined the responsiveness of olfactory receptor neurones and progenitor cells to the potent growth factor GDNF. We have data suggesting that GDNF may be target-derived for olfactory receptor neurones, being supplied to them by olfactory bulb cells and being crucial for their terminal differentiation. GDNF availability may be a key factor in regulation of the processes of apoptosis and regeneration of the ON. Olfactory neuronal progenitor cells are also GDNF responsive. This growth factor may prove therapeutically valuable in anosmias and other olfactory disorders due to receptor cell damage and deficiency.

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RHINOMANOMETRY IN CLINICAL PRACTICE

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Since the turn of the century, clinicians have recognised the need for an objective measure of nasal function. Early workers used a variety of ingenious devices including the frosting on mirrors held under the nose which had marked calibrations to give an indication of air flow.

Early Rhinomanometers were dogged with the problem of inaccuracy, reproducability and the requirement of continual calibration. The new generation of digitalised computerised Rhinomanometers mainly developed by the Europeans are accurate, easy to use and give much information on the state of the airway resistance as well as the likely causes.

Most clinicians use active anterior Rhinomanometry to measure nasal function. Other types available are active posterior Rhinomanometry, passive anterior Rhinomanometry and head out body Plethysmography.

All utilise a pneumotachometer to measure air flow and pressure transducers to measure pressures in front and at the back of the nose.
By utilising Ohm's Law for current flow (1=PD/R ie: R=PD/I=Δ P/V°) the equipment is able to instantly calculate the resistance within the nose at any given pressure and flow. Different graph results before and after nasal decongestion can also give a clue as to the likely pathology.

A recent extension is to combine acoustic Rhinometry with Rhinomanometry. Modern equipment is able to utilise the same computerised soft ware to drive both modalities.

For 15 years, the author has used Rhinomanometry to help differentiate those patients complaining of nasal obstruction from underlying chronic rhinosinusitis who require a Septoplasty or valve surgery as well as FESS from those who merely require FESS alone. Data will be presented to support this concept.

OVERVIEW OF VESTIBULAR FUNCTION

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The ideal vestibular function test battery should have the following characteristics:

- High specificity and sensitivity
- The ability to assess function in all semicircular canals and the otolith organs.
- The ability to give information about specific types of pathology.

In reality however, our currently available tests often lack sensitivity and the results are frequently non-specific, both in terms of accurate localisation and pathological information. Up until recently, there have been no convenient ways to assess otolith function.

Traditional modes of vestibular investigation have involved the assessment of horizontal semicircular canal function using caloric and rotational chair testing. Caloric testing was established early last century by Robert Barany and is still the mainstay of vestibular assessment. While there has been some debate about the method of caloric testing and the most accurate measurement parameters, this test remains the most accurate way of lateralising vestibular pathology.

Rotational chair testing is a useful adjunct to caloric testing. It gives information about vestibular asymmetry (directional preponderance) and follow up studies may give information about the state of central compensation following an acute peripheral vestibular insult. The most useful indication is investigation of the patient with possible bilateral vestibular hypofunction. Mild to moderate degrees of vestibular hypofunction may not be apparent on caloric testing and inadequate caloric irrigation cannot always be excluded as a cause of reduced or absent caloric responses.

In the past, assessment of otolith function has only been possible using expensive and complex laboratory equipment (linear sled, off vertical axis rotation and centrifuge testing). Newer tests of otolith function have been developed and are simple and easy to perform. Utricular function is very much dependent on gravitational forces. It has been demonstrated that perception of the visual vertical and horizontal is a function of utricular or graviceptive pathways. Most normal subjects are able to correct the position of a vertical or horizontal light bar in darkness to within +/- 2 degrees on average. The patient with an acute loss of peripheral vestibular function will usually tilt the light bar towards the side of the lesion. This abnormality is thought to correlate with the static effects on eye movements (abnormal ocular tilt reaction) and postural instability. Such abnormalities may result from central lesions as well, therefore an isolated positive test result does not localise the site of the lesion to central or peripheral pathways.

Loud clicks (at 95-100 dB) produce a vestibulocollic response in the ipsilateral sternomastoid muscle. These vestibular evoked myogenic potentials are mediated by saccular afferents. Recording of averaged responses in sternomastoid reveals a positive/negative short latency response (p13n23). The test requires voluntary

activation of sternomastoid usually by flexion of the cervical spine. A response which is <30% in amplitude compared to the opposite side is deemed to be indicative of an ipsilateral loss of saccular function. It is important to realise that a conductive hearing loss may be responsible for a false negative response. A pathologically reduced threshold to click activation (< 70 dB) has been demonstrated in patients with the Tullio phenomenon and is considered to be a useful diagnostic feature.

NEW TECHNOLOGY DIRECTIONS FOR COCHLEAR IMPLANTS

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Rapid changes in cochlear implant technology have resulted in improved outcomes, expanded candidacy criteria and broad application in hearing-impaired adults and children. Recent technological developments have included improved speech processing, cosmetic BTE speech processors, active noise suppression and telemetry capable of measuring Electrical Compound Action Potentials (ECAP) of the peripheral auditory nerve from electrodes within the cochlea to aid in device programming.

This presentation will focus on four key areas of technology evolution into the near future. These areas include: 1) Bilateral Implantation, 2) Hybrid Acoustic-Electric Devices, 3) Combination Hearing Aid and Cochlear Implant and 4) Totally Implantable Cochlear Implant (TIKI).

The benefits of bilateral cochlear implantation are being investigated in both adults and children through a series of collaborative investigational programs conducted globally. Two studies conducted in the U.S. are currently exploring speech in quiet and in noise, sound localization, subjective preference and cost utility in sequentially and simultaneously implanted adult subjects. Preliminary data demonstrate improved localization and head shadow benefits with two implants compared with one.

As CI selection criteria have broadened to include patients with more residual hearing there has been advent of research interest in hybrid devices, which deliver low frequency acoustic amplification and high frequency electrical information to subjects with high frequency sloping hearing loss. Preliminary work at the University of Iowa has demonstrated the ability to preserve low frequency residual hearing in subjects implanted with a short (10 mm) intracochlear electrode. In addition, preliminary evidence will be reviewed demonstrating improved speech perception in the bimodal (acoustic/electric) condition compared with either acoustic or electric stimulation alone.

Similarly, combining a hearing aid in the contralateral ear with a traditional cochlear implant has demonstrated improved long-term outcomes in children. These data will be reviewed.

The final area to be reviewed is the development work towards a totally implantable cochlear implant (TIKI). Issues associated with design tradeoffs and clinical management of a TIKI will be discussed.

PROTECTIVE EFFECTS OF COCHLEAR IMPLANTATION ON THE DEAFENED AUDITORY SYSTEM

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Cochlear implants electrically stimulate discrete populations of residual auditory nerve fibres in profound and severely deaf patients in order to provide important temporal and pitch cues for speech perception. In this paper, we will review the effects of a neonatal hearing loss on the cochlea, and discuss the potential protective effects of chronic electrical stimulation of the auditory nerve. The implications of these effect for cochlear implants will also be discussed.

A profound sensorineural hearing loss initiates a gradual, ongoing degeneration of spiral ganglion neurons (SGNs) - the target neural population for stimulation via a cochlear implant. The loss of these cells is due, at least in part, to the withdrawal of neurotrophins normally expressed by hair cells [1, 2]. The degenerative changes observed in SGNs are associated with a loss of peripheral processes and demyelination of the soma of surviving auditory neurons [3]. These changes have been shown to affect neural response properties to electrical stimulation, including elevated thresholds, a reduction in the security of action potential propagation and altered refractory properties [3]. Deafness induced degeneration of SGNs may adversely affect the clinical performance of implant subjects, as evidenced by the strong negative correlation between duration of deafness and speech perception [4]. Moreover, the SGN degeneration may place limitations on the development of more advanced cochlear implants.

Recent *in vitro* studies have shown that neural depolarisation provides a strong trophic influence on SGN survival [5]. The possibility of reducing or preventing SGN degeneration via depolarisation, using electrical stimulation, has important implications for cochlear implant research. We have examined the extent of this trophic effect on SGNs *in vivo*, by chronically stimulating neonatally deafened cats, via a cochlear implant, for periods of up to eight months. Preliminary results, illustrating the long-term physiological response of the auditory pathway to electrical stimulation, and the extent of survival of SGNs, will be compared with results from unstimulated deafened controls. In a second study, we have developed an electrode array capable of simultaneously delivering neurotrophic agents into the cochlea and electrically stimulating the SGNs. Initial studies examining both the functional and anatomical efficacy of combining depolarisation and neurotrophic delivery to SGNs will be described.

These studies are designed to develop techniques that will enhance the survival of auditory neurons, using procedures that can be applied to the clinical setting. Our work also examines the plastic response of the cental auditory pathway to both deafness and reactivation via a cochlear implant. Clinical experience consistently shows improved performance with implant use, and suggests that such improvement can be attributed to a reorganisation of the central auditory pathway [6].

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CONTROL OF INNER EAR AND BRAINSTEM PROCESSING BY DESCENDING NEURAL PATHWAYS

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The inner ear not only sends information about environmental sounds to the central nervous system, but it receives signals from the brain via efferent nerves of the olivocochlear system. This neural pathway originates in the superior olivary complex of the auditory brainstem and is the final output stage of a chain of descending pathways that begins in the auditory cortex. How is the olivocochlear pathway organized anatomically, what effects does it have on the cochlea and brainstem when activated, and what might its role be both in normal hearing and in the genesis of various pathologies?

There are two divisions of the olivocochlear pathways one of which make synaptic contact with the hair cells and the other with the afferent dendrites of the cochlea. These two systems are well disposed to be able to control independently the sensitivity of the cochlea to sound and the background, spontaneous firing rate of the afferent neurons in the absence of acoustic stimulation. In the normal cochlea these pathways may be required for instant-by-instant regulation of these important aspects of inner ear function. Experimental data in animals shows that activation of these pathways can improve the ability to discriminate stimuli in the presence of background sounds, serving as a possible basis of selective attentional processes at the most peripheral level of the auditory system (2,6). Consistent with this notion, recent results show that the pathway may be excited by higher centres in the auditory midbrain and cortex (3,4).

It has been shown that the deafening effects of loud sounds can be reduced by the presence of an intact efferent system (5). In addition, the action of the efferent nerves on the hair cells causes an increased drain of ionic current from the scala media and results in a drop in the large steady voltage that exists in this fluid compartment. Such an action could serve to control the endocochlear potential and hence regulate many downstream aspects of peripheral auditory function.

The most recent information from animal studies shows that in addition to acting in the cochlea, the efferent pathways send nerve branches to the cochlear nucleus in the brainstem and that these branches have an action that is antagonistic to that of the endings in the cochlea (1). Hence the action of this system in auditory processing depends on a reciprocal interplay between central and peripheral effects. From a pathological standpoint, abnormally high or low levels of activity in the efferent pathways could be involved in some forms of hearing loss, and in the genesis of tinnitus.

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OXIDATIVE STRESS IN NOISE-INDUCED HEARING LOSS

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Noise-induced hearing loss is a major problem in the industrialized world. In some countries it is the leading cause of work-related disabilities with a significant cost to the economy. Noise may cause damage by two mechanisms: mechanical and metabolic. Cellular structures can be physically disrupted by excessive mechanical stimulation, initiating necrosis or apoptosis (programmed cell death). On the other hand, metabolic overstimulation of any cell is associated with more subtle biochemical traumatic processes, most notably the generation of reactive oxygen species (ROS; free radicals) which also may trigger necrotic or apoptotic reactions. Several independent lines of evidence suggest that excessive noise exposure can cause such oxidant stress. For example, we have reported the elevation of isoprostanes in the cochlea after noise exposure, suggesting that intense noise produces ROS and induces lipid peroxidation. These stress responses are seen in almost all structures of the cochlea, suggesting that the specific detrimental effects of noise on hair cells depend on the down-stream pathways of gene activation that occur in the different cell types. In hair cells, apoptotic cell death is the consequence of noise trauma but the actual time course from initial acute injury to final hair cell loss can range from days to weeks.

Consistent with a mechanism of free radical-induced damage, antioxidants can attenuate noise trauma in experimental animals. In particular, investigations have focused on enhancing anti-oxidant enzyme activity, increasing glutathione availability, or administering free radical scavengers or trophic factors. These studies have provided the framework for the development of new strategies to protect the inner ear from noise-induced hearing loss.

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ATP RECEPTORS IN THE NOISE-DAMAGED COCHLEA.

Peter Thorne, on behalf of
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Adenosine tri-phosphate (ATP) is recognised as a source of energy in cells. In addition it acts as a signalling molecule between cells in many different tissues. Acting on specific ATP receptors (the P2X and P2Y receptors) on the surface of cells, ATP has been shown induce a variety of cellular reactions, ranging from neuronal signalling in the central and peripheral nervous systems to inducing cell proliferation or death. These receptors have been identified in various cells and tissues of the cochlea and there is accumulating evidence that purinergic signalling pathways involving ATP regulate the normal electrochemical homeostasis of the endolymph and act as a neurotransmitter or neuromodulator in the auditory nerve. ATP is a potent vasoactive agent in the cochlear vasculature and it is thus likely to also be involved in regulation of blood flow. Extracellular ATP enzymes (ectoNTPDase) that degrade the ATP to AMP and adenosine regulate the concentration of ATP in the fluids of the cochlea. In order to better understand the role of these purinergic signalling pathways in the cochlea we have undertaken studies to determine what happens to the elements of these pathways as the cochlea is stressed by loud sound or noise. Our studies to date have shown that ATP concentrations increase in the fluids and that there is a complex change in the expression of the receptors and the ectoenzymes with noise exposure. Expression of the $P2X_2$ receptor subtype, the dominant ATP receptor in the cochlea, and the ectoNTPDase increases as sound levels rise. However expression of the $P2X_7$ receptor that is found in the auditory afferent nerve fibres and which has been implicated in initiating cell death, decreases. Changes in the cellular expression of the receptors points to an adaptive or protective role of these purinergic signalling pathways as sound levels increase, at least with moderate levels. We hypothesise that ATP plays a major role in regulating the sensitivity of the cochlea to sound, possibly working synergistically with the efferent or descending pathways to the inner ear to maintain the dynamic range of the cochlea over the range of physiological sound levels. As levels of sound increase ATP secreted into the fluids of the cochlea acts on P2X receptors on sensory and supporting structures lining the endolymphatic compartment. These open cation channels, providing an electrical shunt between the endolymph and perilymph effectively short-circuiting the endolymphatic compartment and reducing the sensitivity of the hair cells to sound stimulation. This may be manifest as a temporary threshold shift or TTS. However, at higher noise levels the ectoNTPDase may be insufficient to maintain cochlear ATP levels and the high levels of ATP in the extracellular space may become toxic contributing to the degeneration of hair cells and nerve fibres through an interaction with P2X receptors that intitiate cell death. In this way ATP could be likened to glutamate which acts as a neurotransmitter in the auditory afferent nerves but at high levels is toxic and initiates neuronal degeneration; the phenomenon of excitotoxicity.

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MECHANISMS OF RHINITIS: PLASMA EXUDATION AS DETERMINANT OF MUCOSAL MILIEU *IN VIVO* AND AS INFLAMMATORY INDEX

Lennart Greiff, on behalf of
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"Rhinitis" may comprise a variety of conditions such as allergic rhinitis, upper respiratory tract infections, "chronic rhinosinusitis" as well as nasal polyposis. "Rhinitis" is characterised by nasal inflammation and is some cases, notably allergic rhinitis, this process is of primary pathogenic importance. The inflammation features interactions between disease-factors and immune-competent cells, production of pro-inflammatory cytokines, recruitment and activation of inflammatory cells, and altered "end-organ" responses (e.g., plasma exudation).

Plasma exudation is produced by a variety of inflammatory mediators and disease-factors. It is also part of the mucosal restitution-response to epithelial damage. It involves extravasation, lamina propria distribution, and non-injurious luminal entry of bulk plasma. Together, the wide-spread distribution of the plasma exudate and its multipotent adhesive, leukocyte-activating, growth-factor active, complement active, and otherwise biologically active peptides and proteins makes a key contribution to the mucosal molecular milieu *in vivo*.

There are often significant correlations between luminal levels of cell products, e.g., cytokines and mediators, and plasma proteins. Furthermore, experimental challenges that produce plasma exudation but not inflammatory cell activation may, by a mechanism likely involving specific binding proteins of plasma, transport cytokines and mediators from the airway tissue into the lumen. Accordingly, luminal levels of these cell products may not properly be interpreted unless the plasma exudation process is assessed concomitantly.

In contrast to other end-organ responses (e.g., vasodilatation, glandular secretion, increased mucociliary activity), plasma exudation is a specific inflammatory response. Thus, it may be used to indicate whether or not an inflammatory process affects the airway mucosa. We have shown that exudative indices are quantitative measures of the intensity of airway inflammatory processes at provocations and at natural disease, and that these indices can be used assess the efficacy of anti-inflammatory drug treatments.

In summary, plasma exudation (extravasation, lamina propria distribution, and luminal entry of plasma) is a non-injurious defence mechanism of the airway mucosa, but also a potentially pro-inflammatory factor. It is a major determinant of the mucosal milieu *in vivo*, and, in combination with nasal lavage techniques, it can be employed to indicate whether or not an inflammatory process affects the airway mucosa.

THE HEALING OF THE NASAL MUCOSA AFTER ENDOSCOPIC SINUS SURGERY IN THE SHEEP ANIMAL MODEL AND THE EFFECTS OF DIFFERENT NASAL PACKS ON THIS PROCESS.

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Introduction

There is a paucity of knowledge about the healing of the nasal respiratory mucosa after endoscopic sinus surgery (ESS). Nasal packs are often placed after ESS in an attempt to reduce adhesions but the effect of these packs on the healing of the nasal mucosa is not known.

Methods

A standardised normal animal model (the sheep) was used to examine the healing of the nasal epithelium after ESS. A full thickness wound was created in the nasal mucosa. This wound was packed with either a Polyvinyl chloride nasal pack, a dissolvable hyaluronic acid based pack, a dissolvable hyaluronic acid pack impregnated with the growth factor IGF 1 or left unpacked to serve as control. The wounded area were biopsied at 28, 56, 84 and 112 days post-injury and epithelialisation, and cilial regeneration were assessed by light microscopy (LM) and scanning electron microscopy (SEM).

Results

The wounds with the polyvinyl chloride pack and dissolvable hyaluronic acid based packs showed no differences in re-epithelialisation up to 54 days post-wounding. However a significant increase in re-epithelialisation was observed at day 24 in IGF1 impregnated hyaluronic packs compared to both hyaluronic packs and controls. At day 84 wounds packed with hyaluronic packs showed an increased rate of healing compared to unpacked controls indicating a significantly improved rate of healing at that time point. In addition, there was a significant increase in the epithelial height in the packed wounds on day 28 indicating that packing was effecting the epithelial maturity of the mucosa. No significant difference was observed in cilial regeneration between the packed and control wounds.

Conclusion

Application of the hyaluronic nasal packs and IGF1 impregnated hyaluronic nasal packs to wounds following ESS may improve re-epithelialization of the nasal mucosa but appears to have minimal effect on re-ciliation at the time points studied.

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Abstracts for Poster Presentations

POSTER 1: THE PATIENT'S PERSPECTIVE: QUALITY OF LIFE AND POSTOPERATIVE SYMPTOMS AFTER ACOUSTIC NEUROMA SURGERY

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Acoustic neuroma (AN) is a life threatening condition. Untreated tumour growth in the cerebellopontine angle leads to hearing loss, dizziness, tinnitus, cranial neuropathy and death. Surgery is hazardous because the cerebellopontine angle contains vitals vessels and cranial nerves. We report on a preliminary evaluation of postoperative quality of life (QOL) as perceived by AN patients.

51 patients who had surgery between 1998-2001 completed a Glasgow Benefit Inventory (GBI) Questionnaire; this was designed for otorhinolaryngological interventions, but as yet seldom applied to AN surgery. Demographics and perioperative findings were obtained from clinical records. Descriptive statistics were calculated and analysed using non-parametric statistics.

As expected postoperative QOL was decreased in 70% of patients. GBI QOL scores were decreased for general health (-22) and physical functioning (-10.8) but increased for social functioning (32.4). Females and non-Caucasians had a significant correlation ($p<0.05$) to social and physical functioning respectively. Tumour size and operative approach did not significantly correlate to QOL.

Modern microsurgery of the ear and brain has dramatically improved morbidity and mortality outcomes following surgery. As has been demonstrated in other studies, quality of life after AN surgery is reduced. In view of the lack of pre-operative symptoms in patients with smaller tumours, these studies indicate that certain tumours maybe suitable for "conservative management". Further work will compare patient QOL data to clinical findings, and QOL with conservative treatment.

Besides providing a patient perspective, this information is valuable for postoperative management for ENT specialists and general practitioners as primary care providers.

POSTER 2: A NOVEL MECHANISM FOR ENHANCEMENT OF SIGNAL TO NOISE RATIO IN A HAIR CELL SYSTEM

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Hair cells in the auditory, vestibular and lateral line systems transduce mechanical stimuli from the environment into electrical currents in the cells via mechanosensitive channels at the tips of their stereocilia. With the change in cell potential, the hair cells release quantal amounts of neurotransmitter to activate excitatory glutamate receptors on afferent fibers and ultimately generate action potentials that deliver information to the brain. In general, discharge patterns in these afferent nerve fibers are thought to reflect the hair cell's response to mechanical stimulation tempered by the kinetics of vesicle release at the synapse, however, it is becoming increasingly evident that there are other contributing processes. Responses in afferent fibers are stochastic and several strategies have been proposed for higher order neurons to extract signal from noise.

A possible example of a strategy to enhance the signal to noise ratio can be seen in *Xenopus laevis* lateral line organ afferent fibers. Physiologically, when an action potential propagates antidromically at a branch point in the afferent fiber it antidromically invades terminals with hair cells innervated by the same afferent fiber and reduces the excitability of all action potential generation sites. Antidromic electrical stimulation has also been shown to suppression afferent activity. Our experiments use an electrical stimulation paradigm, and we measure a half time of recovery of 75.0 ± 2.9 ms for this antidromic suppression, which is

much too long to be attributed to a relative refractory period in the afferent fiber or to collision of antidromic and orthodromic spikes. This antidromic suppression occurs due to stimulation of afferent fibers, and can be separated from suppression due to stimulation of efferent fibers since it occurs at lower voltages and is not blocked by atropine, gallamine or strychnine.

Our work shows for the first time that antidromic suppression is modulated by afferent synaptic input. Exogenous application of glutamate receptor agonists (kainate and NMDA) blocks antidromic suppression, whereas exogenous application of glutamate receptor antagonists (CNQX and kynurenic acid) enhances antidromic suppression. The physiological correlate of this is that antidromic suppression is blocked at strong synapses and enhanced at weak synapses. This, coupled with the stochastic nature of afferent discharge, means that the weak synapses get weaker and the strong synapses get stronger. Therefore, antidromic suppression could improve the signal to noise ratio, implicating the afferent fiber as playing an active role in stimulus coding and not simply functioning as a passive conduit for the transfer of signal to the brain.

Pharmacological investigation indicates that antidromic suppression is probably produced through activation of a TEA-sensitive potassium channel. The time course of antidromic suppression is very similar to that described for a TEA-sensitive, calcium-activated potassium channel in vestibular neurons. In addition, certain large conductance calcium-activated potassium channels can exhibit voltage-dependent activation and inactivation, which would fit with activation by antidromic action potentials.

Therefore, this work provides evidence for the existence of a physiological mechanism that could play a useful role in stimulus coding in that it could act as a strategy for higher order neurons to extract signal from noise. Anatomical and pharmacological similarities between the lateral line organ and many other hair cell systems imply that this mechanism may be broadly applicable.

POSTER 3: FINDINGS OF THE UNIVERSAL NEWBORN HEARING SCREENING PROGRAM IN WESTERN AUSTRALIA

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Aim: To report on the preliminary findings of a pilot program to screen newborns for congenital bilateral permanent hearing loss.

Setting: About 25,000 births occur in Western Australia each year. Approximately 45% of these occur in the five largest maternity hospitals in Perth. Newborn hearing screening was introduced at these hospitals between February to August 2000. Hearing screening was commenced in the level 3 neonatal nursery at the children's hospital in June 2001. Screening is thus offered at all level 2 and level 3 nurseries in the state.

Participants: All babies born at the five largest maternity hospitals after the introduction of hearing screening until 31st December 2001.

Methods: One or both of two automated screening devices were used: one measuring transient evoked otoacoustic emissions (TEOAE) and the other automated auditory brainstem responses (AABR). If a pass response was not obtained in both ears, the screen was repeated at follow-up. All babies who did not pass in either ear at follow-up were referred for audiological assessment.

Results: Of about 18,500 eligible babies, about 18,000 (97.2%) received screening. Eleven babies were diagnosed with bilateral permanent hearing losses (0.59/1000, 95% CI 0.30,

1.01). Ninety-one percent of those babies with a permanent bilateral hearing loss had a risk factor for hearing loss and 63% had been in a level 2 or 3 neonatal nursery.
Conclusions: A low prevalence of hearing loss has been detected. If the prevalence of hearing loss in Western Australia is truly as low as initial program results suggest and as many babies have a risk factor, the introduction of universal newborn hearing screening throughout Western Australia may not be the best use of health resources. A screening program targeting level 2 and 3 nursery admissions and those identified as having a risk factor may be more appropriate.

POSTER 4: GENETIC ASPECTS OF EARLY ONSET PRESBYACUSIS

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Hearing loss significantly affects the quality of life in 10% of the population by the age of 60 years and 50% by the age of 80 years. With the ageing Australian population, presbyacusis is increasingly becoming a major concern. Genetic and environmental factors, or a combination of both, can cause hearing loss. In the majority of cases involving deafness in newborn infants and children the underlying causes are believed to be genetic. Recent genetic studies have led to the identification of over 20 genes for non-syndromic as well as over 20 genes for syndromic deafness. We and others have shown that mutations in the gene coding for the gap junction protein connexin 26 (Cx26) are the major cause of recessively inherited prelingual hearing loss. We estimate that approx. 2% of Australians are "unaffected" carriers of Cx26 mutations. Mutations in the Pendred syndrome (PDS) gene cause a variety of clinical conditions, including recessive non-syndromic hearing loss, enlarged vestibular aqueduct syndrome or Pendred syndrome, and PDS mutations may account for more than 10% of inherited deafness. We estimate that over 1% of Australians are "unaffected" carriers of mutations in the PDS gene.
In contrast to the extensive studies of childhood hearing loss, remarkably little is known about genetic factors and the changes in gene expression associated with presbyacusis. Our study investigates the genetic contribution of single gene mutations in the Cx26 and PDS genes to early onset presbyacusis. We have so far collected DNA samples and audiological data from 120 people with early onset presbyacusis. Ten of these are carriers of a Cx26 mutation, ie we detect a fourfold increase in carrier frequency when compared to the general population. Studies have suggested that some Cx26 mutations may act in a dominant or semi-dominant fashion. In 3 of the 10 carriers the Cx26 mutation was M34T, suggesting that certain Cx26 mutations predispose to early onset presbyacusis. We have also investigated 22 parents of deaf children homozygous for Cx26 mutations. Our preliminary results show that although there is no difference in pure tone threshold levels between these people and controls, there are significant differences in the amplitude values of distortion product otoacoustic emissions at 3kHz and 4kHz.
The identification of genes contributing to onset of presbyacusis is significant, as this will allow us to identify at risk groups who might then be able take preventative measures to delay or prevent the hearing loss. It will also enable us to better study how environmental factors contribute to hearing impairment.

POSTER 5: VIDEO SEQUENCES OF THE TYMPANIC MEMBRANE: DIGITAL COMPRESSION FOR TELEMEDICINE

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Background: Pneumatic otoscopy is an important tool in the diagnosis for otitis media to determine the mobility of the eardrum. When digitised, image sequences can be very large;

a 10 second sequence can be 100 MB in size, too large to transmit efficiently through telecommunication networks. Therefore an effective image compression format must be selected, and a suitable compression level determined.
Aim: To establish a maximum compression level for tympanic membrane sequences. This compression will retain the important diagnostic information.
Methods: 5 to 15 second image sequences of 12 subjects (including 5 subjects with some pathology) were digitised and stored in a non-compressed format. The sequences were compressed to 5 different levels (1:100, 1:200, 1:300: 1:500, 1:700) using an MPEG format, and presented to 4 assessors in a random order. These assessors were asked to judge image quality (including colour, blocking and blurriness), and record any pathology they observe. The assessments of pathology of the forth assessor were used as the gold standard. The Wilcoxon Rank was used to analyse the image quality data, and the chi-squared test was used to analyse the pathology assessments.
Results: Only for sequences compressed to 1:100 were the all the assessments of image quality statistically the same as the assessments from the uncompressed sequences. However, overall image quality and colour were assessed to be more tolerate of compression (to 1:200). An analysis of the assessments of pathology showed that the ability to separate normal from abnormal eardrums was possible through to the 1:500 compression level. If a compression level of 1:200 is chosen, then 100MB image sequences can be compressed to about 500Kb while still retaining good image quality and diagnostic information.

POSTER 6: RUPTURE PRESSURE OF PORCINE TYMPANIC MEMBRANE

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Aim: To determine the rupture pressure of porcine tympanic membranes (TM) as an animal model for future tympanic membrane and tissue engineering studies.
Background: The relative sizes of the pars tensa and flaccida vary greatly amongst species, as does the attachment to the malleus, size, orientation and attachment. The human and porcine TMs are similar in size, and nearly identical histologically as well. As the porcine model is often used in skin studies, and pigs are readily available, they were used in this study.
Methods: A number of pig heads were obtained from an abattoir, and the temporal bone was excised by a bandsaw and a bone drill. The pieces were about 80x80x80mm, and contained the middle ear and a section of the canal. After drying, a 4mm tube was inserted into the canal and glued in place. A tympanometer was used to measure the compliance of the TM, and the pressure threshold was determined by attaching a syringe and pressure gauge to the tube in the ear canal. In total 9 TMs were tested.
Results: By tympanometry, the ear canal volume was 1 +/- 0.2 cm^3, and the compliance peak 1.5 +/- 0.9 cm^3. The rupture pressures were 1.2 +/- 0.3 atmospheres (over ambient). These results are in same order of magnitude of other studies, appear to be more consistent, and are similar to that for humans.

POSTER 7: "DEFINING THE CHARACTERISTICS OF HIGH-FREQUENCY (~1000 HZ) TYMPANOGRAMS FOR NEONATES, 2-MONTH-OLD AND 4-MONTH-OLD INFANTS, IN RELATION TO THE DEVELOPMENT CHANGES IN INFANTS' MIDDLE EARS."

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The importance of hearing integrity in the first 3 to 4 years after birth for normal acquisition of speech and language has long been appreciated. During this sensitive and critical period, speech and language will almost always develop rapidly, and normally, if the auditory and language regions of the brain are adequately stimulated by the sounds of communication.

Unfortunately, by the time hearing loss in infancy and early childhood is suspected, evaluated by an ENT specialist and an audiologist, and appropriately managed, two or more of these communicatively important years have elapsed, and the child has lost an enormous developmental advantage.

Consensus on the crucial impact of early auditory experience on language development has now been reached and unequivocally articulated in North America and most Western European countries. Screening the hearing of all infants within the first 3 months after birth, with equally prompt and appropriate intervention, is perhaps the only way to ensure that all children receive a rich and adequate auditory experience.

Related to the communicative benefits of early intervention, and perhaps more compelling when presented to administrators, legislators and business, is the economic impact of hearing impairment. Estimation of these statistics include a wide variety of factors, ranging from the cost of education and ancillary personnel required for education or everyday communication (e.g., interpreters) to the inevitable increase in unemployment and decrease in wage-earning ability. There is no comprehensive data compiled in Australia, but it is estimated to run into the millions.

This kind of statistics, coupled with the finding that early identification and intervention (by 6 months after birth) can lead to normal language development, provides practical and powerful arguments for an upfront investment in universal newborn hearing screening.

Current procedures used for infant hearing screening programs include otoacoustic emisssions (OAEs) and auditory brainstem responses (ABR). These procedures produce a refer rate which varies from 2 to 10 %. It is possible that some of these "refer" cases may have a congenital middle ear dysfunction (transient or permanent). Recent studies have shown that a large proportion of young infants, diagnosed to have a hearing loss, have a mild to moderate conductive hearing impairment. Hence, it would be an advantage to have a screening test of middle ear dysfunction for neonates who fail a hearing screen, if not for all neonates. However, such a screening test for detection of middle ear dysfunction in neonates has not been widely available.

Conventional tympanometry with a probe tone of 226 Hz has been routinely used since the early 1970's as a clinical tool in the differential diagnosis of middle ear dysfunction in children and adults. Its effectiveness in the detection of middle ear dysfunction has been widely acknowledged. Normative data for adults using conventional tympanometry has been well established.

However, despite its successful application to adults and children, the use of conventional tympanometry for infants aged less than 7 months has been questioned. It has been shown to have poor sensitivity and poor specificity to detect middle ear dysfunction in young infants.

Developmental changes in infant's middle ear system have been suggested to account for the above spurious results. A number of studies have shown that the use of a higher frequency probe tone (such as 1000 Hz) is more sensitive to middle ear effusion in young infants than the conventional probe tone. However, the generalisation of this finding is limited due to the small sample sizes of the studies. Furthermore, normative data for specific groups have, to date, not been established. Hence, clinicians must either wait until the infant is old enough (usually 6-7 months) to perform the conventional procedure or use time-consuming electrophysiologic methods for such investigations (e.g., comparing air- and bone- conducted auditory brainstem responses). In addition, otoscopy is not always possible for neonates and young infants.

Our study examines the findings of high-frequency tympanometry. This will be used in conjunction with recordings of otoacoustic emissions of auditory outer hair cells and whenever, if possible, will be interpreted together with the otological findings of the neonates / infants.

With the present push for universal neonatal hearing screening in Australia, there is a great demand for a time-efficient tool for assessing middle ear function in very young infants. It is recommended that the following research tasks need to be accomplished as soon as possible:

To describe the characteristics of the high - frequency tympanograms for neonates, 2-month-old and 4-month-old infants, in relation to the developmental changes in infants' middle ears;

To establish normative data for the three age groups.

POSTER 8: E2F-1 AND pRb EXPRESSION PREDICT REDUCED DISEASE FREE SURVIVAL IN SQUAMOUS CELL CARCINOMA OF THE ANTERIOR TONGUE

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Purpose: Overexpression of E2F-1 is associated with increased invasiveness in Head and Neck Squamous Cell Carcinoma (HNSCC) cell lines *in vitro*. The significance of increased expression of E2F-1 has not been assessed in HNSCC *in vivo*. In this study, we have sought to determine the relationship between E2F-1 and retinoblastoma protein (pRb) expression to disease outcome and clinicopathological parameters in squamous cell carcinoma (SCC) of the anterior tongue.

Experimental Design: pRb and E2F-1 protein expression was assessed using immunohistochemistry (IHC) in a cohort of 145 patients with SCC of the anterior tongue with known outcome. The relationship between E2F-1 expression and pRb expression to clinicopathological parameters (nodal status, stage and treatment), molecular markers (cyclin D1, $p16^{INK4A}$ and Ki67) and disease outcome were analyzed.

Results: Increased expression of E2F-1 was associated with improved disease-free survival (DFS) (P=0.03) and overall survival (OS) (P=0.04). Reduced expression of pRb was associated with improved DFS (P=0.03) and OS (P=0.05) on Kaplan Meier analysis. Lymph node status (P=0.0009), E2F-1 (P=0.03) and pRb (P=0.03) were independent predictors of DFS on multivariate analysis.

Conclusions: Increased expression of E2F-1 and reduced expression of pRb are independent prognostic markers for SCC of the anterior tongue when co-segregated with a longer DFS.

Research Support: Garnett Passe and Rodney Williams Memorial Foundation Research Fellowship (R.A.K.)

POSTER 9: MUSIC PERCEPTION OF COCHLEAR IMPLANT USERS

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Formal research into the perception of music by cochlear implant (CI) users is relatively scarce when compared with studies of speech understanding. The lack of objective data has been addressed in two recently completed experiments. In the first, CI users' identification of 16 complex sounds was assessed. The sounds, which comprised speech, environmental noises, single musical instruments, and musical ensembles, were presented in a closed-set format without any non-auditory cues. The second experiment, using a similar procedure, assessed the identification of 16 musical instruments. The subjects were 10 postlingually deaf adult users of the Speak sound-processing scheme. They had no formal music training. For experiment 1, sound identification scores ranged from 23 - 89%, with a mean of 54%. Speech was the most accurately identified sound category (mean score 64%), whereas the category of environmental noises was the least accurately identified (mean score 45%). For experiment 2, scores ranged from 24 - 84%, with a mean of 44%. The drums and male singer were the most accurately identified sounds (mean score 74%). In contrast, a group of normally hearing adults, whose recognition of complex sounds was tested using the same procedures, obtained much higher average scores (96% for experiment 1, and 97% for experiment 2). For the CI users, the average scores from the two experiments were correlated (r^2 = 0.77), as were the scores from each experiment and a separate test of speech recognition (r^2 = 0.91 and 0.61 for experiments 1 and 2,

respectively). However, no significant correlations were found between scores from either test and the variables of age, length of experience with the CI, or duration of deafness. Overall, the results suggest that for CI users, the perceptual mechanisms associated with good speech understanding are similar to those related to their ability to recognise complex sounds in general. As the perceptual performance of CI users is known to be highly dependent on the techniques used to process sounds for delivery as patterns of electric stimulation, the findings of this research emphasise the need to develop and evaluate improved methods of processing complex signals, particularly musical sounds, in cochlear implant systems.

This work was supported by the Garnett Passe and Rodney Williams Memorial Foundation.

POSTER 10: PATIENTS' 3-D EYE-MOVEMENT RESPONSE TO SURFACE GALVANIC VESTIBULAR STIMULATION

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We have shown in previous studies that normal subjects respond to maintained galvanic vestibular stimulation (GVS) delivered via large surface electrodes with reflexive eye-movement patterns which are: variable between subjects, but quite repeatable within subjects (idiosyncratic); that the magnitude of eye-movement response is a linear function of the magnitude of the current; that normal subjects show symmetrical responses to GVS delivered to the left or right mastoid and to anodal or cathodal current polarities; that the response to bilateral GVS stimulation seems to be a simple sum of the responses to unilateral stimulation of each side; and that responses adapt, and return to baseline after overshooting at stimulus offset, with long time constants of hundreds of seconds. These principles have formed the basis of a heuristic model of eye-movement responses that uses a simple linear addition of the weighted contributions from each vestibular sensory region. This model, which matches the eye movement responses of normals, can be modified to predict idealized responses that might be expected due to various kinds of vestibular dysfunction by modifying the contribution of the sensory regions affected (e.g. minimizing the contribution of all end organs on the left in the case of a patient with surgical unilateral loss on the left). Patients diagnosed with some of these conditions have been tested, and their responses to GVS have been consistent with the model's predictions, supporting the idea that surface GVS tends to stimulate all end organs to various degrees in an idiosyncratic, yet predictable, fashion. It should be noted that a lack of response to GVS could result equally from normal variations in factors such as impedance paths between delivery at the surface and stimulation at the spike trigger zone, or abnormal disruptions anywhere along the path from the spike trigger zone through central processes and oculomotor output. The absence of eye-movement response to GVS does not, therefore, necessarily implicate end organ dysfunction (just as a lack of response to a caloric test might have a non vestibular cause). In contrast, the presence of an eye-movement response to GVS indicates effective delivery of the stimulus as well as entailing the function of everything upstream from the site of activation. For example, patients may show a GVS response from end organs even after undergoing surgical ablation, if some primary afferent terminals have been spared. These results suggest that despite the variability of eye movement responses, GVS might provide a source of evidence for an accurate diagnosis based on the results from a range of vestibular tests.

POSTER 11: INCREASED CELL DEATH IN AFFECTIVE PSYCHOSIS: EVIDENCE FROM OLFACTORY NEUROEPITHELIAL CULTURE FROM ADULT PATIENTS

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Evidence suggests that neurodevelopment is altered in schizophrenia, however the underlying mechanisms for these changes remain unclear. The adult olfactory epithelium provides an available "window" on neuronal development because new neurons are formed there throughout life. Our previous study demonstrated several differences in olfactory cultures from schizophrenia patients versus well controls. The present study aimed to replicate previous findings concerning schizophrenia and examine the specificity of the finding by including patients with affective psychosis. Biopsies of olfactory mucosa were collected under local anesthetic from individuals with non-affective psychosis (schizophrenia, atypical psychosis, n=11), affective psychosis (bipolar disorder, major depression with psychosis, n=11), or well control (n=10). Tissue was sliced at 200 μm, and cultured for 3 weeks in serum-free medium. Cell nuclei were stained with bisbenzimide and counts were made of total cells, mitotic figures, and apoptotic/necrotic cells. Our analysis demonstrates that in bipolar cultures there was a 2-3-fold increase in cell death compared to both control and schizophrenia. Schizophrenia cultures had a slightly increased proliferation of neural cells compared to controls and bipolars. However this difference did not reach significance. Our current findings in schizophrenia neither support nor contradict our previous findings. Our finding of increased cell death in bipolar disorder demonstrates for the first time an alteration of cell cycle control in this disease. These results provide evidence for a neurodevelopmental aetiology of psychotic disease acting at the level of cell cycle control. Subtle changes in the timing of cell cycle regulation could account for the brain pathologies observed in this disorder.

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POSTER 12: PERCEPTUAL CHARACTERISATION OF CHILDREN WITH AUDITORY NEUROPATHY

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Auditory neuropathy (AN) is a form of hearing deficit in which some cochlear (outer hair cell) function is preserved, but neural conduction in the auditory brainstem is disordered. Affected children and adults can present with normal or near-normal hearing levels, but often show very poor speech understanding. This study sought to characterise the perceptual abilities of a group of 13 children with AN, correlating their results on frequency resolution, temporal resolution and frequency discrimination tasks with open-set speech perception performance. Data were also obtained from a cohort of age and hearing level matched subjects with sensorineural (SN) hearing loss, and from a group of normally hearing children. Results of the frequency resolution experiment revealed that the ability of AN subjects to resolve frequencies in static complex sounds was equivalent to that of the normal-hearing subjects, and was superior to that of the children with SN hearing loss. AN children with poor speech perception did however show significantly poorer frequency discrimination ability than their counterparts with normal hearing or SN loss. In particular, the AN children did not appear to be able to make use of temporal frequency cues for low-frequency discrimination. Temporal resolution (amplitude modulation detection) was also significantly impaired in AN subjects with poor speech perception. The results, together with electrophysiological findings in subjects with auditory neuropathy (which point to a de-synchronisation of neural discharges in the auditory brainstem), suggest that AN involves a disruption of central temporal processing of neural inputs, leading to inability to detect changes in frequency and/or intensity in complex stimuli. We hypothesise that a reduced phase locking of neurons to auditory stimuli could lead to impaired temporal processing and hence poor speech understanding. In summary, the findings of this study provide some insights into the specific processing abnormalities associated with AN type hearing loss. The application of this knowledge will lead to better habilitation strategies (including speech-processing hearing aids) and clinical management of affected children.

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POSTER 13: OLFACTORY REGENERATION IS INACCURATE AFTER INJURY

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Do primary olfactory neurons target topographically correct loci following degradation of the olfactory neuroepithelium? It is known that the olfactory neuroepithelium is a highly plastic region of the nervous system that undergoes continual turnover of primary olfactory neurons throughout life and is capable of regeneration. Even after deafferentation, primary olfactory neurons regenerate and reinnervate the olfactory bulb. However, is the reinnervation in appropriate regions that would allow re-establishment of the normal repertoire of smell? Primary olfactory axons expressing the same odorant receptor gene sort out from axons expressing unlike receptors and converge to topographically fixed glomerular targets in the olfactory bulb. We have examined the guidance of axons expressing the P2 odorant receptor using P2-tau-LacZ mice (Mombaerts et al., 1996) when they were challenged with different cellular environments *in vivo*. We have developed a technique of chemically degrading the entire olfactory neuroepithelium in mice, but which leaves the basal layer of stem cells unharmed. By giving 2 intraperitoneal injections of dichlobenil over 4 days, the olfactory neuroepithelium is totally degraded after one week. We examined the targeting of the regenerating P2-LacZ axons over the next 18 weeks. While some P2-LacZ axons did terminate in the topographically correct glomeruli, many P2 axons terminated in inappropriate glomeruli that were distributed over a wide region of the olfactory bulb. We also examined the convergence and targeting of P2 axons following bulbectomy. After the olfactory bulb is removed from neonatal mice, the developing cortex fills the cavity left by the olfactory bulb and regenerating primary olfactory axons penetrate the cortex. We found that P2-LacZ axons converged and terminated together in loci dispersed over this region of the cortex. Together, these results indicate that convergence and targeting of olfactory axons are separate events mediated by different guidance cues and that following injury it is likely that the insufficient guidance cues are present to enable correct regeneration of the primary olfactory system.

POSTER 14: BIOFILMS IN ABORIGINAL CHILDREN WITH OTITIS MEDIA WITH EFFUSION

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AIM: The aim of this study was to demonstrate the presence of bacterial biofilms in the ears of aboriginal children with otitis media with effusion (OME)

INTRODUCTION: Biofilms are a community of interacting bacteria attached to a surface and encased in a protective proteoglycalyx or matrix of exopolysaccharides. They are increasingly found in the human body, both on implanted devices and on native tissue. They are difficult to detect on routine microbiological assessment and extremely resistant to antibiotics.

Biofilms have been demonstrated in experimentally induced *H. Influenzae* otitis media in chinchillas. This is thought to explain some of the phenomena of OME including culture negative effusions, the inefficacy of antibiotics in the treatment of OME and the efficacy of tympanostomy tubes.

PROCEDURE: Biopsies were taken from the middle ears of several aboriginal children undergoing ventilation tube insertion for OME.

The specimens were processed and studied under electron microscopy. Several biopsies showed evidence for bacterial biofilm formation.
CONCLUSION: The detection of bacterial biofilms in the ears of aboriginal children with OME shifts the aetiological paradigm of this massive public health issue and leads to many new avenues of research into the problem

POSTER 15: THE ANTI-INFLAMMATORY EFFECT OF MACROLIDE ANTIBIOTICS

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Introduction. Long-term, low-dose macrolide therapy is effective in the treatment of chronic rhinosinusitis. The mechanism of the anti-inflammatory effect of macrolides and how this differs from corticosteroids remains unclear.
Methods. *In vitro*: Chronic sinusitis nasal mucosa was cultured for 24 h in the presence of clarithromycin or prednisolone. Cytokine levels were determined by enzyme-linked immunoassay. In addition immunohistochemical staining for TGF-*B* and nuclear factor kappa *B* was performed.
In vivo: Patients were treated with a 3-month course of clarithromycin. Pre- and post-treatment biopsies were stained immunohistochemically for inflammatory cells, TGF-*B* and NF-*kB*.
Results. Clarithromycin produced significant reductions in IL-5, IL-8, GM-CSF and TGF-*B in vitro*. These reductions were equal to that produced by prednisolone. Neutrophil numbers were reduced in post-treatment biopsies *in vivo*.
Conclusion. Macrolide antibiotics have been shown to exhibit an anti-inflammatory activity as potent as prednisolone. This mechanism is likely to be at least partly responsible for the clinical efficacy of macrolide antibiotics in chronic rhinosinusitis.