# 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 3rd to 5th January 2001. It included a symposium on 'The evolution of developmental mechanisms' and the Annual General Meeting of the Society. The following are abstracts of communications and posters presented at the meeting.

## TALKS

**1 Embed in an iodinated polymer: a new paradigm for histology via backscattered electron imaging.** By A. BOYDE[1] and L. H. KOOLE[2]. [1] *Department of Anatomy and Developmental Biology, University College London, UK;* [2] *Center for Biomaterials Research, University of Maastricht, The Netherland.*

Iodine has been used as a contrast agent in prior applications in x-ray and electron microscopic imaging. Here we used methacrylate copolymers with iodine covalently linked to their backbone structure as embedding medium. These materials were prepared from methylmethacrylate (MMA) and 2-[4'-iodobenzoyl]-oxo-ethylmethacrylate (4-IEMA) (Kruft et al., *J. Biomed. Mater. Res.* **28**, 1994). Mouse and horse bone samples fixed in NFS or 70% ethanol were embedded using a molar ratio of 4-IEMA:MMA of 1:3.6 giving an empirical formula of $C_{31}H_{41.8}O_{11.2}I$. Block surfaces were finished by diamond ultramilling and carbon coated. Digital backscattered electron (BSE) images were recorded using a Zeiss DSM962 SEM. Images at nominal 500X and 2048* pixel resolution at 20 kV showed no evident beam damage. At 30 kV, increasing the BSE yield and thereby the working distance and field of view, we could image wide fields (e.g. 15 mm) in a single scan. Image contrasts in cells and soft tissues reflect the inverse of the mass concentration of organic solids retained after the embedding protocol: proteins, DNA, RNA are retained; lipids, being soluble in ethanol and methyl methacrylate monomer are lost, but this is exactly as in conventional histological procedures. Advantages over the latter concern our ability to prepare very flat and undeformed samples. Our material is a solid block, with one or more surfaces flattened to $< 0.1$ μm roughness. At 20 kV, the information depth is of the order of 0.5 μm. It is not possible to generate extensive physical sections that are so thin. Furthermore, sections of mixtures of tissues with varying degrees of hardness are deformed and do not lie within a conventional optical section plane. Thus the new method may have significant applications in discreet problem areas.

**2 Comparison of expression patterns of Sox group E in the developing chick central nervous system suggests specific roles in different glial lineages.** By Y.-C. CHENG, C.-J. LEE and P. J. SCOTTING. *Institute of Genetics, University of Nottingham, UK.*

At least 30 *Sox* (*S*RY-b*ox* related) genes have been identified in different species to date and divided into 7 groups, designated A–G. Members of the same group usually share $> 80\%$ amino acid identity within the HMG domains. *Sox* subgroup E comprises *Sox8*, *Sox9*, and *Sox10* which showed high degree of homology and appear to have conserved intron and exon boundaries. Mutations in *SOX9* causes campomelic dysplasia and autosomal XY sex reversal, mutations in *SOX10* cause Waardenburg-Shah syndrome and Yemenite deaf-blind hypopigmentation syndrome, and recently SOX8 has been suggested a candidate gene for contributing mental retardation phenotype in ATR-16. All 3 genes have been shown to be highly expressed in the developing CNS but this expression has not been studied in detail. In order to study the biological functions of this highly conserved subgroup in developing CNS, we here initially analysed the expression patterns of these genes in the chick by in situ hybridisation. Serial analysis of gene expression (SAGE) was also used. The results show that all 3 genes are expressed in neuroglia in the developing CNS but with different degrees of expression in astrocytes and oligodendrocytes.

**3 Cell movement and differentiation during secondary myogenesis in the rat.** By S. PINTO CARDOSO, P. MUTCH and P. M. WIGMORE *School of Biomedical Sciences, University of Nottingham, UK.*

During development, 2 successive waves of muscle fibres are formed. The first wave between E13–E15 produces primary fibres and the second wave leads to the formation of secondary fibres (E17–birth). Secondary fibres use the primaries as a scaffold to form on their surface. We are interested in cell position, movement and differentiation on the surface of primary fibres during secondary myogenesis, to understand the mechanisms responsible for bringing differentiating cells to the correct position to fuse. Previous work showed that cells are randomly distributed in early stages of secondary fibre formation and then become clustered beside existing secondary fibres (Wigmore et al., *Dev. Dyn.* **207**, 1996). BrdU was injected into E20 pregnant Wistar rats 2 and 24 h before killing them by cervical dislocation. 2 h after injection, cells can be classified in 3 different positions. Cells in position A were found on the surface of primary fibres but were not in contact with secondaries. 30 % of these cells were BrdU-positive. Cells in position B were found in contact with both primaries and secondaries. The majority were BrdU-positive. Cells in position C are wedged between a primary and a secondary fibre and none where found to be BrdU-labelled. We hypothesise that cells move from position A or B, where they are still dividing, to position C, where they differentiate. 24 h after BrdU-pulse labelling, we found BrdU-positive

https://doi.org/10.1017/S0021878201357977 Published online by Cambridge University Press

cells in position C. This indicates that dividing cells move to position C within 24 h. Cells in position C seem to be differentiating and will fuse with adjacent fibres. We are currently trying to correlate this labelling pattern with the expression of myogenin and p21 in order to show that cell differentiation is correlated with cell position.

**4 High glucose potentiated vascular smooth muscle cell chemotaxis is mediated by the PDGF-$\beta$ receptor via PI(3)kinase and MAPkinase.** By M. CAMPBELL, E. R. TRIMBLE and W. E. ALLEN. *Department of Clinical Biochemistry, The Queen's University of Belfast, UK.*

In atherosclerosis it is known that vascular smooth muscle cells (VSMCs) change from a contractile to a secretory phenotype, and that they proliferate and migrate from the tunica media into the subendothelial area. The aim of this study was to investigate the effects of elevated glucose concentrations on the migratory and chemotactic behaviour of cultured porcine and human VSMC using the Dunn chemotaxis chamber assay. This assay allows the behaviour of small numbers of cells to be directly monitored in a stable gradient of a chemoattractant. In this study a gradient of fetal calf serum (FCS) was used. Porcine and human VSMCs were incubated for either 24 h or 10 d in 25 mM glucose before being placed in the gradient of FCS within the chamber, or were cultured in normal glucose (5 mM) and placed in 25 mM glucose only within the chamber. Control assays were run with 5 mM glucose with a FCS gradient and with 25 mM glucose in the absence of a FCS gradient, and with 25 mM L-glucose. Control assays showed no chemotaxis to the FCS, while all 25 mM glucose assays in a FCS gradient showed positive chemotaxis towards the source of the FCS. To determine at what concentration glucose induces this behavioural change, assays were run with concentrations of 10 mM, 15 mM and 20 mM glucose. Chemotaxis to FCS was induced at all these concentrations. Immunofluorescent staining of human VSMCs showed that 25 mM glucose induced an increase in the PDGF-$\beta$ receptor and the c-fms receptor for CSF-1. Western blotting confirmed this observation. Porcine VSMCs pre-incubated with an antibody against the PDGF-$\beta$ receptor in 25 mM glucose failed to show chemotaxis indicating that this receptor mediates the glucose potentiated chemotaxis to FCS. Use of the inhibitors H7 (PKC), wortmannin and LY-294002 (PI(3)K) and PD-98059 (MAPK) showed that inhibiting PI(3)K and MAPK but not PKC inhibited the glucose potentiated chemotaxis to FCS. This study has shown that high concentrations of glucose induce VSMC to become more sensitive to serum factors than in normal glucose concentrations and that this can lead to altered migratory behaviour, which may contribute to the increased incidence of atherosclerosis seen in diabetes.

**5 The complex evolution of the hominid cranial venous system.** By J. M. GRIFFITHS[1], (supervised by M. M. GUNTHER[2]). [1] *Evolutionary Anthropology Research Group, Department of Anthropology, University of Durham; and* [2] *Primate Evolution and Morphology Group, Department of Human Anatomy and Cell Biology, University of Liverpool, UK.*

Human evolution is often said to be characterised by the evolution of the brain and bipedal gait. Dural venous sinuses should therefore be key structures in the study of hominid evolution, being intimately related topographically to the brain and physiologically to body posture. Whilst the methodology of recent studies have been based on the study of vestigial impressions in fossil hominid and macerated human crania, these are unlikely to be an entirely accurate representation of sinus morphology in life. Anatomically modern humans should, by virtue of their habitual orthograde posture and large brain size, require a specially adapted cranial venous system. Yet the range of variation in dural sinus morphologies, i.e. the capacities of individual sinuses, their connections and drainage sites, is extraordinary. An extensive review of what is known about the primate cranial vascular anatomy indicates that intraspecific variation is present in all primate groups and not restricted to *Homo sapiens* alone. As such there appears to be no evidence that dural venous sinus systems need have 'fixed' morphologies in order to function consistently between members of a species. Moreover, the evolution of the cranial venous system must be consistent with the development and function of the skull and its associated soft tissues. Changes in the pattern of growth and development of the brain, skull and masticatory system may lead to changes in dural venous sinus morphology. Features of the cranial venous systems of certain hominid species previously considered adaptations toward habitual orthograde posture may therefore instead be a side effect of other distinct evolutionary processes.

**6 A novel homeobox gene *Atx* may specify the midbrain and pretectum.** By R. N. GOGOL, F. SCHUBERT, A. SIMEONE and A. LUMSDEN. *MRC Centre for Developmental Neurobiology, Guy's Campus, King's College London, UK.*

Specification, patterning and regionalisation of the brain poses a developmental problem. Current evidence suggests that the hindbrain is segregated along the rostrocaudal axis into a number of developmental compartments known as rhombomeres. The positional identity of each rhombomere is defined by the combinatorial expression of highly conserved transcription factors as a 'Hox Code' of information.

The patterning of the rostral brain is far less understood. The neural segmental paradigm offers a model for the development of the rostral brain, however information regarding the molecular mechanisms for specification of individual forebrain and midbrain domains are still elusive.

The fundamentals of forebrain specification have been identified. Knockout studies show that mouse *Otx2* is required for anterior neural plate specification and the development of the anterior brain. It is therefore possible that mouse *Otx2* is a pivotal gene in a hierarchy of related genes that specify and impart regional identity to the

developing midbrain and forebrain in a combinatorial manner.

We have isolated a novel chick homeobox gene '*Atx*' that may be involved in specifying the midbrain and posterior diencephalon. Sequence analysis has shown that the homeodomain is highly concordant to mouse *Otx2*, *dOtd*, *Crx* and other $K_{50}$ paired-type homeobox genes. *Atx* may be the founding member of a family of *Otx*/*Otd* related paired-type homeobox transcription factors.

Chick *Atx* expression is observed at the time of neural induction in a subdomain of chick *Otx2* in the rostral brain anlage. Later in development *Atx* expression is observed rostral to the midbrain–hindbrain boundary, sharing the same caudal boundary as *Otx2*. The anterior limit of expression is at the prospective boundary between the pretectum and dorsal thalamus.

Chick *Atx* may be involved in defining the pretectum–dorsal thalamus boundary and may specify regional identity to the midbrain–pretectum in a combinatorial manner with other homeobox transcription factors such as *Otx2* and *En-2*.

**7 Temporal and spatial expression of cNgn1 and cNgn2 in the developing chick retina.** By D. LEROUËDEC, M. CHEUNG, P. J. SCOTTING and P. M. WIGMORE. *School of Biomedical Sciences*, *University of Nottingham*, *UK*.

Neurogenesis is a process whereby an undifferentiated cell turns into a fully differentiated neuron. Neurogenins are members of the basic helix-loop-helix (bHLH) family and have been shown to play an active role in neurogenesis. We are focusing on 2 members of this family, Ngn1 and Ngn2. These early transcription factors are expressed in a variety of neuronal tissues such as the developing CNS, the neural tube, and the peripheral sensory neurons. Targeted inactivation of these genes has indicated that they are required early in different subsets of sensory neurons (Fode et al., *Neuron* **20**, 1998) and ectopic expression of the Ngns in neuronal and non-neuronal tissue can induce the expression of sensory neuron markers (Perez et al., *Development* **126**, 1999).

In order to understand their role in more depth in the developing CNS, we have studied the expression pattern of Ngns in the developing chick retina. This study has revealed that cNgn1 and cNgn2 are both expressed in chick retina, in contrast to the developing mouse retina, where only Ngn2 has been detected. In addition, these 2 genes seem to be expressed in a complementary pattern throughout maturation of the retina, as they exhibit overlapping or partial overlapping expression patterns in some regions at different stages of development. This is similar to the developing mouse CNS where Ngns exhibit partial or complementary overlapping expression pattern in some areas (Sommer et al., *Molecular and Cellular Neuroscience* **8**, 1996).

To further define the role of Ngns in neuronal maturation we have combined endogenous detection of these genes with BrdU labelling. This marker labels specifically the progenitor cells population. Our study suggests that Ngns are expressed in late dividing/early differentiating neurons, and seem to be transiently required in all neuronal cell types that progressively appear throughout development. The extent to which their redundancy reflects functional compensation in common or distinct precursors in not yet clear. Ectopic expression of Ngns is under way to unravel their role as neuronal determination factors in the retina and ultimately the CNS.

**8 Role of chick Sox11 in central nervous system development.** By M. CHEUNG, M. TAHMASEB, D. LEROUËDEC, M. ABU-ELMAGD and P. J. SCOTTING. *Division of Genetics*, *Queen's Medical Centre*, *Nottingham University*, *UK*.

Sox (*Sry*-related *box*) genes belong to a transcription factor family, which contain a conserved HMG domain responsible for sequence specific DNA binding. All Sox genes that have been identified so far can be placed into one of 7 subgroups (A–G) according to their sequence similarities both within and outside the HMG box. Many of these genes have been shown to be expressed in the central nervous system. Examples of these genes are Sox1, Sox2 and Sox3 which belong to group B and have been shown to exhibit overlapping expression in the ventricular zone which is rapidly downregulated when cells migrate and differentiate, while Sox4 and Sox11 group C Sox genes are upregulated in the region where neuronal cells undergo differentiation. Thus, neural differentiation is characterised by an ordered switch from one group of Sox proteins to the next.

Expression analysis of Sox11 in mouse and chick CNS has suggested a role of this gene in neuronal maturation. Since Sox11 null mutants have not been generated, the role of Sox11 in regulating neural development is still poorly understood. In order to study the function of Sox11 in the CNS, we have misexpressed chick cSox11 (cSox11) GFP expression vector into the chick spinal cord region by electroporation. Preliminary results showed that overexpression of cSox11 generated a distorted neural tube, which may be due to an effect on neuronal differentiation.

**9 Quantitative genetics of shape in the mouse mandible.** By C. P. KLINGENBERG[1] and L. J. LEAMY[2]. [1] *Laboratory for Development and Evolution*, *University Museum of Zoology*, *Cambridge*, *UK; and* [2] *Department of Biology*, *University of North Carolina at Charlotte*, *USA*.

The mouse mandible has long served as a model for complex anatomical structures in developmental and genetic studies. We use it to examine genetic and phenotypic variation in shape and size. We use the new methods of geometric morphometrics in the context of quantitative genetics. We characterise mandible shape as the geometric configuration of 11 morphological landmarks, using a Procrustes superimposition to compute a set of shape variables amenable to standard multivariate analyses. Comparison of shape variation in samples of mice aged 30, 90 and 150 d suggests that growth and bone remodelling do not cause major changes in the patterns of variation of shape over this period. A parent-offspring design allows us to estimate the additive genetic covariance matrix of shape. We use principal component analysis to extract the dominant features of variation from the genetic and phenotypic covariance matrices. Much of the variation is concentrated

in the angular and coronoid processes, in line with findings from studies of quantitative trait loci and of the phenotypes resulting from gene knockouts. Finally, we use the estimates of phenotypic and additive genetic covariance matrices to compute those features of mandible shape that would respond most easily or would be most resistant to natural selection, illustrating the genetic constraints imposed by the development of the mandible.

**10 Investigation of the evolutionary developmental genetics underlying hypercephaly in stalk-eyed flies.** By I. HURLEY, K. FOWLER, A. POMIANKOWSKI and H. SMITH. *Biology Department*, *University College London*, *UK*.

Little previous knowledge exists concerning the developmental genetics of sexually selected traits. We investigated this through the study of hypercephaly in the stalk-eyed flies *Cyrtodiopsis dalmanni* and *Spyracephala beccarri*. We adopted an evolutionary developmental method that enabled us to take advantage of the broad understanding of *Drosophila* genetics. We have shown that the difference in adult head morphology between *Drosophila* and stalk-eyed flies is mirrored in the contrasting shape of their eye-antennal discs. Through the use of immunohistochemistry and in situ hybridisation, we have examined key regulators of *Drosophila* head development, in stalk-eyed flies. Our expression data establishes the regional identity of the stalk-eyed fly eye-antennal disc and provides an insight into the genetic basis of hypercephaly, a sexually selected trait.

## POSTERS

**P1 Regionalisation of the brain as an evolutionarily conserved developmental mechanism.** By E. GALE, M. ZILE and M. MADEN. *Centre for Developmental Neurobiology*, *King's College London*, *UK*.

Comparative studies of chordate neural connectivity and gene families have provided evidence for evolutionary conservation of the patterning mechanisms in brain development (review Holland & Holland, *Curr. Opin. Neurobiol.* **9**, 1999). Based on expression patterns of ascidian and amphioxus homologues of the *Otx* gene and the *Hox1* gene and of the ascidian *Pax-2/5/8*, the chordate brain has been suggested to have tripartite development (Wada et al., *Development* **125**, 1998; Kozmik et al., *Development* **126**, 1999). Primitively, the chordates have regions homologous to the vertebrate forebrain, anterior midbrain and posterior hindbrain while the posterior midbrain/anterior hindbrain region seems to be a vertebrate innovation. The extent of the homologies within each of these regions between the vertebrates and their ancestors is not fully determined but the similarity of *Hox* gene expression patterns suggests organisational constants over evolutionary time within the posterior hindbrain region.

Identification of the posterior hindbrain region as a developmental unit in vertebrates is demonstrated in the retinoid-deficient quail. Embryos laid by quails fed a retinoid-deficient diet have no posterior hindbrain while the anterior hindbrain is specified normally. Through DiI cell lineage tracing and a temporal analysis of gene expression characteristic of this region (*Krox-20*, *Hoxb-1*, *mafB*, and *fgf3*), we have followed the development of this region of cells. From the initial formation of the neural plate phenotype in the retinoid-deficient quail, there is no evidence of a posterior hindbrain. This region is never specified and all the cells of the hindbrain participate in an anterior hindbrain fate. A single retinoid injection in ovo during early development completely rescues the posterior hindbrain ensuring that the phenotype was the result of a single stimulus. Therefore cells from the posterior hindbrain respond in a coordinated regional manner to the presence or absence of a single gene inducer, retinoic acid.

We present evidence of regionalisation of the vertebrate head that is up stream of segment specification. In combination with data from amphioxus and ascidians, this may represent a common mechanism for head development throughout chordate evolution. Interestingly, regional deletion with enlargement of the adjacent region is very reminiscent of the gap gene phenotype in Drosophila. It would be disregarding millions of years of divergent evolution to suggest that vitamin A is identical to a Drosophila gap gene inducer; nevertheless this data supports the hypothesis of common underlying regulation of axial regionalisation and gene hierarchies.

**P2 An immunocytochemical and in situ hybridisation study of embryonic development of the human diencephalon.** By G. CLOWRY[1], S. LISGO[2], D.-M. HAGAN[2], S. ROBSON[3], T. SRACHAN[2] and S. LINDSAY[2]. [1] *Department of Child Health*, [2] *Institute of Human Genetics and* [3] *Department of Obstetrics and Gynaecology*, *University of Newcastle upon Tyne*, *UK*.

In order to understand gene expression patterns in human embryos for a number of genes known to be of importance to normal brain development and to underlie certain developmental disorders, we have supplemented our in situ hybridisation studies with immunostaining for a number of developmental markers. Here, we present expression patterns for the transcription factors *PAX6*, *PAX3* and *OTX2*, the ventralising signalling molecule sonic hedgehog (*SHH*) and immunostaining for nestin (a radial glia marker), $\beta$-tubulin (marker for postmitotic neurons), MAP2 (a marker for differentiating neurons) and GAP43 (a marker for growing axons) in the human diencephalon between Carnegie stages 15 and 21 (33–52 postovulatory days). Human embryos were taken with maternal consent following termination according to Local Ethics Committee Regulations. They were fixed by immersion in paraformaldehyde, embedded in paraffin and oblique transverse serial sections were cut. In situ hybridisation was carried out using radioactively labelled RNA probes, and immunocytochemistry was performed with well characterised, commercially available antibodies using standard methods. *SHH* expression was confined to the basal plate with the exception of the zona limitans intrathalamica (ZLI) where a band of expression formed a boundary between the alar

https://doi.org/10.1017/S0021878201357977 Published online by Cambridge University Press

plates of prosmeres 2 (dorsal thalamus) and 3 (ventral thalamus). The ZLI was bounded by *PAX6* and *OTX2* hybridisations and was the site of unusually strong $\beta$-tubulin immunoreactivity in the ventricular layer. Nestin immunoreactivity was strong in the ventricular zone and also in alar plate regions where a radial arrangement of glial fibres could be seen. In basal plate regions nestin positive glial fibres were not so evident. However MAP2 immunoreactivity was prevalent in basal regions showing that neuronal development is further advanced compared to alar regions. GAP43 immunostaining indicated that development of longitudinal axon pathways occurs predominantly through the ventral regions of the forebrain at these stages. Ventral thalamus development precedes dorsal thalamus development as shown by its thinner ventricular layer and a thicker intermediate layer that is also strongly MAP2 immunoreactive. The outgrowth of the thalamocortical projection from dorsal thalamus through the ventral thalamus had not begun at this age, judging by the lack of GAP43 immunoreactivity. In addition *PAX6* expression was seen in both intermediate and ventricular layers in the ventral thalamus. *PAX3* expression was confined to the roofplate region of prosomeres 1 and 2 corresponding to the pretectum and epithalamus. Therefore our results indicate that by CS21, human diencephalon development is less advanced than the mouse diencephalon at the generally accepted equivalent stage (embryonic day 13–13.5). However development of the human diencephalon was found broadly to conform to the prosomeric model of forebrain development of Puelles & Rubinstein (Puelles, *Trends Neurosci.* **16**, 1993).

**P3 Correlation of BMP-2, BMP-4 and MSX-2 with differentiation and apoptosis in remodelling of the endocardial cushion in the developing heart.** By E. ABDELWAHID[1,2], D. RICE[3], L. J. PELLINIEMI[4] and E. JOKINEN[5]. [1] *Department of Pediatrics, and* [2] *MediCity Research Laboratory, University of Turku;* [3] *Developmental Biology Program, Institute of Biotechnology*3, *Helsinki;* [4] *Laboratory of Electron Microscopy, University of Turku; and* [5] *Department of Pediatrics, University of Helsinki, Finland.*

Cardiovascular malformations are the most common birth defects and the majority of them are due to abnormal septum and valve formation. The bone morphogenetic proteins BMP-2 and BMP-4 and the homeobox gene MSX-2 are required for normal development of many embryonic tissues. To elucidate their possible roles during the remodeling of the tubular heart into a fully septated 4 chambered heart; we have localised the mRNA of *Bmp-2*, *Bmp-4*, *Msx-2* and apoptotic cells in the developing mouse heart from embryonic day (E) 11 to E17. mRNA transcripts of *Bmp-2*, *Bmp-4* and *Msx-2* were localised by in situ hybridisation, and apoptotic cells by TUNEL as well as by transmission electron microscopy. By analysing adjacent tissue sections we demonstrated that the expression of *Msx-2* and *Bmp-2* overlapped in the atrioventricular canal, in the atrioventricular junction and in the maturing atrioventricular valves. Interestingly, *Bmp-4* was expressed in the outflow tract myocardium and in the endocardial cushion of the outflow tract ridges from E12 to E14. *Msx-2* appeared in the endocardial cushion from E11 to E14, while *Bmp-2* and *Bmp-4* were detected between E11 and E14. Apoptotic cells were also detected in the endocardial cushion between E11 and E14. Our results suggest that BMP-2 and MSX-2 are tightly linked to the formation of the atrioventricular junction and valves and that BMP-4 is involved in the development of the outflow tract myocardium and of the endocardial cushion. In addition, BMP-2, BMP-4 and MSX-2 and apoptosis seem to be associated with differentiation of the endocardial cushion.

**P4 Expression analysis of a novel gene expressed in the otic placode of chickens.** By H. MAROON[1], J. CHRISTIANSEN[2], D. WILKINSON[2] and I. MASON[1] [1] *MRC Centre for Developmental Neurobiology, GKT Medical School, London; and* [2] *National Institute for Medical Research, London, UK.*

The otic placode is a vertebrate embryonic structure that gives rise to the inner ear. It is morphologically visible as a thickening of the ectoderm adjacent to the hindbrain by HH10 in the chicken embryo. Expression of various genes have been reported at earlier stages in the presumptive otic ectoderm (POE). However genes such as *pax2*, *sox3* and *gbx2* that are commonly used as otic placode markers are expressed in domains larger than just the POE. Here we report the expression pattern of a novel gene that marks the POE more definitively. *2F6* was cloned in a differential screen. Expression of *2F6* transcripts in POE is first detected as early as HH7 and continues until the placode invaginates to form the otic vesicle. *2F6* transcripts are also detected in extraembryonic tissues, posterior ectoderm and the forebrain.

**P5 Nasal clefting and abnormal apoptosis in *Alx3*/*Alx4* double mutant mice.** By A. BEVERDAM, A. BROUWER, M. REIJNEN, J. KORVING and F. MEIJLINK. *Netherlands Institute for Developmental Biology, Utrecht, The Netherlands.*

Alx3, Alx4 and Cart1 are *paired* related homeodomain proteins that belong to a separate subclass of this family characterised by the presence of a conserved domain at the C-terminal end of the protein, the *aristaless* domain (Meijlink et al., *Int. J. Dev. Biol.* **43**, 1999). *Alx3, Alx4* and *Cart1* are expressed in overlapping domains in the neural crest derived mesenchyme of presumptive craniofacial structures and in the mesenchyme of the limb regions of the developing mouse embryo. The high degree of conservation and the overlapping expression domains of these proteins suggest them to have overlapping functions. *Alx4* mutant mice have mild abnormalities in the skull (Qu et al., *Development* **125**, 1998). *Cart1* mutant mice have open brains due to defective neural tube closure (Zhao et al., *Nat. Gen.* **13**, 1996). *Alx4* and *Cart1* double mutant mice show enhanced limb and craniofacial abnormalities (Qu et al., *Development* **126**, 1999).

We have generated *Alx3* mutant mice by insertion of the *LacZ* gene upstream of the homeodomain. These mice do not show obvious defects, suggesting that *Alx4* and *Cart1*

compensate for the function of *Alx3*. We found that compound mutants of *Alx3* and *Alx4* show severe abnormalities including split face in craniofacial regions comparable to the defects observed in the *Alx4/Cart1* double mutant mice. In newborn *Alx3* and *Alx4* double mutant mice the nose region seems reduced and both lateral halves are wide apart. Most facial bones and many other neural crest derived skull elements appeared malformed, truncated or even absent. We found that the craniofacial defects become evident around E10.5 when the nasal processes become very prominent structures in the facial region of the embryo. The critical event leading to the split face phenotype is a failure of the medial nasal processes to fuse in the facial midline. We studied cellular and molecular mechanisms that underlie this abnormal growth of the nasal processes and detected a significant increase in apoptosis in the outgrowing frontonasal mass of E10.0 double mutant embryos, which might cause the observed defects.

**P6 Temporal contribution of myoblasts to avian limb muscles.** By E. REES and D. J. R. EVANS. *Cardiff School of Biosciences, Cardiff University, UK.*

Skeletal muscles of the limb are derived from myogenic precursor cells (myoblasts) that migrate into the developing limb bud from the adjacent somites. Our previous studies have mapped the source of myoblasts for all limb muscles and defined the distinct patterns of myoblast distribution within the limb. In the present study we focus on whether the time of migration from the somites into the limb dictates the spatial contribution these cells make to the developing musculature, particularly in relation to the proximal-distal and dorsal-ventral axes. Injections of replication-deficient LacZ encoding retroviruses were made into precisely recorded somites of stage 14–18 White Leghorn chick embryos at somite stages I, IV and VII. Embryos were killed by cervical dislocation at stage 36, fixed and stained histochemically for $\beta$-galactosidase in order to identify infected cells and their progeny. Injections into individual somites gave rise to a highly selective pattern of labelling within the developing limb, supporting the suggestion that myoblasts are not randomly distributed throughout the limb mesenchyme. In most instances a single injection resulted in a number of muscles being subsequently labelled, with consistent contributions made by precursors of individual somites to muscles in thigh, shank and digit regions. In most cases there were no obvious differences in the patterns of labelled muscles resulting from progressively later injections, and most resulted in labelling in proximal as well as distally located muscles. This is in marked contrast to other results, which have suggested that later migrating myoblasts are increasingly restricted to more distally placed muscles. Later injections sometimes resulted in less vivid labelling than after earlier injections and this probably reflects the gradually decreasing pool of available progenitor cells in the somite at the time of injection, as some cells would already be migrating into the limb. Overall our results indicate that both early and late migrating myoblasts are capable of contributing to muscles within all regions of the developing limb.

(Supported by the Wellcome Trust).

**P7 Morphogenesis of the muscle-tendon interface during avian hindlimb development.** By S. F. OLDFIELD, J. R. RALPHS, M. BENJAMIN and D. J. R. EVANS. [1] *Cardiff School of Biosciences, Cardiff University, UK.*

Little is known about the intimate interactions involved in the early establishment of the muscle-tendon interface, but it is likely that coordinated development of distinct tissue lineages is required. In the first phase of our study, investigating the formation of the muscle–tendon interface, we have focussed on aspects of muscle and tendon morphogenesis in the embryonic chick limb and in particular the spatial and temporal integration of muscle and tendon precursor populations. Following cervical dislocation, hindlimbs were removed from White Leghorn chick embryos at stages 23–37 (Hamburger & Hamilton, 1951). Limbs were processed for routine histology, serially sectioned at 10 μm and stained with either Alcian blue/haematoxylin-eosin or Masson's trichrome. Detailed examination was made of the shank region, focussing particularly on the cleavage events within muscle masses and tendon blastemas. Our results show that cleavage of the dorsal and ventral muscle masses occurs between stages 27 and 32, when the basic muscle pattern becomes evident. In agreement with other studies we also demonstrate that during this period, the process of conversion of muscle masses into individual muscles can follow several different scenarios. After stage 32, each individual muscle condensation enlarges to accommodate the continued generation of myotubes and becomes further defined by the surrounding and differentiating connective tissues. Detailed examination of the proximal attachment sites of particular groups of muscles revealed unique patterns of attachment that probably result from the preceding delineation events. The separation of tendon blastemas follows a similar scheme to that of the muscle masses, with dorsal and ventral condensations splitting into a distinct pattern of individual immature tendons. Muscle and tendons precursors first interact during stages 25–26 and gradually become increasingly integrated between stages 32 and 34, when the muscle–tendon interface appears overtly organised. This process of maturation continues after stage 34 as the basement membrane begins to form around individual muscle fibres and the myotendinous junction is established. Our study provides essential baseline data for investigating the early interactions involved in the establishment of the muscle–tendon interface.

(Supported by the Wellcome Trust).

**P8 The effects of ethanol on neurodevelopment in the chick embryo spinal cord.** By J. GILES, P. BOEHM, C. BROGAN and J. BANNIGAN. *Department of Human Anatomy and Physiology, and Conway Institute, University College Dublin, Ireland.*

Although central nervous system (CNS) dysfunction is an important feature of the Fetal Alcohol Syndrome (FAS), the effects of alcohol (EtOH) on neuronal development are not clearly understood. We decided to examine the effects of EtOH on a relatively simple and accessible programme of neuronal differentiation and migration, namely that of the chick embryo spinal cord just after neurulation. This is a stage when postmitotic neurons are leaving the neuroepithelium (NE) and migrating to the marginal zone.

Chick embryos were explanted into shell-less culture after 48 h incubation in ovo (H.-H. stage 12–14). They were treated with either 20 µl of 40 % (v/v) EtOH or 20 µl normal saline. Embryos were fixed in Karnovsky fluid and processed into Araldite at 6, 12, 24 and 48 h after treatment. Serial semithin sections of this material through the midthoracic neural tube were examined by light microscopy. Cell proliferation was studied at the above time intervals on autoradiographs prepared 1 h after injection of $^{3}$H-TdR. The occurrence of apoptosis was assessed by TUNEL staining and confocal microscopy.

Weights of EtOH treated embryos were significantly reduced, compared to controls 48 h after treatment. At 6 h abnormal clusters of dissociated cells and cell debris was present in the lumen of the spinal cord. The dorsal region of the cord appeared to be the source of the debris. In many embryos the latter was abnormally shaped. By 24 h the abnormal debris had been cleared from the lumen but the zone of young neurons was reduced in size in the EtOh treated embryos. At 6 h and subsequently the percentage of $^{3}$H-TdR labelled ependymal cells was significantly reduced in the EtOH specimens. TUNEL analysis showed that EtOH did not cause cell death.

In conclusion these results show that treatment of early post-neurulation stage chick embryos with EtOH (1) inhibits cell proliferation in the spinal cord leading to retardation of its development and (2) impairs the normal migration of cells (possibly neural crest) indicating an effect on the extracellular matrix or cytoskeleton.

**P9 Localised changes in expression of calcium binding proteins induced by chronic application of an excitotoxic agent, β-N-oxalyl-L-α,β diaminopropionic acid to the rat motor cortex.** By G. J. CLOWRY[1], C. GOWLAND[2] and S. McHANWELL[2]. [1] *Department of Child Health and* [2] *School of Neuroscience*, *University of Newcastle upon Tyne*, *UK*.

Although amyotrophic lateral sclerosis involves degeneration of both the corticospinal tract and the somatic motor neurones, the relationship between the pathology observed in these 2 regions of the nervous system is unclear. One hypothesis that attempts to explain this relationship is that there is overexcitation in the corticospinal tract that secondarily leads to degeneration of motor neurons. This study examines the effects of chronic release of an excitotoxic agent in the motor cortex. Wistar rats 8–12 wk of age were anaesthetised with a cocktail of 0.3 mg/kg fentanyl citrate, 10 mg/kg fluanisine and 30 mg/kg midazolam. The motor cortex was exposed and Elvax implants containing β-N-Oxalyl-L-α,β diaminopropionic acid (L-ODAP, from Tocris) were applied subdurally. L-ODAP is an excitatory amino acid analogue that has been implicated in toxin-induced motor neuron disease (Ludolph et al., *Brain* **110**, 1987) Elvax is a porous polymer that slowly releases incorporated drug. Elvax implants were prepared by standard techniques using a 100 mM solution of L-ODAP and 100 µm vibratome sections of the polymer were prepared. Saline was incorporated into some implants in place of L-ODAP as a control. After a survival period from 2–18 wk the animals were reanaesthetised and transcardially perfused with fixative. Serial frozen sections of forebrain were either immunostained for nonphosphorylated neurofilament (a marker for projection neurones in the cortex recognised by the monoclonal antibody SMI-32), parvalbumin, calbindin, or histochemically stained for NADPH-diaphorase (all markers for inhibitory interneurones). Application of L-ODAP resulted in clearly detectable reductions in the motor cortex in the numbers of neurons immunostained for parvalbumin, and calbindin, compared to saline-treated controls, contralateral motor cortex or adjacent untreated somatosensory cortex. However there were no major changes in NADPH-d positive neurones or staining for nonphosphorylated neurofilaments. These results share some similarities with a post-mortem study of motor cortex from amyotrophic lateral sclerosis patients (Nihei et al., *Acta Neuropathol*. **86**, 1993) where a reduction in parvalbumin positive neurons but no change in NADPH-d positive neurones was observed. However a reduction in SMI-32 positive cortical projection neurons was observed in this post mortem study.

**P10 Age related changes in nerve growth factor content in primary afferent lumbosacral neurons of the male rat: an enzyme-linked immunoassay study.** By H. MOHAMMED and R. M. SANTER. *Cardiff School of Biosciences*, *University of Wales*, *Cardiff*, *UK*.

Previous studies have shown that the afferent nerve fibres of the rat urinary bladder have cell bodies predominantly located in the L1-L2 and L6-S1 dorsal root ganglia (DRG). Nerve growth factor (NGF) is an essential neurotrophic protein involved in differentiation, survival and functional maintenance of peripheral sensory and sympathetic neurons. The sensory innervation of the bladder is a vital part of the neural circuity responsible for proper filling of the bladder and for the initiation of micturition. As part of an extensive investigation into the effects of ageing on the sensory innervation of the bladder, we have examined the effects of age upon NGF protein level in these lumbosacral DRG neurons. Two age groups of male Wistar rats, 3 mo old and 24+ mo old, were used in this study. Rats were perfused under terminal anaesthesia, fixed in 4% paraformaldehyde and the L1-L2 and L6-S1 DRGs were dissected out bilaterally. The DRGs were weighed and processed for 2-site enzyme-linked immunoassay (ELISA). The average weight of each DRG was $1.0 \pm 0.2$ mg in young rats and $2.0 \pm 0.4$ mg in aged rats. The mean NGF level (pg/mg of DRG) in young rats ($28.5 \pm 2.4$) showed no significant ($P > 0.05$) difference with that in the aged rat DRGs ($26.3 \pm 1.2$). However, the mean level of NGF per DRG (pg/DRG) of young ($28.5 \pm 2.4$) rats was significantly ($P < 0.05$) less than that in aged rats ($52.6 \pm 2.5$). This difference represents an increase of 85% in NGF protein level with age. This may suggest an increase in the uptake of NGF by sensory neurons and/or an increase in NGF expression in the hypertrophied detrusor smooth muscle or the urothelium of aged rats. This maintained level of trophic support may contribute to the almost unchanged density of sensory innervation that we have recently observed in aged rats.

(This study was funded by a Scholarship from the King Abdulaziz University and the Ministry of Higher Education in Saudi Arabia).

**P11 The rat ventral spinal commissure: a precocious glial segregating system.** By J. FRAHER and P. DOCKERY. *Department of Anatomy and Biosciences Research Institute, University College Cork, Ireland.*

The ventral commissure (VC) is a markedly precocious segregating glial element of the presumptive rat spinal cord. It lies ventral to the floorplate. The latter forms the ventral median part of the neural tube and is fundamental to its development. One of its functions is to influence transmedian axon growth. In contrast to the floorplate, the VC has received relatively little attention. It could provide a substrate from which morphogenetically active substances might influence transmedian axon growth. This study examines the organisation of the early VC and its relationship to axons traversing it in rat cervical cords, using light microscopy, electron microscopy and immunohistochemistry.

The VC appeared in the midline around E12. Initially its main constituents were primary processes. These stemmed from perikarya in the floorplate, ran ventrally and contributed to the presumptive glia limitans. They were thin, expanded sheets, orientated in the transverse plane of the cord. At first they were stacked on one another, with few if any intervening axons. When axons first appeared, they were commonly single, many being growth cones. As axon numbers increased, the radial primary (stem) processes gave rise to thin, irregular segregating processes. Together the processes formed a complex matrix. This had a regular appearance and extensively segregated the axon bundles. Morphometric analysis showed that compartment size was small. Each compartment contained few axons. Glial surface area was relatively large. Glial–axon contact was extensive.

The segregating matrix closely resembled that which subdivides the axon bundles of the proximal ventral root segment (PRS) in the prenatal period. The marked precocity of axonal segregation was remarkable. So too was the abundance of glial processes at such an early stage, in advance of the arrival of axons. This indicated provision for the development of axonal guidance channels across the midline in anticipation of the arrival of growth cones at the VC. This sequence of events was the reverse of that which is seen at other sites of axon segregation, such as the CNS–PNS transitional zone or the PRS. At these, segregating processes appear only after the axon bundles are well established.

**P12 Differentiation of neurons and glia from the human pluripotent embryonal carcinoma stem cell line TERA2.SP12.** By S. A. PRZYBORSKI. *Department Biological Sciences, University of Durham, UK.*

Previous studies have used an embryonal carcinoma (EC) derivative of the human teratocarcinoma cell line TERA2 to investigate the development of human neurons (Przyborski et al., *Eur. J. Neurosci.* **12**, 2000). However, there are no similar data describing the differentiation of human glial cells. Existing TERA2 clones were indirectly isolated from a passage 48 culture of the parent line via growth as a xenograft tumour. Recently the EC cell line, clone TERA2.SP12, was derived directly from the earliest existing passage (p15) of the TERA2 parent line. In this study the ability of TERA2.SP12 cells to differentiate into neurons and glia was tested. Undifferentiated human TERA2.SP12 cells were maintained as homogeneous monolayers. Treatment with retinoic acid (RA, 10 μM) for 3 wk induced differentiation and the appearance of morphologically identifiable neurons. After replating the cells and introducing DNA synthesis inhibitors (cytosine arabinoside 0.5 μM; fluorodeoxyuridine 3 μM; uridine 5 μM) to control the proliferation of non-neural cell types, distinct populations of neurons and glial-like cells were observed. Antibodies to neuron growth-associated protein SCG10, neurofilament 68 kD (NF68) and microtubule-associated protein 2 (MAP2) were used to detect neurons whilst glial fibrillary acid protein (GFAP) was immunoreactive for astrocytes. Western analysis did not detect SCG10, NF68 and GFAP in undifferentiated TERA2.SP12 cells, however markers for glia and neurons were detectable after 1–2 wk differentiation. Continued development of both neural lineages was indicated by increasing concentrations of SCG10, NF68 and GFAP that reached maximal levels after 3–4 wk differentiation. Immunocytochemical detection of MAP2 and NF68, and GFAP, confirmed the identity of neuronal and glial cells respectively. TERA2.SP12 cells thus appear to have a greater propensity for neural differentiation since other clones of TERA2 did not produce neurons and glia as readily under the same conditions. Neural differentiation by TERA2.SP12 cells provides an amenable model system to study the development and interaction of neurons and glia in vitro in a manner pertinent to human embryogenesis.

**P13 The cellular and molecular mechanisms of amnion wound repair.** By J. M. MOORE, K. C. McCULLOGH and D. J. WILSON. *Department of Anatomy, The Queen's University of Belfast, UK.*

To examine this reparative process, we created standardised circular wounds in the amnion of 4 d chick embryos. The wounds were dissected out during various stages of development and processed for resin histology, to assess the ultrastructure of the amnion wound edges. Other wounds were stained with FITC-phalloidin and examined using the confocal laser scanning microscope, to visualise intracellular actin.

Transmission electron microscopy revealed that the wound edges have a blunt faced appearance. No lamellopodia were found extending from the wound edge cells, but filopodia were observed. Scanning electron microscopy showed that the ragged edges of the freshly made wound, rapidly smoothed out to give a regular, rounded appearance. On closer examination the wound edge cells appeared raised and braided. Confocal laser scanning microscopy showed the presence of a fluorescently labelled actin cable specific to the cells at the leading edge of the wound. No lamellopodia were observed.

These results confirm that amnion wound closure does not occur by lamellipodial crawling. Contraction of the actin cable may provide the necessary force required to bring about wound closure, as reported in the repair of embryonic chick ectodermal wounds, the blastula of amphibians and adult mouse corneal wounds.

**P14 Microinjected neutrophils retain the ability to release superoxide.** By M. M. BIRD[1] and A. W. SEGAL[2]. [1] *Department of Biomedical Sciences, Queen Mary and Westfield College; and* [2] *Department of Medicine, University College London, UK.*

Recent experiments (Bird & Segal, 2000) have shown that it is possible to microinject human neutrophils in culture with function perturbing proteins and retain their viablility for at least several hours thereafter. In this study we have investigated whether or not cells injected in this manner retain the ability to release superoxide, vital for their normal function. Human neutrophils were plated onto coverslips coated with collagen and/or fibronectin and maintained at 37 °C for 60 min in a Hepes-buffered Ham's F-12 defined culture medium. Controls were processed for an assessment of superoxide production by using the NBT test. The coverslips were then immersed into a solution containing 1.0 μg/ml PMA (phorbol myrcistate acetate), 25 mg/ml NBT (nitro blue tetrazolium) and 2 ml of 20% bovine serum albumin at 37 °C for 15 min. The cells were then rinsed in buffer (PBS) and air dried. Care was taken to make sure the cells were completely dry before they were fixed in methanol for 3–4 min. The neutrophils were then stained in a 1% solution of safranin in water for 1 min, rinsed in distilled water and mounted in Ralmount (BDH). The remaining cultures were treated as follows: fine glass needles were pulled and filled with 5 μl of a solution made up of PMA. Between 50 and 100 neutrophils were injected with this PMA. Following the microinjection experiments the cultures were returned to the incubator for 5 min before immersion in a solution of NBT glucose and bovine serum albumin for 30 min and subsequently processed as described for the control preparations to test for superoxide production.

All the neutrophils in control preparations were stained blue indicating they were producing superoxide. In the injected cell experiments all the noninjected cells were stained red because they had not been exposed to PMA whereas those injected with PMA were the only cells exposed to both NBT and PMA and appeared blue if they were still capable of releasing superoxide. The results demonstrated that microinjected neutrophils retained the ability to produce superoxide.

**P15 Gastrointestinal tumour development in irradiated neonatal and adult *Apc*$^{Min/+}$ CHB6 F1 mice.** By M. ELLENDER[1], M. BARR[2], O. HOUGHTON[2], J. D. HARRISON[1] and K. E. CARR[2,3]. [1] *National Radiological Protection Board, Chilton;* [2] *The Queen's University of Belfast; and* [3] *Radiation and Genome Stability Unit, MRC Harwell, Chilton, UK.*

An important development in research in gastrointestinal tract carcinogenesis has been the characterisation of the *Min* (multiple intestinal neoplasia) mouse as *Apc*-deficient and therefore providing a murine homologue of human familial adenomatous polyposis (FAP). This germ line mutation leads to the development of many adenomas, some progressing to carcinomas. In the current experiments, CBA/H wild-type female mice were mated with C57BL/6J$^{Min/+}$ male mice to produce F1 hybrids, which were irradiated in groups with a single whole body dose of 2 Gy x-rays at 2, 10 or 35 d of age. Irradiated and sham-treated mice were killed 200 d later and lesion incidences compared for 4 small intestinal and 2 large intestinal regions. Procedures involving animals were carried out in accordance with the Animals (Scientific Procedures) Act 1986, with guidance from the local Ethical Review Committee.

Results showed a radiation induced increase in mean lesion incidence in F1 *Apc*$^{min/+}$ mice from 32 after sham exposure to 80, 110 and 65, respectively, after radiation exposure at 2, 10 and 35 d old (based on data for 20 of 50 animals in each group). In all groups most lesions were small intestinal, particularly ileal. However, particularly in mice exposed at 2 d of age, there was an increase in large tumours in the colorectal region. Scanning electron microscopy and histopathological analysis of representative intestinal samples showed the presence of adenomas, carcinomas, atypical crypt foci and hyperplastic lesions. Adenomas and carcinomas were most common and corresponded to larger lesions. Atypical crypt foci were smaller lesions found in the small intestine, while hyperplastic lesions were larger and found in distal segments.

**P16 Visualisation of fused aluminosilicate microparticles taken up by intestinal tissue.** By K. E. CARR[1,2], M. J. YOUNGMAN[3], M. BARR[1], L. COLHOUN[1], C. G. COLLIER[3] and J. D. HARRISON[4]. [1] *The Queen's University of Belfast;* [2] *Radiation and Genome Stability Unit, MRC Harwell, Chilton;* [3] *AEA Technology, Harwell, Didcot; and* [4] *National Radiological Protection Board, Harwell, Chilton, UK.*

It has recently been more widely accepted that some particles with diameters in the micrometre range can cross the small intestinal barrier. The commonly used fluorescent latex model only provides a pointer to mechanisms involved in uptake of material of more environmental relevance, such as fused aluminosilicate particles (FAP). Studies using FAP labelled with $^{57}$Co have provided information on their movement through the gut and evidence of low levels of particle transfer to mesenteric lymph nodes. For information on the route of uptake, morphological techniques are needed, but poor contrast in routine sections hampers their identification in tissue sections. The current work addresses this problem. After overnight fasting, 7-wk-old male Sprague-Dawley rats were exposed by gavage to $4.0 \times 10^7$ FAP particles, 2.2 μm in diameter, administered in 0.25 ml water. Animal procedures were carried out in accordance with the Animals (Scientific Procedures) Act 1986. Tissue was collected 5 min later, when latex particle uptake has been shown to be most apparent. The small intestine was fixed in glutaraldehyde, divided into 9 segments and prepared in 2 ways for identification of FAPs.

Examination of carbon coated resin sections in the scanning electron microscope with a backscattered electron detector allowed identification of particles of the appropriate diameter by differential atomic number contrast. Particles were seen in luminal and tissue compartments. Preliminary counts showed that uptake occurred more proximally and to a lesser extent than for latex particles. In freeze fracture replicas, particle like structures were seen in the lumen, at the microvillous border, within enterocytes

https://doi.org/10.1017/S0021878201357977 Published online by Cambridge University Press

and near but not between the apical intercellular membranes, possibly implicating tight junctions as the initial route of uptake.

**P17 The effect of single and multiple dosing on the uptake of 2 μm latex microparticles in the rat small intestine.** By S. H. SMYTH[1], S. BURNHAM[1], M. DOYLE-McCULLOUGH[1] and K. E. CARR[1,2]. [1] *The Queens University of Belfast;* [2] *Radiation and Genome Stability Unit, MRC Harwell, Didcot, UK.*

The use of different models leads to varying profiles of intestinal microparticle uptake. After multiple dosing Peyer's patch M cells may be the entry route, while single doses may lead to villous uptake. The current study compares single and multiple gavage dosing, using an in vivo in situ model. Groups of male, 7 or 8-wk-old Sprague-Dawley rats (n = 3) were fed ad libitum with standard laboratory food and water. Rats were gavaged with either a multiple dose (MD) of $6.8 \times 10^8$ 2 μm latex microparticles in 0.1 ml of distilled water, each day from d 1 to d 9, or a single dose (SD) of $1.7 \times 10^9$ 2 μm latex microparticles in 0.25 ml of distilled water on d 9. One rat was gavaged with distilled water, for comparison with controls already collected. Animals were killed on d 9 by $CO_2$ asphyxiation, either 30 min or 24 h after gavage. All procedures were carried out according to Home Office guidelines. The small intestine was fixed in buffered glutaraldehyde and divided into 9 equal segments. Particle numbers in several sites from all segments were counted using epifluorescence microscopy of cryosections and analysed using Mann Whitney U statistical tests.

Dealing with 2 variables, such as dose delivery and time of tissue collection, complicates the interpretation of the data sets. With respect to time, total particle uptake is greater at 30 min than at 24 h, making comparison across these 2 groups unhelpful unless through percentage uptake for each site. Comparisons across the dosing schedules are simplified by the fact that the total uptake is similar at each time point, despite the different total delivered dose and the very different doses delivered on d 9. The MD group has more Peyer's patch-associated particles than the single dose group, significant at the 24 h time point, where the percentage figures are 13.9 % and 0.9 % for the MD and SD groups respectively. Multiple dosing and extending the time after gavage thus both increase the proportion of particles taken up at Peyer's patch tissue, but not sufficiently to explain the large differences reported in the literature.

**P18 The use of managed learning environment software to facilitate teaching and learning.** By S. McHANWELL[1] and S. N. FITZPATRICK[2]. [1] *School of Neuroscience and* [2] *Learning and Teaching Support Unit, University of Newcastle upon Tyne, UK.*

There are a range of Managed Learning Environments (MLEs) being investigated by most HEIs in the UK in response to the demand for more intuitive and flexible tools to allow tutors to make more effective use of the World Wide Web. Like most Universities, Newcastle has a number of technology experts dotted around the campus, however, our research had shown that the vast majority of academics, although regular users of the World Wide Web, were less inclined to use the Web more creatively, due to a lack of appropriate tools. In addition the rising number of technology led distance or flexible learning courses suggested a need to investigate delivery systems in more detail. Most commonly academics are competent in the use of a text processor and mail programme. Thus it was felt that a Web toolset was needed which would operate at the same 'natural' level, have a short learning curve and deliver benefits in areas such as assessment and document management. MLEs purport to offer these facilities and typically allow users to upload documents and files without the need to learn a mark-up language, provide sophisticated assessment tools (usually variations on the MCQ), provide synchronous and asynchronous discussion facilities and provide student tracking as well as providing students themselves with a variety of tools. The market for these tools has grown dramatically in recent months, although the market leaders in the UK are probably WebCT and Blackboard, both of which are American products resulting from spin-out activities from Higher Education. The use of one of these products, Blackboard, will be demonstrated and its use in the delivery of an anatomy course for undergraduate and postgraduate students in Speech Sciences will be presented. Some of the institutional issues surrounding the use of MLEs will be highlighted including that of introducing an MLE. In addition some of the pedagogical issues facing academics who may be rethinking their teaching in the light of the new technologies will also be discussed. Finally a preliminary evaluation of the software from a student perspective will also be presented.

**P19 Introducing William Hunter's anatomical specimens to the World Wide Web.** By H. MARR, S. MACKAY, R. A. SMITH, N. BAXTER and L. C. HOOD. *Division of Neuroscience and Biomedical Systems, IBLS, University of Glasgow, UK.*

Glasgow University holds many of Hunter's unique anatomical specimens in its Anatomy Museum. In order to make this valuable resource more widely accessible to anatomists and scholars of the history of medicine we have set up web pages which highlight aspects of the collection (http://www.hunterian.gla.ac.uk/Anatomy/). The long-term aims are twofold: (1) to cover all aspects of the collection including information about the life and times of Hunter and his associates, (2) to investigate how the original dissections were produced and compile and publish a set of dissection instructions which may allow others to recreate specific specimens from the collection. The initial work has been carried out by a group of students as their final year Honours projects. Each chose a topic for special study: the lymphatic system, the larynx and trachea, joints. For example, Hunter's thoracic duct specimen (catalogue number QQ74) was investigated. Hunter had used mercury injections to highlight his specimen, such techniques are not permissible today and so a valid gross dissection was produced
(http://www.hunterian.gla.ac.uk/Anatomy/thoracic.htm)
on which, by transecting the liver, a fine example of the cysterni chyli was displayed. The student was also keen to discover advances which have occurred since Hunter's day

https://doi.org/10.1017/S0021878201357977 Published online by Cambridge University Press

in the methods of studying the lymphatics; there is therefore a most informative page and images on lymphography. Useful links to pages referring to other collections and information on Hunter have been included and will be added to as more are discovered. We believe Hunter would have approved of electronic dissemination of his collection thereby permitting the great teacher to reach a world wide audience.

**P20 A microscopic study of the colon by means of a vascular corrosion casting technique.** By M. BREVET[1], O. PLAISANT[2], C. GILLOT[2], P. COSTIOU[3] and M. D. DIEBOLD[4]. [1]*Faculté de Medecine, Reims;* [2]*Institut d'Anatomie, Paris V;* [3]*Lab d'Anatomie, Ecole veterinaire, Nantes; and* [4]*Lab d'Anatomopathologie, Hôpital Robert Debre, Reims, France.*

Previous studies employing vascular casts and electronic microscopy have been undertaken to study the microvascular architecture of the colonic mucosa in both human and animal tissue (Browning & Gannon, *Acta. Anat.* **126**, 1986 ; Zahner & Wille, *Anat. Histol. Embryol.* 25, 1996 ; Keijiro Araki, *J. Electron. Microsc.* **45**, 1996). The aim of this study was firstly to assess the accuracy of vascular casts obtained at various times after death and secondly to describe the mucosal microvascular architecture of the cat colon.

For our first aim, we injected the digestive system of a nonembalmed subject, 12 d after death. To accomplish this the descending thoracic aorta was ligatured and a catheter was placed in each external iliac artery. The digestive system was washed with water then injected with 500 ml of Altufix (Methylmethacrylate, P100, Atohaas Europe, Paris-La Défense, France) via the right external iliac artery after ligature of the left external iliac artery. The dissection of the colon was undertaken immediately thereafter.

In terms of our second aim, the digestive system of an adult cat was injected immediately following euthanasia by intraperitoneal administration of 10 ml of chloral. By means of an extensive ventral opening into the thorax and abdomen, a catheter was positioned into the thoracic aorta and the abdominal aorta was ligatured. After incising the vena cava, we washed and injected the digestive vascular system with Altufix and then dissected free the colon.

For both parts of this study, the anatomical pieces obtained were immersed in progressive concentrations of hydrochloric acid and left until complete corrosion of tissues had taken place. The vascular casts obtained were subsequently washed and prepared for scanning electron microscopy (SEM).

The human cast presents a very detailed network with microvessels having a calibre of about 15 µm. Major extravasations were not seen. For the cat colon, however, the microvasculature within the colonic mucosa showed vessels having a calibre of about 6 µm. That we were not able to obtain so fine a cast for the human material could have 2 explanations: there might have been a post mortem obstruction of microvessels that prevented the passage of Altufix or there was insufficient amount of Altufix injected. The cast of the cat colonic mucosa presents a regular honeycomb-like network that bounds Lieberkuhn's glands; a finding consistent with the results reported previously. In conclusion, it is possible to obtain a vascular cast of the colon even 1 wk after death, although without obtaining vessels with calibres below 15 µm. Furthermore, the vascular architecture of cat colonic mucosa resembles other mammals previously studied (e.g. the rat, the dog and human).

The following communications, which have been expanded into review articles in the present volume, were also presented at the Symposium:

**The elusive 'Head Organiser'.** By C. D. STERN[1,2] and A. FOLEY[1,3]. [1]*Department of Genetics and Development, Columbia University, New York, USA*; [2]*Department of Anatomy and Developmental Biology, University College London, UK*; *and* [3]*Department of Cell Biology, Harvard Medical School, Boston, USA.*

**Asymmetry and photoreceptive nuclei in the developing forebrain.** By S. WILSON. *Department of Anatomy and Developmental Biology, University College London, UK.*

**Otx genes in evolution: are they involved in instructing the vertebrate brain morphology?** By D. ACAMPORA, P. P. BOYL, J. P. M. BARBERA, A. ANNINO, M. SIGNORI and A. SIMEONE. *International Institute of Genetics and Biophysics, Naples, Italy*; *and MRC Centre for Developmental Neurobiology, King's College London, UK.*

**The vertebrate segmentation clock.** By O. POURQUIE, K. DALE, J. DUBRULLE, C. JOUVE, M. MAROTO and M. McGREW. *Laboratoire de genetique et de physiologie du developpement, Developmental Biology Institute of Marseille, France.*

**A possible role for the notochord in segmentation of the Zebrafish vertebral column.** By A. FLEMING, R. KEYNES and D. TANNAHILL. *Department of Anatomy, University of Cambridge, UK.*

**Evolutionary origins of vertebrate appendicular muscle.** By P. D. CURRIE. *Comparative and Developmental Genetics Section, MRC Human Genetics Unit, Western General Hospital, Edinburgh, UK.*

**Delamination, migration and the control of cell identity by *Hox* genes.** By A. GOULD, P. ELSTOB and V. BRODU. *MRC National Institute for Medical Research, London, UK.*

**Homeobox gene clusters: the more the merrier?** By P. W. H. HOLLAND. *School of Animal and Microbial Sciences, University of Reading, UK.*

**Genes involved in skeletogenesis.** By R. HILL. *University of Edinburgh, UK.*

**Evolutional aspects of limb development.** By K. TAMURA and S. YONEI-TAMURA. *Biological Institute, Graduate School of Science, Tohoku University, Japan.*

https://doi.org/10.1017/S0021878201357977 Published online by Cambridge University Press

**Regeneration as an evolutionary variable.** By J. P. BROCKES. *Department of Biochemistry and Molecular Biology*, *University College London*, *UK*.

**A population of caudally migrating cranial neural crest cells in the developing chick embryo: functional and evolutionary implications.** By I. M. McGONNELL[1], I. J. McKAY[2] and A. GRAHAM1. [1]*MRC Centre for Developmental Neurobiology*, *Guy's Campus*, *King's College London*; *and* [2]*Department of Adult Oral Health*, *Royal London School of Medicine and Dentistry*, *UK*.

**Derivation of the mammalian skull vault.** By G. M. MORRISS-KAY. *Department of Human Anatomy and Genetics*, *University of Oxford*, *UK*.

**Evolution and development of teeth.** P. T. SHARPE. *Department of Craniofacial Development*, *GKT Dental Institute*, *King's College London*, *UK*.

**The development and evolution of the pharyngeal arches.** By A. GRAHAM. *MRC Centre for Developmental Neurobiology*, *King's College London*, *UK*.

**Conserved developmental constraints in development of lungfish dentitions.** By M. M. SMITH[1] and R. REISZ[2]. [1]*Department of Craniofacial Development*, *Dental Institute*, *King's College London*, *UK*; *and* [2]*Department of Zoology*, *University of Toronto*, *Canada*.

**The evolution of crossvein development in insect wings.** By J. MARCUS. *Department of Biology*, *Duke University*, *North Carolina*, *USA*.

**Amphioxus and the evolution of neural crest and placodes.** By L. Z. HOLLAND. *University of California San Diego*, *USA*.

**Induction of the otic placode.** By A. STREIT. *Department of Craniofacial Development*, *Guy's Campus*, *King's College London*, *UK*.

**Early development of the neural plate, neural crest and facial regions in marsupials.** By K. K. SMITH. *Department of Biology*, *Duke University Durham*, *USA*.

**Neural crest, jaws, and joints.** By C. B. KIMMEL[1,2], C. T. MILLER[1] and R. J. KEYNES[2]. [1]*Institute of Neurosciences*, *University of Oregon*, *USA*; [2]*Department of Anatomy*, *University of Cambridge*, *UK*.

Dr N. Le Douarin (*CNRS*, *Paris*, *France*) also delivered a talk entitled: 'Patterning of the facial skeleton by the foregut endoderm.'

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