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Infant bedsharing vs solitary sleeping: behavioural and physiological aspects. S A Baddock, A A Phillips, B C Galland, B J Taylor, D P G Bolton, C A Makowharemahihi. Department of Women's & Children's Health, Dunedin School of Medicine, University of Otago, Dunedin.

Bedsharing between infants and mothers who smoke has been identified in many studies as increasing the risk of Sudden Infant Death Syndrome. We aimed to monitor infants (bedsharing or cot sleeping) in the natural setting of their own home to identify physiological and behavioural indices that might stress a vulnerable infant.

Overnight video and physiological recordings of 40 bedshare infants, aged 5–27 weeks, were compared with 40 age-matched cot infants in their own homes. Video data provided a log of infant/parent sleep positions, movements and interactions. The physiological recordings measured respiratory pattern, respiratory airflow, inspired CO₂, oxygen saturation (SaO₂), heart rate, and core, peripheral and environmental temperatures. The results presented here are the data analysed for infant exposure to CO₂ >3% and consequent physiological responses.

Sleep practices There was no significant difference between the number of bedsharing (BS) or cot infants sleeping prone although BS infants spent less hours/night prone (BS range: 1.6–3.5 h, cot range: 8.9–10.2 h). BS babies spent more time with their faces covered: BS (median: 1.3 h, interquartile range: 0–2.6 h, max: 8 h) vs cot (0, 0–0, 6.4) and 90% of BS babies spent some time (1.7, 0.4–4.5, 7.9) exposed to their mother's expired breath. Maternal checks were more frequent in the BS group (10 per night, 7–23, 55) than the cot group (4 per night, 3–6, 16). These behaviours were often associated with increased CO₂ at the infant's face. BS infants (18) were exposed to >3% inspired CO₂ (range: 1–60 min) and 1 swaddled, cot-sleeping infant was exposed to >3% for 63 minutes. These levels of CO₂ significantly (p<0.05) elevated breathing rate with the result that normal SaO₂ was maintained.

Thus, potentially dangerous sleep behaviours, such as head covering, occurred in both situations, but more commonly in bedsharing. This often resulted in exposure to levels of CO₂ that stimulated increased respiration. All infants maintained normal oxygen saturation through the night – whether vulnerable babies would behave similarly is unknown.

The iron-chelating compound pyoverdine as a signaling molecule: regulation of pyoverdine synthesis in *Pseudomonas aeruginosa*. P A Beare, I L Lamont. Department of Biochemistry, Otago School of Medical Sciences, University of Otago, Dunedin.

Synthesis of the siderophore pyoverdine by the human pathogenic bacterium *Pseudomonas aeruginosa* requires the presence of a protein, PvdS, that directs expression of a set of genes required for pyoverdine production. PvdS is a member of a family of sigma factor proteins whose activities are regulated by anti-sigma factor proteins. The aim of this study was to examine the regulation involved in the synthesis of the iron-chelating compound, pyoverdine.

The DNA sequence of *P. aeruginosa* was analysed for genes likely to be involved in regulating pyoverdine synthesis. Candidate genes were mutated or overexpressed in *P. aeruginosa* and the effect on pyoverdine synthesis was measured. Pyoverdine production was analysed by measuring the absorbance of pyoverdine at 405 nm in a spectrophotometer. Pyoverdine synthesis was measured using a reporter construct that has the promoter for a pyoverdine synthesis gene (*pvdF*) linked to the β -galactosidase gene. Promoter activity was determined by measuring the amount of o-nitrophenyl- β -D-galactopyranoside cleavage reflecting the amount of β -galactosidase.

A candidate gene encoding an anti-PvdS protein was identified in the *P. aeruginosa* genome. Overexpression of this gene, named *fvpR*, suppressed pyoverdine production and inhibited transcription from a PvdS-dependent promoter (*pvdF*). Expression of *pvdS* was unaffected, consistent with the hypothesis that FvpR suppresses the activity of PvdS.

Proteins with the highest sequence similarity to FvpR span the cytoplasmic membrane and, as well as interacting with sigma factors, interact with siderophore receptor proteins located in the outer membrane. Mutation of the ferri-pyoverdine receptor protein FpvA resulted in reduced pyoverdine synthesis. The activity of PvdS is also pyoverdine-dependent; *pvdF* expression in a pyoverdine-deficient mutant was greatly reduced (74.7 ± 10.6 enzyme units, E.U) and addition of exogenous pyoverdine to this mutant strain resulted in expression levels comparable to wild-type bacteria (521.6 ± 62.9 E.U versus 526.3 ± 49.2 E.U). Collectively these data show that pyoverdine controls the activity of PvdS through a signaling pathway involving the FpvA and FvpR proteins and hence regulates its own production.

Adenoviral receptor expression in neoplastic and normal cells. H S Chong, J Royds. Department of Pathology, Dunedin School of Medicine, University of Otago, Dunedin.

Adenovirus can be used in gene therapy, especially in cancer treatment. Adenovirus uptake in cells is dependent on receptors including CAR (coxsackie adenovirus receptor) and $\alpha\beta 1$ integrins, both plasma membrane proteins. It has been shown that adenovirus receptor density influences virally mediated gene transfer and therapeutic response. p53 is a regulator of cell growth and when damaged may result in uncontrolled cell division. p53 status (mutant, wild type, dysfunctional) may affect the expression of viral receptors. The aim was to compare p53, $\alpha\beta 1$ integrin and CAR expression in different cell lines using immunohistochemical techniques.

Paraffin-embedded cell blocks of all the cell lines in the Pathology Department were cut into $7\mu\text{m}$ sections and mounted on APES coated slides. These sections were stained for p53, integrin and CAR with a Vectastain Elite ABC mouse or rabbit kit (dependent on the primary antibody). For p53 and integrin we used commercial

mouse monoclonal antibodies, and for CAR a rabbit monoclonal and polyclonal antibody. These stained sections were graded according to the percentage of cells stained and the strength of the staining. When p53 status of the cell line was compared to the expression of CAR and integrin, it was evident that the amount of p53 within a tumour type affected the expression of viral receptors. Results also showed that cells from the same tumour type displayed similar amounts of integrin and CAR. Osteosarcomas, lung and thyroid tumours were high in both integrin and CAR and would be good candidates for gene therapy. However, cervical tumours with mutant p53 status and retinoblastoma tumours would be poor.

These results suggest that the expression of viral receptors may vary in tumour with the p53 status, which has implications when looking at adenoviral gene therapy, as some types of tumour will be more difficult to infect due to a smaller number of receptors.

The signal for 'stop' of protein synthesis in higher organisms: evidence for a sequence element rather than a single stop codon. A G Cridge, E S Poole, W P Tate. Department of Biochemistry, Otago School of Medical Sciences, University of Otago, Dunedin.

Bioinformatic analysis in prokaryotes has identified a bias in the usage of nucleotide bases surrounding stop codons. Bias in nucleotide usage has been shown to correlate with termination efficiency, indicating that translation termination in prokaryotes is controlled by a termination signal element. The aim of this investigation was to ascertain if a similar bias exists in the nucleotide sequence surrounding eukaryotic stop codons and to determine if any bias is correlated to efficiency of translation termination.

Bioinformatic analysis of non-redundant cDNA coding sequences from *Saccharomyces cerevisiae*, *Arabidopsis thaliana*, *Caenorhabditis elegans*, *Drosophila melanogaster* and *Homo sapiens*, was undertaken to identify potential eukaryotic translation termination signal elements. 'Highly' expressed genes were identified from analysis of proteins on 2DPAGE gels and from Codon Adaptation Indices (CAI). Sequences were extracted and analysed for nucleotide bias surrounding the stop codon. Correlations were identified between nucleotide bias surrounding the stop codon in 'highly' expressed genes and termination signal abundance (four nucleotide sequences *S. cerevisiae* $r=0.16$, *A. thaliana* $r=0.39$, *C. elegans* $r=0.49$, *D. melanogaster* $r=0.60$ and *H. sapiens* $r=0.25$). This correlation was common to all organisms investigated, indicating the probability of a conserved termination signal across eukaryotes.

Termination signals predicted to be efficient or inefficient from the bioinformatic analysis were cloned into vectors between two luciferase reporter genes, and the constructs assayed *in vitro* in a mammalian translation system to determine the efficiency of termination. Initial results indicate a relationship between predicted termination signal efficiency and observed termination efficiency, suggesting that specific nucleotides beyond the stop codon modulate the efficiency of translation termination in eukaryotes.

Transfection of MDCK cells with aquaporin-2 cDNA: a preliminary study to investigate water flux across cells. R Dempsey, J Bedford. Department of Physiology, Otago School of Medical Sciences, University of Otago, Dunedin.

Water movement across cells, in those cells which transport an isotonic fluid, occurs by transcellular rather than intercellular routes. To demonstrate this unequivocally, it is first necessary to induce the expression of water channels in the apical membranes of transporting epithelia.

Messenger RNA was extracted from mouse kidneys and reverse transcribed using Superscript II to produce cDNA. A 760 bp fragment coding for aquaporin-2 (AQP2) was amplified by polymerase chain reaction (PCR) from this DNA. The resulting fragment was separated by agarose gel electrophoresis, extracted from the gel, precipitated and transfected using Lipofectamine 2000 into cultured epithelial cells (MDCK). Transient transfections were carried out for 24-, 48- and 72-hour periods.

Proteins were extracted from transfected and nontransfected (control) MDCK cells. These were separated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE), and then electroblotted onto Immobilon PDVF membranes (western blotting). The presence or absence of AQP2 was detected with antibodies and enhanced chemiluminescence. AQP2 was clearly seen in those cells that were transfected. No AQP2 was seen in the control cells. Furthermore immunohistochemistry of the transfected and nontransfected MDCK cells showed the presence and absence of AQP2 respectively.

The successful transfection of AQP2 into epithelial cells (MDCK) that normally do not express this water channel has been achieved and verified. This will now enable the next stage of this project to proceed: namely the measurement of the flux of water across cells.

Caspase-3 mediated follicular degeneration in the mouse ovary. M A Fenwick, P R Hurst. Department of Anatomy and Structural Biology, Otago School of Medical Sciences, University of Otago, Dunedin.

The fate of any mammalian cell to live or die is controlled by a number of regulatory pathways consisting of many highly-conserved proteins. Caspase-3 is one such protein which represents a downstream convergence point and thus its activation serves as a useful marker for the identification of apoptotic (dying) cells. We sought to determine whether caspase-3 is detectable in the mouse ovary and if activation of the expressed enzyme occurs in cells of degenerating follicles. In order to further investigate the role of caspase-3, we injected purified Fas antibody, an apoptosis inducing agent, beneath the ovarian bursa (n=9 animals) and assessed the effect on follicle demise in comparison with saline treated counterparts (n=8).

Mouse primers were designed and reverse transcriptase-polymerase chain reaction (RT-PCR) was used to confirm the presence of an mRNA transcript for the inactive form of caspase-3 in the normal ovary. The active form of caspase-3 was localised using immunohistochemistry and was found predominantly in the granulosa cells of large secondary and larger developing (antral) follicles. Ovaries treated with an antibody specific to Fas revealed a marked increase in caspase-3 activity, particularly within granulosa cells of large antral follicles. An increase in the number (>6%) of

morphologically degenerate oocytes from smaller secondary follicles was observed in ovaries treated with the Fas antibody. Degenerate oocytes showed no sign of caspase-3 activity, suggesting that an alternative mechanism for apoptosis may exist in oocytes. These results indicate that caspase-3 is present and active in granulosa cells of large degenerating follicles. Degeneration of small follicles may be governed by the oocyte via a caspase-3 independent mechanism.

The effects of catechins on a murine air pouch model of chronic granulomatous inflammation. L Frampton, R Rahman, C Bolger, I Hall, I Appleton. Department of Pharmacology and Toxicology, Otago School of Medical Sciences, University of Otago, Dunedin.

Green tea has been ascribed to have anti-oxidant, anti-carcinogenic and anti-inflammatory properties. These effects have been attributed to the catechins, the predominant group of substances in green tea. In previous *in vitro* studies the effects of the catechins on parameters of inflammation have indicated positive effects. However, their effects *in vivo* have not been established. Therefore, in this study we have determined the effects of the catechins *in vivo* using a murine model of chronic granulomatous inflammation.

In the model of chronic granulomatous inflammation, an air pouch was initiated by injecting 3 ml of air into the dorsal subcutaneous tissue of female BALB/c mice (25 ± 5 g). This was followed 24 hours later by injection of 0.5 ml Freund's complete adjuvant containing 0.1% croton oil. The catechins, epigallocatechin (EGC), epicatechin gallate (ECG) and epigallocatechin gallate (EGCG), at a dose of 50 mg kg^{-1} i.p., were studied (n=8 per group). Animals were dosed 24 hours prior to air pouch formation and for 7 days following the initiation at which point they were sacrificed by CO₂ exposure. Granulomatous tissue dry weight, nitrite formation (the stable breakdown product of nitric oxide), nitric oxide synthase (NOS) activity and arginase activity were assessed. In addition, protein levels of NOS and arginase were measured by western blotting.

The results demonstrated that ECG significantly ($p < 0.05$) reduced inflammation (granulomatous dry weight), but significantly increased the levels of nitrite ($p < 0.05$), NOS ($p < 0.01$) activity and arginase ($p < 0.01$) activity. However, EGC and EGCG did not show significant results. The results clearly illustrated that for ECG *in vivo*, NOS activity and protein levels were increased but inflammation was reduced. It is well established that nitric oxide has cytostatic effects. Thus, the increase in NOS activity could account for the reduction in granulomatous tissue dry weight observed with ECG administration.

In conclusion, the present study suggests that ECG may have many future practical implications in the treatment of chronic inflammation.

An autoimmune diabetes locus (*Idd21*) on mouse chromosome 18. R J Hall, G Williams, T R Merriman. Department of Biochemistry, Otago School of Medical Sciences, University of Otago, Dunedin.

Autoimmune diabetes occurs spontaneously in the non-obese diabetic (NOD) mouse and is used to model human type 1 diabetes (T1D). The condition is precipitated by

the action of multiple insulin-dependent diabetes (*Idd*) genes, which allow a loss of immune self-tolerance and subsequent destruction of insulin-secreting pancreatic β -islet cells in the NOD mouse. Genes of the major histocompatibility complex (MHC) are the major genetic determinant of disease in addition to at least twenty other non-MHC loci contributing to a lesser extent. A meta-analysis of genome-wide linkage scans suggests the presence of an autoimmune disease locus on distal mouse chromosome 18 with the peak of linkage orthologous to human chromosome 18q12-q21 (which contains the T1D locus *Iddm6*) and rat distal chromosome 18 (which contains the T1D locus *Iddm3*). Furthermore, there is evidence for linkage of mouse chromosome 18 to diabetes in a (antibody high; ABH x NOD)F1 x NOD backcross.

A strain of NOD with chromosome 18 derived from the diabetes-resistant strain ABH was generated (NOD.ABH-Chr18). Urinary glucose levels were measured at 10 day intervals from 2 months to 7 months of age to determine diabetes incidence in both congenic (n=40) and NOD (n=106) mice. Congenic mice show a delayed onset and an overall reduced frequency of diabetes when compared to the parental NOD mice (40% versus 82% at 7 months of age, $p < 0.0001$). These data confirm the presence of a diabetes susceptibility locus on mouse chromosome 18, which we have named *Idd21*. Inflammation within the endocrine pancreas (insulinitis) was assessed by microscopy of paraffin-embedded sections stained with haematoxylin and eosin. There was no difference in insulinitis severity or frequency between congenic and NOD strains at 8-9 weeks of age suggesting that *Idd21* acts after insulinitis onset by retarding the destruction of insulin secreting β -islet cells.

Expression of T β RII-b: a potential motor neuron survival factor receptor. J A Harley, K Koishi, I S McLennan. Department of Anatomy and Structural Biology, Otago School of Medical Sciences, University of Otago, Dunedin.

Transforming growth factor-beta 2 (TGF- β 2) is being trialled as a therapeutic agent for motor neuron diseases. TGF- β 2 has traditionally been thought to share a common receptor with two other growth factors, TGF- β 1 and TGF- β 3. However, a novel splice variant of the TGF- β receptor (T β RII-b) has recently been described and suggested to be a specific mediator of TGF- β 2 responses *in vivo*. We have therefore determined whether T β RII-b is expressed in motor neurons.

Messenger RNAs were isolated from the spinal cords of rats and mice and examined by RT-PCR. The classical form of the TGF- β receptor (T β RII-a) was detected in both rats and mice. In contrast, T β RII-b mRNA was detectable only in the murine spinal cord. We then developed a method to determine whether T β RII-b mRNA was present in the motor neurons and/or the glia of the spinal cord. Pure populations of glia or motor neurons were isolated from cryostat sections of spinal cord, using laser capture micro-dissection. Messenger RNA from the isolated cells was examined by RT-PCR using [α - 32 P]-dCTP. A house-keeping gene, glyceraldehyde-3-phosphate dehydrogenase, was detected in these samples but the method was insufficiently sensitive to detect T β RII-b expression. It should be possible to overcome this limitation by amplifying the mRNA before assaying it.

In summary, these results indicate that T β RII-b is present in the central nervous system of mice, but not rats. Consequently mice may be a more appropriate model of

human motor neuron diseases than rats, as humans express both TβRII-a and TβRII-b in various peripheral tissues.

Immunisation with bone marrow-derived dendritic cells presenting HPV-16 E2 protects mice from virus challenge. L Heinemann, S Dillon, M Hibma. Department of Microbiology, Otago School of Medical Sciences, University of Otago, Dunedin.

Cervical cancer is the second most common cause of cancer-related death in women. Previous infection with certain human papilloma virus (HPV) types (especially 16 and 18) is associated with cervical cancer and as such provides an opportunity to target the virus to prevent this disease. We have assessed E2, an early protein of HPV-16, as a possible vaccine immunogen. Here we examine the ability of dendritic cells to deliver E2 to lymphocytes and to protect against viral challenge in a mouse model.

Mice were immunised twice with 5×10^4 bone marrow-derived dendritic cells (bmDC) either transduced with a replication deficient E2 retrovirus or pulsed with soluble recombinant E2 protein. Following immunisation, mice were challenged intranasally with 1×10^8 plaque forming units of recombinant vaccinia virus expressing E2 and the reporter β-galactosidase. Body weight loss was recorded daily as a measure of infection and expressed as percentage decrease of pre-challenge weight. On day five post-challenge, lung suspensions were prepared and analysed for β-galactosidase positive (vaccinia infected) cells using flow cytometry.

Mice immunised with E2 protein bmDC lost less body weight ($23.02 \pm 2.32\%$) than mice immunised with protein control bmDC ($25.54 \pm 0.42\%$) and had fewer vaccinia infected lung cells (79.38% compared to 86.58%). Mice that received retroviral E2 bmDC developed a vaccinia infection of reduced severity, with a body weight loss of $16.83 \pm 3.22\%$ and 40.65% vaccinia positive lung cells. Mice immunised with retroviral control bmDC lost $20.23 \pm 3.28\%$ body weight and had 79.59% vaccinia infected lung cells.

These results indicate that immunisation with E2 bmDC is capable of protecting mice against viral challenge. Dendritic cell-based vaccines presenting early HPV genes may therefore have application in humans to protect against cervical cancer.

Biochemical investigations into NMDA receptor numbers following long-term potentiation. J T T Kennard¹, D Guevremont¹, S E Mason-Parker², W C Abraham², J M Williams¹. ¹Department of Anatomy and Structural Biology, Otago School of Medical Sciences; ²Department of Psychology, University of Otago, Dunedin.

The *N*-methyl-D-aspartate (NMDA) receptor is central to the induction of hippocampal long-term potentiation (LTP), a molecular model of learning and memory. The functional NMDA receptor consists of core subunit proteins, as well as associated signal transduction and structural proteins. Previous studies from our laboratory found that expression of NMDA receptor subunits was elevated in protein extracts isolated from rat dentate gyrus after the induction of LTP at perforant path synapses. To determine the functional significance of these findings, we investigated

whether changes in NMDA receptor subunits, and associated proteins, were localised to dentate gyrus synapses. Synaptic membrane fractions (synaptoneurosomes) were isolated from tetanised and control dentate gyri and the expression of the NMDA receptor subunits NR1 and NR2B, and receptor-associated proteins neuronal nitric oxide synthase (nNOS), post-synaptic density protein of 95 kilodaltons (PSD-95) and α -calmodulin-dependent kinase II (α CaMKII), analysed by western blot. In this study we found significant increases in expression of NR1 ($30 \pm 7\%$, $n=5$; $p<0.05$, 2 tailed t-test) and NR2B ($60 \pm 22\%$, $n=5$; $p<0.05$, 1 tailed t-test) at 48 hours following tetanisation of the perforant path. Significant increases in nNOS expression ($104 \pm 31\%$, $n=7$; $p<0.05$, 2-tailed t-test) α CaMKII ($11 \pm 4\%$, $n=6$; $p<0.05$; 1 tailed t-test) and PSD-95 expression ($33 \pm 13\%$, $n=7$; $p<0.05$; 2-tailed t-test) were also detected at this time point. These results suggest that there is an increase in the number of NMDA receptor complexes presented to post-synaptic membranes at perforant path synapses after the induction of LTP. This increase may be an aspect of the formation of new post-synaptic spines that has been shown to occur following LTP induction in potentiated hippocampal pathways.

Appropriateness of and outcome after colonoscopy. A Lin, M Schlup, R Lübecke, G Barbezat. Department of Gastroenterology and Medicine, Dunedin School of Medicine, University of Otago, Dunedin.

Colorectal cancer is the second most common form of cancer in New Zealand. Colonoscopy and barium enema are the two main methods of large bowel investigation. Colonoscopy is more accurate and offers the chance of removing neoplastic lesion in one procedure. Strict access criteria are used to maximize limited resources. This study investigates the appropriateness of indications for colonoscopies and the outcome of patients following colonoscopy over a three-year period.

All consecutive colonoscopies performed in 1997 at Dunedin Hospital were assessed for indications, results and outcomes over a three-year period. The indications were categorized into appropriate, inappropriate, and indeterminate according to a predetermined list agreed among local experts. Any further interventions in the following three years were also noted.

Of the 598 colonoscopies performed in 1997, 543 (91%) were carried out appropriately, 586 (98%) were defined as complete and 252 (42%) of the examinations were normal. Tumours and polyps were found in 36 (6%) and 156 (26%) of colonoscopies respectively. There were no major differences in the rate of significant findings across the three groups of indication. During the three-year follow up period, 70 colonoscopies were performed after index colonoscopy. Fifty-seven of these were planned surveillance and one tumour was found. Nineteen barium enemas were performed, including 5 to complete colonoscopy, 3 for same indication, and 11 for different indication to the index colonoscopy. There were no relevant new findings. One patient who had normal colonoscopy in 1997, was found to have widespread tumour disease during an operation for bowel obstruction.

Most colonoscopies were performed appropriately, reflecting adherence to access criteria. Overall there was a high rate of relevant findings (42%). The lack of differences in significant findings across the different groups may be related to small

numbers, but if confirmed in larger studies the value of access criteria may need to be reviewed.

Microvenous valves in venous disease. M N Phillips^{1,2}, A M van Rij², M Zhang¹, G T Jones². ¹Department of Anatomy and Structural Biology, Otago School of Medical Sciences; ²Section of Surgery, Department of Medical and Surgical Sciences, Dunedin School of Medicine, University of Otago, Dunedin.

Venous valves have been known to exist in the large veins of the human body since the late 1500s. Very recently venous valves have been identified in the small superficial veins of the human lower limb. The role of these valves in normal physiology and pathophysiology remains unclear. This study assessed differences in microvenous valve size and distribution in the microcirculation of normal and diseased limbs.

The superficial venous systems of six normal lower limbs (with no evidence of venous reflux; four males, two females; 59–92 years old) and five amputated lower limbs (with confirmed venous ulcers; three males, two females; 61–86 years old) were filled with resin by perfusion (Batson's #17 corrosion casting resin, PolySciences). Superficial tissue (20 x 20 mm) from the gaiter, lower, mid and upper calf was excised and macerated (15% NaOH). The resulting resin casts of the vasculature were viewed by light and scanning electron microscopy.

Microvenous valves were present in all regions from the limbs with venous ulceration. The regional distribution and size distribution of veins containing valves was not different between the normal and diseased limbs. Limbs with venous ulceration had an increased density and tortuosity of veins filled with resin compared with normal (153.8 vessels/cm³ vs 15.1 vessels/cm³ respectively). Unlike the normal limbs, in the venous ulcerated limbs the resin passed retrogradely from the large veins through incompetent tributaries to the dermal capillaries.

This study shows, for the first time, that the distribution and size of veins containing microvenous valves did not differ between normal and venous ulcerated limbs. However, microvenous valves in the venous ulcerated limbs permitted reflux from the large veins to the dermal capillaries. This novel observation has great relevance to our understanding of the microcirculation in venous disease and may aid in developing treatment strategies for this condition.

Aquaporin-1 is expressed in cultured IMCD cells in response to external hypertonic shock. J Short-Rollo, J P Leader. Department of Physiology, Otago School of Medical Sciences, University of Otago, Dunedin.

Aquaporins (AQPs) are a class of membrane-intrinsic protein channels predominantly permeable to water, found in all living things. It has been widely believed that cells in tissue culture fail to express AQPs. However, two recent reports have suggested that cultured renal cell lines will express AQPs when subjected to osmotic stress. The present work aimed to confirm and extend these findings by investigating the ability of a cell line from mouse inner medullary collecting duct (IMCD) to express AQPs.

IMCD cells were grown to confluence in DMEM supplemented with 5% foetal calf serum, in 10 cm petri dishes, with sterile coverslips added. The osmotic pressure of the bathing medium was made progressively hyperosmotic (50 mOsmol/day), by the addition of urea, sodium chloride or mannitol. Twenty-four hours after the bathing osmolarity had reached 600 mOsmol/L, the cells were drained, treated with SDS in Tris buffer, and scraped free of the substrate. All proteins were separated using SDS-polyacrylamide gel electrophoresis (SDS-PAGE). After separation, proteins were transferred onto an Immobilon PVDF membrane, and aquaporin-1 (AQP1) was detected with the appropriate antibody using enhanced chemiluminescence. Cells grown on coverslips were fixed and examined by immunohistochemistry for the presence of AQP1.

Both western blotting and immunohistochemistry confirmed the presence of AQP1 in the cells exposed to media made hyperosmotic by the addition of NaCl or mannitol, with significantly higher expression shown by the NaCl-treated cells. In contrast, no AQP1 expression was detected either in isosmotic control samples or in cells from media made hyperosmotic with urea. It is concluded that AQP1 expression can be induced in IMCD cells by exposure to osmotic stress using impermeant solutes.