

Proceedings of the Anatomical Society of Great Britain and Ireland

The Winter Meeting of the Anatomical Society of Great Britain and Ireland was held at the Royal Holloway College, Egham, Surrey from 5th to 7th January 2000. It included a symposium on 'Phenotypic changes in epithelial development' and the Annual General Meeting of the Society. The following are abstracts of communications and posters presented at the meeting.

TALKS

1 Three dimensional behaviour of microcracks in compact bone. By F. J. O'BRIEN^{1,2}, D. TAYLOR², G. R. DICKSON³ and T. C. LEE^{1,2}. ¹*Department of Anatomy, Royal College of Surgeons in Ireland;* ²*Department of Mechanical Engineering, Trinity College Dublin, Ireland;* ³*Department of Anatomy, Queen's University of Belfast, UK.*

Fatigue damage in bone occurs in the form of microcracks. These microcracks contribute to the formation of stress fractures, act as a stimulus for bone remodelling and accumulate with age leading to fragility fractures. Microcracks in bone are normally analysed using 2 dimensional transverse sections of bone but a detailed knowledge of their 3 dimensional shape is required for analysis of stress intensity factors and fracture prediction.

In this study microcracks produced *in vivo* in human ribs were stained with basic fuchsin and viewed in longitudinal sections and, using 2 different computer based reconstruction methods, in 3 dimensions. In the first method laser scanning confocal microscopy was followed by reconstruction of microcracks into a 3D image. In the second methyl-methacrylate embedded sections were serially sectioned, microcracks identified using UV epifluorescence and individual microcracks reconstructed using Surfdriver software.

The size and shape of microcracks were found to be similar using both reconstruction techniques. The data obtained from the serial sectioning method showed microcracks to have a mean length in the transverse direction of 404 μm (s.d. 145 μm), mean width of 97 μm (s.d. 37 μm) and an aspect ratio of 4.6 ($n = 9$). Using epifluorescence microscopy of 92 microcracks, mean microcrack length was 370 μm (s.d. 211 μm). The lengths obtained were consistent with other results (Burr & Martin, *J. Biomech.* **26**, 1993; Lee et al. *J. Anat.* **193**, 1998). The 3D measurements were also consistent with the theoretical prediction of an elliptical crack shape with axis ratio (longitudinal:transverse) of 5:1 deduced from analysis of random 2D sections (Taylor & Lee, *J. Biomech.* **31**, 1998). The results obtained provide new data on the nature of microcracks in bone and the method has the potential to become a useful tool in the prediction of stress intensity factors which indicate the probability of an individual microcrack propagating to cause a stress or fragility fracture.

This work was supported by the Health Research Board, Cappagh Hospital Trust and the Research Committee of the Royal College of Surgeons in Ireland.

2 Axonal regeneration, astrocyte morphology and the expression of the extracellular matrix molecules tenascin-C, tenascin-R, chondroitin sulphate proteoglycan stubs and NG2 following dorsal rhizotomy in adult rats. By Y. ZHANG¹, K. TOHYAMA², A. R. LIEBERMAN¹, J. WINTERBOTTOM¹, M. SCHACHNER³, W. B. STALLCUP⁴ and P. N. ANDERSON¹. ¹*Department of Anatomy and Developmental Biology, University College London, UK;* ²*Department of Neuroanatomy, Center for Electron Microscopy and Bio-Imaging Research, Iwate Medical University Morioka, and Neural Architecture, BSI, RIKEN, Wako, Japan;* ³*Centre for Molecular Neurobiology, Hamburg, Germany;* ⁴*The Burnham Institute, La Jolla, California, USA.*

Few sensory axons regenerate back into the cord following dorsal rhizotomy and it is thought that regeneration may be limited by astrocyte processes and extracellular matrix molecules in the dorsal root entry zone (DREZ). Adult Sprague-Dawley rats were deeply anaesthetised with Halothane, the left L5 dorsal roots transected and reanastomosed, and the animals killed after 3 d to 24 wk. Some dorsal roots were teased and immunoreacted for GFAP and GAP-43 to identify astrocyte processes and regenerating axons. Sections from other injured roots were immunoreacted for GFAP, neurofilaments, or the putative axonal growth inhibitory molecules tenascin-C, tenascin-R, NG2 or chondroitin sulphate proteoglycan stubs (CSPG, Chemicon polyclonal), or used for *in situ* hybridisation to detect tenascin-C mRNA. Many processes from astrocytes grew up to 700 μm from the CNS/PNS boundary into the basal lamina tubes in the PNS part of the roots, ending as bulbshaped expansions. Regenerating axons were present within basal lamina tubes containing astrocyte processes and in some cases could be shown to turn back into the root as they approached the DREZ, passing between 2 basal lamina tubes as they did so. Tenascin-C mRNA and protein were upregulated in the roots by 3 d after rhizotomy, and strongest at 2 wk but there was little tenascin-C within the DREZ. CSPG stub immunoreactivity was also greatly upregulated in the PNS part of injured roots and only slightly upregulated in the DREZ. Tenascin-R immunoreactivity was always confined to CNS tissue, including the DREZ, and was unaffected by rhizotomy. NG2 immunoreactive cells were ramified and GFAP negative. They were widespread in the intact spinal cord and DREZ, but took the form of large rounded cells in the degenerating dorsal column and occupied much of the DREZ following dorsal rhizotomy. Astrocyte processes projecting into the roots were tenascin-R and NG2 negative. Hence NG2 and tenascin R are appropriately situated to inhibit regeneration through the DREZ but 2 putative axonal growth inhibitory molecules, tenascin-C and CSPG, were more strongly

expressed in the PNS part of the root where axons regenerate than in the DREZ where they do not regenerate.

This work was supported by the Wellcome Trust and MRC.

3 LABH-1: cloning of a novel alpha/beta hydrolase fold protein from mouse lung. By A. J. EDGAR and J. M. POLAK. *Department of Histochemistry, Division of Investigative Science, Imperial College School of Medicine, London, UK.*

To identify changes in gene expression associated with emphysema, differential display was used to compare RNA extracted from human distal lung tissues of emphysematous and donor lungs taken at the time of transplant. A differentially expressed clone DD03 appeared to be 44% down regulated in emphysema. The sequence of this clone was similar to that of a mouse expressed sequence tag. 5' and 3' rapid amplification of cDNA ends generated 2 overlapping murine clones. Together they form a 1357 bp transcript (GenBank accession no. AF189764). The 3' untranslated region of this cDNA overlaps with the 3' UTR of the DNA binding protein PREB (GenBank AF150808) encoded by the opposite strand. The predicted open reading frame encodes a 411 residue protein (45.7 kDa) with an isoelectric point of 6.59. There is a predicted alpha/beta hydrolase fold encompassing residues 118–342, and by comparison with nonhaem bromoperoxidase-a2 the catalytic triad is Ser 210, Asp 336 and His 365. Lung alpha/beta hydrolase fold protein-1, LABH-1, is a member of the prosite UPF0017 family. Mammals appear to have 3 UPF0017 containing genes. The 3 genes appear to have distinct tissue distributions. A search of the expressed sequence tag database suggested that LABH-1 was associated with lung and reproductive tissues, PHPS1-2 being most abundant in testis, and the third, an unnamed gene (GenBank AF007152), being most abundant in brain. Family members are found in diverse phyla including *Escherichia coli* and the white spruce. Mouse LABH-1 protein has 22% identity and 50% similarity with the *Saccharomyces cerevisiae* EHT1 protein, which produces ethylhexanate from ethanol and hexanoylCoA. We aim to clone the human LABH-1 gene and further examine its expression in emphysematous and donor lung and investigate the possible enzymatic activity of the recombinant protein.

This work was supported by GlaxoWellcome and the Julia Polak Lung Transplant Fund.

4 Ultrastructure and function of oviducal glands in elasmobranchs. By W. C. HAMLETT and C. K. HYSSELL. *Department of Anatomy and Cell Biology, Indiana University School of Medicine, USA.*

Elasmobranch fishes (sharks, skates and stingrays) possess a diverse array of reproductive strategies ranging from oviparity to placental viviparity. A consistent feature of the female reproductive tract is the presence of oviducal glands, previously referred to as shell or nidamental glands. Oviducal glands of elasmobranchs are unique specialised regions of the anterior oviduct modified to perform various

functions: (1) lubrication to facilitate movement of the fertilised egg to the uterus; (2) production of various jelly coats that envelope and support the egg; (3) secretion of a cementing layer between the egg jelly and egg envelope; (4) production of the various investing tertiary egg envelopes; and (5) storage of sperm. Oviducal glands have a consistent zonation from anterior to posterior: club, papillary baffle and terminal. The club and papillary zones elaborate the various hydrophilic components of the egg jelly that surround and support the fertilised egg. Merocrine secretory products are released from columnar cells and moved to the gland lumen by ciliary activity. The terminal papillary zone lamella elaborates a periodic acid-Schiff and Alcian blue positive bonding material that attaches the egg jelly to the lamellated enveloping egg envelope produced by the baffle zone. Baffle zone secretory tubules produce egg envelope constituents via merocrine secretion in the manner of a blown extrusion die. Liquid crystal components are secreted and transferred from the gland tubule by ciliary activity to a spinneret region of the gland where paired baffle plates extrude the pliable material into transverse grooves that extend across the full width of the gland lumen. Each transverse groove is responsible for the production of a single lamella of the egg envelope with each lamella oriented at right angles to the next. The terminal zone harbours bundles of sperm prior to their release into the gland lumen where they move to the anterior oviduct for fertilisation.

5 Short duration exposure to high glucose alters distribution of VE-cadherin in human endothelial cells. By B. A. I. PAYNE and L. LEACH. *School of Biomedical Sciences, Faculty of Medicine, University of Nottingham, UK.*

Diabetes mellitus is associated with vascular complications including increased vascular leakage. High glucose incubation for 30 min has been shown to elicit a permeability increase in endothelial cells. Other studies suggest that several day incubations are required. The rapid increase in endothelial permeability induced by such agonists as histamine and thrombin has been associated with focal loss of the integral adherens junction protein VE-cadherin (vascular-endothelial cadherin) from cell-cell contacts. We therefore investigated whether brief exposure of endothelial cells to high glucose leads to redistribution of VE-cadherin. HUVEC (human umbilical vein endothelial cells) were isolated from normal term placentae (n = 3) obtained by elective caesarean section. They were grown to confluence for 48 h on gelatin coated coverslips at 37 °C and 5% CO₂. Confluent cultures were treated with standard growth medium containing normal glucose (5.5 mM D-glucose), high D-glucose (25 mM) or high L-glucose (5.5 mM D-glucose with 19.5 mM L-glucose as an osmotic control) for 30 or 120 min. Further HUVEC isolates (n = 3) were exposed to these media for 2 h in the presence or absence of 10⁻⁸ M Gö6976, an inhibitor of classical protein kinase C isoforms. Cells were fixed (1% paraformaldehyde), permeabilised (0.5% Triton-X100) and immunolabelled for VE-cadherin. Systematic randomised counts were employed on the photomicrographs generated to analyse differences in VE-cadherin immunoreactivity. All experimental conditions showed a junctional VE-cadherin distribution that consisted predominantly of continuous labelling of the para-

cellular clefts. However, the percentages of paracellular clefts showing breakage in labelling i.e. a discontinuous distribution were (as mean \pm S.E.):

	Normal glucose	High D-glucose	High L-glucose
30 min exposure	17.3 \pm 2.5 %	17.9 \pm 3.7 %	15.6 \pm 4.9 %
120 min exposure	19.0 \pm 1.1 %	31.7 \pm 3.0 %	18.8 \pm 1.8 %

Analysis of data by randomised block ANOVA showed that exposure to high D-glucose for 120 min gave significantly more disruption of junctional VE-cadherin compared with other treatments ($P = 0.014$). Gö6976 had no significant effect on VE-cadherin distribution. In conclusion it appears that a 2 h exposure to high D-glucose causes a protein kinase C independent perturbation of adherens junctions, which is not simply an osmotic effect. This is the first study showing that glucose can affect junctional adhesion molecules of human endothelial cells. Alterations in junctional integrity may help to explain the increased endothelial permeability observed in diabetes.

6 Ultrastructural identification of spontaneously regenerating retinal ganglion cell axons in the lesioned optic nerve of the adult rat. By H. R. SHOTTON and G. CAMPBELL. *Department of Anatomy and Developmental Biology, University College London, Gower Street, London, UK.*

Retinal ganglion cell (RGC) axons of the adult mammal do not normally regenerate. However, we have recently shown using light microscope pathway tracing that following optic nerve crush a small number of RGC axons regenerate across the crush site and grow very slowly towards the optic chiasm (Campbell et al. *NeuroReport* **10**, 1999). Here we investigate the nature and cellular relationships of the regenerating RGC axons at the ultrastructural level in order to understand how these axons navigate the possibly hostile terrain of the degenerating optic nerve and why they regrow so slowly. Adult Sprague–Dawley rats (250–350 g) received an intraorbital left optic nerve crush under deep halothane anaesthesia. After 5 d to 2 mo 5 μ l N-hydroxysuccinimidobiotin (Sigma) was injected under halothane anaesthesia into the vitreous body of the left eye. Three days later animals were perfused with fixative under deep Sagatal anaesthesia. Vibroslice sections of the optic nerve were taken and reacted with avidin-biotin complex and DAB and processed for electron microscopy. Ultrathin sections cut at various points proximal to the crush site (towards retina) and distal to the crush site (towards chiasm) were viewed in the electron microscope. Small nonmyelinated axons labelled with electron dense reaction product were consistently observed in the proximal optic nerve and at least 500 μ m from the crush site in the distal optic nerve, confirming the unexpected finding that at least some RGC axons regenerate following optic nerve crush. These axons were generally round and either in small clusters of similar axonal sprouts or isolated. They were usually enmeshed in astrocyte processes and occasionally apposed to degenerate

myelin or oligodendrocyte processes. Other labelled regenerating RGC axons were observed in the meningeal covering. This shows that some regenerating RGC axons are not totally impeded in their regrowth along the optic nerve by oligodendrocytes, degenerate myelin or astrocyte processes.

7 Changes during maturation in the regulation of neurotrophin receptor expression in rat sympathetic neurons. By C. D. SULLIVAN¹, C. THRASIVOULOU¹, G. J. MICHAEL² and T. COWEN¹. ¹*Department of Anatomy and Developmental Biology, Royal Free and University College Medical School, London;* ²*Department of Anatomy, Queen Mary and Westfield College, London, UK.*

Until recently ageing in the nervous system was generally thought to coincide with large scale neuron death. In the PNS changes range from significant loss of innervation in some targets to increases in others. For example projections to the iris from the superior cervical ganglion are largely unaffected by age whilst projections from the same ganglion to some of the cerebral blood vessels, pineal gland and sweat glands show age related losses. No single cause for age related neurodegeneration of peripheral neurons has been identified. What is clear, however, is that certain neurons are sensitive to ageing events whilst others are not impaired, suggesting selective vulnerability of subpopulations of neurons. Preliminary evidence suggests that changes in target synthesis of neurotrophins and/or in their receptor signalling during maturation influence selective neuronal atrophy or damage in ageing. The maturational changes in the expression of p75 and trkA receptors, which regulate responses to neurotrophins, as well as the effects of treatment with exogenous neurotrophins were studied in the hope that this may lead us closer to discovering the causes of selective vulnerability. Exogenous NGF and NT-3 in varying doses were injected into the anterior chamber of the eye under appropriate anaesthetic, followed by the retrograde tracing dye fluorogold. The expression of p75 and trkA neurotrophin receptors was then assessed in iris projecting sympathetic neurons of the superior cervical ganglion from 15 d and 12 wk old rats ($n = 10$ for each group). Immunohistochemistry for p75 and trkA receptors and image analysis were used to detect changes in receptor protein and cell size. Unexpectedly NGF and NT-3 had no significant effect on p75 receptor staining at physiological doses in young or mature sympathetic neurons. NGF also had no effect upon trkA receptor protein. However large increases (almost 100%) were observed with 200 ng NT-3 and 10 ng NGF + 200 ng NT-3 treatment in mature neurons. No increases in trkA receptor staining were seen in young neurons with any treatment. Cell size increased considerably as a result of the treatment. Taken together, these results suggest that NT-3 becomes important during maturation in regulating trkA expression or distribution. This occurs after survival dependence on NGF is lost, and may represent a novel mechanism for the regulation of growth in the mature neuron. Additional studies are currently being undertaken to explore whether receptor expression (protein or mRNA) alters with neurotrophin treatment in 24-mo-old rats compared with younger animals.

8 An investigation into the effect of cytoskeletal inhibitors on neuronal apoptosis in HT-22 cells. By J. JOYNER and J. P. BENNETT. *Division of Biomedical Sciences, Imperial College School of Medicine, London, UK.*

The aim of this project was to investigate apoptosis resulting from glutamate induced excitotoxicity in neuronally derived HT-22 cells. Previous experiments had shown that specific cytoskeletal changes took place during apoptosis, namely breakdown of the microtubule cytoskeleton, maintenance of the submembrane actin cytoskeleton and the possibility that myosin dependent contractile activity contributed to blebbing. The effect of colchicine (affects microtubules), cytochalasin D (affects actin filaments) and 2,3-butanedione monoxime (BDM, inhibits myosin ATP hydrolysis) was investigated. Immunofluorescence experiments showed that these drugs inhibited the cytoskeletal morphology of HT-22 cells.

Time lapse video microscopy was used to investigate the time course of the blebbing process. Duration of blebbing was significantly reduced with BDM (41 ± 9 min, mean \pm s.d., $n = 10$) when compared to glutamate alone (84 ± 28 min, $P < 0.01$). Duration of blebbing was greatly increased with both cytochalasin (490 ± 95 min, $P < 0.01$) and colchicine (502 ± 265 min, $P < 0.01$). In both cases the change was due to earlier onset of blebbing, with the time of cessation of blebbing not greatly changed.

To investigate whether the change in blebbing related to a change in overall cell death, a cell viability assay was used which measures mitochondrial function. Cells treated with colchicine were affected earlier following glutamate addition than untreated cells with a significantly different reading at 6 h ($P < 0.05$). Cytochalasin and BDM showed no significant effect. In the absence of glutamate colchicine caused no decrease in cell viability at the concentration used.

The data imply that colchicine speeds up the process of apoptosis. It is possible that microtubule disassembly is a necessary stage in the onset of membrane blebbing and loss of cell viability and that this is the mechanism which colchicine accelerates. The data also suggest that altering the duration of membrane blebbing in HT-22 cells via other mechanisms does not change the underlying time frame of the apoptotic pathway.

9 Morphometric analysis of follicle-associated and non-follicular small bowel in the CD-1 mouse. By C. J. TANSEY, O. S. MANSOUR and R. R. ETTARH. *Department of Human Anatomy and Physiology, University College, Dublin, Ireland.*

Peyer's patches are follicle-associated lymphoid tissue which appear randomly in the wall of the small intestine in the mouse, but with greater frequency in the distal parts of the bowel. In studies of the small bowel which entail a morphometric analysis of enteric tissue, reference is seldom made to the fate of these lymphoid aggregates during the sampling process. It is not known whether or not the presence of these Peyer's patches have any influence on the outcome of estimations and calculations that are derived on the basis of quantitative data obtained from small intestinal samples. This study therefore examined the number of crypts of Lieberkuhn in follicle-associated and nonnodular parts of the small intestine in the mouse. Five male adult

CD-1 mice were killed by cervical dislocation and the small intestine delivered from the abdomen. The length of the small intestine was measured and the bowel quartered into 4 segments of equal length. Tube samples containing Peyer's patches, as well as an adjacent nonfollicular sample, were taken from each intestinal site. All samples were fixed in formol saline, processed and embedded in paraffin and stained with haematoxylin and eosin for light microscopy. Crypt profiles were counted in whole circumference sections of the intestine and the width of these crypt profiles was measured; whole crypt numbers for each intestinal quarter segment were then calculated. The results show that there were no statistically significant differences in crypt parameters examined between lymphoid and nonlymphoid samples across all intestinal sites sampled. This finding suggests that (a) the presence of Peyer's patches in the wall of a small intestinal sample does not affect the number of crypts within the specimen (b) these nodules may be considered space-occupying tissue elements which displace but do not obliterate other structural elements within the wall of the small intestine.

10 Molecular dissection of the tumour suppressor activity of E-cadherin: the role of adhesion versus β -catenin sequestration. By C. J. GOTTARDI and B. M. GUMBINER. *Department of Cellular Biochemistry & Biophysics, Memorial Sloan-Kettering Cancer Center, New York, USA.*

E-cadherin is down regulated or mutated in many epithelial cancers and has been shown to have tumour suppressor activity. E-cadherin is a calcium dependent adhesion molecule that together with β - and α -catenin is important for the physical association of adjacent epithelial cells. In this context it has long been reasoned that the tumour suppressor activities of E-cadherin would be mediated through maintaining cell-cell adhesion. However the recent finding that β -catenin is an oncogene and that constitutive signalling activity is associated with a number of cancers has led to the suggestion that an equally important role for E-cadherin in tumour suppression might be through sequestering and consequently antagonising the nuclear signalling activity of β -catenin. To understand how E-cadherin mediates tumour suppression we have generated chimeric constructs to distinguish between an adhesive versus β -catenin signalling-dependent mechanism. Using the SW480 cell line which expresses little endogenous E-cadherin protein and has high levels of nuclear and cytoplasmic β -catenin we show that stable over expression of the wild-type E-cadherin, or the cytoplasmic domain of E-cadherin fused to a heterologous transmembrane protein the interleukin-2 receptor (IL2R/E-cad cytoplasmic domain), significantly inhibits the growth characteristics of this cell line. Cadherin constructs that rescue adhesion (using a laminar flow assay) but do not interact with β -catenin (E-cadherin fused directly to α -catenin, or an E-cadherin juxtamembrane construct) fail to inhibit growth. Thus adhesion is neither necessary nor sufficient to mediate growth inhibition in the SW480 cell line. Both wild type E-cadherin and the IL2R/E-cad cytoplasmic domain can interact with endogenous β -catenin and inhibit β -catenin signalling activity using the TCF/ β -catenin-dependent reporter gene TOPFLASH. However this reduction in β -catenin signalling does not involve

obvious changes in β -catenin levels or nuclear localisation. This suggests that β -catenin signalling activity may in certain circumstances be regulated independently of protein levels or nuclear import. Whether the observed growth inhibition is mediated through reduction in a β -catenin signalling pathway will be determined by rescue experiments using a constitutively active form of the presumptive β -catenin dependent transcription factor TCF.

11 Epithelial cell plasticity in morphogenesis and tumour progression. By J. P. THIERY. *Institut Curie, Paris, France.*

Epithelial sheets are formed very rapidly during early embryonic development in a wide variety of metazoans. These specialised cell assemblies undergo extensive regional reorganisations leading to the formation and shaping of tissues. Remodelling of epithelial sheets and transient or definitive conversion of epithelial cells to a mesenchymal state is particularly spectacular during gastrulation. These morphogenetic events also occur in many other tissues in the vertebrate including somites, heart and kidney, the neural tube and most prominently the neural crest. In this presentation I will discuss some of the mechanisms governing different steps in the complex modulations of cell shape. In an attempt to study the molecular basis of epithelial-mesenchymal interconversions, our laboratory has used a malignant epithelial cell line (NBT-11) which can convert reversibly into motile fibroblast-like cells upon exposure to several growth factors, including acidic FGF (FGF-1), EGF and Scatter Factor/Hepatocyte Growth Factor. We have analysed the specific mechanisms whereby FGF-1 can act either as a mitogen or a scatter factor. I will describe early events in the FGF-1 transduction pathway which signals via a tyrosine kinase surface receptor. The scatter activity, which is observed only in subconfluent cultures, is characterised by an early and transient activation of c-src. Transient or stable transfectants expressing a constitutively active c-src are sensitised to the scatter factors and cells at the periphery of the clusters undergo an epithelial-mesenchymal transition (EMT). By contrast cells expressing a dominant-negative mutant of c-src do not respond to the scatter inducing activity of the growth factors. Slug, a transcription factor related to snail in *Drosophila*, is also induced early prior to the dissociation of epithelial cells. Cells expressing slug do not fully convert into fibroblast-like cells but have lost their desmosomes. I will discuss the respective contributions of c-src and slug in EMT. NBT-11 epithelial cells can also undergo EMT upon exposure to native collagens. Activation of the $\alpha 2\beta 1$ integrin by type 1 collagen induces specific tyrosine phosphorylation of FAK and paxillin. Transient expression of single point mutated paxillin in 2 critical tyrosines in NBT-11 cells strongly inhibits their motility on a collagen coated substrate. Type 1 collagen and FGF-1 act synergistically to promote scatter and locomotion of the NBT-11 cell line, most likely through an upregulation of the $\alpha 2\beta 1$ integrin. I will present a model to integrate the complementary roles of these 2 EMT inducers. Transfection of an expression vector coding for FGF-1 into epithelial cells converts them into fibroblast-like cells, endowed with motile, invasive and highly tumorigenic and metastatic properties. In vivo the FGF-1 transfected NBT-11 cells allow untransfected cells to

grow and metastasise as rapidly as the transfected cells, providing us with the opportunity to unravel the role of certain subsets of highly malignant cells in the progression of a heterogenous tumour cell population. Finally I will present very recent data obtained with human bladder carcinoma specimens of different grades and stages. These studies emphasise the multifunctional properties of some growth factors acting positively through enhanced autocrine loops or negatively as suppressors of tumour progression.

12 Desmosomal adhesion: its regulation and role in epithelial morphogenesis. By D. R. GARROD. *School of Biological Sciences, University of Manchester, UK.*

Much is now known about the molecular composition of desmosomes but there has been little advance in understanding their structure since the 1960s. To address this we have carried out quantitative immuno-electron microscopy with domain specific antibodies and have constructed a molecular map of the desmosomal plaque. The map adds weight to some conclusions reached by the study of molecular interactions but casts doubt on others. It also raises new questions about the molecular organisation of desmosomes. We have shown by blocking the adhesion of transfected cells with synthetic peptides that the putative cell adhesion recognition (CAR) sites of desmosomal cadherins are functional. Subsequently we have used anti-adhesion peptides to investigate the role of desmosomes in epithelial morphogenesis. The luminal epithelial cell line FSK 7-1 undergoes morphogenesis to form alveoli, and separated primary luminal and myoepithelial cells reaggregate to adopt the same relative positions as found in vivo. Both of these processes are specifically blocked by CAR site peptides, demonstrating for the first time a role for desmosomes in epithelial morphogenesis. The adhesive state of desmosomes in epithelial cell sheets is modulated in response to wounding through a signalling pathway involving protein kinase C. Depletion experiments with antisense oligonucleotides show that the α isoform of PKC is involved. This PKC isoform is localised to desmosomes in MDCK cells and cultured primary human keratinocytes and may play an essential role in regulating adhesion in epithelia.

13 Junctions and Wingless signalling in *Drosophila*. By J.-P. VINCENT. *National Institute for Medical Research, London, UK.*

Armadillo, the *Drosophila* homolog of vertebrate β -catenin, is required for cadherin-based cell adhesion. It is also a key transducer of Wingless signalling. We have been investigating to what extent these 2 functions are related. One view is that Armadillo exists in 2 pools, one dedicated to cell adhesion and the other to signalling. Junctional composition affects signalling since overexpressed Cadherin decreases Wingless signalling activity. Using a genetic screen, we have confirmed the influence of cell junctions on Wingless signalling. We screened for dominant modifiers of phenotypes due to decreased or increased Wingless signalling and have recovered mutations in genes known to be involved in cell adhesion. We also recovered a mutation in fasciclin3 a gene encoding an immunoglobulin domain protein localised at septate junctions. We find that fasciclin3 is required for the proper localisation of adherens junction

components such as Armadillo and Cadherin. Moreover many defects in fasciclin3 mutants are phenocopies of Cadherin-deficient animals. We would like to understand how a component of septate junctions affects adherens junction functions. It is also unclear how junctional structure modulates Wingless signalling.

Another important issue for the future is to find out whether Wingless signalling regulates junctional properties. Segmentally repeated indentations form transiently in the epidermis of stage 10 *Drosophila* embryos. Wingless is required for these morphological indentations, presumably by regulating local cell adhesion properties. We have yet to discover the cell biological basis of this control. However it is known that these indentations correspond to clonal boundaries, boundaries that cells or their progeny do not cross, and I will demonstrate that unidirectional cell movement imposed by the boundaries is crucial for pattern formation.

14 Regulating cell division and morphogenesis in a tubular epithelium of *Drosophila melanogaster*. By H. SKAER, A. GAMPEL, S. WAN and V. P. SUDARSAN. *Developmental Genetics Programme, Krebs Institute, University of Sheffield, UK.*

The renal or malpighian tubules of the fly arise from the embryonic hindgut as 4 buds of primordial cells. The pattern of cell proliferation in each tubule primordium is precise and reproducible and occupies only a short period of embryogenesis. We have identified components of the regulatory network governing the patterning of cell division and have shown a requirement for the activity of 2 signalling pathways: one mediated by *wingless* and the other by *spitz*, through the activation of the *Drosophila* EGF receptor. These pathways act at different stages in the programme of cell division, in distinctive spatially restricted patterns, and affect different stages of the cell cycle. The segregation of a specific cell lineage in the tubule primordia is required, first to activate signalling by *spitz*, and later for the morphogenetic movements characteristic of the tubules as they elongate and navigate through the body cavity to take up their mature configuration.

15 Positional information in gut endoderm. By F. BECK^{1,2}, J. TUCCI¹, R. PLAYFORD¹ and K. CHAWENGSAKSOPHAK². ¹*University of Leicester, UK* and ²*The Howard Florey Institute, University of Melbourne, Australia.*

We disabled the mouse homeobox gene *Cdx2* by homologous recombination. The gene is normally expressed in trophoblast, paraxial mesoderm and gut endoderm during early embryogenesis but is restricted to the post-gastric gut epithelium during later development and into adulthood. *Cdx2*^{-/-} animals die between 3 and 4 d post coitum because of a failure to implant. Heterozygotes exhibit an anterior homeotic shift involving the axial skeleton. Multiple intestinal polyps are present, most frequently in the proximal colon. These consist of fore-stomach epithelium, between which and the surrounding colon an orderly succession of tissues is intercalated to restore continuity of gut phenotype. This consists of histologically normal epithelium characteristic of gastric

cardia, gastric body, pyloric antrum and small intestine as one passes from the central area to the surrounding colonic tissue. The lesions are initiated around focal heterotopic areas of fore-stomach epithelium which later represents an anterior homeotic transformation involving gut epithelium. Secondly intercalatory growth takes place to restore continuity resulting in ordered 'filling-in' of tissue types between fore-stomach and colonic epithelia. Additionally polyps show isolated regions of low grade dysplasia and hyperplasia. Preliminary results indicate that while histological markers of cytodifferentiation within the polyps unequivocally identify their content of gastric endoderm, Hox code markers appear to remain related to intestinal position.

16 Branching morphogenesis in the developing kidney. By J. DAVIES. *University of Edinburgh, UK.*

The urine produced by excretory nephrons of the metanephric kidney is drained away into the ureter by a tree like system of collecting ducts. The collecting duct system develops from initially unbranched epithelial rudiment, the ureteric bud, which invades the area of the presumptive kidney. Once there it begins a programme of growth with reiterated branching, mainly terminal but occasionally internodal, to generate the tree, and while it does so it also induces cells in the surrounding mesenchyme to undergo a mesenchyme-to-epithelial transition and to form excretory nephrons.

The more we study collecting duct development, the more apparent it becomes that arborisation is controlled by a large number of signals and receptors. Work in this and other labs, using a variety of culture and transgenic methods, has shown the importance of growth factors (e.g. GDNF, neurturin, persephin, HGF, BMP4, BMP7, FGF7), receptor tyrosine kinases (e.g. c-Ret, c-Met, FGFRs), matrix components (e.g. laminin, nidogen, sulphated proteoglycans), matrix receptors (e.g. integrin α 3, integrin α 8), matrix metalloproteinases (e.g. MMP-2, MMP-9), adhesion molecules (e.g. L1) and transcription factors.

Cells of the developing collecting duct must combine these varied environmental influences to produce a coherent integrated morphogenetic response. To investigate how and where these influences converge we are now investigating how particular signal transduction systems in the cell control morphogenesis. We have so far found evidence that both the MAP kinase pathway and PKC control branch initiation, while PI-3-kinase can regulate morphogenesis of growing branch tips; we hope in the near future to outline the regulatory links between these pathways and the extrinsic factors listed in the preceding paragraph.

17 Signal transduction in mammary apoptosis. By C. STREULI, Y.-J. LEE, A. GILMORE, J. OLIVER and A. VALENTIJN. *School of Biological Sciences, University of Manchester, UK.*

In adherent cells, growth factors and extracellular matrix (ECM) cooperate to regulate signalling pathways and gene transcription. In the mammary gland, both the expression of milk protein genes and the suppression of apoptosis is controlled by crosstalk between signals derived from hormones and the specialised ECM known as basement

membrane (BM). This talk will focus on the adhesion mediated signals that regulate apoptosis in the mammary gland.

Survival of mammary epithelial cells requires adhesion to specific types of ECM. They undergo apoptosis after culture for several days on plastic or collagen but not on BM. The signal from BM is mediated by the integrin class of adhesion receptors and we have demonstrated that both $\alpha\beta1$ and $\alpha3\beta1$ integrins act as survival receptors. Furthermore, BM controls the ability of insulin to deliver PI 3-kinase regulated survival signals. Although insulin induced autophosphorylation of its receptor is ECM independent, downstream signals including tyrosine phosphorylation of IRS-1 and its association with PI 3-kinase are significantly lower in cells cultured on collagen than on BM. Thus a novel ECM dependent restriction point in insulin signalling exists in normal mammary cells, whereby the phosphorylation cascade downstream of insulin receptor activation requires adhesion to BM.

To understand events that link adhesion with the intracellular apoptotic machinery we investigated the possibility that the function of Bcl-2 family proteins is controlled by ECM mediated signalling. The proapoptotic protein Bax was largely present in the cytosol of adherent nonapoptotic mammary cells. Detachment from ECM induced rapid apoptosis in conjunction with translocation of Bax to mitochondria and a concurrent conformational change resulting in the exposure of its BH3 domain. Bax translocation and BH3 epitope exposure was reversible and occurred prior to commitment to apoptosis. Kinase cascades were critical in adhesion regulated survival signalling and conformation of the Bax BH3 epitope was dependent on both pp125FAK and PI 3-kinase. Thus integrin mediated adhesion regulates apoptosis through a control on Bcl-2 family protein dynamics.

Our results indicate that apoptosis suppression is mediated by crosstalk between integrin and growth factors and suggests that pp125FAK has a central role in linking decision events at the plasma membrane with the Bcl-2 family of apoptosis regulators.

This work is funded by the Wellcome Trust.

18 The basement membrane is necessary for polarisation of the epiblast and cavitation during early embryonic development. By P. MURRAY and D. EDGAR. *Department of Human Anatomy and Cell Biology, University of Liverpool, UK.*

During embryogenesis programmed cell death (PCD) plays a critical role in formation of the body plan. The formation of the pro-amniotic cavity is the first instance in mammalian development where PCD sculpts an embryonic structure. It is believed that this cavity forms because the cells at the centre of the inner cell mass (ICM) undergo PCD. To investigate this process, we are using embryoid bodies derived from mouse embryonic stem cells that have been genetically modified by knocking out the gene encoding laminin $\gamma1$. This defect renders the cells incapable of assembling a functional laminin trimer and hence no basement membranes are produced (Smyth et al. *J. Cell Biol.* **144**, 1999).

The current theory on PCD in the ICM is that visceral endoderm cells secrete a death signal that induces ICM cells

to undergo PCD. The basement membrane that lies between the endoderm and the ICM is thought to counteract this death signal by instructing cells in direct contact with it to live (Cocouvanis & Martin, *Cell* **83**, 1995). Consequently the only ICM cells to survive are those aligned against the basement membrane, which become the epiblast.

Our results disprove the above theory because although visceral endoderm cells form in basement membrane deficient embryoid bodies there is no evidence of any cavity formation involving PCD. Moreover, despite the fact that the basement membrane is not required for differentiation of visceral endodermal cells, we have found that it is essential for differentiation of the polarised epiblast cells. Additionally wherever polarised epiblast cells are observed in normal embryoid bodies dead cells are invariably present in their close vicinity. We propose therefore that PCD in the ICM is induced by a signal emanating from the epiblast and not the visceral endoderm.

19 Development of the scanning near field optical microscope as a tool in biological microscopy. By M. C. DENYER^{1,2}, R. MICHELETTO³, M. SCHOLL⁴, K. NAKAJIMA², S. BRITLAND¹, M. HARA² and W. KNOLL⁵. ¹*School of Pharmacy, University of Bradford, Bradford, UK;* ²*Frontier Research System, The Institute of Physical and Chemical Research (RIKEN), Japan;* ³*University of Kyoto, Faculty of Engineering, Division of Material Science, Yoshida, Sakyo, Japan;* ⁴*Institute of Physiological Chemistry and Pathobiochemistry, Johannes Gutenberg University, Mainz, Germany;* ⁵*MPiP, Mainz, Germany.*

Application of light to the underside of a glass substrate results in the generation of a zero energy evanescent field. This evanescent field decays exponentially with distance from the sample so that by a distance equivalent to a third of the wavelength of light from the surface the evanescent field is negligible. If a sharpened optic fibre is dipped into the evanescent field, photons will tunnel through the substrate directly into the probe. This allows the probe to sample the optical properties of the substrate with a lateral resolution dependent on the probe tip diameter. This forms the basis of the scanning near field optical microscope (SNOM). SNOM systems have a lateral resolution equivalent to that of electron microscope (EM) and atomic force microscope (AFM) systems. However, because of difficulties positioning the scanning probe within the near field in a liquid medium, SNOM systems have not been used to examine live biological samples. We used the SNOM to examine rhythmically beating cultured cardiac myocytes, fixed hippocampal neurons immersed in a physiological saline and also fluorescently labelled rat cardiac myocytes immersed in physiological saline using a custom made SNOM system mounted on an Olympus 1 \times 70 inverted fluorescence microscope. We found it was possible to monitor the contractile activity of the live cardiac myocytes at a sub micrometre lateral resolution, clearly image hippocampal neurons at resolutions equivalent to the earliest AFM measurements of liquid immersed biological samples and resolve fluorescently labelled rat cardiac myocytes. This work clearly shows that SNOM systems have the potential of being developed into very powerful tools in biological microscopy.

20 Mouse metrial gland cell rearrangement on an extracellular matrix material, in vitro. By P. FUSSING, D. D. Y. MUKHTAR and I. J. STEWART. *Departments of Human Morphology and Obstetrics & Gynaecology, University of Southampton, UK.*

It is increasingly apparent that the extracellular matrix plays a modulating role in supporting cell function, both in vivo and in vitro. Recent studies have shown that epithelial cells, including in our laboratory human ovarian granulosa cells, have the ability to rearrange extracellular matrix and thereby maintain the viability of the cultured cells. Mouse bone marrow derived pregnancy specific granulated metrial gland (GMG) cells fail to thrive when cultured in isolation. In contact with stromal cells GMG cells survive for longer but still show a steady decline in numbers so that few remain by d 7 of culture. In an attempt to improve culture conditions and increase the viability of cultured GMG cells we have determined the effect of the extracellular matrix material Matrigel on the organisation of cultures of metrial gland cells. Single cell suspensions of metrial glands from d 9 or 12 pregnant mice were cultured in Lab-Tek II 8 well glass chamber slides with or without a thin coating of Matrigel. Cells were cultured at densities of 1×10^5 , 2.5×10^5 or 5×10^5 cells in each well (area = 120 sq mm) for up to 7 d. At 1×10^5 density cells grown on Matrigel grew singly or in very small groups. At 5×10^5 density cells were largely confluent. However at 2.5×10^5 density stromal cells formed large interconnected clusters, evenly distributed across the culture well. GMG cells were found in, on, adjacent to, or spatially independent of the stromal cell clusters. Counts of GMG cells on d 1, 3 and 7 of culture showed that they declined in numbers in a similar pattern to GMG cells in cultures of metrial gland cells cultured on glass without Matrigel. Thus Matrigel supports rearrangement of stromal cells into a more 3-dimensional network but this does not provide for increased long term viability of GMG cells.

P. Fusing was supported by the Wellcome Trust (ref. VS/99/SOU/217).

21 A preliminary study on the effect of aspirin on the developing chick embryo. By W. VORSTER, D. J. LIZAMORE and D. KOTZE. *Department of Anatomy, University of Pretoria, South Africa.*

The aim of this study was to evaluate the abnormalities resulting from exposure of chick embryos in ovo to the nonsteroidal anti-inflammatory drug aspirin (acetyl salicylate). Aspirin is widely used and is safe at therapeutic doses but may cause abnormalities if taken at overdose levels.

Fertile eggs from a White Leghorn flock were randomly divided into 4 groups. The 3 experimental groups were injected into the air sac after 48 h embryonic development, receiving 0.03 µg, 0.12 µg and 0.24 µg aspirin dissolved in 50 µl normal saline respectively. The control received 50 µl saline. The embryos were killed on the 8th day of development and gross abnormalities, body weight and total length determined.

None of the control group showed any abnormalities, whereas 8%, 5% and 23% of the embryos were affected from the lowest to the highest dosage groups. The abnormalities encountered were ectopia cordis (5.4%),

cyclopia and other eye defects (2.7%) and beak and tailbud defects (2.7%). There were no statistical significant growth differences between the groups ($P < 0.05$ level). A slightly increased mean beak-tail length in group 2 could correspond to an increase found in growth and neonatal weight in the offspring of mothers who took moderate doses of aspirin daily (Beningi et al. *New Engl. J. Med.* 1989).

It thus seems that aspirin at therapeutic doses might be beneficial to fetal growth, but teratogenic at overdose levels.

22 Functional analysis of transcription factors in the chick retina using square pulse electroporation. By D. LE-ROUËDEC¹, P. J. SCOTTING² and P. M. WIGMORE¹. *School of Biomedical Sciences¹ and Nottingham Children's Brain Tumour Research Centre, Institute of Genetics², Queen's Medical Centre, University of Nottingham, UK.*

Transcription factors play an important role in cell migration and differentiation during neurogenesis. We are studying the role of the basic helix-loop-helix (bHLH) and Sox genes, 2 families of transcription factors implicated in this complex process, and are using the retina as a model for neuronal development.

First we have carried out functional analysis by transfecting genes suspected of being involved in the process of neuronal maturation. This was achieved using square pulse electroporation, a powerful technique of transfection into living tissues. The genes of interest were cloned into a plasmid containing the GFP reporter gene in order to detect their expression in the transfected cells. Our results show that neurogenin2 (Ngn2), a member of the bHLH family, thought to be a determination factor in the process of neurogenesis, can activate NeuroD, another bHLH gene known as a differentiation factor. Therefore Ngn2 can trigger neurogenesis in the retina. Ngn2 has also displayed similar results in other tissues such as the neural tube (M. Cheung, personal communication) and the peripheral nervous system (Perry et al. *Development* 126, 1999) and has thus a conserved role in neuronal maturation.

We are now investigating the interaction of Sox genes and bHLHs. Early results suggest a relationship between some members of the Sox and bHLH families during maturation of the retina, indicating for the first time a close link between these 2 families in the sequence of gene activation during neurogenesis.

23 Investigation of the potential role of Sox4 in developing muscle. By S. PINTO CARDOSO¹, S. POOL¹, M. CHEUNG², P. J. SCOTTING² and P. WIGMORE¹. *School of Biomedical Sciences¹ and Institute of Genetics², Queen's Medical Centre, Nottingham University, UK.*

Sox4 belongs to the Sox gene family, a new class of transcription factors characterised by the presence of a DNA-binding domain, the High Mobility Group (HMG) box. Sox4 has a very broad expression pattern in murine embryogenesis. In adult mice Sox4 is restricted to immature B and T lymphocytes. Sox4 knockout mice show an expansion of pro-B lymphocytes and a defect in cardiac outflow tract formation, which causes embryonic death at E14.

Using a degenerate PCR based method, we have identified

Sox4 expression in embryonic muscle tissue and C2C12 myoblasts. In situ hybridisation of whole mount E13.5 mouse embryos showed strong expression in differentiating cells in the neural tube, in the connective tissue in the limb bud, and in the embryonic heart. In developing muscle Sox4 showed a punctate expression, being found in single cells but not in muscle fibres. This expression pattern may indicate that Sox4 marks a stage of differentiation in neural and myogenic tissue.

To investigate the role of Sox4, construction of an eukaryotic expression vector was achieved by subcloning Sox4 into pcDNA3 (full-length Sox4 cDNA cloned into pBluescript SK +/- was kindly given by Hans Clevers). All cloning steps were carried out as standard protocols.

This construct is currently being transfected into C2C12 myoblasts, by lipofection, to test the effect of constitutive expression of Sox4 on the proliferation and differentiation of these cells.

24 Location of dividing cells during skeletal myogenesis. By P. M. WIGMORE, P. MUTCH, C. SLOUGH and C. BOWEN. *School of Biomedical Sciences, Queen's Medical Centre, Nottingham University, UK.*

All skeletal muscle fibres form by the fusion of myogenic cells and prior to fusion the cells exit mitosis and undergo a program of differentiation. Fibre formation occurs in 2 successive waves which produce first large diameter primary fibres, and then smaller secondary fibres. Secondary fibres are produced by the fusion of cells on the surface of primary fibres. After formation and growth secondary fibres detach from the primary fibre and form a halo of secondary fibres around each primary. At any given time during this period there are 1–2 secondary fibres and numerous myogenic cells on the surface of each primary fibre. Cells on the surface of a primary fibre can be classified as being located either in contact with or not in contact with a secondary fibre. As myogenesis progresses the distribution of cells on the surface of primary fibres changes such that an increasing proportion of cells are found in contact with secondary fibres. This change in distribution may indicate that cells differentiate in contact with secondary fibres possibly prior to fusing with these fibres.

To test this, vibratome slices of developing rat muscle were stained with Dil to show cell and fibre outlines and with an antibody against Ki67 to show the location of dividing cells. Slices were viewed by confocal microscopy and cells scored according to position (whether in contact with a secondary or not) and whether they were dividing. Results showed that fewer dividing cells were found in contact with secondary fibres indicating that this location may trigger differentiation.

POSTERS

P1 Morphological responses to scatter factor/hepatocyte growth factor in 3-dimensional epithelial cell cultures. By M. J. WILLIAMS and P. CLARK. *Biomedical Sciences Division, Imperial College School of Medicine, London, UK.*

Scatter factor/hepatocyte growth factor (SF/HGF) is a multifunctional polypeptide growth and motility factor that also has morphogenetic properties. SF/HGF together with

its receptor c-met plays essential regulatory roles during embryonic development, adult tissue growth and repair, and is involved in a number of pathologies. It is known that epithelial cells form fluid filled, inwardly polarised cysts when cultured in a 3-dimensional matrix, and that cysts in type 1 collagen form long, branching tubules (tubulogenesis) in response to SF/HGF exposure. Although several models of tubule formation have been proposed, many of the cellular events that take place during these SF/HGF-induced responses largely remain unclear. Using the MDCK epithelial cell line as an in vitro model we investigated the time course of morphological changes after SF/HGF treatment. Changes to cytoskeletal and cell–cell contact organisation and cell ultrastructure were examined at various times after exposure to SF/HGF using confocal microscopy, transmission electron microscopy (TEM) and scanning electron microscopy (SEM). Within the first 6 h of SF/HGF exposure an increase in paracellular space with the acquisition of highly irregular lateral cellular protrusions was found. This was followed by the formation of basal protrusions consisting of closely apposed extensions from 2 or more cells, which extended from the most basal points of contact between neighbouring cells. These protrusions formed nascent tubules by 24 h and contained large paracellular spaces. The invasion of the matrix by the extending nascent tubules was associated with a distortion of the cyst in the region of the protrusion, drawing the cells and luminal space into the extending tubule. By 72 h these extensions became large tubules, some of which had started to branch. The complexity of the tubular branching increased with time. SF/HGF treatment led to an increase of F-actin levels at the apical and lateral membranes. In addition we have found a change to the distribution of the apical marker gp-114 which, together with the altered F-actin arrangement, suggests a modification of cell polarity. Junctional plaques such as adherens junctions, desmosomes, and tight junctions persisted at the lateral membranes indicating maintenance of strong cell adhesion. Our findings suggest that SF/HGF induces a dramatic modification of the characteristically closely apposed lateral membranes and a partial loss of cellular polarity, while close cell–cell contact is maintained during these morphogenetic changes. This mechanism of tubulogenesis resembles reported in vivo epithelial tubulogenesis.

M. J. Williams is supported by the Anatomical Society of Great Britain and Ireland.

P2 Inducible expression of alpha smooth muscle actin in human placental microvascular endothelial cells correlates with a migratory phenotype. By J. DYE¹, P. CLARK¹, L. LEACH² and J. A. FIRTH¹. ¹*Division of Biomedical Sciences, Imperial College School of Medicine, London;* and ²*Faculty of Biomedical Sciences, University of Nottingham, UK.*

In sprouting angiogenesis endothelial cells may undergo a reversible transition between an epithelial phenotype and a migratory fibroblastoid phenotype. In cultures of Human Placental Microvascular Endothelial Cells (HPMEC) such transition may occur as cells are stimulated to proliferate and then to adopt a more quiescent state. Previously we have described the expression of the marker alpha smooth muscle actin (α SMA) in homogenous cultures of micro-

vascular endothelial cells, though these cultures were atypical of endothelial cells (Galustian et al. *In Vitro Cell Dev. Biol. Anim.* **31**, 1995). We now investigate whether the change of HPMEC from typical endothelial 'cobblestone' to spindle morphology is associated with the expression of α SMA.

HPMEC were cultured from isolated capillary fragments released from chorionic villi, obtained from caesarian section delivered placentae. Cells were fixed with paraformaldehyde and reacted with antibody against α SMA or markers of cell–cell adhesion, which were detected with Cy3-secondary antibodies. In some cases specimens were examined with laser scanning confocal microscopy. In accordance with previous work we found that all the primary cell outgrowths from isolated capillary fragments of chorionic villi were fibroblastoid and expressed α SMA. However subpopulations of these cells formed a cobblestone morphology which was stable through repeated passage. These cobblestone cultures were negative for α SMA but expressed adherens junction and tight junction components. When established monolayers of cobblestone cultures were overlaid with a type 1 collagen gel the monolayer was disrupted and cells were stimulated to migrate into the gel. The migrating cells showed induction of α SMA and reduced junctional localisation of cell–cell adhesion components such as cadherin, occludin and ZO-1.

In conclusion, under conditions in which HPMEC are induced to migrate they show induction of α SMA and a marked loss of cell–cell adhesion molecules. This behaviour may be relevant to angiogenesis in the placenta.

This work is supported by the Wellcome Trust and the British Heart Foundation.

P3 Uptake of microparticles in the small intestine during lactation. By S. H. SMYTH¹, M. ARASARADNAM¹, P. BROWN¹ and K. E. CARR^{1,2}. ¹*The Queens University of Belfast*; and ²*MRC Radiation and Genome Stability Unit, Harwell, UK.*

Pregnancy may affect uptake and motility in the gastrointestinal tract due to altered levels of circulating gastrointestinal hormones (Chang et al. *Gynecol. Obstet. Invest.* **45**, 1998). Another state in which hormonal levels are greatly altered is during the lactation period (Altemus et al. *J. Clin. Endocrinol. Metab.* **80**, 1995). This study aims to investigate whether lactation affects the uptake of large microparticles in the small intestine, a process that can have toxicological as well as pharmaceutical relevance.

Eighteen female 10 wk old Sprague–Dawley rats were randomly divided into 3 groups: early (d 1 and 2), mid (d 10) and late (d 20) lactation, with d 1 defined as the day the neonates were born. All adults were fed 0.25 ml fluorescent latex microparticles, 2 μ m in diameter, by gavage. Animals were killed by CO₂ asphyxiation 30 min after gavage and the small intestine divided into 9 equal segments. All procedures were carried out according to Home Office guidelines. Particle numbers in various sites were counted using epifluorescence microscopy of cryosections. The levels of uptake of these animals were compared with those for nonpregnant females (n = 6) and for pregnant animals in the 3rd trimester of their pregnancy (n = 6).

The results were analysed statistically using Mann–Whitney U tests. The total uptake of microparticles was higher in the pregnant group (547 particles) and lower during lactation when compared with the values from the nonpregnant group (330 particles). The total uptake of microparticles in the early lactation group (418 particles) was found to be statistically significantly higher than both the mid and late lactation groups (173 particles and 190 particles respectively).

P4 Influence of latex microparticle size on initial uptake in the rat small intestine. By M. CAMPBELL¹, S. H. SMYTH¹, R. A. HAZZARD¹ and K. E. CARR^{1,2}.

¹*The Queens University of Belfast*; and ²*MRC Radiation and Genome Stability Unit, Harwell, UK.*

Little has been known about the initial wave of microparticles crossing the intestinal wall since reports have concentrated on time periods when uptake was well established, such as 30 min after administration. This time point was used as a baseline for a study of localisation of tissue particles at 2 and 5 min. Fluorescent latex particles of 2 or 6 μ m diameter were fed by gavage to male Sprague–Dawley rats aged 7–8 wk old. Controls received distilled water. Asphyxiation at 2, 5, or 30 min was followed by removal of the small intestine and its subsequent division into 9 segments. All animal procedures were carried out in accordance with Home Office regulations. Complete circumference cryosections were cut. Epifluorescence microscopy was used to collect particle numbers for luminal and tissue based sites, although the latter were more relevant to uptake. Even 2 min after gavage 2 μ m particles had already progressed through the wall in the first 3 segments. By 5 min particles of both sizes had penetrated the wall, with the 2 μ m particles seen in greater numbers, more distally, and deeper in the wall than the larger particles. At 30 min there were more luminal particles but fewer in the wall for both particle sizes. Uptake at villous enterocytes occurred earlier and to a greater extent than at epithelium associated with Peyer's patches. The results therefore confirmed a peak in uptake 5 min after administration, with size determining initial entry time, rate of movement through the wall and distance reached down the intestine. Each of these uptake parameters may be associated with different aspects of the size differential.

P5 Substrate effects on neutrophil morphology in culture. By M. M. BIRD and A. W. SEGAL. *Biomedical Sciences, Queen Mary and Westfield College, London and Department of Medicine, Rayne Institute, University College London, UK.*

Neutrophils, phagocytes in blood, are the first cells to reach sites of infection and have the capacity to move rapidly over or through a variety of substrates. It is now established that the mechanisms for gliding or crawling locomotion involve the actin cytoskeleton. We are interested in investigating the role of the cytoskeleton in these cells by microinjecting molecules to perturb and/or label cytoskeletal components and the present study was undertaken to determine the optimal conditions in vitro for obtaining settled and spread cells suitable for microinjection experiments. Neutrophils

were purified from human blood by dextran sedimentation followed by centrifugation through a Ficoll/Hypaque gradient (Segal & Jones, *FEBS Lett.* **110**, 1980). The neutrophils (1×10^6 cells/ml) were resuspended in a serum free, HEPES-buffered Ham's F-12 culture medium and plated out onto glass coverslips either uncoated or coated with one of the following substrates: collagen, fibronectin, laminin, poly-L-lysine and maintained in culture for 60 min. The number of cells settling onto the substrate, the shape of the cells and the arrangement of the actin cytoskeleton were then assessed using fluorescent phalloidin. The results showed substantial differences in neutrophil morphology and settling ability for each of the substrates used. The vast majority of neutrophils settled onto collagen and flattened into a 'fried egg' shape in which the nucleus formed the central bulge. These cells were filled with actin stress fibres. A few cells remained rounded and contained only small numbers of actin stress fibres. The fibronectin substrate also resulted in the widespread settling and spreading of most neutrophils which were filled with actin stress cables but the cells were less flattened than on collagen. Fewest neutrophils settled onto laminin but those which did so settled well and contained abundant actin stress fibres. Many cells however remained rounded. An additional population of smaller star like cells also settled on the substrate. With poly-L-lysine the majority of cells remained rounded and settled only lightly. The actin cytoskeleton was distributed in a punctate manner, forming small interconnected aggregates rather than stress fibres. On uncoated glass, neutrophils were similar in appearance to those on poly-L-lysine but fewer cells survived the fixation procedure. The results show that neutrophils exhibited the greatest settling and flattening ability on the 2 substrates they most commonly encounter in vivo i.e. collagen and fibronectin. Actin stress fibres, transient contractile bundles anchored to the plasma membrane, allow cells to exert tension on the substratum when they contract. This process is important both in wound healing and morphogenesis and is thought to be important in coordinating neutrophil movement.

P6 Changes in neutrophil actin cytoskeleton with time in culture. M. BIRD and A. W. SEGAL. *Biomedical Sciences, Queen Mary and Westfield College, London and Department of Medicine, Rayne Institute, University College London, UK.*

Neutrophils in culture on a fibronectin and/or collagen substrate rapidly settle on the substrate and become filled with actin-stress fibres. These fibres are transient contractile bundles of actin filaments anchored to the plasma membrane at focal adhesions (Burridge & Chzanowska-Wodnicka, *Annu. Rev. Dev. Biol.* **12**, 1996). The neutrophil has intracellular stores of both membrane proteins and soluble proteins that may be incorporated into the plasma membrane and exocytosed at different times to meet the demands for assisting the neutrophil in adhesion, migration and phagocytosis. The purpose of this study was to investigate the distribution of actin with time within neutrophils in culture. Neutrophils were purified from fresh human blood by dextran sedimentation followed by centrifugation through a Ficoll/Hypaque gradient (Segal & Jones, *FEBS Lett.* **110**, 1980). They were then resuspended in HEPES-

buffered, Ham's F-12 culture medium and plated out onto glass coverslips coated with collagen and fibronectin. Neutrophils were maintained at 37 °C for periods ranging between 60 min and 24 h. Fluorescent phalloidin was subsequently used to investigate changes in the actin cytoskeleton. After 60 min in culture 95% of the settled neutrophils were filled with actin stress fibres. After 3 h the appearance of about 1% of the neutrophils had begun to change with the dispersal of the actin stress cables as the neutrophils developed processes. These processes radiated out from the cell body and sometimes extended far into the surrounding medium. The actin was differently distributed in these cells. Actin either migrated and collected at the tips of processes or was concentrated in a mass within the cell body. Gradually, with further time in culture, the distribution of actin changed in the majority of cells. Few were packed with stress fibres. Instead a network of small almost spherical aggregates of interconnected actin was spread throughout the cytoplasm. This distribution of actin was retained over the remaining period in culture. The actin cytoskeleton provides the protrusive and contractile forces required for cell adhesion and migration through a combination of cross-linking and interaction of myosin-based motors with actin filaments. Neutrophils are the phagocytes of circulating blood and are the first cells to arrive at sites of infection. Shape changes and motility are therefore essential for host defence and require the dynamic reorganisation of the actin cytoskeleton by the reversible polymerisation of G-actin into filaments. The results of this investigation indicate that actin stress fibres are essential for adhesion to the substrate and that the subsequent changes in the actin cytoskeleton are necessary for cell migration and the maintenance of cell shape.

P7 The release of mercury from fresh and aged abraded dental amalgam and its effects on sensory neurons. By M. SWEENEY^{1,2}, S. L. CREANOR², R. A. SMITH¹ and R. H. FOYE². ¹Laboratory of Human Anatomy/IBLS, University of Glasgow; ²Hard Tissue Group, Glasgow Dental School, UK.

Mercury has been used as a dental restorative material for over 150 y. Dental amalgam however is not inert. This has caused continued concern since mercury may be released into the intraoral air from where it is inhaled or swallowed to be distributed throughout the body. Since its pathological actions on the nervous system are well documented, the present study aimed firstly to determine the release of mercury from amalgam in the different conditions of fresh, abraded, dry and when covered with saliva. Secondly, in order to test for neurotoxicity, cultures of adult sensory neurons were treated with mercury at representative concentrations comparable with those present in aqueous fluid during polishing an amalgam. Air mercury levels were estimated from 8 fresh and 25 aged abraded amalgam pellets over a 20 min period by use of a vapour analyser. Total mercury content of saliva applied to the pellets was analysed by high sensitivity spectrometry. Dorsal root ganglia (DRG) cultures were prepared from 9 adult mice, killed humanely by CO₂ asphyxiation, and following 24 h treatments with 0–10 µM mercuric chloride they were monitored and stained for neuropeptide Y (NPY) immunoreactivity as previously

described (Baxter & Smith, *Neurosci Lett* **246**, 1998). Mercury levels of $13.5 \pm 4.5 \mu\text{g}/\text{m}^3$ were recorded in air samples from dry fresh amalgam pellets compared with $6.75 \pm 2.5 \mu\text{g}/\text{m}^3$ when covered with saliva. Higher levels were recorded in air from aged abraded pellets: $36 \pm 8.7 \mu\text{g}/\text{m}^3$ in dry conditions (above the recommended industrial limit) and $10.2 \pm 3 \mu\text{g}/\text{m}^3$ when coated with saliva. Total mercury in saliva exposed to fresh amalgam varied from 15–298 $\mu\text{g}/\text{l}$, with higher levels for abraded amalgam

ranging from 46 to 7774 $\mu\text{g}/\text{l}$. These levels were used in planning the neuronal study. Neuronal cultures treated with 1 μM mercuric chloride did not differ in their morphology or in NPY staining compared with untreated control cultures. 10 μM doses however caused a reduction in cell adhesion, neurite shrinkage and cell loss. NPY-immunoreactive neurons were still observed. These preliminary findings suggest care should be taken in polishing abraded amalgam, with preference for a moist atmosphere.