The human tympanic membrane: preliminary studies in tissue engineering.

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Introduction: The function of the tympanic membrane (TM) in transferring energy to the middle ear is largely due to the cellular arrangement. Three layers are distinctly recognized, namely the outer epidermal, middle lamina propria and inner mucosal layer. To maximize the functional hearing outcomes after tympanoplasty a tissue-engineered TM must closely replicate the normal human TM. The rupture pressure of the TM is an indicator of tympanic membrane strength. The mean rupture pressure of human TMs is 1.2-1.6 atm, which is significantly higher than measured in previous animal studies. Two studies were undertaken as part of a project to tissue engineer a human TM.

Methods: TMs were obtained from patients having surgery where the drum was sacrificed. TM structure was examined using light and electron microscopy. The TMs of nine porcine temporal bone specimens were ruptured by a calibrated pressure delivery system.

Results: Our study is consistent with previous studies showing the outer radial and inner circular fibres of the lamina propria, the keratinising stratum corneum with underlying stratum granulosum, stratum spinosum, and stratum basale layers in the epithelium, and the simple cuboidal cells in the mucosa. The rupture pressure of the porcine TM was measured at 1.2 ± 0.3 atm.

Conclusions: Successful tissue engineering of a TM will require further work on structure and function. Immunohistochemistry will be performed to determine cytokeratin expression and collagen types. The mean rupture pressure in pigs is comparable to humans, and represents a suitable animal model for testing our tissue engineered TMs.
Tissue engineering of vascularized adipose tissue with the use of adult human bone marrow and lipoaspirate derived mesenchymal stem cells.

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Introduction: The need for tissue engineered vascularized adipose tissue has arisen due to the poor results of autografting fat. A cellular source is as essential for tissue engineering as an instructive matrix, a blood supply and space for tissue to grow into. This study compares the utility of adult Mesenchymal Stem Cells (MSC), multipotential cells capable of differentiating into fat, cartilage and bone, derived from bone marrow and lipoaspirate in the tissue engineering of vascularized adipose tissue.

Methods: Cells were isolated from human bone marrow and lipoaspirate samples using an established protocol and these were expanded in-vitro in conditions favouring MSC. In-vitro assays for bone, cartilage and fat were performed to confirm that MSC were present in this population. We use an in-vivo SCID mouse model of tissue engineering which involves placement of silicone chambers around the skeletonized superficial inferior epigastric vessels bilaterally in the mouse groin. The chambers contain a synthetic extracellular matrix containing growth factors (Matrigel) and are wax sealed to exclude mouse fat. The control chamber contains only the Matrigel while the test chamber contains Matrigel with MSC of bone marrow or lipoaspirate origin. Chambers were harvested at 4, 6, 8, 12, and 16 weeks and assessed morphologically as well as immunohistochemically to determine the type and source of tissue formed.

Results: Control chambers without a cellular source did not support adipogenesis in contrast to the test sides. Both bone marrow and lipoaspirate derived MSC were capable of promoting adipose tissue formation, thought the former was much more effective than the latter. This correlated with their in-vitro behaviour. Angiogenesis was seen at 4 weeks with little evidence of adipogenesis. Adipogenesis commenced at 6 weeks and increased from this time onwards with a maximal amount of fat tissue formed at 12 weeks. Some regression of adipose tissue formation was evident at 16 weeks. Human specific labelling proved that the human MSC contributed to tissue within the chamber including blood vessels.

Conclusions: Our chamber supports the growth and differentiation of adult MSC derived from wither bone marrow or adipose tissue thereby providing a method of assessing the in-vivo differentiation capacity of these cells. The production of vascularized adipose tissue with the addition of MSC is possibly a means of tissue engineering fat for clinical use. This is also the first time that MSC have been shown to contribute in-vivo to blood vessels and this suggests further use of these cells in engineering blood vessels.
Utilisation of tissue engineering techniques to produce large volumes of tissue for reconstructive surgery - A porcine model.

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Introduction: The study was undertaken to show that amounts of tissue suitable for reconstructive surgery (e.g. breast reconstruction) can be produced using tissue engineering techniques.

Methods: A porcine model of tissue engineering, using a bilateral polycarbonate chamber (78ml volume) around a ligated pedicle containing the thoracodorsal vessels was developed in the axillae to simulate in-situ breast reconstruction using wild-type pigs. Tissue growth was tested in the presence or absence of a matrix scaffold and different stem cell sources within the chamber. All animals had their chambers removed at 6 weeks. Magnetic Resonance Angiography (MRA) was assessed for its ability to non-invasively monitor tissue growth and vascularisation within the chamber as such monitoring would be necessary for application of these techniques in humans.

Results: MRA was capable of monitoring vessel patency, tissue perfusion and could distinguish between the new tissue in the chamber and the matrix scaffold compared with histological sections at harvest. At six weeks post-chamber insertion, the entire chamber was filled with new tissue with a small amount of residual scaffold. Histology confirmed the growth of new adipose tissue around the central vasculature with granulation tissue filling the remainder of the chamber. There was significant capsule formation around the polycarbonate chamber but no other apparent adverse effects.

Conclusions: This study demonstrates that tissue engineering techniques are capable of producing significant amounts of vascularised and transferable tissue. Research efforts are now directed towards producing stable tissue for breast reconstruction. Vascularised adipose tissue will be the most appropriate product.
Eve-3, a liver specific inhibitor of cell proliferation: A new clue for an old mystery

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Introduction: The liver is the only organ that can regulate its growth and mass. What controls this process, in particular what prevents the liver from continuing to proliferate, is still largely a mystery. We have identified a new protein, Eve-3, which is expressed uniquely in the liver and which is a potent inhibitor of cell proliferation.

Methods:

Results: We have identified a protein Eve-3, containing a single Ena Vasp homology (EVH1) domain that can potently block activation of the Ras/MAPK pathway. Differential splicing of the Eve-3 transcript yields another protein, Spred-3, similar in structure to the Spred proteins, which are known inhibitors of Ras/MAPK activation. Expression of Eve-3 is restricted to the liver. Eve-3 is specific in inhibiting the Ras/MAPK pathway, however Spred-3 can also inhibit the c-Jun amino-terminal kinase, JNK pathway. Eve-3 and Spred-3 have differential effects in blocking cellular differentiation, whilst both can block cell cycle progression.

The time course of Eve-3 expression is closely matched to the proliferation status of the liver throughout development. Eve-3 expression is absent from proliferating liver tissue and strongly induced in non-proliferating liver tissue.

Conclusions: Eve-3 may be involved in modulating the unique regenerative capacity of the liver. A targeted inhibitor of Eve-3 function may be clinically useful in boosting liver regeneration in clinical conditions where this would be greatly advantageous.
Delivery of neurotrophin-3 to the cochlea using alginate beads

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Introduction: Neurotrophin-3 (NT-3) is a growth factor promoting survival of auditory spiral ganglion cells (SGCs). It may have therapeutic potential in slowing programmed cell death sensorineural hearing loss, and may have a role as an adjunct to the current cochlear implant. We have investigated alginate beads as a novel delivery vehicle for NT-3 to the cochlea and tested two surgical delivery methods, at the round window and intracochlear.

Methods: Alginate beads with and without heparin were manufactured by an ionic gelation technique. These beads were subsequently loaded by soaking them in a solution of NT-3. ELISA studies were performed to quantify the loading and release kinetics of NT-3. Beads were then tested in vivo for local tissue reaction and degradation in adult guinea pig cochleae. Subsequently, NT-3 loaded beads and unloaded controls were placed either on the round window or though into the basal turn of the cochlea in one week deafened animals. The animals were sacrificed at 28 days and cochlea tissue analyzed.

Results/ Conclusions: ELISA studies demonstrated a 98-99% loading of NT-3 with a partial release over 5 days in Ringer's solution. Furthermore, the addition of heparin to the alginate delivery system modulated a more sustained release of NT-3. We subsequently used alginate-heparin beads in vivo. Histological analysis revealed minimal tissue reaction. The NT-3 loaded beads had significantly higher SGC survival effect in both the round window and intracochlear arms of our study, This pilot study has established alginate beads as a biocompatible and effective delivery system for NT-3 to the cochlea.
Electrical stimulation of the visual cortex in the cat with a prototype visual prosthesis for blind patients

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Introduction: An in-vivo model for stimulation of the visual cortex with a surface electrode array was developed in the cat to evaluate a prototype medical device to restore visual perceptions to blind patients.

Methods: In anaesthetised cats, a craniotomy was performed and the dura removed. An electrode array consisting of 21 0.7 mm diameter disc electrodes, arranged in three rows, was placed over a gyrus in one hemisphere, overlying the border region between area 17 and area 18 of the visual cortex. Electrical stimulation of an electrode in this array was carried out, and recordings of the transcallosal evoked potentials were made from an electrode placed in a homologous region of the contralateral visual cortex.

Results: Electrical stimulation of the visual cortex in the cat with the electrode array elicited predictable transcallosal responses. The threshold current for production of a transcallosal response decreased with increased duration of the pulse. Strength-duration relationships for electrical stimulation of the visual cortex with anodal and cathodal monopolar stimulation were obtained. It was found that electrical stimulation with anodal pulses required a higher current threshold for a given stimulus pulse duration.

Conclusions: This in-vivo cat model is useful for evaluating electrical stimulation of the visual cortex. It allows us to evaluate prototype electrode arrays and stimulation parameters in the development of a visual neuroprosthesis for blind patients. Electrical stimulation of the visual cortex with monopolar cathodal pulses requires less current than stimulation with anodal pulses for a given pulse duration.
Comparative neurovascular anatomy of human and animals

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Introduction: The neurovascular anatomy of the skin and the deep tissues of the body is fundamental to the design of flaps for local and distant transfer. Although closely related in the deep tissues, these structures especially the arteries and veins, often course at a distance from each other.

Methods: The arterial and venous anatomy of the integument was examined in 68 human cadavers and in a large series of 24 different adult animals after perfusion with lead oxide, as well as their relationships to the cutaneous nerves. The neurovascular patterns were studied in the forelimb of 1500 developing quail embryos to investigate the establishment of this relationship.

Results and Conclusions:

1. There is a close match between the "blueprint" of the cutaneous nerves of the human and the animals.

2. The vascular pattern is similar between species, however the size, number and length of vessels varies, especially in relation to the mobility of the skin.

3. A primary, secondary and tertiary development of the vascular system was seen in the embryo which helps explain the adult relationships between the arteries and the veins which is "fickle" in the skin but "wedded" in the deep tissues. It helps explain also: (i) the development of the angiosomes of the body; (ii) the development of the cutaneous perforating arteries, which course either through or between the deep tissue; (iii) the coexistence of the dual mechanism of venous drainage of the integument, namely horizontal and perforator, and (iv) the vascular basis for the currently popular "perforator flaps" in clinical use.
Molecular mechanisms associated with the resistance of cartilage to malignant invasion.

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Introduction: Hyaline cartilage is a barrier to osteosarcoma invasion, however the mechanisms behind this resistance remain unclear. The aim of this study was to examine the temporospatial pattern of osteosarcoma growth and invasion, and to investigate the molecular mechanisms behind the resistance of cartilage to malignant invasion.

Methods: An *in vivo* mouse model of osteosarcoma was used, whereby osteosarcoma cells were orthotopically injected into the tibiae of nude mice. Animals were sacrificed at weekly timepoints, enabling histological analysis of tumors at different stages of disease progression. Routine Haematoxylin & Eosin staining and immunohistochemical staining using antibodies against pro-angiogenic vascular endothelial growth factor (VEGF) and anti-angiogenic pigment epithelium-derived factor (PEDF) was performed.

Results: Hyaline cartilage of the growth plate and articular surface was resistant to osteosarcoma invasion in all cases, despite increasing tumor size and extensive intra- and extra-osseous destruction. In the most advanced cases, only the lowermost layers of the hypertrophic zone of the growth plate were eroded. These layers displayed strong immunostaining for the potent angiogenic factor VEGF, and absent immunostaining for PEDF. In contrast, the resting, proliferative and upper hypertrophic layers, which were consistently resistant to osteosarcoma invasion, showed high expression levels of the potent anti-angiogenic factor PEDF. These results confirm that the balance of angiogenesis, influenced by pro- and anti-angiogenic factors, determines tumour growth and invasion.

Conclusions: The resistance of epiphyseal cartilage to osteosarcoma invasion is likely to be due to the expression of anti-angiogenic factors. Work-in-progress involves testing the therapeutic application of one such factor, PEDF, in osteosarcoma.
Validation of virtual endoscopy of the maxillary sinus

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Introduction: To assess the predictive value of virtual endoscopy for planning surgical procedures of the maxillary antrum. Virtual endoscopy is increasingly available on radiological workstations, and has the potential to assist surgeons plan their sinus surgery. A method is described for setting up, and validating, a virtual endoscope so that it accurately reflects the view obtained from a real endoscope.

Methods: Six human cadavers were scanned by CT and the images were used to construct 3D images of the head and sinuses. Virtual endoscopy of the antrum were performed in the Analyze™ (Mayo Clinic) image analysis program. The virtual endoscopic videos were compared with videos of real endoscopic examinations of the antrum of the same cadavers.

Results: A realistic rendering of a virtual surgical environment requires careful “calibration” of the virtual endoscope. This involves setting the correct field of view, and if the program will allow it, the lens size. Most virtual endoscopes simulate a zero degree scope so rotation of the endoscope's tip is required to see the perspective of an angled endoscope.

Conclusions: Virtual endoscopy of the sinuses can be of predictive value for planning endoscopic sinus surgery, provided that the virtual endoscope is properly calibrated.
Can surgical delay augment a pedicled flap in an irradiated field?

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Introduction: The dimensions of pedicled flaps can be increased by "surgical delay". Delayed pedicled flaps provide an alternative to free flap reconstruction, particularly in head and neck cancer patients. These patients may require radiotherapy as part of their cancer treatment. Irradiated tissues are notoriously difficult to reconstruct when chronic radiation damages have become established. However, there may be a window of opportunity after radiotherapy when regional reconstructive options, aided by surgical delay, may be used successfully.

Methods: New Zealand white rabbits are used as they have consistent and symmetrical vascular territories. X-ray superficial radiotherapy is used. The tolerance dosage for the rabbit skin is found to be between 20 and 22 Gy by a preliminary study. Standardised radiotherapy of 22 Gy was administered to two "choke vessel" zones in each animal. Each animal had a 3-territory L-shaped skin flap raised with a preliminary surgical delay. Experiment Group A (n=11) - the delayed 3-territory flaps were raised 4 weeks after radiotherapy. Experiment Group B (n=11) - identical flaps were raised 4 months after radiotherapy. Control Group - animals had identical flaps raised without preceding radiotherapy. All animals were assessed clinically, by intravenous fluorescein studies and by post-mortem lead oxide angiographic studies.

Results: Radiotherapy side-effects were variable despite identical dosage and administration. Experimental Group A - all the flaps survived without necrosis. The fluorescein studies showed good vascularity of the third vascular territory and lead oxide studies showed dilated choke vessels in both choke vessel zones. Experimental Group B - variable areas of the third territory became ischaemic and necrotic (10-100%). Fluorescein studies confirmed lack of perfusion to the ischaemic areas. Lead oxide studies showed dilated choke vessels in zone 1 and variable defined choke vessels in zone 2. Control animals had complete flap survival and dilated choke vessels in both zones.

Conclusions: After radiotherapy, there is a window of opportunity during which surgical delay can augment the dimensions of a pedicled flap in the irradiated field. This period may last up until 4 months post radiotherapy. This knowledge may benefit patients in whom pedicled flaps represent better reconstructive options, than free flaps, after ablative surgery. Referees: Mr Mark Ashton, The Royal Melbourne Hospital; Prof G Ian Taylor, The Royal Melbourne Hospital.
Mechanisms by which CD44v2-10, lacking the v6 domain, exerts a dominant negative effect on the malignant phenotype

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Introduction: The CD44v6 domain is considered critical to the metastatic phenotype in human solid organ tumours, and is thought to act via cooperation between CD44v6, hepatocyte growth factor (HGF), c-met and the cytoplasmic protein, ezrin. We have shown that CD44v2-10 mediated metastasis is dependent on v6, and expression of CD44v2-10 lacking v6 (CD44v2-10delv6) reduces cell proliferation and tumourigenesis in SKHep1 cells. The aim of this study was to determine the mechanisms for these phenomena.

Methods: 1. To determine CD44v6-c-met complexing, we immunoprecipitated met from SKHep1 cells and probed with CD44 monoclonal antibody in Western blots. Cells were stimulated with exogenous HGF, and tyrosine phosphorylation was measured.

2. Gene array analysis was used to compare differential gene expression between SKHep1, SKHep1/CD44v2-10 and SKHep1/CD44v2-10delv6 cells. Expression of promising genes was quantitated using real-time RT-PCR.

Results: HGF stimulation resulted in CD44-met complexes, but none contained CD44v6. HGF stimulation did not increase tyrosine phosphorylation, suggesting maximal c-met activity exists in SKHep1 cells. This was confirmed by RT-PCR and DNA sequence analysis of Tpr-met, an oncologic transformation in SKHep1. Gene arrays/ real-time PCR confirmed down-regulation of the heparin binding growth factors VEGF, PDGF and HB-EGF. SKHep1/CD44v2-10delv6 conditioned media assays showed reduced HUVEC proliferation in vitro. Importantly, SKHep1/CD44v2-10delv6 demonstrated 1000-5000 fold up-regulation of the recently described metastasis suppressor KISS-1.

Conclusions: These results show that expression of CD44v2-10 lacking v6 in SKHep1 likely exerts its repression of the malignant phenotype not by interfering with CD44v6/c-met cooperation, but by reduced growth factor expression and up-regulation of KISS-1.


**ErbB2 in Gliomas**

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**Introduction:** Gliomas represent the most common malignant primary brain tumour and the high grade glioblastomas still have a median survival of only 12 months following surgery. The most common genetic abnormality in glioblastomas is amplification of the Epidermal Growth Factor Receptor (EGFR), a member of the ErbB family of receptor tyrosine kinases. A frequent mutant form of EGFR, missing part of the extracellular domain, increases tumour growth through increased activity and upregulation of signalling pathways. We have examined the role of the second member of the ErbB family, ErbB2 (HER2/neu) in gliomas. Monoclonal antibody therapy (Herceptin) against ErbB2 is in use for breast cancer.

**Methods:** We have screened a large series of human glioma specimens using a variety of molecular biology techniques for expression of ErbB2 and presence of potential oncogenic mutations.

**Results:** ErbB2 was overexpressed in 63% of high grade gliomas and 30% of lower grade tumours. A mutant form designated ΔErbB2, which has a deletion of 187 amino acids in its extracellular domain, was identified in transcripts from a glioblastoma. Although it retained in the endoplasmic reticulum, the mutant appears to be constitutively active, can interact with signalling components and enhances tumour growth both in vitro and in vivo.

**Conclusions:** ErbB2 is overexpressed in a significant proportion of high grade gliomas and a novel oncogenic mutation is identified, which enhances tumour cell growth through ligand-independent activation. This represents another example in a growing body of evidence that receptor tyrosine kinases can signal from the intracellular compartment as well as at the cell surface. ErbB2 may prove to be a potential target for molecular therapies in glioma.
The effect of glutamine supply on rates of apoptosis and glutathione concentration of prostate cancer cells

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Introduction: Glutamine is a substrate for the synthesis of glutathione, a tripeptide, that protects cells from damage caused by oxidative stress. The linkage between glutamine and glutathione suggests disruption of glutamine metabolism might enhance anti-tumour therapies that kill cells via oxidative stress. The aim of this experiment was to examine the effect of glutamine supply on rate of apoptosis and glutamine metabolism of prostate cancer cells.

Methods: DU-145, LNCaP and MaTLyLu prostate cancer cells were grown in media containing either 0.04, 0.08, 0.15 or 2.00 mM glutamine for 48 hours before assessment of: cell numbers; rate of apoptosis; glutathione content; and activity of glutaminase and glutamine synthetase. Analysis of variance was used to examine the effect of glutamine supply on cellular growth and glutamine metabolism.

Results: There was a significant positive association between glutamine concentration and (1) cell numbers and (2) glutathione concentration for DU 145 (p1=0.00; P2=0.001) and MAT-LyLu (p1=0.02; P2=0.005) cells but not LNCaP cells (p1=0.92; p2=0.48). Glutamine deprivation induced an increase in numbers of apoptotic/necrotic cells for DU 145 (p=0.004) and MAT-LyLu (p=0.02) cells but not LNCaP (p=0.27) cells. The activity of glutaminase was increased by low glutamine concentrations in DU 145 cells and glutamine deprivation induced an increase in glutamine synthetase activity in all cell lines.

Conclusions: Glutamine deprivation kills some, but not all, prostate cancer cell lines. The resistant cells are able to maintain cellular glutathione concentrations during glutamine deprivation. These data suggest that targeted disruption of glutamine metabolism may have a variable effect on prostate cancers in vivo.
The effect of Indole-3-carbinol and Sulforaphane on proliferation of a colon and prostate cancer cell lines

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Introduction: The consumption of cruciferous vegetables has a protective effect on the development of colorectal and prostate cancer. The phytochemicals Indole-3-carbinol and Sulforaphane are found almost exclusively in cruciferous vegetables. We have studied the effect of Indole-3-carbinol and Sulforaphane on cell proliferation of both an HT-29 colon cancer cell line and a PC-3 prostate cancer cell line.

Methods: Colon or prostate cancer cells were cultured in 96 well tissue culture plates. Indole-3-carbinol (in concentrations ranging from 0.1 - 0.7mmol) and Sulforaphane (in concentrations ranging from 0.01 - 0.1mmol) were added to the wells. Cell proliferation was measured using the colorimetric assay technique utilizing the 2% WST-1 cell proliferation kit.

Results: Significant inhibition of cell proliferation was observed in both HT29 colon cancer cells and PC3 prostate cancer cells exposed to Indole-3-carbinol at doses greater than 0.1mmol and Sulforaphane at doses greater than 0.2mmol respectively.

Conclusions: Both compounds inhibited the proliferation of colon and prostate cancer cells in a dose-dependent manner. These findings may help explain the observed protective effect of cruciferous vegetables in both colon and prostate cancer.
Integrin-kinase signalling in cancer

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Introduction: We have previously reported that the αvβ6 integrin induces its own expression in an autocrine manner with cell crowding and proposed a self-perpetuating model of colon cancer progression regulated through αvβ6-mediated matrix metalloproteinase (MMP-9) secretion\(^1\). In addition, it is now well accepted that mitogen-activated protein (MAP) kinases play an important role in cancer growth and we have identified a direct link between the β6 integrin subunit and a member of the MAP kinase family, extracellular signal-regulated kinase (ERK2)\(^2\). The aim of the present study was to examine the effect of β6 lacking the ERK binding domain on tumour cell growth, integrin expression, and MMP-9 secretion.

Methods: SW480 colon cancer cells that lack constitutive αvβ6 expression were stably transfected with the β6 gene construct lacking the ERK2 binding domain. β6 expression on cancer cells was determined by FACScan and Western Blotting under different cell density conditions. MMP-9 secretion was determined by means of gelatin zymography and in vivo studies performed using SW480 xenografts established in athymic mice.

Results: Loss of the ERK2 binding domain on the β6 integrin subunit abolished β6-mediated MMP-9 secretion, β6 upregulation with cell confluence, and β6-mediated tumour growth in vivo compared with cancer cells expressing wild-type β6.

Conclusions: The ERK2 binding domain on β6 is necessary for colon cancer progression. Targetting the β6-ERK2 interaction may prove useful as an anticancer strategy in colon cancer.

**Evaluation of novel genes identified by transcript profiling utilizing microarray analysis in the development and progression of pancreatic cancer**

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**Introduction:** Pancreatic cancer (PC) remains the fifth highest cause of Western cancer mortality, highlighting crucial needs for better diagnostic and therapeutic strategies to improve survival. Assessment of mRNA expression of over 44K genes with microarray technology demonstrated upregulation of secreted frizzled related protein 4 (sFRP4) and beta-catenin in PC compared to normal pancreata (NP). Their pathway - Wnt signalling is integral to transcriptional regulation and aberrations in these molecules are critical in the development of many human malignancies.

**Methods:** Total RNA was extracted from frozen tissue (11 PC and 6 NP) followed by synthesis of double stranded cDNA by reverse transcriptase PCR. *In vitro* transcription generated cRNA for hybridisation to Affymetrix Genechip® HG-U133A and B oligonucleotide microarrays. Immunohistochemistry using polyclonal sFRP4 and monoclonal beta-catenin antibodies was evaluated by two independent examiners for differences in expression associated with survival patterns in 94 patients with resected PC.

**Results:** Array data analysis identified aberrant expression of Wnt pathway members, including sFRP4 and beta-catenin, that were upregulated in all PC compared to NP samples. Immunohistochemistry demonstrated increased sFRP4 membranous expression (>20%) in 32/94 PC specimens and this was correlated with improved survival (p=0.03). Decreased membranous staining of beta-catenin was demonstrated in PC compared to NP (p=0.011). Increased disease specific survival was associated with beta-catenin cytoplasmic expression (p=0.02), whilst increased membranous beta-catenin trended towards improved survival (p=0.09) in 48/102 PC specimens.

**Conclusions:** A central tenet of contemporary cancer research is that an understanding of the genetic and molecular abnormalities that accompany the development and progression of cancer is critical to further advances in diagnosis, treatment and eventual prevention. This study has identified sFRP4 and beta-catenin as potential novel prognostic markers.
Methylation of MT-3 promoter in oesophageal squamous cell carcinoma.

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**Introduction:** The metallothioneins (MT) are a group of small, cysteine-rich proteins that bind heavy metals such as zinc and copper. They have been proposed to play a role in metal detoxification, metal homeostasis and to protect against oxygen radical induced DNA damage. Methylation of the promoter regions of genes can lead to gene silencing by preventing initiation of transcription. We investigated the prevalence of MT-3 DNA methylation in oesophageal squamous cell carcinoma (ESCC).

**Methods:** MT-3 expression and promoter methylation were determined in the oesophageal carcinoma cell lines, OE-19, OE-33 and TE-7, before and after treatment with the demethylating drug 5-aza-2'-deoxycytidine, by RT-PCR and combined bisulphite restriction analysis respectively. Methylation status of MT-3 was measured in DNA extracted from formalin-fixed paraffin-embedded primary tumour, metastatic lymph nodes and margins from 14 patients with ESCC.

**Results:** MT-3 mRNA expression was detected in OE-19 and TE-7, but not in OE-33 which had a methylated MT-3 promoter. Treatment of OE-33 with 5-aza-2'-deoxycytidine demethylated the MT-3 promoter, and was associated with re-expression of MT-3. MT-3 methylation was present in 3/14 primary ESCC tumours and 6/14 metastatic lymph nodes. MT-3 methylation was not detected in the histologically normal squamous epithelium, but was detected in hyperplastic epithelium.

**Conclusions:** Methylation of the promoter region of MT-3 correlated with transcriptional silencing of MT-3 expression in cell lines. Methylation of the MT-3 promoter was detected in ESCC primary tumours and metastatic lymph nodes.
Proteomic Analysis in the investigation of a Deep Dermal Burn Injury in the Ovine Fetus

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Introduction: It has been demonstrated previously that the ovine fetus heals a deep dermal burn injury (DDBI) in a scar-less fashion, and a lamb heals the same injury with scarring. In an attempt to identify the factors responsible for scar-less wound healing, we extracted both protein and total RNA from control and experimental animals, to determine unique proteins and gene activation responsible for scar-less wound healing.

Method: Twenty one Merino fetal lambs (80 day gestation) and twenty one Merino lambs (30 days) each received a standard DDBI, as previously described by our group. In groups of three, animals were serially euthanased at day 1,3,5,7,14,21,60. At post mortem, sections of burned and control tissue was taken from both lamb and fetus. Tissue samples were prepared to extract both protein and RNA. Protein is first separated by charge along a pH gradient, followed by separation by molecular mass in a second dimension. The gels are then stained with a silver stain, and the resulting pattern is mapped. Unique "spots" are then identified by use of mass spectrometry.

Results: There are consistent and reproducible differences between fetal and lamb protein gels at Day 1 and Day 14 post injury.

Conclusion: This method has provided unique information in the differences between fetal and post natal wound healing response. We aim to determine protein unique to the fetal wound healing process, with a view to developing these for treatment in patients with burns.
**The potential use of Seldi-TOF MS for the early diagnosis of pancreatic cancer**

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**Introduction:** Pancreatic cancer is one of the most aggressive and rapidly growing cancers. Early detection is paramount for improving the chance of survival, as only around 70% of patients that present with clinical symptoms of pancreatic cancer are resectable. Novel ProteinChip® technology has allowed for high throughput screening of protein expression within tissue in such a way that rapid comparisons can be made between cancer tissue and normal samples.

**Methods:** Invasive pancreatic cancer tissue (n=10), with adjacent normal tissue, was collected immediately after pancreatic resection and stored at -80°C. The tissue was lysed, homogenised and placed on either an hydrophobic (H50), immobilized metal affinity capture (IMAC30-Cu) or a strong anion exchange (SAX2) ProteinChip® array. The ProteinChip® was analysed using a surface-enhanced laser desorption/ionisation time of flight mass spectrometer (SELDI-TOF MS). Pancreatic cancer and normal tissue protein expression profiles were generated and compared. Significance was accepted at p≤0.05.

**Results:** When compared with normal tissue, the tumour samples showed significantly increased expression of specific proteins. Both the H50 and the SAX2 ProteinChip® array revealed two differentially expressed protein peaks at around 9830Da and 10040Da (p=0.02 and p=0.03; p=0.05 and p=0.04 respectively), while the IMAC30-Cu ProteinChip® array displayed a more highly expressed protein at around 10774Da (p=0.04).

**Conclusions:** This study has demonstrated the potential role that SELDI-TOF MS may play in detecting specific malignant protein profiles from pancreatic cancer samples. Although further validation is required, these protein profiles may provide the means for early detection.
Maternal and postnatal vitamin D ingestion influences aortic elastogenesis in rats.

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Introduction: Subtle abnormalities of fetal nutrition appears to predict hypertension and atherosclerosis in adults. It has been hypothesised that impaired aortic elastogenesis is the initiating step in adult hypertension and abdominal aortic aneurysms. Vitamin D has been shown to inhibit elastin synthesis by cultured smooth muscle cells. Here we have investigated the hypothesis that increased exposure to vitamin D during gestation and in the postnatal period alters aortic elastin content.

Methods: Nine breeding pairs of Sprague-Dawley rats were allocated to one of three diets containing 3,000 (control group), 6,000 (low dose) or 12,000 (high dose) IU/kg vitamin D during pregnancy and lactation. Male pups were continued on the same diet until six weeks of age. Aortic elastin content was assessed by measuring desmosine + isodesmosine content using capillary zone electrophoresis. Transverse aortic sections were used for quantification of elastic lamellae and morphometric analysis.

Results: The desmosine + isodesmosine content of the abdominal aorta of 6 week old male pups, was 14.1, 10.0 and 10.1 nmoles/mg dry wt in the control (n=20), low (n=23) and high-dose (n=15) groups respectively (p=0.007). The median number of aortic elastic lamellae was 8.25, 7.13 and 6.88 in the control, low dose and high dose groups respectively (p<0.001). There were no significant differences in aortic cross-sectional areas.

Conclusions: In rats, exposure to modest amounts of vitamin D during gestation and early life results in a reduction of elastin content and number of elastic lamellae in the aorta. A similar effect in man might be of relevance to the pathogenesis of abdominal aortic aneurysms.

Acknowledgements: This research was funded by a Grant from the Royal Australasian College of Surgeons.
Comparative study of the management of acute pancreatitis

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Introduction: Guidelines have been published regarding the management of acute pancreatitis [1]. The aim of this paper is to compare the management of patients with acute pancreatitis in a tertiary referral medical centre and a regional health centre during 2001 and evaluate compliance with these published guidelines.

Methods: Patients with a primary diagnosis of acute pancreatitis were identified retrospectively. Eighty-four admissions from Austin and Repatriation Medical Centre (ARMC), a tertiary referral centre, and 83 from The Geelong Hospital (TGH), a regional health centre were treated in these two hospitals. Their histories were collected and evaluated in accordance to the recommendations by the British Gastroenterology Society (BSC) [1].

Results: Only 38% of patients from these two centres had all the investigations performed on them for the severity stratification recommended by BSC. Otherwise, ARMC and TGH had managed these patients with acute pancreatitis according to the recommendations. The overall mortality rate from acute pancreatitis was 3.0%, and within the group of severe acute pancreatitis the mortality rate was approximately 23%. Two-thirds of patients from ARMC with gallstone related acute pancreatitis had a cholecystectomy or sphincterotomy and extraction of gallstones within four weeks after presentation. There were five readmissions to ARMC in year 2001 due to non-operated gallstone-related acute pancreatitis. In contrast, 84.3% of patients from TGH had definitive treatment within 4 weeks and there were 3 re-admissions to TGH.

Conclusions: A tertiary referral centre and a smaller regional hospital in Australia both comply with recently published guidelines with regard to management of acute pancreatitis. With regard to management the guidelines emphasise the importance of expertise in hepatopancreatobiliary surgery, availability of ICU/HDU and dynamic CT scanning. The recommendations for definitive treatment on patients with gallstone-related pancreatitis within four weeks after presentation reduced the morbidity and mortality in this group of patients. However compliance with guidelines on severity stratification of acute pancreatitis was poor, but this lack of formal severity assessment did not appear to influence the outcome.

Radiosurgery in acoustic neuroma management: Is this the best option currently?

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Introduction: Two major treatment options are available for patients with acoustic neuroma: microsurgery and radiosurgery. Debate continues as to whether stereotactic radiosurgery results in lowered cranial nerve morbidities, better hearing preservation and good quality of life without the complications of microneurosurgery. Our objective is to compare the outcomes of gamma knife radiosurgery and microsurgery by reviewing the recent literature to observe if there is a significant difference between these interventions.

Methods: A MEDLINE literature search was conducted from Jan 1990-June 2002 using 'acoustic neuroma', 'vestibular schwannoma' as primary keywords. Combinations with, 'microsurgery' and 'neurosurgery' yielded 117 and 39 papers respectively. Combinations with 'stereotactic radiosurgery' and 'gamma knife surgery' found 282 papers. Excluding duplicate publications, 26 papers in microsurgery and 18 papers in Gamma Knife radiosurgery were examined. Outcome data were extracted for tumour control, hearing preservation, facial nerve function, certain other complications, and quality of life.

Analysis: No papers were found which directly compared outcomes for microsurgery vs. radiosurgery. In particular no randomized control trials were found. Since a classical meta-analysis is not possible in this situation our analysis compares rates for particular outcomes between studies of the same intervention, and includes tests for heterogeneity between studies using a random effects analysis. Comparisons of different interventions can only be made where the patient groups are judged sufficiently similar, although the interpretation of these comparisons must be made with extreme caution.

Results and Summary: This review will be completed in August 2003. The most recent review comparing both modes of therapy, published in November 2000, suggested that surgery remain the therapy of choice, since long term results from gamma knife radiosurgery using low dosimetry were not reported by that time. Our aim is to update the recent results of gamma knife radiosurgery and compare with microsurgery results to see if radiosurgery (in particular with low doses) is superior in acoustic neuroma management.
Progressive necrosis following focal hyperthermia treatment of colorectal liver metastases

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Background: The exact mechanism of focal hyperthermia induced tissue necrosis is undetermined. There is some evidence to suggest that tissue injury progresses after cessation of the initial heat stimulus. The aim of this study is to investigate the time sequence and mechanisms of focal hyperthermia induced necrosis in the treatment of colorectal cancer liver metastases.

Methods: Focal hyperthermia produced by a Nd-YAG laser source was applied to the normal liver and colorectal cancer liver metastases in CBA mice. The volume of direct lethal thermal injury was assessed histochemically by a vital stain for nicotinamide adenine dinucleotide (NADH) diaphorase immediately after laser application. The time sequence of tissue necrosis was determined by routine histological examination of injury in different groups of animals on subsequent days. This was compared to the extent of immediate lethal thermal injury.

Results: Thermal injury immediately following the application of 100 Joules of energy was greater in tumour tissue than normal liver (mean (s.d.), measuring 16.3(9.9) mm$^3$ and 23.9(9.2) mm$^3$ respectively (P = 0.042). There was however a significantly greater increase in the volume of necrosis in normal liver than tumour tissue after the initial injury. In normal liver, the peak volume of necrosis was 147.1(42.1) mm$^3$ and occurred at 3.2 days, whilst in tumour tissue the peak was 49.0(12.3) mm$^3$ at 4.5 days (P < 0.001).

Conclusion: Focal hyperthermia produces tissue necrosis that extends considerably beyond that caused by its immediate lethal thermal effects. Normal liver and tumour tissue respond differently to focal hyperthermia. In tumour tissue the direct lethal heat injury is more pronounced, whilst the progression of injury is more rapid and far greater in normal liver.
**Slow transit constipation (STC): Is resection necessary? Lessons from paediatric surgery.**

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**Introduction:** STC affects both adults and children. Despite numerous new treatment modalities, colectomy remains a common end-point for adult STC. In comparison, colectomy is rarely performed for paediatric STC. We have examined new modalities of investigation and treatment for paediatric STC.

**Methods:**
1. Laparoscopic seromuscular colonic biopsies have been taken for fluorescence immunohistochemistry. Staining for established excitatory and inhibitory neurotransmitters has been performed.

2. Colonic manometry, via an appendiceal stoma, has been performed on six children with STC. Studies were carried out for a 24-hour period to establish responses to waking and feeding.

3. Transcutaneous electrical therapy has been performed on nine children with STC. Twelve sessions over four weeks were complemented by a detailed stool diary before, during and after the period of treatment.

**Results:**
1. Over 180 children have been biopsied. A significant proportion of the children has demonstrated abnormalities upon immunofluorescence imaging.

2. Children with STC exhibited significant abnormalities in pre- and postprandial colonic motor function. Many of the changes were similar to those seen in adult STC.

3. Eight of the nine children had substantially improved symptoms following the twelve sessions. Improvements were sustained in four of five children followed at six months.

**Conclusions:** Despite differences in gender distribution and aetiologies, there are similarities between adult and paediatric STC. Advances in the investigation and treatment of paediatric STC may be applicable to adult colorectal practice. The lower rate of colectomy observed in the paediatric population may be possible in the adult population.
**Epidemiology of Major Trauma in the "Top End": a retrospective study of 132 cases between 2000-2001**

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**Introduction:** Major trauma is known to be one of the most important contributors to hospital bed stays, deaths and patient morbidity. This study aimed to describe the demographics, nature, severity and outcomes of major trauma in the "Top End": to consider patterns of injury and correlations between aetiology and outcome; and to compare the findings with national and international studies of major trauma.

**Methods:** We designed a cross-sectional retrospective analysis of all cases of major trauma referred to the Royal Darwin Hospital over a 24 month period. Information regarding age, gender, ethnicity, occupation, type of injury, mechanism, body part, severity, involvement of alcohol, location, time till arrival, length of stay (including ICU) and outcome was considered. 350 case notes were reviewed, patients with an injury severity score (ISS) > 15 were included. The total number of patients admitted to the study was 132.

**Results:** The incidence of major trauma in the Top End was 46.6 per 100,000 for the total population and 67.4 per 100,000 for the Aboriginal population. The average age was 34.8 and 72% were males. The average ISS was 28.6. Blunt trauma compromised 94.5% of all injuries, penetrating trauma being relatively rare. The predominant mechanism of injury was motor-vehicle accidents (MVA's), accounting for 61.4% of injuries. Assaults compromised 15% of all cases of major trauma. All victims of motorcycle accidents that resulted in major trauma were Caucasian males. 73% of Aboriginal females who were involved in MVA's were pedestrians struck by motor vehicles. 31% of those involved in MVA's were unrestrained, 41% were known to have been under the influence of alcohol at the time of injury. On average, the delay between injury and arrival at RDH was 6.3 hours. The average length of hospital stay till discharge or death was 27 days. 53% had a period of stay in the ICU, the average length of time being 6.72 days. Hospital mortality was 30.1%.

**Conclusions:** The study revealed that incidence of major trauma in the Top End is considerably in excess of that experienced elsewhere in Australia and Europe. The major trauma admissions were characterised by young males, with a high incidence of major head injury and with poor outcomes, MVA's being the predominant mechanism of injury. There was an over-representation of indigenous victims. Major trauma in the Top End has a unique complication that affects immediate survival and clinical presentation in that the time between injury and arrival at the RDH was so prolonged.
Transplanted porcine islet cell clusters survive and function in inbred Westran pigs

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Aim: As a means to treat diabetes, transplantation of neonatal porcine islet cell clusters (NICC's) have yet to be shown to survive and provide function long-term without immunosuppression. We evaluated the rejection process of NICC's from Westran and outbred pigs transplanted into Westran pigs and determined if this tissue could survive and provide function long-term.

Method: Pancreata were removed from neonates at 1-3 days of age, dissociated, enzymatically digested, washed and plated out in HAMs F10 then incubated at 37°C in 5% CO² for 6 days. Media changes were performed second daily. On the day of transplantation tissue was separated from the media, counted, viability assessed and a biopsy taken. NICC's were transplanted beneath the capsule of the spleen and kidney of Westran pigs. Wedge biopsies were taken from each of the transplant sites on days 3, 7, 10, 14, 21, 28, 60 & 120 and were processed for histology; selected stains included insulin, somatostatin, chromagranin, keratin, CD3, CD4, CD8 & CD79a.

Results: A total of 50 donors were used to transplant 17 Westran pigs. Westran pigs with Westran grafts macroscopically demonstrated an increase in transplanted NICC tissue volume. Histological analysis of early biopsy time points of Westran pigs with Westran NICC graft tissue stained positive for somatostatin and chromagranin indicating the presence of active endocrine cells within them. By days 60 and 120 NICC tissue stained positive for insulin, somatostatin and chromagranin and some discrete foci of endocrine nests had formed. Histological analysis of the grafts in 4 pigs with third party grafts showed a distinct pattern of rejection and graft loss within 14-21 days.

Conclusions: Westran NICC's transplanted into Westran pigs are accepted whilst outbred pig NICC's were rejected. These results suggest we have a model suitable for studying the efficacy of NICC's as a method for correcting diabetes without the influence of toxic immunosuppression.
The natural history of chronic allograft nephropathy after simultaneous pancreas and kidney transplantation (SPK)


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**Purpose:** Initial justification for pancreas transplantation in patients with end-stage diabetic nephropathy included the potential benefit of preventing recurrent primary disease after renal transplantation. Subsequently, with improvements in immunosuppression and early allograft survival, chronic allograft nephropathy (CAN) has proven to be the dominant cause of kidney transplant failure after SPK and not recurrent disease. Its aetiology is uncertain.

**Methods:** The natural history CAN was evaluated longitudinally from 868 prospective protocol kidney transplant biopsies from 120 patients after SPK taken regularly until 10 years after transplantation and scored by the Banff schema.

**Results:** CAN comprised two distinctive phases of injury. Early tubulointerstitial damage resulted from ischemic injury (P<0.05), prior severe rejection (P<0.01) and subclinical rejection (SCR, P<0.001) leading to grade I CAN in 94.3% by 1 year. SCR was common early (42.0% at 3 months), exacerbated by prior severe rejection and reduced by tacrolimus and mycophenolate mofetil therapy (both P<0.05), but gradually abated after 1 year. Chronic rejection, implying continuous immunological injury and defined as persistent SCR≥2 yrs duration, was uncommon (5.8% of patients). Both chronic rejection and SCR exacerbated CAN and tubulointerstitial damage (P<0.05-0.01). Beyond one year, the pattern altered to microvascular and glomerular injury. Progressive high-grade arteriolar hyalinosis, luminal narrowing, increasing glomerulosclerosis and further tubulointerstitial damage accompanied late calcineurin inhibitor nephrotoxicity. Nephrotoxicity was primarily implicated in late ongoing injury becoming almost universal at 10 years (92.4%), even in grafts with essentially normal 1-year histology. By 10 years grade II CAN occurred in 84.7% and 35.4±14.7% glomeruli were sclerosed. Tubulointerstitial and glomerular damage were irreversible once established and resulted in functional decline and late graft failure.

**Conclusion:** CAN represents the common end-pathway of cumulative and incremental nephron damage from time-dependent immunological and nonimmunological causes. Current long term calcineurin based immunosuppression protocols are not beneficial to long term kidney allograft function after SPK.
Autologous tumour/autologous dendritic cell vaccine trial therapy – results and ongoing programmes.

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Introduction: We recently published\(^1\) the results of a Phase I trial on 19 patients. Of 12 patients who finished the trial, 3 have complete ongoing remissions. A subsequent series of 19 patients completing an initial course of treatment had 4 complete responses.

Methods: Overall 31 patients, 7 had complete responses, 6 of which were durable. Low plasma S-100B levels prior to treatment were predictive of objective clinical response. Further trials are being planned. (1) A Phase I/II trial comparing surgical or radiotherapeutic debulking with vaccine comparing to patients with lower bulk disease only. (2) A Phase II trial of Stage IV patients with low serum 100B levels prior to therapy, compared with those of more elevated levels. (3) A Phase III double blind trial of Stage III AJCC patients involving 200 patients is currently underway.

We have also commenced a Phase I trial using autologous glioma cells with autologous dendritic cells post surgery in adult and paediatric patients. The atrocious results of treatment of this aggressive tumour and the common neuroectodermal origin of melanoma and glial cells has stimulated our hopes and interest in this group.

Nine advanced prostate cancer patients have been randomized to intradermal (ID) or intravenous (IV) injections of dendritic cell (DC)-based vaccine using prostate specific membrane (PSM) peptides as the antigen. Of 7 men followed for 6 months, 0/4 given vaccine ID compared with 2/3 given vaccine IV showed evidence of disease progression. In a second study, we are emulating the above melanoma phase I trial using autologous tumour as the source of antigen in men the hormone refractory prostate cancer.

Profile of oxidative injury in experimental colitis: Model of necrotizing or ulcerative colitis?

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Introduction: The type of injury produced by oxidative stress in colitis is unclear yet reactive oxygen species are upheld to be central to the process of ulcerative colitis through formation of peroxy-nitrite from nitric oxide. The aim was to establish the type of colitis produced by hydrogen peroxide and nitric oxide, derived from S-nitrosoglutathione, in vivo and in vitro.

Methods: Enemata (2.0 mL) of hydrogen peroxide (1.0%, 1.4%, 1.7%, 3.0%) with or without 2 mM S-nitrosoglutathione were administered to Sprague Dawley rats. Cell respiration (CO₂/aceotacetate) was measured in isolated rat colonocytes with [1-14C] butyrate and exposed to hydrogen peroxide (0.25-2.0%) with and without S-nitrosoglutathione (2.0 mM).

Results: Four days after hydrogen peroxide administration 1% H₂O₂ produced no histological mucosal changes. At 1.4 and 1.7% H₂O₂ 6/12 animals revealed acute transmucosal necrosis and at 3% H₂O₂ all animals revealed transmucosal necrosis. Without mucosal necrosis subacute mucosal changes were not seen and S-nitrosoglutathione (a nitric oxide donor) did not increase the damage. Isolated colonocytes revealed 19% reduction of 14CO₂ and 23% reduction of acetoacetate with 2.0% hydrogen peroxide. Respiration was diminished by S-nitrosoglutathione but colonocytes retained viability.

Conclusions: Hydrogen peroxide in vivo produced transmucosal necrotizing colitis. In vitro gradual but not lethal inhibition of respiration occurred with peroxide/nitric oxide. Reactive oxygen species in the colon produce necrotizing colitis rather than a profile of ulcerative colitis for which other injurious mechanisms need to be sought.
**Distal colonic flushing with butyrate and saline reduces aberrant crypt formation**

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**Introduction:** Dietary fibre is postulated to be protective against colorectal neoplasia, but data on this topic are contradictory. Varying physicochemical characteristics of different fibres may explain these findings. The aim of this study was to assess the direct luminal effects of butyrate (a fermentation product of fibre) and saline on carcinogenic events in the distal colon.

**Methods:** Male Sprague Dawley rats were operated upon to place a polyethylene tube into the start of the distal colon. Rats were maintained on a fibre-free diet in order to minimize endogenous butyrate production, and administered Azoxymethane (15mg/Kg) as a carcinogen. Following recovery, rats were randomised to receive no infusions, normal saline or 80mM butyrate at two separate dose levels (0.5ml BD or 1ml x 5/ d). Rats were sacrificed after 4 weeks of infusions, and colonic aberrant crypt foci per cm² quantified by dissection microscopy. Results were analysed by ANOVA, with subgroup analysis by unpaired t-tests.

**Results:** There were no differences across the groups with respect to well-being or weight gain. Compared with the non-infused control group, saline reduced ACF formation to a similar degree for low and high doses (45% & 38% respectively; p<0.01, t-test). In contrast, butyrate at the lower dose had no additional effect over saline alone, but, at the higher dose reduced ACF to a greater extent (64%, p<0.05).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>ACF per cm²</th>
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</thead>
<tbody>
<tr>
<td>Control</td>
<td>9.0 ± 1.4</td>
</tr>
<tr>
<td>Saline</td>
<td>5.0 ± 0.7</td>
</tr>
<tr>
<td>Butyrate</td>
<td>5.4 ± 0.8</td>
</tr>
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**Conclusions:** Delivery of butyrate to the distal colonic lumen suppresses aberrant crypt formation in a dose-dependant manner, whereas luminal flushing does so without an apparent dose effect. Thus, there is evidence for at least two mechanisms whereby dietary fibre may suppress the early tumorigenic events in the distal colon: exposure of the epithelium to fermentation products, and alterations to the physical luminal contents.