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The Annual General Meeting of the Anatomical Society of Great Britain and Ireland was held at the St George's Hospital Medical School, University of London, from 17th to 19th December 1996. A symposium on 'Neural Crest Development' was held on 18th December. The following are abstracts of communications and posters presented at the meeting.

COMMUNICATIONS

1 Characterisation of gene expression in the developing chick heart. By S. P. Allen, P. H. Francis-West*, N. A. Brown and M. K. Richardson. *Department of Anatomy, St George's Hospital Medical School, London and Department of Craniofacial Development, UMDS, London*

Congenital cardiac and arterial malformations are one of the major causes of infant death. For this reason it is important to understand the development of the fetal heart, particularly the mechanisms controlling the development of the major structures, e.g. the septa and valves. We have therefore set out to characterise the expression of various genes which may be important in heart development. We used wholemount in situ hybridisations with digoxigenin labelled probes on chicken embryos between Hamburger and Hamilton stages 7 and 24. Results so far have shown 3 genes which are expressed in cardiac tissue in developmentally regulated patterns. In early heart development (Stages 7–8) both *bmp-7* and *Nkx 2.5* expression were observed in cardiac tissue, the domain of *Nkx 2.5* being larger than that of *bmp-7*. By stage 11 both of these genes are expressed throughout the whole heart. In later stages of development (stage 18 or greater) cardiac expression of both genes was limited to the inflow and outflow tracts. There was, however, also expression of *bmp-7* in the pharyngeal arches and *Nkx 2.5* in the 3rd aortic arch at these later stages. There was no visible expression of *msx-1* in cardiac tissue until stages 20 and greater when expression was seen throughout the endocardial cushions of the AV canal and the outflow tract. The expression of these genes in specific regions of the heart at different times during development suggests they may be important in certain aspects of heart development.

2 Morphological asymmetry of the embryonic mouse heart before looping. By N. A. Brown and D. Bellomo (introduced by R. H. Anderson). *Department of Anatomy and Developmental Biology, and MRC Experimental Embryology and Teratology Unit, St George's Hospital Medical School, University of London*

In vertebrates, it has been thought that looping of the heart tube to the right is the first morphological sign of left–right (LR) asymmetry in the embryo. There is much current interest in the development of LR asymmetry, following the first identification of genes involved, and the direction of looping has been used as an indicator of laterality in many studies. Proper lateralisation is essential for normal heart development, and a range of cardiac malformations can result from defective LR asymmetry. We studied the anatomy of the mouse heart to look for prelooping asymmetry for 2 reasons. First, looping involves the ventricular portion of the heart, which is a surprising location for a primary LR asymmetry since the ventricles are not intrinsically lateralised (i.e. the L and R ventricles develop in series). Second, the TGFβ-related genes, *nodal* and *lefty*, appear to be distal steps in the pathway leading to asymmetry, but are expressed in left lateral plate mesoderm, not in the ventricular loop. We examined C57BL/6J × CBA F1 wild-type and SI/Col *iv/iv* embryos explanted on d 8, by scanning electron microscopy and gross morphology. Heart looping began at about the 8 somite stage with a rightwards displacement of the rostral heart tube. However, at 5–6 somites, we observed a left-sided tissue growth at the junction where the medial portion meets the caudal horns of the heart tube. Following this site to later stages suggests it is the precursor of the atrioventricular canal. In 5–8 somite *iv/iv* mutant embryos (50% of which will develop inverted looping, and some AV canal and atrial isomerism malformations), this caudal asymmetry was absent in many hearts and inverted in some. Using BrdU incorporation, we compared proliferation in L and R horns of wild-type hearts between 2 and 4 somites, before the morphological caudal heart-tube asymmetry. A higher proportion of proliferative cells was found on the left, and the rostrocaudal pattern also differed between left and right. The splanchnopleure of this region overlaps with that of left-sided TGFβ gene expression, providing a link between molecular and morphological asymmetry. We suggest that the asymmetric location of the putative AV canal is the crucial event in the development of heart asymmetry, and that a symmetrical location may lead to atrial isomerism and other malformations.

3 The mouse heart close to term with deficient atrioventricular septation due to trisomy 16. By R. H. Anderson, S. Webb and N. A. Brown. *Department of Paediatrics, National Heart and Lung Institute, Imperial College School of Medicine, London, and MRC Experimental Embryology Unit, St George's Hospital Medical School, London*

Part of the 16th chromosome of the mouse is known to be syntenic with the 21st chromosome of man, and trisomy of the latter chromosome is known to produce Down's

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syndrome. Cardiac malformations are frequently associated with Down's syndrome and the commonest malformation is an atrioventricular septal defect associated with a common atrioventricular junction. Mice with trisomy 16 also develop with deficient atrioventricular septation and have thus been used to explore the mechanisms of abnormal fusion of the atrioventricular endocardial cushions. This has long been presumed to be the cause of the deficient septation. There is some evidence, however, to suggest that morphology of the abnormal murine hearts is not directly comparable with their human equivalents. To explore this possibility further, we studied cardiac morphology in 55 trisomic and 46 normal mice fetuses at full term, using scanning electron microscopy and serial histological sectioning. All trisomic mice had deficient atrioventricular septation and a common atrioventricular junction, but the arrangement was, indeed, dissimilar from that typically seen in man. In almost half the mice, the common junction was exclusively connected to the left ventricle, and guarded by a valve with 3 leaflets. In the other mice, the junction was bridged minimally to moderately to the right ventricle, but always with separate right and left atrioventricular orifices. A ventricular septal defect was typically present beneath the superior bridging leaflet. In none of the hearts was the aorta connected within the left ventricle, as is typically the case in man. Instead, the aortic orifice was overriding, both arterial trunks were connected to the right ventricle, there was a common trunk, or else the ventriculo-arterial connections were discordant. The findings indicate that, while the mouse unequivocally has abnormalities in development of the atrioventricular junctions, these are combined with abnormal development of the ventriculo-arterial junctions. The mouse model should prove useful in exploring the mechanisms underscoring these abnormalities, but does not replicate precisely the situation found in humans with trisomy 21.

4 Atrial septation in the mouse – the role of the dorsal mesocardium. By S. Webb, R. H. Anderson* and N. A. Brown. *Department of Anatomy and Developmental Biology*, *St George's Hospital Medical School*, *London*; *and *National Heart and Lung Institute*, *Imperial College*, *London*

During the formation of the heart tube in the mouse (d 8; 0–8 somites), the heart is attached dorsally, throughout its length, to the body of the embryo by reflection of the myocardial wall. By mid-gestation (d 10) this attachment is reduced to a stalk-like pedicle at the back of the common primary atrium – the dorsal mesocardium. We are studying the role of the dorsal mesocardium in heart morphogenesis, and here we report on its involvement in septation of the atria. Day 9, 10 and 11 embryos, from a CBA × C57BL6/J cross were killed by cervical fracture, and microdissected in various planes to expose the dorsal part of the developing atrium, and examined by scanning electron microscopy. In addition, a group were serially sectioned for orthodox histological evaluation. In 24 somite embryos we see 2 ridges, which are symmetrical about the midline, either side of a blind 'pit' in the dorsal part of the common primary atrium. These ridges are continuous with the dorsal mesocardium and rostral to the sinus venosus. The ridges were termed the 'pulmonary ridges' and the pit considered the origin of the pulmonary vein in early studies, but have received little recent attention. By 38 somites, the developing septum primum can be seen as the broad ridge in the back of the common primary atrium. Rostrally, the septum primum is in continuity with the superior endocardial cushion. Gradually, at base of the septum, there is a bifurcated spur of tissue, adjacent to the inferior endocardial cushion. The tissue of the bifurcated spur is contiguous with the dorsal mesocardium, and was termed the spina vestibuli by His (His, W. *Anatomie Menschlicher Embryonen* **1**, 1880). The most rightward part of the spur is continuous with the developing left venous valve. The spina vestibuli appears to be separate from the mesenchyme which is known to cover the crest of the developing septum primum. Thus, it appears that there are 2 components to the formation of the atrial septum; the septum primum and the tissue of the spina vestibuli derived from the dorsal mesocardium, which is also involved in the transfer of the pulmonary vein to the left atrium. Our results show the morphological transitions of atrial septation: formation of the pulmonary ridges and the spina vestibuli from the dorsal mesocardium; appearance of the septum primum; and incorporation of the pulmonary vein into the left atrium.

5 An investigation of the vertebral level termination of the spinal cord between the period of fetal development and adulthood. By M. A. Malas, A. Salbacak, M. Büyükmumcu*, M. Seker* and B. Köylüoglu**. *Department of Anatomy*, *S.D. University*, *Isparta/Turkey*, *Department of Anatomy* and Radiology***, *Selçuk University*, *Konya/Turkey*

The spinal cord is situated at the end of the vertebral canal at the 3rd month of intrauterine life. It is reported to extend to the level of the 3rd lumbar vertebra at birth, because vertebrae are more developed than the spinal cord at this stage. The present investigation aims to determine the changes in the level of the conus medullaris from fetus to adulthood in a total of 305 individuals. These cadavers had no defects in the central or peripheral nervous system and were obtained from the Faculty of Medicine and Konya Maternity Hospital between 1992 and 1995. The age distributions were as follows: 36 fetuses (22 male and 14 female), 20 premature and 20 neonates (10 male and 10 female), 101 children aged 0–7 y (66 male and 35 female) and 128 adults aged 15–68 y (72 male and 56 female). In all samples except adults, the level of the conus medullaris was determined using ultrasonography (Toshiba SAL 77B US). In addition, microdissection was used in fetuses and Nuclear Magnetic Resonance (NMR) in adults to confirm the results obtained from the above technique. The fetus ages were estimated from measurements of Crown Rump Length (CRL) and ranged from 55 to 230 mm. Their conus levels extended from L3 to S4 vertebrae. The tip of the conus medullaris of the prematures (aged 32–37 postmenstrual weeks as determined by the Dubowitz method) and neonates ranged from L1 to L4. The tip of the conus medullaris in the children lay between T12 and L3 and in the adults it was between T12 and L2. There was no change between the prematures and neonates in terms of the vertebral level termination of the spinal cord. Consequently, we concluded that the determination of the tip of the conus medullaris especially in prematures and infants is important for preventing post-operative neuralgical complications.

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6 Somatotopy in the developing mouse trigeminal ganglion does not depend on target contact. By L. Scott and M. E. Atkinson *Department of Biomedical Science, University of Sheffield*

The specific arrangement of peripheral vibrissal follicles in the rodent snout is somatotopically represented at all levels of the trigeminal system in the brainstem, thalamus and somatosensory cortical barrel fields. Somatotopy in the trigeminal ganglion is not as distinct but cell bodies innervating specific peripheral structures are discrete. Removal of vibrissal follicles perinatally leads to progressive loss of organisation at succeeding levels with higher levels becoming organised later than lower levels, each level depending on input from the peripheral vibrissae. It might therefore be expected that the first level of organisation within the trigeminal ganglion would not occur until peripheral nerves actually made contact with specific vibrissal follicular precursors. To test this hypothesis, 2 contrasting carbocyanine dyes were inserted into the maxillary and mandibular arches of fixed mouse embryos and traced using confocal microscopy to establish when somatotopy within the ganglion occurred in relation to peripheral target innervation. Embryos between E9 and E11 were delivered by caesarian section from time-mated female mice killed by cervical dislocation. They were accurately aged by somite counting, beheaded and the heads fixed in 4% paraformaldehyde/0.2% glutaraldehyde; 24 h later, DiO (which fluoresces green) was inserted unilaterally into the maxillary arch and DiI (which fluoresces red-orange) into the mandibular arch via a micropipette. The heads were maintained in fixative in the dark for up to 4 wk to allow the dyes to be transported through the nerves to the trigeminal ganglion and brainstem. Ganglionic labelling was first detected at E10 in the rudimentary maxillary division and cell bodies in the ganglion and at E10.25 in the mandibular division and cell bodies. The 2 groups of cell bodies were discretely and separately localised within the ganglion from the outset of labelling. Central branches from both peripheral divisions were labelled as 2 distinct bundles running to the brainstem at the same stage. The trigeminal motor nucleus was first labelled at E10.75. With increasing age, more cell bodies supplying axons to each peripheral division were labelled within the ganglion. Somatotopy of the maxillary and mandibular cell bodies within the trigeminal ganglion is thus established prior to the earliest target contact which does not occur until E10.5 in the mandibular and maxillary arches. It is suggested that somatotopy is determined by factors intrinsic to ganglionic neurons and is independent of nerve contact with their targets.

L. Scott was in receipt of an Anatomical Society studentship.

7 The involvement of actin in annulus formation in the rat lumbar intervertebral disc. By A. J. Hayes, M. Benjamin and J. R. Ralphs. *Anatomy Unit, School of Molecular and Medical Biosciences, University of Wales, Cardiff*

The annulus fibrosus of the intervertebral disc is composed of lamellae of parallel collagen fibres arranged concentrically around a semifluid nucleus pulposus. Within individual lamellae, collagen fibres lie at a constant angle to the long axis of the spine. The inclination of this angle alternates in adjacent lamellae allowing the annulus to resist compression and torsion. In development, the lamellar organisation of collagen fibres in the annulus is preceded by a similar arrangement of sheets of fibroblasts. These sheets are precisely oriented into a complex lattice, which is of key importance in the development of a functional annulus. (Rufai et al. *Anat. Embryol.* **192**, 1995). Here we examine the possible roles of actin and adherens-type junctions in the orientation process. Spines from Wistar rat embryos of embryonic day (E) 15–21 and from neonates were cryosectioned and labelled with FITC-conjugated phalloidin for filamentous actin, or immunolabelled for adherens junctions and focal contacts using an anti-vinculin monoclonal antibody. Sections were counterstained with propidium iodide and examined with a confocal microscope. Actin was consistently present in notochordal tissue. The prominence of actin in the outer annulus, however, was intimately associated with its development. At E15 the discs consisted of dense cell condensations interposed between developing vertebral bodies. At this stage actin label was diffuse in the disc anlagen. At E16, following differentiation of the annulus into distinct inner and outer parts, actin label was strong only in the cell sheets of the outer, fibroblastic part. In this region, long arrays of filamentous actin bundles coursed through the elongated fibroblasts. In later stages actin remained prominent in the outer annulus, but the cell sheets became more separated with extracellular matrix (ECM). This pattern persisted until birth, after which actin label decreased rapidly in the outer annulus. In all stages investigated vinculin gave punctate staining in similar regions of the disc and at similar stages to actin. Actin distribution correlates with the formation of cell layers in the annulus, and with the presence of vinculin-containing adherens junctions. The fact that its organisation reaches prominence only in the outer annulus, becoming greatly reduced after birth when the lamellar pattern of cells and ECM is fully established, suggests that it plays an important role in establishing the pattern. Actin is generally involved in cell shape and motility through interaction with myosin. Such interaction might be important in the dynamics of cell orientation during lattice formation here. The actin cytoskeleton may also act as a temporary scaffold, facilitating the organisation of an oriented matrix on cell surfaces. Vinculin-containing adherens-type junctions may play a role in mechanically integrating cells to each other and to the ECM during annulus formation.

8 The behaviour of myoblasts, and myotube formation, on micropatterned substrata. By P. Clark, D. Coles* and M. Peckham*. *Department of Anatomy and Cell Biology, Imperial College School of Medicine at St. Mary's, London and *Randall Institute, King's College, London*

During development, the myotubes of skeletal muscle are formed from the fusion of single cells, the myoblasts. This process can be mimicked using myoblast cultures, and usually results in randomly oriented linear myotubes adherent to the culture surface. We attempted to control myotube orientation and diameter by culturing conditionally immortalised myoblasts, whose differentiation

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can be tightly induced, on micropatterns of laminin. Before differentiation, myoblasts show some preference for laminin surfaces over adjacent serum attachment factor-coated surfaces. After differentiation, this preference becomes more apparent, and increases with time. Alignment of myoblasts and developing myotubes to alternating laminin/non-laminin tracks is dependent on track spacing and duration of differentiation. In more mature cultures, most myotubes are aligned and confined to laminin tracks, suggesting loss of cells from non-laminin regions. Although myoblasts preferentially adhered and clustered to the laminin tracks, the width of the tracks had no apparent effect on the width of the myotubes formed after differentiation: myotube width was uniform and wider tracks contained more than one myotube. When myoblasts were cultured on ultrafine grooved substrata (i.e. topographic guidance), they were highly aligned and elongated but, paradoxically, became organised into aggregates of aligned cells. These aggregates were most commonly arranged at 45° or perpendicular to the groove direction. Myotubes formed in the groove direction, but not at these other angles. These data suggest a mechanism inhibiting lateral adhesion and fusion of differentiating myoblasts which will promote the formation of linear myotubes, and inhibit them from forming irregularly shaped structures.

9 The anatomy of the hindfoot and its implications in the surgical treatment of calcaneal fractures. By S. Duff, H. D. Nicholson, B. J. C. Freeman* and R. M. Atkins*. *Department of Anatomy*, *University of Bristol and *Department of Orthopaedics*, *Bristol Royal Infirmary*

Surgical treatment of calcaneal fractures is undertaken using an incision on the lateral surface of the hindfoot. The traditional approach is a curved incision behind the lateral malleolus and carries with it the risk of sural nerve damage and poor wound healing. Recently an extended lateral approach has been described which involves an L-shaped incision posterior to the traditional incision. This has been reported to reduce the incidence of sural nerve damage and skin bruising and result in prompt wound healing. This study was performed to investigate the anatomical reasons behind the improved surgical outcome with the new incision. Dissections were performed on 15 cadavers and photographed to demonstrate the structures of the posterolateral aspect of the foot. Measurements of the positions of relevant structures were made. The sural nerve was identified in all specimens and found to run 2.1 cm (1.5–2.6 cm) behind the lateral malleolus. Deep to the nerve the anatomy could best be described as 2 triangles, one deep to the other. The superficial triangle was bounded inferiorly by the superior border of the calcaneum, anteriorly by the peroneal tendons and posteriorly by the tendo Achilles. It contained fat and the posterior peroneal artery (PPA). The deep triangle lay on the posterior surface of the tibia and was bounded inferiorly by the superior border of the calcaneum, laterally by the peroneal tendons and medially by flexor hallucis longus. The apex lay on the tibia a mean of 4.1 cm above the posterior surface of the ankle joint, which was itself within the triangle. The PPA passed down through the deep triangle and crossed the calcaneum a mean of 3.1 cm posterior to the lateral malleolus. The PPA supplied the skin of the posterolateral heel in all cases and in 13 specimens arose as a continuation of the peroneal artery and in 2 cases as a branch of the posterior tibial artery. Thus, in its distal course the artery passed beneath the sural nerve from anterior to posterior. This study shows that in the traditional approach the poor wound healing is likely to be due to damage to the PPA. The traditional incision also lies very close to the sural nerve. The extended lateral incision avoids neurovascular damage, allows good wound healing and also provides good access to the lower tibia, talus and calcaneum.

10 The anatomical basis for the possible use of the long head of biceps femoris and sartorius muscles in the construction of a dynamic perianal neosphincter. By D. A. Shanahan and R. K. Jordan. *Anatomy & Clinical Skills Centre*, *The Medical School*, *University of Newcastle*, *Newcastle Upon Tyne*

Surgical attempts to restore faecal continence have focused on the repair of the anal sphincters or the transposition of gracilis around the anal canal to create an artificial anal sphincter. Contraction of this neosphincter led to transient retention of rectal contents. Electrical stimulation of the nerve to the transposed gracilis created a neosphincter capable of actively opposing intrarectal pressures. A problem associated with this procedure was sepsis of the distal part of the graciloplasty. This was overcome by the delay procedure, i.e. ligating and dividing the minor vascular pedicles to the transposed section of gracilis 4–6 wk before transposition (Patel et al. *J. Anat.* **176**, 1991). The transposed section is that part of the muscle removed from its anatomical position. In a small group of patients, gracilis may be unsuitable or unavailable for transposition, therefore the surgeon would require a suitable alternative muscle. A preliminary study of the thigh musculature and perianal regions of 5 cadavers was undertaken to define the criteria a thigh muscle must fulfil for it to be used to create a dynamic perianal neosphincter (DPN). Following this the long head of biceps femoris (LHBF) and sartorius muscles seemed to be suitable alternatives to gracilis. Eighty cadaveric thighs were dissected to elucidate the neurovascular anatomy and length of these muscles and 14 biceps femoris and sartorius muscles were dissected to display their intramuscular arterial anatomy. The majority of LHBF muscles (71.25%) fulfilled the anatomical criteria for them to be used to create a DPN. This finding agrees with an earlier study of the possible use of the LHBF as a DPN in which the neurovascular anatomy and length of 24 LHBF muscles were elucidated (Shanahan et al. *Plast. & Reconstr. Surg.* **92**, 1993). In the present study, dissection of the intramuscular arterial anatomy of the LHBF revealed potential intramuscular arterio-arterial anastomoses between the arterial pedicles to its nontransposed and transposed sections. Although the neurovascular supply and length of the majority of sartorius muscles (83.75%) permitted its transposition to the perianal region, a nerve trunk onto which the stimulating electrode could be sutured was not found. Dissection of the intramuscular arterial anatomy of sartorius revealed potential intramuscular communications between the arterial pedicles of this muscle. Following delay, intramuscular communications between the arterial pedicles of a transposed muscle may prevent

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necrosis of its distal part. Therefore, the LHBF is a feasible alternative to gracilis in the creation of a DPN and sartorius could possibly be used with modified or implanted stimulators.

11 Midfacial morphology and morphometry in subjects with mandibular prognathism. By G. D. Singh, J. A. McNamara Jr. and S. Lozanoff*. *Department of Dental Surgery and Periodontology, University of Dundee, Department of Orthodontics and Pediatric Dentistry, University of Michigan, Ann Arbor, USA, and *Department of Anatomy & Reproductive Biology, University of Hawaii, Manoa, USA*

The purpose of this study was to determine whether the morphology of the midface differed in subjects with a prognathic mandibular facial appearance (Class III malocclusion) using a combination of morphometric and cephalometric analyses. After obtaining appropriate consent, lateral cephalographs of 133 children of European–American descent aged between 5 and 11 y were compared. The cephalographs were traced and subdivided into 7 age- and sex-matched groups. Average geometries, scaled to an equivalent size, were generated based upon 7 nodes (pterygoid point, PTS; orbitale, O; posterior nasal spine, PNS; midpalatal point, MPP; anterior nasal spine, ANS; subspinale, A; prosthion, Pr). Bivariate analyses utilising 11 linear and 7 angular measurements were also undertaken. Graphical analysis using a colour-coded finite element programme was used to localise differences in morphology. Results indicated that although the overall midfacial configurations differed statistically ($P < 0.05$), only 4 of the linear (PNS-MPP, MPP-ANS, A-Pr and PTS-O), and only 2 of the angular parameters (PTS-O-ANS and ANS-A-Pr) tested were significantly different ($P < 0.05$). Comparing normal and midfacially retruded groups for size-change, finite-element analysis revealed that there were local size changes in the maxillary incisor and palatal regions. For shape-change, major differences in shape over the entire midface were not as evident, with an isotropic midfacial morphology in normal and mandibular prognathic subjects. It is concluded that the appearance of Class III malocclusion may be influenced by the morphology of the midface as well other craniofacial components such as the mandible and cranial base.

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12 Evaluation of anatomy teaching in a problem based curriculum. Investigations of medical students and recently qualified doctors. By J. Drukker, H. Croonen, T. Leiner, A. J. B. Wanders, M. Verwijnen*, C. P. M. Van der Vleuten* and H. Van Mameren. *Department of Anatomy and Embryology and *Department of Educational Development and Research, University of Maastricht, The Netherlands*

A permanent discussion is going on about the role of so called basic disciplines in the medical curriculum. There are some reports about the opinion of medical students (e.g. Pabst, *Anat. Rec.* **237**, 1993) but we could not find any about the relevance in medical practice of the material presented during the curriculum. A special problem is that departmental contributions are not easily recognisable in a multidisciplinary problem based curriculum. Two, partly different, questionnaires were constructed to investigate the experience of all 6 cohorts of medical students in the academic year 1995–6 and of 3 cohorts of doctors, graduated at Maastricht University in 1995, 1992 and 1989. The material taught by the Department of Anatomy comprises gross anatomy, microscopic anatomy, embryology and neuroanatomy. Preclinical students, students during their clerkships and doctors report in a vast majority that the various divisions of anatomy and embryology, as presented in the Maastricht Medical School curriculum, are indispensable for the understanding of the majority of other basic disciplines and of almost any clinical discipline. There are no significant differences between the older student cohorts and the respective 3 cohorts of qualified doctors. This type of questioning permits the identification of gaps in the contents of the material offered; moreover it gives suggestions about improvement of the presentation. In this way the results of this investigation may contribute to optimising the teaching of anatomy. Examples of suggested alterations are: (1) increase in the number of computer assisted instruction programmes, (2) teaching of (clinical) anatomy during the clerkships, (3) a more intense relationship with the teaching of the modern imaging disciplines and (4) more opportunities for dissection.

13 Use of the VOXEL-MAN dissectable atlas in teaching anatomy. By M. Lowndes, J. F. Morris and R. Schubert*. *Department of Human Anatomy, University of Oxford and *I.M.D.M., University Hospital Eppendorf, Hamburg, Germany*

Interactive 3-dimensional computer-based atlases of anatomy are now being realised. Here we explore the use of one such tool in the presentation of anatomy. The use of virtual anatomy in the classroom would have several important advantages over traditional anatomical models and cadaver dissections. Most importantly, each object may have linked to it, information (a knowledge base) which is instantly accessible to the student. This allows students to learn without the presence of a qualified anatomist to provide information about the structures being explored. Students are, therefore, not limited to the time that such staff have available and can access the information at will. These atlases are usually based on CT or MRI data and the ability to relate information from such noninvasive techniques to the 3-dimensional structure of the body is becoming an essential part of the armoury of both clinicians and neuroscientists. The Institute of Mathematics and Computer Science in Medicine, Hamburg, is developing an electronically dissectable, knowledge-based, 3-dimensional atlas of the human body, VOXEL-MAN (V-M). The Department of Human Anatomy in Oxford is collaborating to develop the knowledge base and add information and interpretation. This involves defining the identity, connections, vascular supply, known function and dysfunction for each anatomical unit segmented from the data set. We are also exploring the use of this system in the teaching of anatomy to preclinical medical students and investigating how this information may best be made available. V-M is approached either by direct dissection of the data set or by

use of hierarchical lists based on morphology, function and blood supply. The atlas in its present form is ideal for free, unstructured exploration of neuroanatomy, which is useful for the experienced anatomist but can be daunting for a newcomer. We have therefore developed an online textbook to provide a structure to the knowledge, which is dynamically linked to the 3-dimensional atlas. Our knowledge bases have also been modified for use on the Internet as interactive tutorials on anatomy. The textbook presents the information that V-M provides in a linear format so that the student is able to learn in a structured manner. Knowledge-based information is viewed through a single, consistent window. The text is divided into 'pages', each of which is linked to relevant resources such as diagrams, histological photographs and sections from the Visible Human data-set. Each page is also linked to the 3-dimensional atlas such that an object of interest can be visualised and the student can flip between the 3-dimensional view, the text and other information. Within the knowledge base, hypertext links are being developed to enable the student to access further information on specific topics, in a 2nd window. This approach provides the advantages of a paper textbook (e.g. structure) with the advantages of hypertext (e.g. access to information) and the ability to interact with the 3-dimensional data set.

14 Effect of dose concentration on the uptake of microparticles by the rat small intestine. By O. T. Cox (supervised by R. A. Hazzard and K. E. Carr). *School of Biomedical Science/Anatomy, The Queen's University of Belfast*

Microparticulate uptake by the intestine and translocation to secondary organs have several implications, among which are the possible use of microparticles as drug carriers and their use as a model to study the potential hazards of absorbed radioactive particles. Previous studies have established the uptake profile of 2 μm latex particles by the rat proximal small intestine after oral administration of 0.25 ml undiluted latex suspension. This study uses microscopy to examine the effect of suspension dilution on particle uptake along the length of the small intestine and translocation to the blood, liver, spleen and mesenteric lymph nodes with the aim of establishing a minimum threshold for uptake. Three concentrations were used: the undiluted suspension ($\sim 1.96 \times 10^9$ particles per 0.25 ml), 1:100 and 1:1000 dilutions (in distilled water) of this suspension. Two fixation methods were also compared to examine possible effects on particle numbers obtained. Three groups of male Sprague Dawley rats (n = 3 per particle concentration) were given 0.25 ml of fluorescent polystyrene latex suspension by gavage. Control animals were given 0.25 ml sterile distilled water. The animals were perfusion-fixed with 3% glutaraldehyde in sodium cacodylate buffer, 30 min after particle dosing. The small intestine (divided into 9 equal segments), liver, spleen, mesenteric lymph nodes and a blood sample were collected from each animal. An additional group of animals was given 0.25 ml undiluted particle suspension, killed by carbon dioxide asphyxiation and the tissues removed and fixed in glutaraldehyde. All treatments to animals were carried out by a licensed investigator. Particle numbers were assessed, using epifluorescence microscopy, in 14 μm propidium iodide-stained cryosections obtained from each organ collected. Particle numbers in blood samples were counted in 20 1 μl samples from each animal. For the small intestine, results showed significantly greater numbers of particles in many tissue sites after administration of the undiluted suspension in comparison to either the 1:100 or 1:1000 dilutions. The 1:100 dilution showed many fewer particles and there were only small numbers of luminal particles noted in intestinal tissue from animals given the 1:1000 dilution but these differences were not significant. Only a few particles were seen in the liver, spleen and mesenteric lymph nodes across the 3 groups. Analysis of particle numbers in blood showed an average of 466.7/ml for the undiluted group, 175/ml for the 1:100 dilution and 400/ml in the 1:1000 dilution: differences were not statistically different. Particle numbers were statistically higher in perfusion-fixed tissues for some intestinal sites. The findings indicate that substantial numbers of particles are translocated to the systemic circulation after intestinal uptake but there is little passage to secondary organs. The data also suggest that for substantial uptake of 2 μm latex particle uptake to occur within 30 min of dosing, particle numbers in excess of 10^6 must be administered.

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15 The effects of methylmercury chloride on the neuropeptide content of adult mouse sensory neurons in vitro. By G. J. Baxter (supervised by R. A. Smith). *Laboratory of Human Anatomy/IBLS, University of Glasgow*

Neuronal subsets, with respect to neuropeptide expression, in sensory neurons are thought to have modulatory functions. The present study investigated whether a neurotoxin, methylmercury, altered neuropeptide content in cultured adult sensory neurons, and whether differential changes were discernible. Cultures were prepared from 6 mo old CBA mice, killed humanely by cervical dislocation, with neurons maintained for 8 d in standard Dulbecco's medium containing supplements (Jiang & Smith, *Dev. Brain Res.* **91**, 1995). Control and treated, containing 0.1–1 μM methylmercury chloride (MeHg), cultures were maintained for additional periods of up to 3 d, and monitored by phase contrast microscopy. Although viability decreased with dosage it remained above 80% throughout the treatment intervals studied. At the end of the exposure period, cultures were fixed with 4% formaldehyde and prepared for immunostaining by the sensitive avidin–biotin–peroxidase complex (ABC) method, and with primary antisera against substance P (SP; Incstar, USA), calcitonin gene related peptide (CGRP) and somatostatin (SOM; both from Affiniti Ltd, UK) and followed by biotinylated second antibody and ABC reagent (Vector Laboratories, UK). Data were collected from at least 3 separate experiments for each concentration and time group, with the proportions of immunoreactive (-ir) neurons determined from counts of approximately 500 neurons per well. Statistical analyses (ANOVA and *t* tests) were carried out by Minitab. The proportions of immunoreactive neurons increased in a dose dependent manner for both SP (33 $\pm$1% in controls without MeHg rising to 49 $\pm$3% with 1 μM at 6 h ($P < 0.01$) and 45 $\pm$0.5% at 24 h ($P < 0.05$)) and CGRP

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(44 ± 0.3% in the minus control to 60 ± 2% at 24 h ($P < 0.01$) with a 1 μM exposure). A different response was noted in the proportions of SOM -ir neurons which decreased over a 24 h period (17 ± 2% with 1 μM compared to 24 ± 2% in controls ($P < 0.05$)). The upregulation SP and CGRP -ir neurons may be related to nociceptive responses, whereas the decreases in SOM containing neurons may reflect a differential loss in this subset of sensory neurons.

16 Developmental changes in oligodendrocyte responses to wallerian degeneration in the rat optic nerve. By M. Tran-Dang (supervised by A. M. Butt). *Department of Physiology, UMDS, St Thomas' Hospital, London*

Axons are implicated in the control of development of oligodendrocytes, the myelinating cells of the central nervous system. It has been suggested that competition for axon-derived survival factors may be the mechanism by which the number of oligodendrocytes is matched to the number of axons to be myelinated. The aim of this study was to investigate developmental changes in the role of axons in oligodendrocyte differentiation in the rat optic nerve during wallerian degeneration. Rats of the Wistar strain, 11 (P11) and 21 (P21) d old, were deeply anaesthetised under ether and unilateral enucleation was performed by gently retracting the eyeball and transecting the optic nerve behind the eye; after surgery the eyelids were sutured. At post-enucleation d 1–21, rats were killed by cervical dislocation and optic nerves were removed for immunohistochemical and in situ hybridisation analysis; age-matched rats were used as controls. Anti-carbonic anhydrase II and antibody to proliferating cell nuclear antigen were used to immunolabel oligodendrocytes and dividing cells, respectively, and oligodendrocyte precursors were identified by expression of mRNA for platelet-derived growth factor-a receptors (PDGF-αR); immunolabelling for axons, astrocytes, and microglia/macrophages was also performed. At P11, optic nerves contained all stages of oligodendrocyte differentiation, namely precursor, promyelinating and myelin-forming oligodendrocytes, whereas at P21, oligodendrocyte differentiation was largely completed. There was an almost complete loss of oligodendrocytes following enucleation at P11, whereas enucleation at P21 had no obvious effect on oligodendrocyte cell numbers; interestingly, there were abundant macrophages which effectively removed the oligodendrocyte and myelin debris in the former but not in the latter. Following enucleation at P11, there was an immediate loss of PDGF-αR mRNA expressing cells, but the number of proliferating and promyelinating oligodendrocytes both increased initially, before they fell sharply to below control levels. We conclude that axon-derived factors are essential for both the survival of precursors and to trigger their differentiation into oligodendrocytes. However, once underway their differentiation continued in the absence of axons, but the survival of newly differentiated promyelinating oligodendrocytes over the longer term was dependent on axons, whilst myelin-forming oligodendrocytes were not dependent on axons for their survival.

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17 The connections between the precerebellar relay site nucleus reticularis tegmenti pontis (NRTP) and the cerebellum were studied in the monkey, by means of the tracer wheatgerm–agglutinin horseradish peroxidase (WGA–HRP). By C. Smith (supervised by M. Glickstein). *Department of Anatomy & Developmental Biology, University College London*

Three monkeys received injections of WGA–HRP into the NRTP. In a 4th monkey, the injection was placed in lobule VII of the cerebellar vermis. A 5th monkey did not receive an injection and was used to study the morphology and relations of NRTP, in the uninjected animal. In the 3 cases in which the injections were placed in the NRTP, retrogradely labelled cells were present in all 3 of the contralateral deep cerebellar nuclei, confirming the presence of projections from the deep nuclei to NRTP. In the 4th case, which received a cerebellar vermal injection, retrogradely labelled cells were present bilaterally and symmetrically within NRTP, demonstrating a projection from the NRTP to lobule VII of the cerebellar vermal cortex. The majority of the labelled cells were found in the lateral regions of the nucleus. These findings are consistent with the results of previous studies which used orthograde tracing methods. More significantly, they demonstrate that there is a topographical organisation of the connections between NRTP and both the cerebellar cortex and deep nuclei. Projections to the contralateral, caudal NRTP arise from the caudal lateral fastigial and anterior interposed nuclei, and from the dorsal aspect of the posterior interposed nucleus. The rostral NRTP receives inputs from the rostral lateral fastigial and anterior interposed nuclei, and from the lateral part of the posterior interposed nucleus. The inputs to NRTP from the dentate nucleus arise from the dorsolateral region of the caudal part of the nucleus, which projects to both the caudal and rostral parts of NRTP. The lateral region of the entire rostrocaudal extent of the NRTP projects mossy fibres to lobule VII of the cerebellar vermis. The results are discussed in terms of the possible functions of NRTP in (1) maintaining the excitatory background firing rate of the deep cerebellar nuclei; (2) providing an inhibitory control over movements by means of its projections to the cerebellar cortex.

18 Nuclear and cellular morphology of glutamate induced neuronal death of HT-22 cells. By P. J. Robshaw (supervised by J. P. Bennett). *Department of Anatomy and Cell Biology, Imperial College School of Medicine at St Mary's, London*

HT-22 cells are a neuronal cell line that was originally derived from immortalised mouse hippocampal neurons and that dies in response to glutamate by an oxidative mechanism. In this project, the death of HT-22 cells was examined for the morphological features of apoptosis. Glutamate-treated cells showed several distinct morphological features that were not apparent in normal cells. By time-lapse photography, dying cells were observed to lose contact with neighbouring cells before shrinking and rounding up. This was followed by a period of intense surface blebbing, lasting on average 1 h 15 min, which left cells round and often fragmented. Scanning electron microscopy showed that these surface blebs vary in size and

are extensive, covering the entire surface of the cell. There were also changes in nuclear morphology. Transmission electron microscopy showed that glutamate-treated HT-22 cells contained more intensely stained chromatin that was distributed to a greater extent around the nuclear periphery, indicating that a degree of chromatin condensation had occurred. The nuclear membrane, although intact, was highly irregular in appearance. DAPI staining showed the nuclei to be smaller and measurement of the nuclear area showed that glutamate-treated nuclei had shrunk by at least 50%. A nick end labelling assay indicated that this type of cell death did not involve DNA fragmentation. Pharmacological manipulation showed that these morphological changes correlated with previous measurements of cell death using a mitochondrial function assay. They were observed after treatment with the glutamate analogue, quisqualate, but not after NMDA. Both the selective monoamine oxidase A inhibitor, clorgyline, and the antioxidant, cysteine, were shown to protect HT-22 cells from glutamate-induced morphological changes. These results confirm that the observed changes are associated with glutamate-induced cell death by an oxidative pathway. It is widely believed that apoptosis plays a role in a number of diseases including neurodegenerative diseases such as Alzheimer's and Parkinson's. In particular, oxidative stress is believed to be an underlying mechanism involved in Parkinson's disease. These results show that HT-22 cells die by a process that morphologically resembles apoptosis, therefore, this cell death is likely to be a useful model for studying the cell death of neurodegenerative diseases and for the development of therapeutic agents.

19 Heterogeneity in the migratory responses of endothelial cell lines to *N*-acetyl-(*p*-methylamino)-phenylalanine ethylester (AMAPE, a protease antagonist). By K. McCool (supervised by D. J. Wilson and J. Nelson*). *School of Biomedical Science/Anatomy and *School of Biology and Biochemistry, The Queen's University of Belfast*

The behaviour of endothelial cells during angiogenesis has been of considerable interest and it has been reported that microvascular and large vessel-derived vascular endothelial cells exhibit heterogeneity in their angiogenic responses. SK HEP-1, a transformed human cell line of endothelial origin, and HMEC-1, a human microvascular endothelial cell line, were investigated using an in vitro migration assay to examine their angiogenic potential. The aim of this study was to compare the 2 endothelial cell lines both qualitatively and quantitatively in terms of their migratory capacities. In addition, their response to AMAPE an inhibitor of uPA (generously provided by Dr B. Walker, School of Biology and Biochemistry) was examined. A time-lapse phagokinetic track assay was used to measure the time at which the maximum rate of migration occurred ($n = 60$). Subsequently, migration assays were performed using AMAPE at concentrations of 1, 10 and 100 μM to determine the degree of inhibition. Conventional phase microscopy revealed that the SK HEP-1 endothelial cells were plump and polygonal in contrast to the small vessel HMEC-1 cells, which were elongated and flattened. The optimum migration time was found to be 18 h in both the cell lines. The SK HEP-1 cells were significantly inhibited by AMAPE at 1 μM, whereas the migration of the HMEC-1 microvascular cells was not inhibited at any of the concentrations. These findings support the view that heterogeneity exists between endothelial cells derived from different sources in terms of both their morphology and their response to a putative angiogenic inhibitor. Thus it may be necessary to re-evaluate results of in vitro assays which have examined angiogenic factors using only endothelial cells derived from one source.

20 Developmental regulation of Schwann cell precursors and Schwann cell generation in the rat. By K. R. Jessen and R. Mirsky (introduced by M. Benjamin). *Department of Anatomy, University College London*

Schwann cells arise from neural crest cells via a distinct intermediate cell, which we have characterised and called the Schwann cell precursor (Jessen et al. *Neuron* **12**, 1994; Dong et al. *Neuron* **15**, 1995). Schwann cell precursors are the predominant cell type in newly emergent limb nerves and can be identified by a distinctive set of morphological and molecular properties at E14–15 in the rat and E12–13 in the mouse. They are acutely dependent on neuronal signals for survival and die by apoptosis when deprived of axonal contact in vitro. Conditioned medium from cultured DRG neurons and β forms of neuregulin (GGF, NDF) rescue these cells from death and allow them to convert to Schwann cells on a time schedule similar to that seen in vivo when they are in contact with axons. Neuregulin mRNA is strongly expressed by both motor and sensory neurons in early development and addition of soluble erbB4 protein, a neuregulin specific receptor protein, removes the survival activity from the neuron-conditioned medium. Together with recent data from other laboratories showing that Schwann cell precursors in neuregulin null mice fail to develop properly (Meyer & Birchmeier, *Nature* **378**, 1995) and that early glia in embryonic chick nerves die when their axons are made to degenerate (Ciutat et al. *J. Neurosci.* **16**, 1996), our data establish the neuregulins as axon-associated signals that regulate survival and lineage progression in Schwann cell precursors. Further analysis of the difference in survival regulation between Schwann cells and their precursors has shown that Schwann cells are capable of autocrine support of their own survival while autocrine survival loops are not yet present in precursors. Therefore Schwann cell precursors are precariously dependent on cell-extrinsic (axonal) survival signals (see above) while in developing nerves, Schwann cell survival is likely to depend largely on autocrine mechanisms. The molecular identity of the autocrine survival factor is being investigated.

21 The changing neurotrophin requirements of developing sensory neurons. By A. Davies, V. Buchman, A. Horton, R. Klein*, L. Minichiello*, N. Ninkina, G. Paul and L. Pinon (introduced by M. Benjamin). *School of Biological and Medical Sciences, University of St Andrews, Scotland and *Differentiation Programme, EMBL, Heidelberg, Germany*

Our in vitro studies have shown that many populations of sensory neurons become dependent on brain-derived neurotrophic factor (BDNF) for survival shortly after their axons reach their targets. The onset of BDNF dependence is

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principally controlled by an intrinsic clock in these neurons; neurons with more distant targets that are innervated later in development survive longer in culture without neurotrophins before becoming BDNF-dependent. Grafting experiments in early chicken embryos indicate the clock becomes programmed in neuron progenitor cell populations by signals that act in the immediate vicinity of these progenitors. Whereas some populations of sensory neurons retain dependence on BDNF throughout the phase of naturally occurring cell death, other populations, such as the neurons of the trigeminal ganglion, switch their dependence from BDNF to NGF during the early stages of target field innervation. Consistent with these in vitro findings, there is a substantial increase in the number of dying neurons in the early stages of the development of the trigeminal ganglion in *trk*B-/- embryos, whereas the increased neuronal death occurring in *trk*A-/- embryos peaks later in development, coincident with peak of naturally occurring neuronal death. Unlike the onset of BDNF dependence, which is controlled by an intrinsic timing mechanism that is programmed early in development, the switch to NGF dependence is triggered by target-derived signals that act during the switchover period. The onset of neurotrophin dependence is associated with marked increases in the expression of the corresponding neurotrophin receptor tyrosine kinase. The loss of BDNF dependence is associated with high levels of expression of noncatalytic trkB isoforms in neurons which act as negative suppressors of BDNF signalling, as shown directly by ectopic expression studies in other neurons. In the late stages of naturally occurring neuronal death, trigeminal neurons additionally acquire in vitro responsiveness to ciliary neurotrophic factor (CNTF), leukaemia inhibiting factor (LIF), oncostatin-M (OSM), cardiotrophin-1 (CT-1) and interleukin 6 (IL6).

22 Neurotrophins and neurotrophic activities in neural crest development. By M. Sieber-Blum and J. Min Zhang* (introduced by M. Benjamin). *Department of Cell Biology and Anatomy, Medical College of Wisconsin, Milwaukee, Wisconsin and *Laboratory of Biochemistry, National Cancer Institute, Bethesda, Maryland, USA*

The roles pertinent growth factors play in neural crest cell development were determined by in vitro colony assay in a serum-free culture medium. Stem cell factor (SCF) and neurotrophins affect survival and differentiation in several lineages. SCF is trophic for pluripotent neural crest cells. This survival effect is neutralised by any neurotrophin tested: nerve growth factor (NGF), brain derived neurotrophic factor (BDNF), or neurotrophin-3 (NT-3). Contrary to expectation, survival of committed melanogenic cells is not supported by SCF alone, but by the combination of SCF plus a neurotrophin (NGF, BDNF or NT-3). Moreover, SCF and BDNF promote differentiation of neural crest cells into SSEA-1-immunoreactive sensory neuron precursors. In contrast, NT-3 acts in sympathetic neuron differentiation by selectively promoting high affinity norepinephrine transport. Internalised norepinephrine subsequently promotes terminal differentiation of adrenergic cells by a signalling pathway that includes elevated levels of intracellular free calcium. Adrenergic differentiation is blocked by the norepinephrine uptake inhibitors, desipramine and cocaine. In summary, our observations indicate that growth factors which are required for the survival of neural crest derivatives can also play multiple roles in the early phases of neural crest cell development. Our observations suggest that trophic mechanisms are nonselective, whereas effects on cell differentiation are selective for a particular neurotrophin.

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23 Human neurocristopathies associated with melanin pigmentary abnormalities. By J.-P. Ortonne (introduced by M. Benjamin). *Department of Dermatology, University of Nice, France and INSERM U385*

In mammals, future skin melanocytes differentiate as melanoblasts from the neural crest, proliferate and differentiate subsequently into melanin producing melanocytes at their final anatomical sites (skin, inner ear, choroid, iris, etc.) in the embryo. Several genes including the c-KIT protooncogene, that encodes a transmembrane receptor of the tyrosine kinase family, the MITF gene, encoding a dimeric transcription factor of the basic-helix loop-helix-leucine zipper class and the genes encoding either the endothelin B receptor or its ligand endothelin-3 are known to be involved in survival and/or migration, and proliferation of melanoblasts and melanocytes in mice. The neural crest is also responsible for the development of several other embryonic structures such as the connective tissue of the head and neck, components of the branchial arch muscles, the sensory and autonomic ganglia and the gut enteric neural plexus. Thus, developmental abnormalities of the neural crest (neurocristopathies) predict phenotypes related to pigmentary abnormalities of skin, ear and eyes as well as characteristic abnormalities of other neural crest-derived elements. The characteristic distribution of skin hypomelanosis in piebaldism and Waardenburg syndrome is among the most striking of all human disease phenotypes. Piebaldism is an autosomal dominant disorder characterized by cutaneous hypomelanosis involving the anterior trunk, the central portion of the eyebrows and the midfrontal portion of the scalp with resultant white forelock, but sparing the skin of the middorsal region and of the midlimbs. The depigmented skin typically lacks most or all melanocytes. The involvement of KIT mutations in human piebaldism is well established. About two-thirds of the patients have point mutations, and most of all of the rest appear to have KIT gene deletions. The actual mechanisms by which abnormal KIT function results in the piebald phenotype is not yet known. Waardenburg syndrome (WS), another autosomal dominant disorder characterised phenotypically by piebald like hypomelanosis, hypopigmentation of the iris, lateral displacement of the inner canthi of the eye (dystopia canthorum) and sensorineural deafness shows clinical and genetic heterogeneity. Loss of function mutations of the paired box and homeobox gene PAX3 have been found in WS type 1 and WS type 3 (Klein-WS associated with other abnormalities such as anomalies in the musculoskeletal system of the limb). A subset of individuals with WS 2 (WS 1 phenotype except dystopia canthorum) have mutations of

the MITF gene. Shah–Waardenburg syndrome (WS type IV), an autosomal recessive disorder including the additional feature of Hirschsprung disease (aganglionic megacolon) has been associated with mutations of the endothelin B receptor gene. Several additional human pigmentary genodermatoses due to abnormal embryogenesis of the melanocyte system have been described but not yet associated with specific genes. Congenital dermal melanocytoses such as sacral and aberrant mongolian spots may be due to abnormal melanocyte migration towards epidermis. Whether these sporadic disorders are due to neural crest abnormalities remains to be demonstrated. The complex syndrome phacomatosis pigmentovascularis characterised by the association of extensive oculocutaneous hypermelanosis and extensive vascular malformations may result from alterations involving both the primitive vascular plexuses and the neural crest.

24 New data on the factors controlling patterning and differentiation of neural crest cells. By N. M. Le Douarin (introduced by M. Benjamin). *CNRS Institute of Embryology, Nogent-sur-Marne, France*

Two aspects of neural crest ontogeny will be discussed. First, the capacity of neural crest cells to regulate the deficiencies resulting from extirpation of segments of the cephalic neural fold at the early somitic stages. It will be shown that the neural fold located at the anterior and posterior limits of the removed territory regenerates neural crest cells which migrate longitudinally along the neural axis and fill the spaces normally occupied by the missing crest. Whatever the transverse level they reach at the term of their migration, the neural crest cells keep the *Hox* code corresponding to their rhombomeric origin. This results in abnormal *Hox* gene expression in certain branchial arches. The phenotypic abnormalities resulting from altering the *Hox* code will be examined. The second point concerns the factors which are critical for survival and differentiation of neural crest derivatives. The respective roles of the *steel* factor and of endothelin 3 will be discussed.

25 Neural crest migration and congenital heart defects in the *splotch* mutant mouse. By A. J. Copp, D. J. Henderson and S. Conway* (introduced by M. Benjamin). *Neural Development Unit, Institute of Child Health, University of London and *Developmental Biology Program, Medical College of Georgia, Augusta, USA*

Mice mutant at the *splotch* locus have deletions or point mutations in the *Pax3* gene. The mutant phenotype includes abnormalities of neural crest migration, defective colonisation of the limbs by muscle progenitor cells and defects of neural tube closure. Among the neural crest-related abnormalities are congenital heart defects in which the muscular septum that divides the aorto-pulmonary outflow tract fails to form, yielding the malformation persistent truncus arteriosus (common arterial trunk). Using gene expression markers including *Pax3*, *Crabp1*, *Hoxa3*, *Prx-1/2* and c-met, we have followed the cardiac neural crest to the developing outflow tract in 10.5 d mouse embryos and find that this neural crest sub-population initiates migration but does not reach the outflow tract of homozygous *splotch* embryos (Conway et al. *Development*, 1996, in press). Mutant neural crest cells appear to migrate normally in vitro, suggesting that the genetic defect may reside in the environment through which the neural crest migrates, not in the crest cells themselves. Assessment of the expression of genes encoding a number of extracellular matrix molecules has indicated that the chondroitin sulphate proteoglycan versican (PG-M) is markedly over-expressed in homozygous *splotch* embryos. Moreover, the pattern of versican expression in normal embryos is complementary to *Pax3*. These data suggest that the transcription factor *Pax3* may negatively regulate the expression of versican. Since versican is known to have an inhibitory effect on neural crest cell migration in vitro, its over-expression in *splotch* embryos may constitute the primary molecular abnormality limiting neural crest cell migration.

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26 Odontogenic potential of trunk neural crest in the urodele amphibian *Ambystoma mexicanum*. By A. C. Graveson, M. M. Smith and B. K. Hall* (introduced by M. Perry). *Department of Craniofacial Development, U.M.D.S., London and *Department of Biology, Dalhousie University, Canada*

The odontoblasts of the teeth are derived exclusively from neural crest cells of anterior cranial levels. It has been generally accepted that the potential for odontogenesis is limited to these axial levels. An in vitro system, in which segments of neural folds were cultured in direct contact with known inductors, was used to test directly both odontogenic and chondrogenic potential of the neural crest in the Mexican axolotl. The study included a region for which fate maps are not known, situated between the levels traditionally designated as cranial and trunk. Odontogenic potential was found in neural crest cells from axial levels not normally fated to produce teeth, including all chondrogenic levels, as well as a short segment of neural fold at the posterior end of the 'intermediate' region which did not possess chondrogenic potential. These results necessitate a re-evaluation of the designations of 'cranial' and 'trunk', and a reconsideration of the evolutionary implications of the developmentally distinct crest-derived skeletal populations.

27 The role of Sox genes in the migration and differentiation of neurons in the developing central nervous system. By M. Rex, P. J. Scotting and P. Wigmore*. *Department of Biochemistry and *Department of Human Anatomy and Cell Biology, University of Nottingham*

The transition from dividing undifferentiated epithelial cells to differentiating cells migrating to their final destination is a major event in development of the central nervous system. The mechanisms controlling this transition are, however, currently unclear. We have recently shown changes in the expression of 3 members of the Sox gene family as cells pass from the proliferative epithelial state to a migratory

differentiating state. The Sox genes are a family of genes which encode transcription factors which share a common DNA-binding motif called the HMG-domain. Expression of Sox2 and Sox3 is restricted to the proliferating neuroepithelium, of the early neural tube and later ventricular zone. Cells which have exited mitosis and migrated from the ventricular zone do not express Sox2 or Sox3. In contrast Sox11 is expressed at low levels in the neuroepithelium but its expression increases dramatically in cells as they migrate. The expression patterns of these genes suggest that they may regulate this progression of cells from the proliferative neuroepithelium of the ventricular zone to the migrating and differentiating cells found in the subventricular zone and cortical mantle. These changes in Sox gene expression are a feature common to all neuronal populations we have studied. We have tested the role of these Sox genes in these events by transfection into cells in culture. The constitutive expression of these genes interferes with the transition of cells from proliferating to differentiating states. Their expression also appears to affect the cell–cell adhesion of these cells.

28 Immunohistochemistry of neural crest cells of the rat thymus. By M. D. Kendall, K. Botham, A. G. Clarke, G. Jones and *G. M. Head. *The Thymus Laboratory*, *The Babraham Institute*, *Cambridge and *Biology Department*, *Imperial College of Science*, *Technology and Medicine*, *London*

Many reports exist of a few cells in adult thymuses showing reactivity for tyrosine hydroxylase (TH), neuron specific enolase (NSE), S100, protein gene product 9.5 (PGP 9.5) or acetylcholinesterase. These cells appear epithelial, but their long cytoplasmic extensions make them resemble neuronal cells. If the markers were present in the same cell, these cells would now be called neural crest cells. Neural crest cells are involved in the formation of the thymus, but controversy exists as to their final fate. Targeted disruption of the Hoxa-3 gene (involved in neural crest cell patterning) in mice results in the athymic condition, and pax-1 (the expression of which precedes Hoxa-3 gene activity) mutants are hypothymic. Observations during the development of rat thymic epithelial cultures, and fragments for transplantation under the kidney capsule (where after 2 months, a minute, but fully formed thymus develops), identified relatively undifferentiated neural crest-like cells which are much more frequent in developing transplants. The proliferative cell types selected by primary and subsequent cultures are similar, but of a wider range of morphology. Primary cultures and fragments were cultured from male Wistar rats (6–8 wk) using RMPI or DMEM/F12, both with additions. Cultured fragments were inserted under the kidney capsule of littermates (28+ healthy transplants, conducted with the appropriate Home Office Licences, using Hypnorm and Hypnovel anaesthesia, and killed under Schedule 1 conditions). Examination of frozen sections of implants (8–21 d after surgery) revealed many large, rounded, well spaced-out cells, predominantly towards the outside of the implants, immunoreactive to both TH and L1 (a neural cell adhesion glycoprotein), and singly immunoreactive to PGP 9.5, nerve growth factor, chromogranin A, calcitonin gene-related product, thymulin (using 4β), cytokeratin, vimentin, laminin, glutamate decarboxylase and a neuroectodermal cell antibody (A2B5). Many of these cells were in mitotis. Cell culturing is also highly selective for proliferating cells. However primary cultures are slow to start, suggesting a very low number of stem precursors. After 4–6 wk at least 3 morphological forms of cells immunoreactive to the above panel (NSE and S100, not tested) were observed, as well as flattened large epithelial cells. After subcultures 4–6 the cells are 98% epithelial, still immunoreactive to 4β, TH$\pm$, A2B5, vimentin and laminin but not to L1 or NGF. Further work on the pattern of growth and differentiation potential of these cells is currently in progress.

29 Endothelin-3 promotes the proliferation and delays differentiation in a range of precursor types in quail neural crest cell culture. By J. G. Stone, L. I. Spirling and M. K. Richardson (introduced by D. C. Davies). *Department of Anatomy and Developmental Biology*, *St George's Hospital Medical School*, *London*

The neural crest gives rise to a multitude of different cell types in the vertebrate embryo. For example at the level of the trunk it contributes to the peripheral nervous system and to melanocytes. Much work has been previously undertaken and is still required to determine the many factors which contribute to proliferation and differentiation of these cells. Recent work has identified the peptide endothelin-3 (ET-3) as an important regulator of neural crest development. Transgenic mice with mutations in the gene for ET-3 or its receptor show defects in the development of enteric neurons and melanocytes. It has also been shown that ET-3 is a mitogen for melanoblasts in vitro. We have used an in vitro colony assay to examine the effects of ET-3 on precursor subpopulations in the truncal crest of the quail embryo. Secondary cultures of neural crest were grown in control and ET-3 supplemented medium. These parent cultures were replated into permissive medium at varying time points to give daughter cultures. The colonies which developed in daughter cultures were analysed for the presence of differentiated cells in the adrenergic, sensory and pigment cell lineages. In the absence of exogenous ET-3, crest cells underwent terminal differentiation into neurons and pigment cells after a few days. Colony assay in daughter cultures showed a progressive decline in the number of identifiable cell types per colony, suggesting a loss of developmental potentials in the parent population with time. By contrast, when parent cultures were grown in the presence of 100 ng/ml ET-3, this decline in developmental potentials was inhibited and some neuronal colonies increased in abundance. We are currently trying to identify which population of early migrating cells is responsive to ET-3.

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30 Immortal cell lines representing precursors of melanoblasts, and their molecular markers. By E. V. Sviderskaya, D. J. Easty, S. P. Hill, C. R. Goding* and D. C. Bennett (introduced by D. C. Davies). *Department of Anatomy and Developmental Biology, St George's Hospital Medical School, London and *Marie Curie Memorial Foundation Research Institute, Oxted, Surrey*

We previously established immortal lines of murine melanocytes and melanoblasts (Sviderskaya et al. *Development* **121**, 1995) of various genotypes, with the aim of understanding the cellular and molecular basis of the many known germline coat colour mutations. We have now established 3 lines of a new cell type, derived from neonatal murine skin. We used dermal fibroblasts as feeder cells, and a culture medium designed for melanoblasts. These cells are not, however, melanoblasts as judged by numerous molecular markers as well as morphology and inability to differentiate directly to melanocytes. However, the lines, even when cloned, produce occasional colonies of melanoblasts, which do form melanocytes. We have also observed neuron-like cells in the cultures; however, we do not yet know the full potency of the cells and we provisionally call them melanoblast precursors (PMB). We have used Northern analysis in a broad study of differential gene expression in these PMB as compared to melanoblasts (MB) and melanocytes (MC). PMB have few melanocytic markers but they do, like MB and MC, express mRNA for the transcription factors Pax3, Sox4 and Slug, which are neural crest-related, and Brn2, which is found in brain, melanoma cells, MB and MC. PMB also have a transcript for Ednrb (endothelin receptor B), required for development of melanocytes and enteric neurons. Unlike MB, PMB have mRNA for the receptor tyrosine kinases Pdgfra (PDGF receptor alpha), also implicated in development of melanocytes and other neural crest lineages, and Sek4/HEK2, ectopically expressed by most malignant melanomas. To define this cell type further, we are studying whether the differentiation or commitment of PMB can be manipulated by varying culture conditions and supplements.

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31 A model of the stratum corneum in man, the Rhesus monkey and the rat. By J. E. Malcolm. *Royal College of Surgeons, Lincoln's Inn Fields, London*

Evidence has previously been presented for a liquid crystal (paracrystal) origin of the structure of skin, following a description in mineral terms (Malcolm, *Annals Anat*. Suppl. **178**, 1996). Study of the mineral analogues suggest that skin structure is crystalline, analogous to 't-o-t' structure in phyllosilicates and that it originates from a liquid crystal mesophase as in nerve tissue (Rinne *Trans. Faraday Soc*. **29**, 1933). From a study of hair whorls, Findley and Harris (1977) concluded that human skin has paracrystal properties (*J. Phys. Anthropol*. **46**, 1977). In murine stratum corneum lipids, crystalline and liquid alkyl chains coexist at 25°, as shown by X-ray diffraction (White et al. *Biochemistry* **27**, 1988). Frozen sections of mammalian skin obtained at operation or postmortem were examined unfixed and unstained with a polarising microscope. Photomicrographs of 3 specimens will be shown (1) from the footpad of a Rhesus monkey, showing biaxial birefringence and corneocyte stacking resembling that of paracrystal, smectic texture with continuity of strata (killed by injection; intramuscular ketamin; intravenous Euthatal); (2) hairy skin from the human scalp (obtained at operation), showing uniaxial birefringence on stage rotation and the use of a tinted plate; (3) a rat hair containing spiral, long range cholesteric crystals. From these points and from a study of cleavage planes, a 't-o-t' analogue, paracrystal chemical model of stacking in the stratum corneum can be devised.

32 The 3-D fibromuscular structure of the human fallopian tube. A high resolution scanning electron microscopic study. By P. M. Motta, E. Vizza and S. Correr. *Department of Human Anatomy, Faculty of Medicine, University of Rome 'La Sapienza'*

The present study reveals the 3D organisation of the muscular and associated collagen components in the human fallopian tube by means of chemical maceration techniques (2N-NaOH/6N-NaOH) (Vizza et al. *Hum. Reprod*. **9**, 1995) followed by scanning electron microscopy with the scope to evaluate the biomechanical properties of the myosalpinx. The tubes were taken from women (35–45 y old; with their consent) that were subjected to hysterectomy for metrorrhagia. The 6N NaOH maceration of the extracellular matrix showed that the smooth muscle cells (SMC) of the myosalpinx did not form clear cut longitudinal and circular layers but dichotomised and fused with one another giving rise to a continuous multidirectional network. The SMC bundles appeared thinner and more randomly arranged in the ampulla than in the isthmus. The 2N-NaOH maceration showed that the collagen fibrils form very delicate baskets housing the SMC bundles and connect the ramifications of the muscular network. The folds of the isthmus contained thick collagen bundles with a wavy course, whereas the ampullar folds were very thin, elongated and dichotomised in secondary and tertiary ramifications. The study clearly showed that the collagen skeleton of the myosalpinx is interconnected with the entire collagen skeleton of the tubal wall to form a complex structural system. In particular, SMC are embedded in a series of delicate fibrillar frameworks extending without interruption along the whole tube. Our 3D observations suggest that forces generated by the muscular contraction may diffuse throughout the fibrillar skeleton following the spatial orientation of the collagen bundles. In this way, the forces can be distributed to regions of the tubal walls (i.e. mucosal folds) where the SMC are not present. Moreover, the arrangement of the fibromuscular skeleton, particularly in the mucosal folds, differs in the various segments of the tube. The large and thick folds of the uterotubal junction are formed by dense and apparently rigid collagen bundles, and therefore, it is likely that here they may act as an inner gear, teeth like, sphincter apparatus. On the other hand, the mucosal folds of the ampulla, due to their fine, thin and highly textured collagen skeleton, all around the rich vascular net of this zone may provide support to vessels. Thus, this collagen skeleton, diffusing a contraction wave

may activate blood and lymphatic circulation including permeability followed by tubal fluid secretions. These 3D data together suggest that the microanatomical organisation of each tubal segment is designed for a specific reproductive function which deserves to be studied biochemically and functionally.

This work was supported by funds from MURST and CNR, Italy.

33 Fluorescence-aided detection of microdamage in bone. By T. C. Lee, E. R. Myers* and W. C. Hayes*. *Department of Anatomy, Royal College of Surgeons in Ireland and *Orthopaedic Biomechanics Laboratory, Beth Israel Hospital and Harvard Medical School*

Fatigue damage is a stimulus for bone remodelling. If it accumulates too rapidly and the capacity for repair is exceeded, stress fractures result. If the repair mechanism is deficient, as in old age, damage also accumulates and contributes to fragility fractures. Microcracks generated in vivo are detected by bulk staining with basic fuchsin in ethanol. Sections are then cut and ground in an aqueous medium in which the dye is relatively insoluble. Despite this, partial staining of artefactual damage occurs. The fluorescence properties of fuchsin offer a potential screening tool for differentiating true from artefactual microcracks. The right 6th rib was excised from 10 unembalmed cadavers and a 0.5 cm section cut 5 cm distal to the tubercle. These were fixed in 70% ethanol and bulk stained in 1% solutions of basic fuchsin in 80, 90 and 100% ethanol at −20 psi vacuum. Specimens were rehydrated in distilled water, sections cut and hand-ground to 150 μm. They were examined using conventional transmitted light microscopy and using green (510–560 nm) and ultraviolet (400–440 nm) epifluorescence. Using transmitted light, fuchsin-stained microcracks varied from purple to black in appearance and differentiation of fully stained microcracks sustained in vivo from partly stained, artefactual cracks required variation in light intensity, magnification and depth of focus. Using green epifluorescence, fully stained microcracks shone reddish-orange against the dark field background and, under u.v. epifluorescence, emitted a dark blue light. Partly stained, artefactual cracks failed to satisfy these criteria. Microcrack density ($P = 0.06$) and length ($P = 0.11$) did not differ between the transmitted light and epifluorescence groups (Wilcoxon rank test) and are comparable with published data (Burr, *Clin. Orthop. Rel. Res.* **260**, 1990).

	Density (no./cm² (S.D.))	Length (μm (S.D.))
Transmitted	7.18 (6.35)	76.05 (27.8)
Epifluorescence	13.39 (9.31)	82.22 (28.95)
Burr (1990)	14.25	88 (38)

Fluorescence-aided detection of microcracks proved as accurate as transmitted light microscopy in identifying fully stained microcracks. However, epifluorescence is a simpler and more rapid screening method for differentiating fully stained from artefactual microcracks.

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34 The structure and function of mitral valve chordae tendineae. By C. Millington-Sanders, L. Lawrence and C. Stolinski. *Department of Anatomy and Cell Biology, Imperial College School of Medicine at St Mary's, University of London*

The composite parts of mitral valve chordae tendineae consist of a dense collagenous central core, surrounded by a layer of elastic fibres and subendocardial connective tissue, covered by flat endocardial cells. Chordae tendineae were obtained from anaesthetised, perfused primates (*Macaca mulata*), post operation, and 8 postmortem elderly human subjects. The structure of the chordae was observed using light and scanning electron microscopy. Frozen sections 8 μm thick and 2 μm thick glycol methacrylate resin sections, stained with Miller's or Van Gieson's stain or with Fast Green were examined. In normal relaxed chordae of adult primates, a distinct regular wavy arrangement of collagen fibrils was clearly seen. Under tension this pattern was not observed. The subendocardial layer of these chordae was relatively thin. Chordae originating from elderly human subjects showed a substantial enlargement of this layer and disorganisation of collagen fibril arrangement. In normotensive individuals, the continuous repetitive force exerted at systole on an average size mitral valve is of the order of 8 Newtons. This force is distributed among the chordae and amounts to approximately 0.3 Newtons. In the elderly human subjects, the central collagenous core formed only a fraction of the overall structure responsible for transmitting the force to the valve. The surrounding thick layer of subendocardial loose connective tissue, judging by the disposition of its structural components, is not capable of transmitting force from the papillary muscle. It can be suggested that the ageing process which is taking place within the chordae results in changes of the structural arrangement and reduction of the collagenous core which is accompanied by the formation of excessive amounts of subendocardial tissue.

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35 Temporary paralysis of soleus muscle in rats affects survival and connectivity of motoneurons. By S. J. Lawson and M. B. Lowrie. *Department of Anatomy and Cell Biology, Imperial College School of Medicine at St Mary's, London*

Neonatal motoneurons are critically dependent upon interaction with the target muscle for survival. Disconnection of the motoneurons from the muscle either by axotomy or by pharmacological blockade of the neuromuscular junction causes motoneuron death but the timing is different. After axotomy most motoneurons die by apoptosis within the first 3 d whereas after temporary neuromuscular blockade motoneuron loss is delayed by at least 3 wk. This study investigates the timecourse of motoneuron loss after

neuromuscular blockade and the connectivity of those motoneurons which survive. In the 1st experiment neonatal Wistar rats were anaesthetised with halothane and motoneurons innervating the soleus muscle in both legs were retrogradely labelled by intramuscular injection of the fluorescent dyes fast blue (FB) and diamidino yellow (DY). At the same time a silicone implant containing α-bungarotoxin was placed adjacent to the soleus muscle in one leg. This was replaced by a larger implant 3 d later to produce a total period of paralysis of about 7 d. Control animals received plain silicone implants. Animals were taken at intervals after surgery, terminally anaesthetised with sodium pentobarbitone (0.1 ml/kg) and perfusion-fixed. The number of labelled motoneurons in serial sections of the lumbar spinal cord was counted. In control animals and on the side contralateral to paralysis in the experimental group no difference in motoneuron survival was observed. On the side of paralysis most motoneuron loss occurred between 6 and 10 wk and there was a preferential loss of motoneurons from the caudal region of the motor pool. Comparison of counts with the contralateral side showed that at 10 wk $65.9 \pm 4.5\%$ (mean $\pm$ S.E.) of motoneurons survived on the operated side ($P < 0.05$, paired t test). In the 2nd experiment sequential double labelling was used. Motoneurons were prelabelled with FB only at the time of paralysis then post-labelled with DY at 8 wk. This method revealed a similar number of surviving FB-labelled motoneurons as found at 10 wk (66.4 ± 6.4) but only $40.8 \pm 7.9\%$ (mean $\pm$ S.E.) were additionally labelled with DY. This study confirms that some motoneurons die after temporary postnatal paralysis and also shows that some surviving motoneurons lose functional contact with the muscle. Both processes begin several weeks after the period of paralysis is ended.

S. J. Lawson is in receipt of an Anatomical Society Studentship.

36 The effect of reserpine on intestinal uptake of microparticles in the rat. By R. A. Hazzard, G. M. Hodges and K. E. Carr. *School of Biomedical Science/Anatomy, The Queen's University of Belfast*

Microparticle uptake by the small intestine is a recognised phenomenon which has implications for the uptake of atmospheric particles including radionuclides: there is also increasing interest in the potential use of microparticles as vehicles for the oral delivery of vaccines and drugs. However, for such systems to be fully understood, study of the uptake of microparticles by the intestine and factors which influence this process are required. Previous microscopy and bulk studies have demonstrated the uptake of microparticles by the proximal intestine, although the precise extent of spread of microparticles along the intestine following oral administration is not known. The current study assesses the uptake of 2 μm latex microparticles along the intestine and examines the effect of reserpine on uptake using an established in vivo rat model. Microscopy is used to examine particle uptake as bulk analysis has been shown to over-estimate particle numbers and microscopy can provide information on the position of particles within the tissues. Twelve male Sprague–Dawley rats, aged 7–8 wk were used with 6 animals given an IP injection of reserpine (1 mg/kg in ascorbic acid) 18 h prior to particle dosing. Experimental animals (n = 3 per group) were given 0.25 ml latex particle suspension ($\sim 1.65 \times 10^9$ particles) by gavage. Control animals were given 0.25 ml sterile distilled water. Thirty minutes after dosing, animals were perfusion-fixed in 3% glutaraldehyde and the entire small intestine was removed and divided into 9 equal segments. All treatments to animals were carried out by a licensed investigator. Peyer's patch-containing full circumference rings were dissected from each intestinal segment and 14 μm cryosections prepared. Particle numbers were assessed in propidium iodide-stained sections using epifluorescence microscopy. Results for the nonreserpine group showed particle uptake in all 9 intestinal segments with numbers decreasing from proximal to distal. Greatest uptake was noted in the 1st segment with a small peak at the 4th. In the reserpine-treated group uptake was higher from the 2nd segment onwards: the pattern of uptake was different, with maximal uptake at the 2nd and 5th segments and another smaller peak at the 7th segment. Both groups had negligible particle numbers in the most distal areas. For both groups, particles were noted in association with the villous epithelium, cryptal and deeper tissues, with minimal numbers in Peyer's patches. Statistical analysis showed higher particle uptake in the reserpine-treated group for some cell and tissue types in several intestinal segments. The data indicate that, in addition to a reserpine-induced increase in motility and hence spread of particles along the intestine, reserpine may also enhance total particle uptake by the mucosa. The findings are relevant to the study of microparticles as potential drug carriers, as the bioavailability of such systems could be enhanced by the use of agents which alter intestinal motility and uptake. Also relevant is the possibility that changes in intestinal motility could alter the uptake of atmospheric particles including radionuclides.

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37 Human placental microvascular endothelial cells show distinct morphological responses to gels of type I collagen and matrigel. By J. F. Dye, P. Clark, L. Leach and J. A. Firth. *Department of Anatomy and Cell Biology, Imperial College School of Medicine at St Mary's, London*

Endothelial cells, cultured in vitro with extracellular matrix proteins such as collagen, laminin or fibrin, may develop morphological responses resembling angiogenesis or vasculogenesis in vivo. This process appears to be a reciprocal interaction in which cells orchestrate the reorganisation of matrix proteins into a meshwork which in turn determines the guidance pathways determining cell shape. Two types of extracellular matrix, interstitial (type I collagen) and basement membrane extract (Matrigel) have been widely used in different in vitro models of angiogenesis. We compared the effects of the 2 matrices on the behaviour of human placental microvascular endothelial cells (HPMEC) which have been described to the Society previously. Cells were cultured in 2 different configurations.

(1) Cultures were first seeded onto a gelatin substratum and allowed to reach confluence. Then gelling solutions of either collagen I or Matrigel in medium were overlaid. (2) Gels of collagen I or Matrigel were first prepared, and then cells were overlaid at a density of $1 \times 10^5/cm^2$. Cultures were observed at intervals thereafter and were fed every 2 d. Human umbilical vein endothelial cells (HUVEC) were used for comparison. We found that both matrices, at certain concentrations, caused cell monolayers to reorganise. When cells were overlaid, the differences in morphology between the matrices were more dramatic. Both cell types responded to Matrigel by retraction and alignment into cords which sometimes developed into tube-like structures. Collagen I gels resulted in monolayer retraction concomitant with cells elongating. HPMEC showed alignment of cellular projections along matrix pathways without obvious cord formation. HUVEC showed a similar elongation of cells but contrasted with HPMEC in forming cords. We conclude that different matrices provoke different morphological responses. Interstitial collagen gels cause cells to elongate and tend to weaken or disrupt cell–cell adhesion. Basement membrane gels cause cells to align, maintaining cell–cell adhesion. This favours the development of tubes. Moreover, there are cell-specific differences in the responses to extracellular matrix components. This suggests that although the matrix influences morphology by providing a 3 dimensional structure, adhesion mechanisms, determined by cellular phenotype, shape the angiogenetic process.

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38 The comparative immunohistochemical staining of endothelin and nitric oxide synthase in rat, pig and human rectus abdominus flaps. By R. D. Harper, R. Martin, C. Green and G. Terenghi (introduced by S. Hall). *Blond McIndoe Centre, Queen Victoria Hospital, East Grinstead*

The rectus abdominus myocutaneous flap is widely used in reconstructive surgery for tissue coverage and is also an accepted model for the experimental investigation of flap pathophysiology. The morphological and physiological similarities of the domestic pig to man are widely acknowledged, yet the rat is a more readily accessible alternative. Recent advances in vascular physiology include the identification of the endogenous vasoconstrictor endothelin-1 and the endothelium-dependant vasodilator, nitric oxide. Their relative importance in the components of this flap model has yet to be established. Using indirect immunohistochemical techniques with antisera to endothelin-1 and the constitutive isoforms of nitric oxide synthase (NOS), the present study aims to examine the distribution of these vasoactive substances within post-mortem specimens of skin, panniculus carnosus and rectus abdominus muscle of the rat, pig and the human. Comparative morphological assessment was performed on serial sections stained with a known endothelial cell marker, von Willebrand factor (VWF), and a panneuronal marker, protein gene product (PGP 9.5). The endothelial NOS showed intense staining in the papillary plexus of the cutaneous vasculature in all 3 species although this was more prominent in the pig with the human and the rat showing a more limited arrangement. The rat panniculus carnosus had a dense capillary network staining with endothelial NOS which was less marked in the pig. The rectus abdominus muscle in all 3 species was richly supplied with endothelial NOS stained vessels. Perivascular nerves staining for endothelin and the neuronal NOS were noted in all components of the flap. The study demonstrates the human rectus abdominus flap more closely resembles its pig counterpart in terms of distribution of these vasoactive mediators than the rat.

39 The role of VEGF in embryonic angiogenesis. By M. Aitkenhead, J. Wilting*, A. Eichmann** and D. J. Wilson. *School of Biomedical Science/Anatomy, The Queen's University of Belfast, Northern Ireland, *Anatomisches Institut II der Universität Freiburg, Germany and **Institut d'Embryologie Cellulaire et Moléculaire CNRS, Collège de France, Nogent-sur-Marne, France*

The development of an adequate vascular system is essential to the success of the embryo. Recently there has been interest in the molecules that mediate and regulate vascular growth. In particular, Vascular Endothelial Growth Factor (VEGF), a potent endothelial cell mitogen of high specificity, has been shown to be expressed during developmental, physiological and pathological angiogenesis. We have studied the expression of VEGF and its receptor, QUEK 1, throughout quail embryonic development using in situ hybridisation with digoxigenin labelled RNA probes. This method allows detection of the signal at the cellular level. First expression of VEGF was located in the blood islands of the d 1–2 embryo. On d 3 of incubation VEGF was expressed in the brain, neural tube and, in particular, the developing kidney. The podocytes of the d 4 mesonephros were stained and by d 7 the renal capsule and the parietal layer of Bowman's capsule were additionally positive for VEGF. Staining of alternate sections for the VEGF receptor, QUEK 1, indicated that it was strongly expressed by the renal endothelial cells. Expression of VEGF was also detected in skeletal muscle, bone and cartilage, thyroid, glandular stomach, skin and lung. VEGF expression seemed to correlate very closely with major vascular events in these organs, reinforcing the view that VEGF acts as a paracrine stimulator of angiogenesis. However, a small number of endothelial cells were also VEGF positive, a phenomenon only previously observed in vitro under hypoxic conditions. These observations raise the possibility of autocrine regulation of VEGF-mediated angiogenesis as a result of tissue hypoxia.

POSTERS

D. 1 Ultrastructural evidence for amino acid-mediated presynaptic inhibition of intracellularly labelled glabrous skin afferents in the lumbar dorsal horn of the rat. By D. I. Hughes and A. H. D. Watson. *Anatomy Unit, School of Molecular and Medical Biosciences, University of Wales, Cardiff*

Presynaptic inhibition of afferent input allows contextually relevant responses to be elicited through the barrage of incoming sensory information. Electrophysiological and

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pharmacological evidence supporting the role of GABA in these mechanisms is well documented, while a similar role for glycine has been disputed. Though ultrastructural studies show glycinergic inputs on to type II central glomeruli, there is no electrophysiological evidence supporting a role for glycine in presynaptic modulation of sensory afferents. Glycinergic synapses in the spinal cord have been characterised by the presence of gephyrin, an intermediate molecule which binds a glycine receptor subunit to the underlying cytoskeleton. In the first part of this study, spinal levels L3, L4 and L5 were exposed by laminectomy in male Wistar rats under halothane anaesthesia. Single afferent fibres were impaled in the dorsal horn with glass microelectrodes and intracellularly labelled by iontophoretic application of Neurobiotin. Afferents were characterised by mechanical stimulation of receptor fields, prior to termination by lethal injection of Euthatal followed by intracardiac perfusion with fixative. Vibratome sections of spinal cord were processed to reveal Neurobiotin labelling, then resin embedded for electron microscopy. Postembedding immunogold labelling to show GABA- and glycinergic profiles was carried out on serial ultrathin sections. GABA and glycine-immunoreactive terminals were shown to be presynaptic to both en passage and terminal boutons, as well as to intervaricosity segments of the afferent fibre terminal arborisations. The majority of labelled profiles were immunoreactive for only one of the transmitters, though a significant portion showed colocalisation of both GABA and glycine. The 2nd part of the study combines pre-embedding immunocytochemistry to gephyrin with postembedding immunogold labelling for GABA and glycine to investigate the possible role of glycine in presynaptic inhibition. Though presynaptic GABAergic and glycinergic terminals were identified in type II dorsal horn glomeruli, gephyrin immunoreactivity was not seen within central terminals but was commonly found within dendrites. These results together may imply glycinergic presynaptic inhibition is mediated through nongephyrin associated glycine receptors, or that presynaptic inhibition of sensory afferents is predominantly under GABAergic control.

D. 2 Quantitative ultrastructural study of inhibitory amino acid inputs on to distal dendrites of identified motoneurons from antagonistic hindlimb muscle groups in the rat. By D. I. Hughes and A. H. D. Watson. *Anatomy Unit*, *School of Molecular and Medical Biosciences*, *University of Wales*, *Cardiff*

The main flexor and extensor muscle groups acting on the hip in rat are gluteus superficialis and tensor fasciae latae, and gluteus medius respectively. Both GABA and glycine are inhibitory amino acid neurotransmitters previously shown to play important modulatory roles in gating the activity of lumbar motoneurons, through the activity of either local segmental interneurons or descending brainstem projections from the ventromedial reticular formation. In the abducens motor nucleus in rat, GABAergic inputs are the dominant form of inhibitory inputs on to motoneuron somata, while the most common inhibitory input on to proximal dendrites are glycinergic (Lahjoujui et al. *J. Neurocytol*. **24**, 1995). The spatial arrangement of inhibitory inputs on to distal dendrites has not been addressed, nor have similar studies been attempted on motoneuron populations associated with locomotion. This ultrastructural study quantitates the length of axodendritic synaptic and membrane appositions from GABAergic and glycinergic inputs on to distal dendrites of motoneurons to illustrate the importance of these inputs in modulating activity within antagonistic hindlimb muscle groups. Under halothane anaesthesia, muscle fibres from gluteus superficialis and tensor fasciae latae or gluteus medius from Wistar rats were injected with CBT-HRP. Following a postoperative survival time of 4 d, animals were killed by injection of Euthatal into the liver, then perfused intracardially with glutaraldehyde–paraformaldehyde fixative. Vibratome sections of lumbar spinal cord were developed with the TMB–paratungstate method, before processing for electron microscopy. Postembedding immunogold labelling for GABA or glycine was carried out on ultrathin sections. The results show the majority of axon terminals immunoreactive for GABA and glycine contain pleomorphic synaptic vesicles and make Gray type II (symmetrical) synapses. In both flexor and extensor motonuclei, axodendritic appositions between glycinergic terminals and dendrites comprise higher percentage dendritic coverage than between GABAergic terminals and dendrites. These results are comparable to those found in the abducens motor nucleus (Lahjoujui et al. *J. Neurocytol*. **24**, 1995), and suggest that for lumbar motoneurons, a similar pattern exists whereby axodendritic appositions are also more commonly glycinergic, whereas inhibitory axosomatic interactions are more commonly GABAergic.

D. 3 Gene expression in the posterior neuropore region of C57Bl/6 and *curly tail* mutant mouse embryos. By F. Gofflot, M. Hall and G. M. Morriss-Kay. *Department of Human Anatomy*, *University of Oxford*

Curly tail (*ct*) mice are spontaneous mutants characterised by an abnormal development of the caudal neural tube occuring in 54% of homozygous embryos. The caudal defects are associated with imbalance of growth rate between dorsal (neuroepithelium) and ventral (endoderm and notochord) tissues, leading to increased ventral curvature and delayed closure of posterior neuropore (PNP). Recently, an abnormal pattern of expression of retinoic acid receptors RAR- and RAR- has been described in the PNP region of *ct*/*ct* embryos. In this study, we have investigated whether the defect in the *ct* mutant is associated with defects in the normal pattern of expression of genes related to morphogenesis in the PNP region. The first step has been to analyse in detail the patterns of expression of key genes in the most caudal part of day 10 nonmutant embryos. The list of genes includes *Shh, HNF-3*, *HNF-3*, *Wnt-5a, Wnt-5b*, *Evx-1*, *FGF-8* and *T-gene*, all suggested to be involved in proliferation and/or differentiation in the tail region. The second step of this work has been to compare these patterns with those observed in *ct* mutant embryos at the same developmental stage. *ct*/*ct* embryos were explanted at day 10 (27 to 31 somite stages) and affected embyos were distinguished phenotypically according to the size of PNP. Control (C57Bl/6), *ct*/*ct* unaffected and *ct*/*ct* affected embryos were then processed for whole-mount in situ

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hybridisation. The results obtained in the first part of this study provide a detailed description of the patterns of expression of genes important for proliferation and differentiation in the tail region of C57Bl/6 embryos. Of particular interest is the expression of *Wnt-5a* in the ventral ectodermal ridge, an important zone suggested to be the equivalent of the apical ectodermal ridge in the limb bud, and therefore of likely importance for mechanisms of tail development. We show in the second part of this study that *HNF-3*, *HNF-3* and *Shh* genes show a completely normal expression in the hindgut and notochord of *ct/ct* embryos, as compared to nonmutant embryos. The same result is observed for *T-gene* in the notochord. This suggests that the imbalance in growth rate between the ventral and dorsal tissues is brought about through influence of the mesenchymal tissue surrounding the hindgut and notochord. This hypothesis seems to be supported by the preliminary data of *Wnt-5a* expression, in which the difference between mutant and nonmutant embryos is localised to the surrounding mesenchyme.

D. 4 Changes in perivascular mesenteric nerves in response to hypertrophy of the rat ileum. By R. J. R. Johnson, R. M. Santer* and T. Cowen. *Department of Anatomy and Developmental Biology*, *Royal Free Hospital School of Medicine*, *London and* **Anatomy Unit*, *School of Molecular and Medical Biosciences*, *University of Wales College of Cardiff*

Nerves are able to grow in response to large alterations in the size of the tissues which they innervate, both during development and under certain conditions in maturity. Muscle hypertrophy induced by lumenal obstruction is followed by enlargement of the perikarya not only of intrinsic neurons, but also of extrinsic autonomic and sensory neurons projecting to the affected part of the gut. In this study, we have investigated growth and neurotransmitter changes in the innervation of mesenteric arteries supplying the hypertrophic intestine, by means of conventional immunohistochemistry and computerised analysis of confocal microscopical images. An acetate loop was placed round a portion of the small intestine of 8-wk-old Sprague–Dawley rats; the development of the hypertrophy was monitored by changes in body weight and by palpation of the abdomen. Approximately 2 wk after the operation the animals were killed by an overdose of pentobarbital, and a 10 mm segment of arteries supplying both the hypertrophic (H) portion of the intestine and those supplying other, less affected regions (NH), were removed from midway between the gut wall and the aorta, and cleaned and fixed. Vessels from age matched controls (CON) were also taken. The vessels were processed as wholemounts for the following markers: PGP 9.5 (a panneuronal marker), tyrosine hydroxylase (TH – the specific enzyme for noradrenaline [NA] synthesis) and CGRP (a neurotransmitter in some sensory nerves). Nerve density was measured and expressed as area % (the percentage field area of specific fluorescence) and intercept density (ID/mm, the number of nerve bundles in a field), and differences between groups compared with ANOVA after correction for changes in the width of vessels. No differences in area % or ID/mm were found between H, NH and CON groups using PGP as a marker. However, both ID/mm and area % increased in fibres expressing activity for TH on H and NH arteries, compared to CON. CGRP immunoreactivity (IR) showed a significant decline of ID/mm in H and NH vessels, compared to CON. Increased TH IR may indicate either increased synthesis and hence axonal content of NA and/or increased sympathetic nerve fibre growth, whilst a decline in CGRP IR suggests either increased CGRP utilisation, decreased availability or nerve fibre atrophy. The results are suggestive of differential responses of sympathetic and sensory nerves to an increased functional demand.

We gratefully acknowledge the assistance of Professor G. Gabella with this project.

D. 5 Age-related changes in the morphology of preganglionic neurons projecting to the paracervical ganglion of the female rat. By M. A. Dering, R. M. Santer and A. H. D. Watson. *Anatomy Unit*, *School of Molecular and Medical Biosciences*, *University of Wales*, *Cardiff*

Previous work has shown that the sympathetic postganglionic neurons in the hypogastric ganglion of male rats and the sympathetic preganglionic neurons which project to them are more susceptible to the ageing process than the corresponding parasympathetic populations (Warburton & Santer, *Neurosci. Lett*. **194**, 1995, Dering et al. *J. Neurocytol*. **25**, 1996). We aim to determine if there is also a change in the preganglionic neurons projecting to the female equivalent of the hypogastric ganglion: the paracervical ganglion. The paracervical ganglion of virgin female Wistar rats of 4 and 20 mo of age (n = 5 per age group) was exposed under halothane anaesthesia and 2 µl of 1% cholera toxin B-subunit (CTb) (List Biologicals) injected with a glass micropipette. After a survival period of 3 d the animals were perfused with 4% paraformaldehyde after terminal anaesthesia with sodium pentobarbitone. Spinal cord segments L1–L2 and L6–S1 were removed and postfixed overnight. Vibratome slices (100 µm) were incubated for 21 h in goat anti-CTb (List Biologicals), followed by a 2 h incubation in Cy3-conjugated antigoat antibody (Sigma). Labelled preganglionic neurons were scanned, processed and analysed using the Molecular Dynamics Sarastro 2000 argon confocal laser scanning microscope with ImageSpace software on Silicon Graphics workstations. Measurements were made of the number of primary dendrites, number of dendritic branch points, total dendritic length and soma area of preganglionic neurons. There were significant decreases in the number of primary dendrites, number of dendritic branch points and total dendritic length of sympathetic preganglionic neurons in the aged rats compared to the young adult group. The soma area was not significantly different between aged and young adult animals. In addition, some aged cells exhibited signs of degeneration, such as swelling of the soma and distension of the proximal part of primary dendrites. No significant differences were found in any of the parameters measured for the parasympathetic neurons. The results suggest that there may be a selective impairment of the sympathetic preganglionic control of the lower pelvic viscera in aged female rats similar to that found previously in males.

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D. 6 Lack of coexpression of MBP and PDGF-αR mRNAs in the anterior medullary velum of the rat brain. By M. F. Hornby, S. Kirvell, A. Graham*, M. Berry** and A. M. Butt. *Departments of Physiology,** *Anatomy & Cell Biology and *Experimental Pathology, UMDS, London*

Platelet-derived growth factor (PDGF) is considered a key factor in regulating the development of oligodendrocytes, the myelin-forming cells of the central nervous system. Cells of the early oligodendrocyte lineage express PDGF-α receptors (R) but it is unclear whether terminally differentiated oligodendrocytes also express PDGF-αR in vivo. To address this question we have used double in situ hybridisation with digoxigenin- and fluorescein-labelled riboprobes to relate PDGF-αR mRNA and myelin basic protein (MBP) mRNA expression in the isolated intact anterior medullary velum (AMV) of rats aged postnatal day (P) 10–12 and P30–32. AMV were also labelled with the oligodendrocyte-specific marker Rip to provide information on oligodendrocyte development and the extent of myelination. At P10, the AMV contained tracts in which axons ranged from unmyelinated to fully myelinated and in P30–32 AMV myelination was complete. The first oligodendrocytes to express MBP mRNA or Rip were promyelinating oligodendrocytes which had a 'star-burst' morphology and had not yet begun to form myelin sheaths. As myelination proceeded, MBP mRNA became dispersed throughout oligodendrocyte units, comprising the cell somata, processes and internodal myelin sheaths. By P30–32, MBP mRNA had been redistributed to the myelin sheaths only, reflecting a change in the site of protein synthesis in mature myelinated axon tracts. At no stage of oligodendrocyte differentiation did we observe cellular colocalisation of mRNA for PDGF-αR and MBP. The results indicated that oligodendrocytes lost expression of PDGF-αR prior to gaining that of myelin gene products, and preclude an action of PDGF-AA on Rip+/MBP+ 'star-burst', promyelinating oligodendrocytes. A notable finding was the high expression of PDGF-αR mRNA in the AMV of juvenile rats, localised to cell bodies within myelinated axonal tracts, an observation which strongly suggests that oligodendrocyte precursors persist in the AMV.

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D. 7 Cerebral neuron morphology in the late embryonic trisomy 16 mouse, a possible animal model of Down's syndrome. By M. J. Shaw and D. C. Davies. *Department of Anatomy and Developmental Biology, St George's Hospital Medical School, London*

The trisomy (Ts) 16 mouse has been proposed as an animal model of Down's syndrome (DS), since murine chromosome 16 shares a number of genes with human chromosome 21 and there are phenotypic similarities between the 2 trisomies. However, although neuronal abnormalities are a feature of the DS cortex, little similar information is available for the Ts 16 mouse. Therefore, developing neuronal morphology was investigated in the late embryonic Ts 16 mouse cerebral wall. Ts 16 embryos (approx. 15% of each litter) were obtained by crossing doubly heterozygous Rb(16.17)7BNR/Rb(11.16)C3H males with C57B1/6J females. On E 18 the pregnant females were lethally anaesthetised by fluothane inhalation and the embryos removed by caesarean section. Brains were dissected from 8 Ts 16 embryos (recognised by their characteristic phenotype) and 8 diploid littermates, under cold, phosphate buffered saline and fixed by immersion in cold, half strength Karnowsky's solution for 48 h. The brains were then processed for the rapid Golgi technique, serial sagittal 150 μm sections cut and mounted on glass slides. Impregnated neurons were investigated 'blind' in 130 μm wide strips of the cerebral wall in frontal, parietal and occipital regions of both hemispheres. Cajal-Retzius neurons, local circuit neurons and subplate neurons were observed in frontal, parietal and occipital regions of the cerebral wall. No qualitative differences were apparent in the morphologies of these neuronal types between hemispheres or genotypes. Pyramidal neurons in the cortical plate were poorly developed. Their apical dendrites typically terminated below the marginal zone with a rounded process and possessed few arborisations or dendritic spines. The occasional terminal tuft was observed. Basal neurites were observed, but it was not possible reliably to differentiate axons from dendrites. Morphometric analysis of 434 Ts 16 and 430 diploid pyramidal neurons was performed with the aid of an image analysis system and the data analysed by multivariate ANOVA. There was a significant regional variation in both apical dendrite length and curvature: parietal > frontal > occipital, for both parameters. There was also a significant effect of region on soma surface area: frontal > parietal > occipital. Neither the mean apical dendrite branch order (approx. 2), nor the mean number of extra apical neurites (approx. 1) varied between cerebral region. None of the parameters investigated varied between hemispheres or genotypes. Thus, Ts 16 does not appear to affect cerebral neuronal morphology in the late embryonic mouse. Although pyramidal dendritic fields are reduced in the adult DS cortex, aberrant neuronal morphology is only expressed after birth (Takashima et al. *Brain Res.* **225**, 1981). Similarly, although no information is available for adult Ts 16 mice since they rarely survive birth, neurites of cultured embryonic Ts 16 cholinergic forebrain neurons have been shown to be shorter, smoother and less complex than those cultured from diploid mice (Corsi & Coyle, *Proc. Nat. Acad. Sci. USA* **88**, 1991). Thus, both human DS and mouse Ts 16 appear to involve impaired neuronal development, that is not manifested until after birth.

D. 8 Visualisation of the uptake of rat prolactin and expression of binding sites in rat embryonic tissues. By A. K. Karabulut, H. Ulger* and M. K. Pratten**. *Department of Anatomy, Selcuk University, Turkey, *Department of Anatomy, Erciyes University, Turkey and **Department of Human Anatomy and Cell Biology, University of Nottingham*

The roles of maternally derived factors in the growth and development of the fetal rat are poorly understood. In our previous studies, we have shown that addition of human placental lactogen (Karabulut & Pratten, *Acta Anat.* **152**, 1995) and prolactins from different species (Karabulut & Pratten, *Teratology* **51**, 1995) to depleted rat serum increased

growth and developmental parameters in cultured rat embryos. Amongst those, rat prolactin was found active at much lower concentrations than human or sheep prolactin. To clarify the roles of prolactin in rat embryogenesis we examined the uptake of rat prolactin and expression of binding sites of the hormone in rat embryonic tissues. Postimplantation rat embryos were cultured in vitro for 44 h using the technique of New (*Biol. Rev.* **53**, 1978) in whole rat serum and serum which has low growth supporting capacity (retenate prepared by ultrafiltration of serum for a period of 8 h using Millipore filters with a molecular weight exclusion of 30 kDa). The conceptuses were transferred to phenol red free Medium 199 for 4 h, and 12.8 ng/ml rat prolactin was added to the culture medium for different times (4 h, 2 h, 1 h, 30 min and 15 min) and/or different temperatures (37 °C and 4 °C). As a control tissue, pituitary glands from 11.5 and 18.5 d pregnant rats were excised after decapitation of the animals. An indirect immunofluorescence protocol was carried out to observe uptake and expression of rat prolactin in the embryo and yolk sac. The pituitary glands showed positive stain for antiprolactin antibody whilst there was no stain in the control brain tissue, suggesting that the method used in this study is sufficient to show binding or localisation of prolactin. Prolactin binding sites were detected on the yolk sacs and the embryos grown in rat serum in the presence and absence of additional rat prolactin, suggesting that there are receptors for prolactin in the embryonic tissues. However, the intensity of uptake and binding of prolactin to embryonic tissues was much greater in the presence of exogenous prolactin. There was no detectable uptake and specific binding of prolactin in the embryos grown in retenate showing that ultrafiltration of the serum is an effective method to remove rat prolactin molecules from the serum. Shorter incubations and incubations at 37 °C caused a greater uptake and distribution of prolactin to the rat embryonic tissues, suggesting that this is time and temperature dependent.

D. 9 Teratogenicity of Edoferon Kappa A in cultured rat embryos, differences from the original molecule: salicylate, and interaction with the free oxygen radical scavenging enzymes. By A. K. Karabulut, H. Ulger* and M. K. Pratten**. *Department of Anatomy, Selcuk University, Turkey, *Department of Anatomy, Erciyes University, Turkey and **Department of Human Anatomy and Cell Biology, University of Nottingham*

The effect of Edoferon Kappa A (E-KA), a nonspecific immunomodulatory and antineoplastic substance derived from the methyl form of salicylate, and acetyl salicylic acid (ASA) on mammalian embryos has been studied. Also the interaction of these molecules with antioxidant agents has been investigated. Rat embryos were cultured in vitro from 9.5 d of gestation for 48 h (New, *Biol. Rev.* **53**, 1978). E-KA (0.1–12.8 mg/ml) and ASA (0.1–0.6 mg/ml) were added to whole rat serum. Also the lowest effective dose of E-KA (0.6 mg/ml) and ASA (0.3 mg/ml) for all parameters was added to the culture media in the presence of SOD (30 U/ml) or glutathione (0.5 μmol/ml). The growth and development of embryos was compared (Van Maele Fabry, *Toxic.* **4**, 1990) and the total protein content of embryo and yolk sac was assessed (Lowry, *Biol. Chem.* **193**, 1951). Each embryo was evaluated for the presence of any malformations. When compared to control embryos, E-KA and ASA decreased all growth and developmental parameters in a dose-responsive manner, with an increase in overall dysmorphology (haematoma, open neural tube, abnormal tail torsion, absence of fore limb bud). The effective dose of E-KA was much higher than ASA for growth retardation and malformations. There was no statistically significant difference between the control and embryos grown in the presence of 0.1–0.4 mg/ml E-KA, while the effects of ASA started at the concentration of 0.2 mg/ml on crown rump length and the morphological score. Embryos showed significant growth retardation in all scoring criteria and severe malformations when 0.5–3.2 mg/ml E-KA and 0.3–0.6 mg/ml ASA were added. Embryos showed a considerable growth at the end of 24 h culture, but did not continue to grow when 6.4 mg/ml E-KA was added, and there was no visible growth in the presence of 12.8 mg/ml E-KA. When SOD was added, there was a significant decrease in the incidence of malformations and growth and developmental parameters were increased but never reached the control level. Addition of glutathione did not change the effects of either molecule. We concluded that E-KA has direct toxic effects on the developing embryo but at 2 fold higher concentrations than ASA, and the teratogenic effects of these molecules might be related to free oxygen radicals.

D. 10 Analysis of the embryonic growth supporting fractions of extra embryonic coelomic fluid (EECF). By A. K. Karabulut, R. Layfield*, H. Ulger** and M. K. Pratten***. *Department of Anatomy, Selcuk University, Turkey, *Department of Biochemistry, Queen's Medical Centre, Nottingham, **Department of Anatomy, Erciyes University, Turkey and ***Department of Human Anatomy and Cell Biology, University of Nottingham*

At the early stages of embryonic development, many growth promoting molecules must be provided by the maternal system. The exact source of these molecules is not clear; however, various workers have suggested that factors may be produced locally to the embryo, either by decidua, the placenta or the yolk sac. In our previous study, we showed that addition of EECF to serum which has low growth supporting capacity (retenate) significantly improved embryonic growth. The EECF fraction containing molecules with molecular weights between 30 and 10 kDa were found to be much more active than other fractions. Embryos grown in EECF showed appreciable growth but did not reach the level of those grown in retenate or whole rat serum (Karabulut & Pratten, *Teratology* **53**, 1996). Therefore, as a 2nd stage of the study, we investigated the low molecular weight proteins of the EECF, which may have possible growth promoting potential. 9.5 d old rat embryos were explanted (New, *Biol. Rev.* **53**, 1978) and cultured for 9 d for anembryonic yolk sacs, and then EECF was collected. Sodium dodecyl sulphate polyacrylamide-gel electrophoresis (SDS–PAGE) was performed on whole rat serum and 3 different preparations of EECF according to the modification of the method described by Laemmli (*Nature*

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277, 1970). We observed 4 protein bands less than 30 kDa in the EECF which were not obvious in whole rat serum. The molecular weights of these proteins were estimated as 24, 22, 16.5 and 14 kDa. These proteins were blotted on to polyvinylidine difluoride (PVDF) membrane, bands were excised and amino-terminal protein sequence determination was performed. Three of the 4 proteins were successfully identified; fetal rat alpha-fetoprotein precursor (alpha-fetoglobulin) (22 kDa), apolipoprotein precursor A1 (24 kDa) and fetal mouse haemoglobin epsilon-Y2 chain (14 kDa); these proteins are unlikely to be responsible for the growth promoting activity. To investigate further growth-promoting proteins, EECF was Western blotted to nitrocellulose membrane and probed with antisera against rat prolactin, EGF, IGF I and II, and human placental lactogen. No immunoreactive bands were detected in the EECF, suggesting that either these proteins are not present or are present at levels too low to be detected. Therefore, the molecules in EECF responsible for the growth promoting effect remain uncharacterised.

D. 11 Human placental endothelium expresses an unusual phenotype. By J. F. Dye, P. Clark, L. Leach and J. A. Firth. *Department of Anatomy and Cell Biology, Imperial College School of Medicine at St Mary's, London*

The endothelium of the placenta may be expanding until term and may therefore be in a relatively proliferative state. Previous studies from our laboratory have suggested that the endothelial layer contributes a significant component of the maternofetal barrier to paracellular exchange of macromolecules, a function dependent upon the expression of cell–cell junctions normally associated with quiescent endothelial cells. In order to understand more fully the status of the placental endothelium, we have sought to characterise the histological phenotype with a panel of monoclonal antibodies and related markers. Placentas were obtained from elective caesarean section, and small pieces were excised, washed and fixed in 4% paraformaldehyde. Thereafter tissue was processed for cryomicrotomy. Immunohistochemistry was performed by standard procedures, using the fluorescent dye Cy3 as a detection reagent. Results were visualised by epifluorescent microsopy and recorded photographically using TM400 film. The results demonstrate that most of the constitutive endothelial markers are expressed. Thus vWF, thrombomodulin, CD34, CD36 and PAL-E were intensely stained, and angiotensin converting enzyme was detected. Also constitutively expressed cell adhesion molecules such as PECAM-1 and VE-cadherin were present. However, the endothelial layer also stained strongly for A10–33/1, a marker associated with proliferative endothelium, and for adhesion molecules CD44 and thy1, which are considered to be absent in 'normal' endothelium. It is interesting that cultured placental endothelial cells maintain these later markers whilst downregulating many of the constitutive markers. These findings suggest that placental endothelium may indeed exist in an activated state. However, the placenta was negative for E-selectin and VCAM, which demonstrates that it is not under inflammatory influence.

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D. 12 The growth-promoting effects of platelet-derived endothelial cell growth factor (PD-ECGF) on early embryonic development and yolk sac vascularisation. By H. Ülger, A. K. Karabulut* and M. K. Pratten**. *Department of Anatomy, University of Erciyes, Turkey, *Department of Anatomy, University of Selcuk, Turkey and **Department of Human Anatomy and Cell Biology, University of Nottingham*

Platelet-derived endothelial cell growth factor (PD-ECGF), associated with thymidine phosphorylase activity, is a 45 kDa single chain polypeptide, originally isolated from platelets, which stimulates chemotaxis of endothelial cells in vitro and angiogenesis in vivo (Ishikawa, *Nature* **338**, 1989). The finding that it stimulates other cells, including certain epithelial cell types and choriocarcinoma cells, as well as neutrophils and monocytes, and is present in large amounts in the stromal parts of placenta, suggests that PD-ECGF may play a role in the formation of blood vessels and embryonic development. The embryonic requirement for a constituent of serum may be studied by its removal. Thus, centrifugal filtration of rat serum has been carried out for 8 h using Millipore filters. To obtain serum fractions excluding molecules between 30 and 50 kDa molecular weight, 30 kDa filtrate (F) was freeze dried and added back to 50 kDa retenate (R). Rat embryos were explanted on d 9.5 and cultured for 48 h in fractionated serum presence of different concentrations of human recombinant PD-ECGF (1, 2, 4, 8, and 16 ng/ml) using the technique of New (*Biol. Rev.* **53**, 1978). Visceral yolk sacs (w/o embryo) were also cultured for 96 h in the presence of 4 ng/ml of PD-ECGF, a concentration at which optimum embryonic growth was seen. Embryonic development and visceral yolk sac growth were assessed using a morphological scoring system (Van Maele Fabry, *Toxic.* **4**, 1990) and a protein assay (Lowry, *Biol. Chem.* **193**, 1951). Morphometric analysis of the effect of this factor on yolk sac vascularisation was assessed using stereological methods. A greater growth promoting effect was seen in the presence of low concentrations of PD-ECGF. Embryonic growth was significantly greater than control values at $P < 0.001$ in all criteria when 2 and 4 ng/ml of PD-ECGF was added. However, the higher concentration of this factor was ineffective (16 ng/ml). The visceral yolk sac growth was also significantly increased ($P < 0.05$) in the presence of 4 ng/ml PD-ECGF tested after 48 and 96 h of culture. Morphometric analysis of histological sections showed that PD-ECGF increased both yolk sac vessel number and blood island development ($P < 0.05$).

D. 13 Isolation and culture of embryonic rat yolk sac endothelial cells. By H. Ülger, A. K. Karabulut* and M. K. Pratten**. *Department of Anatomy, University of Erciyes, Turkey, *Department of Anatomy, University of Selcuk, Turkey and **Department of Human Anatomy and Cell Biology, University of Nottingham*

The vascular system is the first to develop. In rats, the primordia of blood vessels appear around d 8 in the wall of the yolk sac, and the circulatory system is established by d 11–12 of gestation, when nutritional and informational molecules are transported via the vessels from the yolk sac to the embryo. We have now succeeded in isolating and

culturing endothelial cells from 11.5 d embryonic yolk sac. Female rats were terminally anaesthetised with ether on d 11.5 using the technique of New (*Biol. Rev.* **53**, 1978). The entire uterus was removed, placed in calcium and magnesium-free Hank's balanced salt solution (CMF-HBSS) and cut into individual conceptuses. The yolk sacs were excised and minced with scissors into 1–2 mm^2 fragments. These fragments were washed 3 times with CMF-HBSS and stirred at 37 °C in CMF-HBSS containing 2 mg/ml trypsin, 100 μg/ml collagenase I and 40 μg/ml DNAse until the tissue was completely dispersed. The digestion effect was then neutralised by the addition of fetal bovine serum (FBS), at 1:3 (v/v) and the cell suspension passed through 100 μm mesh nylon bolting cloth. The filtered mixed cell suspension was pelleted at 300 g × 10 min. The cell pellet was resuspended in Dulbecco's modified Eagle's minimal essential medium (DMEM) with 1.25 mg/l amphotericin B, 25 mg/l gentamycin sulphate, 100 μg/ml endothelial cell growth supplement (ECGS), 100 μg/ml heparin sulphate containing 15% FBS. The resuspended cells were plated in 25 cm^2 culture flasks, which were coated with 5 μg/cm^2 of fibronectin (Sigma) according to the manufacturer's directions, for overnight differential adherence at 37 °C. The nonadherent cells were removed by gentle aspiration and adherent cells refed with fresh medium. Culture flasks were incubated with 5% CO_2 and 95% air at 37 °C until the endothelial cells became confluent. The cells were fed every 2 d with fresh medium. The confluent cells were identified as endothelial by their characteristic 'cobblestone' morphology, positive stain for von Willebrand factor by immunofluorescence, and presence of Weibel–Palade bodies which are a unique ultrastructural marker for endothelium.

D. 14 In human palatine tonsil nitric oxide synthase is expressed not only on the endothelium. By M. E. Perry, M. J. Simon,* H. Al-Asam*, Y. Mustafa and J. E. G. Downing*. *Division of Anatomy and Cell Biology, UMDS, Guy's Hospital and *Department of Biology, Imperial College, London*

Constitutive production of nitric oxide (NO) by endothelium and its role in the control of vascular tone are well established. In addition, expression of the inducible form of nitric oxide synthase (NOS) has been identified in a variety of cells, including airway epithelia. Levels of NO are elevated in the exhaled air of asthmatics but may derive in part from upper respiratory tract, particularly in chronic bacterial infections. Thus, tonsillitis may also contribute to the production of NO at this anatomical site. We examined 15 palatine tonsils from children undergoing routine tonsillectomies. NADPH-diaphorase histochemistry performed on 200 mm thick sections proved to be an excellent panendothelial as well as epithelial marker. The staining outlined especially clearly the high endothelial venules (HEV) and the stratified squamous nonkeratinised oropharyngeal epithelium (OE). Immunocytochemistry was used to identify which of the 3 known isoforms of NOS [b (neuronal), i (inducible) and e (endothelial)] were present. Although the OE and reticulated crypt epithelium (RE) stained weakly with b and eNOS, iNOS gave the strongest reaction. All tonsillar microvasculature (including follicular capillaries) stained moderately with eNOS but was heavily labelled with iNOS, especially the HEV. In addition, iNOS also labelled follicular macrophages. This pattern of NOS expression may be associated with the following functions. (1) chemical defence of the OE surface; (2) use of NO as a signalling molecule within the honeycomb network of RE; (3) extravasation of recirculating lymphocytes as a result of changes in regulation of endothelial cell junctional permeability, through responses of the surrounding pericytes; (4) pronounced phagocytic activity of follicular macrophages.

D. 15 Calbindin-D_{9K} protein and mRNA localisation in bovine placenta. By L. Nikitenko, G. Morgan and F. B. P. Wooding. *Babraham Institute, Cambridge*

The fetus must transport considerable and increasing amounts of calcium across the placental trophoblast epithelium to support bone formation. Active calcium transport across epithelia has been shown to correlate with calbindin-D_9 or $_{28K}$ content for example in gut and kidney. The present study examined the distribution of calbindin-D_{9K} (9CBP) protein and mRNA throughout pregnancy in cow placenta to determine its possible role in calcium transport in this system. Placentas of 12 cows (55–280 d post coitum) were used. Animals were killed in an abattoir with a captive bolt pistol, the uterus removed within 5 min and perfused with fixative. We used immunogold methods at light and electron microscope level, ^{35}S-labelled 45 mer oligonucleotide probe in situ hybridisation (ISH) and double labelling technique for simultaneous detection of protein (bovine placental lactogen) and 9 CBP mRNA. In the interplacentomal (IP) region of bovine placenta at early stages of pregnancy (50–100 dpc) the 9CBP immunoreactivity is restricted to the fetal binucleate cells (BNC), some of uninucleate cells (UNC) and uterine epithelium (UE). Later the number of immunoreactive IP UNC increases to term (270–280 dpc), when the level of 9CBP in these cells is about 8–10 times more than in any other regions of bovine placenta at this stage. In contrast the immunoreactivity of UE decreases until it disappears at 220 dpc. In the underlying gland epithelium only a few cells show immunoreactivity at any stage of pregnancy. In placentomal (P) regions of bovine placenta no differences in 9CBP localisation through pregnancy have been detected, the immunoreactivity is restricted to the fetal BNC and maternal UE, fetal UNC are not 9CBP-positive. ISH and double labelling results showed distribution of 9CBP–mRNA predominantly in IP trophectoderm (both BNC and UNC) and placentomal BNC. There is a steady rise in mRNA level in the IP trophoblast throughout pregnancy; the UE and gland epithelium maintain a constant low level. The placentomal fetal BNC show the highest concentration of 9CBP mRNA found in the placenta between 100 and 150 dpc; towards term the level decreases to that found in the IP trophoblast. Sheep and goat BNC contain no 9CBP, it appears to be a unique feature of cow BNC development and not involved in transcellular calcium transport. Increase in fetal calcium demand in the 2nd half of pregnancy correlates with the increase in 9CBP protein only in the IP trophoblast as we have found also in sheep and goat. This 9CBP is distributed uniformly in the cytosol and nu-

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cleoplasm supporting a role in facilitated diffusion of calcium through the cell rather than by vesicular shuttle. The IP trophoblast would thus provide a major route for active calcium transport by the ruminant placenta.

D. 16 The effect of indomethacin on small intestinal crypt kinetics in the mouse. By R. R. Ettarh. *Department of Anatomy, University College, Dublin*

Administration of a high ulcerogenic dose of indomethacin in mice is associated with a reduction in the number of crypts in the nonulcerated parts of the small intestine (Ettarh & Carr, *J. Anat.* **189**, 1996). This decrease in crypt numbers may be related to the process of ulcer formation or of tissue regeneration during repair. Alternatively, the reduction may be related to direct effects, on intestinal crypt kinetics, of the ulcer-inducing agent. In order to examine the latter possibility, the effect of indomethacin on intestinal crypt cell production was investigated using a stathmokinetic technique with vincristine. Groups of male CD-1 mice were given either a high dose of indomethacin (2 administrations of 85 mg/kg body weight intraperitoneally, 2nd injection 20 h after the first) or a low dose of indomethacin (1 mg/kg body weight intraperitoneally at 12 h intervals). Control animals did not receive any treatment. In animals that received high dose indomethacin, the presence of ulceration at the gastroduodenal junction was confirmed at laparotomy. Vincristine (1 mg/kg body weight) was administered intraperitoneally to all animals 40 h after the start of the experiment; thereafter, animals were killed by cervical dislocation every 30 min for 2.5 h. In all animals, the small intestine was removed, its length measured and then divided into 4 equal segments. Samples were taken from the midpoint of the 1st segment. Following fixation and staining (Feulgen reaction), mitoses and crypt cell numbers were counted in microdissected crypts and mitotic indices determined. Both groups of indomethacin treated mice showed reductions in the duration of mitosis (low dose 0.44 h, high dose 0.61 h) when compared to the values obtained in control mice (1.52 h); the finding of a greater degree of reduction in the duration of mitosis in the low dose treated group of mice is suggestive of a dose-dependent effect. The calculated birth rate was higher in the low dose treated group when compared to the control values (low dose 2.52% cells/h, controls 1.82% cells/h) but the cell production rate per crypt remained unchanged in all groups. These results for the 1st quarter segment of the small intestine indicate that indomethacin affects intestinal crypt kinetics and show these perturbations to be dose dependent.

D. 17 The motile responses elicited by the cytokine CSF-1 in a stable transfection model of cellular migration. By J. Hyde-Dunn and G. E. Jones (introduced by W. E. Allen). *Anatomy and Human Biology Group, The Randall Institute, King's College, University of London*

The experimental Rat-2^{fms} is a fibroblastic cell line, transfected in a stable manner with the murine *c-fms* receptor. CSF-1 is a mitogen for Rat-2^{fms} fibroblasts. Quantitation of cellular proliferation measured via 2 assays (Brd-Urd incorporation and dual immunofluorescence PCNA / Hoescht detection), reveal that over the concentration range applied, CSF-1 had no effect upon stimulating serum-starved Rat-2 fibroblasts to re-enter the cycle. In contrast, serum-starved Rat-2^{fms} exhibit a pronounced concentration-dependent induction of mitotic activity. Two minutes post stimulation with 1.32 nM human recombinant CSF-1, serum starved Rat-2^{fms} fibroblasts demonstrate membrane ruffling with the formation of motile cell surface projections rich in β-actin and a transient loss of F-actin stress fibres. No such change in membrane or cytoskeletal architecture was observed in Rat-2 fibroblasts after identical cytokine stimulation; these cells remained quiescent, extremely flat and with prominent arrays of stress fibres. Immunocytochemistry showed that serum deprived Rat-2^{fms} stimulated with CSF-1 for 10 min, had distinct and robustly labelled phosphotyrosine plaques with specifically oriented distribution, polarised fibroblasts displaying an asymmetry of signal with greater label intensity at the leading edge. After an identical cytokine challenge Rat-2 fibroblasts remained extensively spread with small, weak phosphotyrosine plaques restricted to the periphery, comparable with the picture seen in a serum starved population. An elevation of proteins phosphorylated on tyrosine residues was detected through Western blot analysis in Rat-2^{fms} stimulated cells. Tyrosine phosphorylation levels remained basal in Rat-2 fibroblasts following CSF-1 stimulation. Immunoprecipitation and Western blot examination revealed that in Rat-2^{fms}, CSF-1 stimulation causes an increase in tyrosine phosphorylation of the 68 kDa focal adhesion protein paxillin. There was no elevation above the basal level of phosphorylation in Rat-2 fibroblasts after cytokine stimulation. Using the Dunn chemotaxis chamber the movement of serum starved and stimulated fibroblasts was monitored over a 10 h period. Rat-2^{fms} stimulated in an isotropic environment of 1.32 nM CSF-1 showed a high degree of translocation but no directional bias in their displacement. In a linear concentration gradient of CSF-1, serum starved Rat-2^{fms} demonstrated a strong preference for the majority of the cells to migrate towards the source of CSF-1. This direct observation confirms the proposition that CSF-1 is both a chemokinetic and positive chemotactic agent for Rat-2^{fms} fibroblasts. In contrast, Rat-2 fibroblasts show no directional bias challenged in a gradient of CSF-1 or elevation of their translocation within an isotropic environment of the cytokine.

D. 18 Cdc42-, Rac- and Rho-induced changes in the actin cytoskeleton of Bac1.2F5 macrophages. By W. E. Allen*,** G. E. Jones* and A. J. Ridley**. **Randall Institute, King's College London and* ***Ludwig Institute for Cancer Research, University College London*

The actin cytoskeleton is of great importance to many cellular functions, one of which is cell migration. Cdc42, Rac and Rho are small GTPases with a high degree of sequence homology to the Ras oncogene product. It has been shown that microinjection of RhoA into serum-starved Swiss 3T3 fibroblasts induces the formation of actin stress fibres and focal adhesions, while Rac1 induces the formation of actin rich membrane ruffles. These observations suggest that these proteins are important regulators of cell mi-

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gration. To test their effects on cell locomotion, Bac1 2F5, a CSF-1-dependent murine macrophage cell line was studied. In the absence of CSF-1, Bac1 cells lose their motile behaviour and round up. When re-stimulated with CSF-1, cells became highly motile and showed a chemotactic response to this cytokine. Following injection of activated RhoA (V14RhoA) into cells cultured for 24 h in the absence of CSF-1, actin polymerisation and cell contraction were rapidly (< 10 min) induced. However, this actin polymerisation did not lead to the formation of stress fibres. When Bac1 cells growing in the presence of CSF-1 were injected, retraction fibres were observed. The Rho-induced actin reorganisation persisted for 6 h after injection, but reverted back to normal by 18 h. Microinjection of activated Rac (V12Rac1) induced the formation of a large circular lamellipodial structure around the cells (< 10 min). This response persisted for 4–6 h, and did not significantly differ between growing and cytokine-starved cells. This Rac induced morphology resembles that induced by the addition of CSF-1 to cells cultured in its absence. Activated Cdc42 (V12Cdc42) induced the formation of a large number of long filopodia (< 10 min) in both growing and cytokine-starved cells. These results suggest that Cdc42, Rac and Rho will affect macrophage motility. To determine the nature of cell adhesion structures, immunofluorescent staining was carried out for vinculin, paxillin, $pp125^{FAK}$ and $\beta 1$ integrin. These proteins which are all associated with focal adhesions in fibroblasts, were found to be present in the Bac1.2F5 focal complexes. It was found that both Cdc42 and Rac1 mediated the formation and distribution of these focal complexes while RhoA did not.

D. 19 Distribution of pancreatic hormones, neuropeptides and cytoskeletal proteins in the pancreas of the one-humped camel. By E. A. Adeghate and D. J. Pallot. *Department of Human Anatomy*, *Faculty of Medicine & Health Sciences*, *United Arab Emirates University*, *Al Ain*, *United Arab Emirates*

The patterns of distribution of insulin (INS), glucagon (GLU), atrial natriuretic peptide (ANP), neuropeptide-Y (NPY), cholecystokinin-octapeptide (CCK-8), neurofilament-200 protein (NF), S-100 protein (S-100) and vimentin (VIM) in the pancreas of the one-humped camel (*Camelus dromedarius*) were investigated using immunohistochemical techniques. INS-immunoreactive cells were observed in the central and peripheral parts of the islets of Langerhans but some solitary INS positive cells were found outside the islets. INS positive cells constituted 44.26–90.91% (mean ± standard deviation (S.D.) 67.34 ± 14.20) of the total number of islet cells. GLU immunopositive cells were located mainly in the peripheral region of the islets and they constituted 11.43–44.44% (mean ± S.E., 23.54 ± 8.27) of the total number of islet cells. ANP and CCK-8 immunoreactivity was observed in neurons and perivascular nerves fibres. NPY was identified in pancreatic neurons and in some peripheral and central cells of the islets of Langerhans. VIM immunoreactivity was observed in the endothelial cells of blood vessels and the nerves located in the perivascular, interlobular and periacinar regions. VIM was also detected immunohistochemically in the periductal nerves of the pancreas. NF occurred only in nerves. S-100 was discerned mainly in the nerves of the interlobular connective tissue and in nerves lying close to blood vessels and acinar tissue. It is concluded that INS, GLU, ANP, NPY, CCK-8, NF, S-100 and VIM are well distributed in the pancreas of the camel. These bioactive substances may play significant roles in maintaining adequate pancreatic function especially during the fluctuations that occur in the dry and cold seasons of the arid region.

D. 20 Nitric oxide synthase immunoreactivity in the jejunum of the camel (*Camelus dromedarius*). By E. P. K. Mensah-Brown, P. Lawrence, **A. Tinson, E. Adeghate, D. J. Pallot and *A. Garner. *Department of Anatomy*, *Faculty of Medicine and Health Sciences*, *U.A.E. University*, *Al Ain*; **Department of Pharmacology*, *Faculty of Medicine and Health Sciences*, *U.A.E. University*, *Al Ain and* **Camel Research Institute*, *Al Ain*

Nitric oxide (NO) is an inhibitory nonadrenergic non-cholinergic neurotransmitter important for the autonomic innervation of the gastrointestinal tract. Its pharmacological action is to relax the circular muscles of the gut. Nitric oxide synthetase (NOS), the synthesising enzyme for NO, has been identified in neurons immunohistochemically and also by diaphorase histochemistry as the enzyme has NADPH diaphorase activity. The jejunum (which forms about 75% of the length of the camel small intestine) was resected from a 2–3-y-old healthy male camel (*Camelus dromedarius*). The animal was anaesthetised with Dipravin (propofol) 2 mg/ml after an intravenous dose of 50 mg xylazine and 50 mg of ketomine nitrochloride as premedication. Sections of Zamboni's fixed and paraffin wax embedded small intestines were immunolabelled for nitric oxide synthetase. The primary antibody used was mouse monoclonal anti-bNOS at a concentration of 1 mg/ml in tris-BSA buffer solution. Peroxidase activity was demonstrated with diaminobenzidine. NOS immunohistochemistry revealed immunoreactive monoaxonal nerve cells and fibres present in the myenteric ganglia, and ganglia and nerve fibres within the submucosal plexus. Whilst 25–30% of the perikarya of all the neurons present in the myenteric plexus labelled for NOS, only 10% of the cell bodies of neurons in the submucosa stained positive for the enzyme. Immunoreactivity was not observed within either the circular or the longitudinal muscle layers of the muscularis externa. It is concluded that the enteric nervous system of the camel small intestine possesses NOS positive cells. It is further suggested that these NOS immunoreactive cells be used as inhibitory neurotransmitters of neuromodulators as occurs in the gastrointestinal tract of all mammals so far studied.

D. 21 The presence of light, dark and pyknotic cells in the human carotid body: fact or artefact? By M. Seker, D. J. Pallot. and J.-O. Habeck*. *Department of Human Anatomy*, *University of the UAE*, *United Arab Emirates and * Department of Pathology State Hospital*, *Chemnitz*, *Germany*

It is said that the carotid body in man, unlike that in other animals, contains 3 varieties of Type I cells. Clear Type I cells are the variants found in most animals. The dark cell variant is characterised by increased nuclear size and increased eosinophilia of the cytoplasm whilst pyknotic cells

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contain little cytoplasm and a small intensely darkly stained pyknotic nucleus. One of the problems associated with the study of human tissue is the delay between death of the patient and fixation of the tissue. We have examined the Type I cells in rat carotid bodies fixed in Zamboni fluid at the time of death from overdose of anaesthetic and also at 1, 2 and 4 h after death; human carotid bodies obtained at routine autopsies at accurately known times after the death of the patient have also been studied. H&E stained sections of the carotid bodies were used to classify Type I cells as either clear, dark or pyknotic. The percentage of each Type I cell variant was then correlated with the time between death and fixation of the tissue. In the rat carotid body fixed within minutes of death more than 95% of cells were of the clear variety. With increasing delay in fixation, firstly dark cells and then cells with intensely pyknotic nuclei appeared. The percentage of clear cells within a given carotid body was inversely related to the delay between death of the animal and fixation whilst the percentage of pyknotic cells was directly related to the fixation delay. In the human carotid body at 2 h fixation delay there were some dark Type I cells within the carotid body. With increasing fixation delay the number of clear cells decreased whilst the number of pyknotic cells increased. Furthermore, if 2 carotid bodies were divided in half and each half fixed at different times after death, there was an increase in the number of pyknotic cells in the same carotid body as a consequence of the increased delay. There was a significant negative correlation between the percentage of clear cells within the carotid body and a significant positive correlation between the percentage of pyknotic cells with increasing fixation delay. The fact that pyknotic cells are not found in freshly fixed rat carotid body and that the same relationship between percentage occurrence of clear and pyknotic cells is found in the human and rat carotid body, suggests that they are not true variants within the organ but the result of postmortem change.

D. 22 An ultrastructural study of the human carotid body. By M. Seker and D. J. Pallot. *Department of Human Anatomy, University of the United Arab Emirates, Al Ain*

There are very few studies of the human carotid body obtained from young subjects in good health. As part of another study we were fortunate to obtain 3 specimens from road traffic accidents who underwent autopsy within 2 h of death. Specimens were fixed in 3% glutaraldehyde/2% paraformaldehyde and processed by routine techniques for embedding in Epon. Semithin and ultrathin sections were produced using a Reichert Autocut. In the light microscope the cell clusters consisted of 2 types of cells, the Type I and Type II cells which have been shown to be present in all mammalian carotid bodies. Some cells possessed nuclei with light staining chromatin whilst others had smaller and more dense nuclei. A 3rd variety of Type I cell with very small intensely staining nuclei which appeared pyknotic were also found. In addition to the Type I & Type II cells a 3rd cell variety with large nuclei was also seen. In the electron microscope the type I cells were seen to contain electron dense cored vesicles the size and distribution of which varied from cell to cell and in different areas of the same cell. The clear cells found by light microscopy had large complex cell processes and possessed vesicles scattered throughout the cytoplasm; cytoplasmic vacuoles were rare. The dark cells seemed to contain fewer vesicles and contained small numbers of cytoplasmic vacuoles. In cells with pyknotic cells the nuclei were crenated rather than the round or ovoid nuclei seen in dark and clear cells and the cytoplasm was dense and characterised by large numbers of vacuoles. Examination of the vacuoles in dark and pyknotic cells suggested that they represented exploded mitochondria. In addition to the normal specific cells and nerve fibres isolated groups of epithelioid cells were also found. These cells, located in groups and associated with nerve fibres and Type II cells, lacked electron dense cored vesicles. In this postmortem material it was not possible to study the nerve endings adequately; large numbers of nerve fibres, however, were found associated with Type II cells. The increasing degree of vacuolation from clear to dark cells and from dark cells to pyknotic cells suggests that they may represent postmortem damage rather than provide evidence for 3 varieties of type I cells within the human carotid body as has been previously claimed.

D. 23 Intestinal uptake of microparticles in the rat – an extended time course study. By G. Johnston, R. A. Hazzard, G. M. Hodges and K. E. Carr. *School of Biomedical Science/Anatomy, The Queen's University of Belfast*

Study of microparticle uptake is relevant for an understanding of the fate of ingested radioactive or drug-loaded particles. Previous microscopy and bulk (maceration) studies by the group have shown the uptake of 2 μm latex microparticles by the proximal rat small intestine as early as 5 min after oral administration, with continued uptake at 15 min, 30 min and 2, 4, 8 and 24 h. The present report extends this study to examine uptake, not only in the proximal intestine, but in 9 equal segments from proximal to distal at time points of 5 min, 30 min, 24 h, 48 h, 7 d and 5 wk. The study uses microscopy to examine particle uptake as evidence has shown that bulk analysis over-estimates particle numbers: microscopy also gives additional information on the precise position of particles within the tissues. Thirty-six male Sprague–Dawley rats, aged 7–8 wk, were used ($n = 3$ per time point plus 1 control). Animals were given, by gavage, 0.25 ml fluorescent polystyrene latex suspension containing approx. 1.65×10^9 2 μm particles. Control animals were given 0.25 ml sterile distilled water by gavage. At the appropriate time points, animals were perfusion-fixed using 3% glutaraldehyde in sodium cacodylate buffer. All treatments to animals were carried out by a licensed investigator. The small intestine was dissected from each animal and divided into 9 equal segments from proximal to distal. A Peyer's patch-containing full circumference ring was dissected from each segment: tissue samples were frozen and cryosectioned at 14 μm. Propidium iodide-stained sections were examined using epifluorescence microscopy and the numbers of particles associated with each intestinal cell and tissue type were counted. Particle uptake for many tissue sites in the 1st (most proximal) and 2nd intestinal segments was greatest 5 min after administration. By the 3rd segment, uptake after both 5 and 30 min was greater than the later time points and in the 4th segment, particle uptake was greatest in the 30 min group. There were no statistically significant differences for the

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other intestinal segments: however, particle numbers remained high for the 30 min group in segments 5 and 6 but decreased in the more distal segments where uptake was highest 24 h after administration. Particles within tissue for the 7 d and 5 wk groups were limited to negligible numbers in the Peyer's patch lymphoid tissue. The data indicate that in the 24 h following oral administration, particle uptake occurs along most of the length of the small intestine. Findings also suggest that Peyer's patch tissues may contain small numbers of particles as long as 7 d to 5 wk after administration. Despite inter-animal variation, this study confirmed previous findings for the proximal intestine and provided additional data on particle uptake along the entire small intestine at the five time points studied.

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D. 24 Distribution of MyoD and myogenin in developing fast and slow muscle fibres of the fetal mouse. By E. H. Walters, N. C. Stickland and P. T. Loughna. *Department of Veterinary Basic Sciences*, *The Royal Veterinary College*, *London*

The 4 myogenic regulatory factors (MRFs) MyoD, myogenin, Mrf-4 and Myf-5 are known to have vital roles in the proliferation and differentiation of myoblasts, which when fully differentiated form skeletal muscle. Myf-5 and MyoD are expressed in proliferating undifferentiated myoblasts whereas myogenin is expressed when muscle differentiation has been initiated. MRF-4 is usually expressed in mature skeletal muscle. The multiple roles of MRFs during muscle development suggests that each member may regulate a distinct group of genes, and that differential expression of MRFs may contribute to the diversity of the myogenic cells (Weintraub et al. *Science*, **251**, 1991), namely the expression of fast and slow myosin isoforms. Work on rabbit muscle satellite cells has shown that MyoD and myogenin are present in slow satellite cells on d 6 of culture whereas fast satellite cells express MyoD from d 8 and, myogenin is expressed from d 12. Hughes et al. (*Development*, **118**, 1993) have shown in adult muscle that muscle fibres expressing fast myosin isoforms preferentially accumulate MyoD transcripts and slow fibres accumulate myogenin. The purpose of this investigation was to investigate whether MyoD and myogenin distribution was related to the expression of specific myosins in prenatal muscle. Pregnant Balb C mice were time mated and the fetuses removed at specific time points from E13 until E18. The fetuses were snap chilled and 10 μm sections of the upper forelimb were cut on a cryostat. The sections were incubated with the primary antibody in 1% BSA in PBS for 2–3 d at 4 °C. Anti-MyoD and anti-myogenin polyclonal antibodies were used as well as an antibody to slow MyHC. The sections were washed in 3 changes of PBS with 0.1% Tween 20. The FITC labelled secondary antibody in PBS with 1% BSA was applied for 3–5 h at room temperature, and the staining was visualised using fluorescence microscopy. At d 13 Slow Myosin Heavy Chain (MyHC) is present in newly formed primary fibres, MyoD and myogenin are present in the nuclei of cells in discrete areas of the limb bud, corresponding to those areas expressing slow MyHC. At 15 d Slow MyHC is present in all primary fibres while MyoD and myogenin are present in all muscle fibres. At 18 d slow MyHC is present in a proportion of muscle fibres whereas MyoD and myogenin are again present in all muscle fibres. From these observations we conclude that there is no evidence of MyoD or myogenin distribution being related to the development of specific fibre types, as determined by the distribution of slow MyHC.

D. 25 Differences in muscle differentiation of Atlantic salmon (*Salmo salar* L.) embryos in response to temperature and oxygen levels at a late developmental stage. By T. W. Matschak and N. C. Stickland. *Department of Veterinary Basic Sciences*, *The Royal Veterinary College*, *University of London*

Fish muscle increases in size by either muscle fibre hypertrophy, an increase in cross-sectional area of individual fibres, or by fibre hyperplasia, an increase in the number of fibres constituting the muscle. During embryonic development these processes are influenced by the incubation temperature and, at least during late development in Atlantic salmon, by the amount of oxygen available. By the time of hatching a difference appears in the number and the cross-sectional area of muscle fibres when eggs are reared under different temperature and oxygen regimes. It is also known, for example, that in herring temperature can lead to asynchronous development of certain organs. In fish, as in other animals, it has been found that a sequence of myosin heavy chain isoforms is expressed during development. Additionally, embryonic fish possess a superficial layer of muscle fibres which initially often exhibit a fast character and later change to a slow fibre type. Frozen cross-sections of Atlantic salmon embryos, at a developmental stage just after hatching, which had been subjected to 5 °C for about 1 mo and 11 °C for about 2 wk, and normoxia and hyperoxia (200% normoxia) were stained with an antibody against slow myosin heavy chain. The embryos had been kept at the different conditions from stage 25 to stage 32 after Gorodilov (*Sb. Nauchn. Tr. GosNIORKh*. **200**, 1983), i.e. for 40 d at 5 °C and for 17 d at 11 °C. It was found that, while there was no obvious difference between oxygen treatments within each temperature group, the fish subjected to the lower temperature exhibited staining throughout their superficial layer of muscle fibres, whereas in the high temperature group staining only occurred in the area near the lateral line and at the epaxial and hypaxial extremes. This suggests that, whilst at 5 °C all fibres of the superficial layer had switched to the slow type, this was only the case in certain areas at 11 °C. It is also known that during early muscle development new muscle fibres are added to the inner muscle fibres in 'growth zones' located towards the surface of the fish and at the epaxial and hypaxial extremes. Later in development new fibres are added throughout the muscle. Serial sections of the above experimental groups were therefore stained with an antibody against proliferative cell nuclear antigen (PCNA), a nuclear proliferation marker. Again no obvious difference was found between oxygen treatments, but in the 5 °C group staining was observed throughout the muscle whereas in the 11 °C embryos staining occurred only in areas corresponding to the above-mentioned growth zones. The muscle in the 11 °C group of fish therefore appeared to

be less mature than that in the 5 °C group at the time of hatching.

D. 26 Changes to murine enteroendocrine cells containing cholecystokinin and gastrin following exposure to ionising radiation. By J. S. McCullough, G. A. Burke, P. Abram*, C. F. Johnston* and K. E. Carr. *Schools of Biomedical Science/Anatomy and *Clinical Medicine, The Queen's University of Belfast*

Irradiation of the gut may give rise to symptoms including vomiting, diarrhoea, anorexia, abdominal cramps and gastric stasis. Peptides produced by the enteroendocrine (EC) cells are known to regulate much of the functional activity of the gastrointestinal tract and it has been proposed that damage of these cells may contribute to radiation enteritis. This study reports on the response of EC cells containing cholecystokinin (CCK) and gastrin to radiation. Eighty female, 12 wk old, BSVS mice were allocated at random to one of 3 groups: control, irradiated or sham-irradiated. Mice to be irradiated were given a single whole body dose of 5, 10 or 20 Gy gamma rays at a dose rate of 1 Gy/min from a Theratron ^{60}Co unit. Mice were killed by cervical dislocation at 6, 24 and 72 h post irradiation and pieces of duodenum were removed and fixed in modified Susa's fixative before processing for wax immunohistochemistry. Whole circumference sections (5 μm thick) were produced and incubated firstly in either rabbit anti-CCK or rabbit antigastrin antibodies and subsequently in swine antirabbit IgG FITC conjugated secondary antibody. The numbers of labelled cells per section were counted (4 sections/animal), and adjustments made to the data to take account of the 100% cross-reactivity between CCK and gastrin cells when using the CCK antibody. Differences between groups were assessed by one way analysis of variance. There was a decline in the number of CCK-positive cells 6 h after 5 Gy with a return to normal thereafter. Irradiation with 10 Gy produced no changes to numbers of CCK-positive cells whereas there was an apparent increase 24 h after 20 Gy. Numbers of gastrin-positive cells showed no response after 5 or 10 Gy but an apparent increase was evident 72 h after 20 Gy. Apparent changes to the numbers of cells are probably related to increased/decreased production of CCK and gastrin by the cells, rather than proliferation or death of the cells themselves. Such findings may suggest important roles for these cells in radiation-induced gastrointestinal dysfunction. The inappropriate release of CCK, for example, may cause the secretion of excessive amounts of bile salts leading to increased gut motility and water secretion which may be manifested as symptoms of diarrhoea and abdominal cramps.

D. 27 Measuring the tilt and the period of a double helix in sectioned material. By M. Kidd. *Department of Anatomy and Developmental Biology, St George's Hospital Medical School, London*

In thin sections of nonorientable tissue examined with the electron microscope, filamentous structures such as the paired helical filaments of Alzheimer's disease are rarely found lying perpendicular to the electron beam. Thus, accurate measurement of their periodicity is difficult. This problem can be circumvented by using 'negative staining' or 'shadowing'. However, with these methods the material has collapsed onto a substrate after extraction procedures, giving rise to a variety of artefacts. Moreover, the structures are no longer in situ in their tissue. When paired helical filaments were first observed in the electron microscope, it was noticed that those helices that did not lie perpendicular to the electron beam appeared asymmetric. This asymmetry can be measured and used to estimate the 'tilt' of paired helices and hence the true period. One method is to measure the distance from the central axis to the point where the 2 filaments cross each other. The formulae for the image of a vertical pair of helices in orthogonal projection are shown below, with θ the tilt of the helix from the vertical, p the *observed* period and d the diameter or maximum width of the pair of helices. Where the 2 helices cross each other, the above 2 functions will be equal and this equation can be solved for θ, using x, the distance of the crossover point from the axis of the pair of helices.

$$y_1 = \frac{p}{2\pi}\cos^{-1}\left(-\frac{2x}{d}\right) + \sin\theta\sqrt{(d^2/4 - x^2)}$$

$$y_2 = \frac{p}{2}\left(1 - \frac{1}{\pi}\cos^{-1}\left(-\frac{2x}{d}\right)\right) - \sin\theta\sqrt{(d^2/4 - x^2)}$$

$$\sin\theta = p\frac{\left(1 - \frac{2}{\pi}\cos^{-1}\left(-2\frac{x}{d}\right)\right)}{4\sqrt{(d^2/4 - x^2)}}$$

In this way the true period of randomly oriented paired helical filaments in thin sections can be calculated.

D. 28 Multi-organ damage in a porcine peritonitis model of the systemic inflammatory response syndrome (SIRS). By R. F. Moss, D. J. Tighe*, D. C. Davies** and E. D. Bennett***. *Department of Electron Microscopy, *Department of Physiological Medicine, **Department of Anatomy and Developmental Biology and ***Intensive Care Unit, St George's Hospital Medical School*

The systemic inflammatory response syndrome (SIRS) associated with septicaemia and multiple organ failure has a high morbidity and a mortality approaching 100%. We have developed a porcine model to study the anatomical and physiological changes that affect organs including the lungs, liver and brain. Anaesthesia was induced in 12 adolescent Middle White pigs (25–30 kg) by intramuscular injection of Azoperone and Metomidate. During surgery a mixture of 50% O_2, 48% NO_2 and 2% halothane was given. For the remainder of the experiment anaesthesia was maintained with Propofol and Rapifen. The lungs were ventilated with room air. Physiological parameters were monitored by catheters inserted into the internal jugular vein and descending aorta. A Swan–Ganz catheter for monitoring cardiac output and pulmonary artery occlusion pressure was advanced into the pulmonary artery. A mid-line laparotomy was performed and an ultrasound flow probe placed round the portal vein. Six pigs had 35 ml caecal contents aspirated and spread around the peritoneum and their incisions closed. The other 6 pigs (shams) were left

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uncontaminated and their incisions closed. The wedge pressures of both groups of pigs were maintained at baseline by infusion of saline or starch solutions. All pigs were killed 8 h after surgery and tissue samples taken from various organs and processed for examination by both transmission and scanning electron microscopy. In the peritonitis group, hepatic sinusoids were occluded by swollen red blood cells, neutrophils, lymphocytes and platelets. The sinusoid endothelial lining showed oedematous change and the breakdown of sieve plates. Occlusive damage was also seen in the lungs, presenting as blocked alveolar capillaries. The frontal cerebral cortex showed substantial perivascular oedema and slight qualitative changes to the endothelium. There was little other apparent structural change to the surrounding neural tissue. Tissues taken from sham pigs showed less hepatic occlusion, more patent lung vasculature and a less oedematous cerebral cortex. The results of this study provide evidence of a structural basis for multi-organ failure resulting from SIRS. Preliminary investigations of biopsy tissue revealed that similar structural changes to those occurring in the porcine peritonitis model, also occur in human SIRS patients.

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