Proceedings of the Anatomical Society of Great Britain and Ireland

The Summer meeting of the Anatomical Society of Great Britain and Ireland was held at the Anatomy Department, Edinburgh University from 15 to 17 July 1997. It included a symposium on 'Computer Modelling for Anatomists and Clinicians' on Wednesday 16 July. The following are abstracts of communications and demonstrations presented at the meeting.

COMMUNICATIONS

1 Electrophysiological and histological examination of mice expressing varying levels of human peripheral myelin protein 22 (PMP22). By P. K. Thomas, J. F. Pellisier*, S. Huston**, A. Manson**, E. Passage*, A. M. Robertson, B. Youl, M. Fontés* and C. Huxley**. *Royal Free Hospital School of Medicine, London, *Laboratoire de Pathologie Neuro-musculaire and INSERM U*406, *Faculté de Médecine de la Timone, Marseille, France, and **Imperial College School of Medicine at St Mary's, London*

We have made 5 transgenic mouse lines carrying the human PMP-22 gene, one of which has been published (Huxley at al. *Hum. Mol. Genet*. **5**, 1996). The different lines carry 1–19 copies of the transgene and the level of expression of the human gene is roughly proportional to the copy number. Nerve conduction velocity and peripheral nerve histology were also analysed in the various lines. In mice with up to 2 copies of the transgene, or about 0.6 times as much human as mouse mRNA, the histology of nerves was normal and nerve conduction velocity was also normal (> 34 m/s). With 4 copies, or approximately equal amounts of human and mouse mRNA, nerve conduction velocity was reduced to 20–30 m/s and histological examination showed demyelination/hypomyelination. With 7 copies of the transgene, about 1.6 times as much human as mouse mRNA, nerve conduction velocity was reduced to less than 8 m/s or no response to nerve stimulation could be obtained. In these mice, the peripheral nerves were severely demyelinated. Thus the extent of demyelination and consequent reduction in nerve conduction velocity is increased with increased expression of the human gene with no effects detectable until the human and mouse levels are approximately equal. The level of expression does not affect the type of demyelination but influences the severity of involvement.

2 A new manual power grip. By N. M. Dawson, P. Felle and D. K. O'Donovan. *Department of Human Anatomy and Physiology, University College Dublin*

The power grip commonly used in racquet sports (handshake or oblique grip) involves the use of the flexed fingers to hold the object obliquely across the palm against the buttress of the partially abducted thumb. The main pressure is exerted by the radial (index and middle) digits, and so this grip can be referred to as the radial grip. A possible alternative, increasingly seen in empirical use by sports professionals, is to use all digits except the index finger. This 'relaxed index' grip has not previously been analysed. Wrist movement is an integral part of the full function of the hand, but is decreased or abolished with increasing grip strength by increased tension in the long digital flexors. This apparent incompatibility of flexibility with power might not apply if the power of the ulnar 3 digits is combined with relaxation of the flexor tendons to the index finger. This study was directed to comparing the relative power and wrist mobility of the relaxed index grip with the conventional oblique power grip as applied to a cylindrical object. Having given fully informed consent, 21 young adult subjects with no disease or injury of the upper limb were recruited to this study. A strain gauge dynamometer in the shape of a racquet handle was designed for grip strength testing. Output from the dynamometer was relayed to a storage oscilloscope. Following calibration, grip strength was recorded in kilograms of pressure applied. Standard instructions were given to each subject – to copy 2 grip positions demonstrated by the investigator (oblique and relaxed index) and to squeeze as hard as possible when directed. Testing was conducted with the wrist in the neutral position and in self selected comfortable adduction (mean 7°). To examine the degree of radioulnar deviation attainable in each grip, one arm of a 6 inch goniometer was fixed with surgical tape to the ventral forearm in line with the long axis, and the other was allowed to slide in contact with the dynamometer which was held in the hand as before. Subjects were instructed to exert their maximal grip in the neutral position as described above. While maintaining maximum grip strength they were directed to maximally adduct, and then abduct the wrist. The degree of deviation in each direction was recorded for both grip types. Mann–Whitney U testing was performed on each set of results. The self selected position of adduction gave power comparable with that obtained in the neutral position in all tests. Overall results show that the relaxed index grip gave significantly greater power than the traditional oblique radial grip. In female subjects ($n = 10$), the relaxed index grip only gave equivalent power to the radial grip in the neutral position, indicating some gender effect on grip strength. Female grip strength was significantly weaker than male grip strength, approximating 50% of the male value in each grip. The relaxed index grip allowed significantly greater wrist movement than the radial grip at subjective maximum grip strength. The findings of this study are significant in that this newly described relaxed index grip offered significantly more wrist mobility than the

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traditionally employed racquet grip with equivalent – increased power. This greater freedom of the wrist without any loss of power may explain its empirical use by top class racquet players. Further testing is required to assess the impact of relaxation of the index finger on the element of precision or directional control in the power gripping of cylindrical objects.

3 Compartment syndrome following intramedullary nailing of the tibia. By A. Devitt, P. Diggin*, P. Felle* and D. McCormack. *Departments of Orthopaedic Surgery and * Human Anatomy and Physiology, University College Dublin*

Following intramedullary nailing for fracture of the tibia, there is a high incidence of compartment syndrome. The top of the nail, just above the tibial tuberosity, is closed off with a 'Leissling plug' to prevent bone growing into the nail (which would render its subsequent removal more difficult). Some of these plugs are cannulated, others are solid. The object of this experiment was to ascertain whether the type of plug used to close off the top of the intramedullary nail might affect development of compartment syndrome. Twelve formalin-fixed cadaveric legs were studied. The tibia was fractured and the intramedullary nail inserted just above the tibial tubercle, after splitting the patellar ligament. A solid Leissling plug was screwed home closing off the upper part of the nail. Two cannulae were inserted into the deep posterior compartment. An electronic pressure transducer was connected to one and 60 ml of normal saline with methylene blue was infused through the other. The pressure in all cases settled at approximately 30 cm H_2O. The limb was left for 10 min and the Leissling plug was removed. In all cases, methylene blue-stained fluid started rising through the aperture at the top of the intramedullary nail within 20 s, and the pressure in the posterior compartment dropped to about 5 cm H_2O. This study indicates that some fluid from the fracture site might pass through the medullary cavity of the bone via the centre of the hollow medullary nail. Use of a hollow Leissling plug might aid this process, reducing the risk of developing a compartment syndrome following intramedullary nailing for fracture of the tibia.

4 Joint shape: a criterion for establishing the identity of 'isolated' fossil hominid limb bones. By B. Wood, L. Aiello*, C. Wood and C. Key*. *Hominid Palaeontology Research Group, Department of Human Anatomy and Cell Biology, University of Liverpool and *Department of Anthropology, University College London*

Specimens which preserve more than one body part from the same individual are especially important for taxonomic and functional analyses. This study concentrated on the subset of associated skeletons which preserve the reciprocal surfaces of a joint. Laser scanning was used to explore whether the shapes of the reciprocal surfaces of a joint of an individual are significantly more congruent than are the surfaces of randomly matched pairings taken from the same species. Laser scanning was used to capture the distal articular surface of the left tibia of OH35 and the trochlear articular surface of the talus of OH8, both from Bed I, Olduvai Gorge, Tanzania. The degree of congruency between those articular surfaces was matched against the congruency of the talocrural joint of AL 288-1 (*Australopithecus afarensis*), and the congruency of associated talocrural joints and randomly matched pairs of tibiae and tali of modern man, chimpanzees and gorillas. The results suggest that OH35 and OH8 do not come from the same individual and may not come from the same species. This analysis demonstrates the potential of laser scanning for capturing the shapes of 3-dimensional surfaces in palaeoanthropology. It also demonstrates the potential for using the relative congruency of reciprocal joint surfaces as a test of the likelihood that isolated limb bones are components of a single individual and for examining the taxonomic affinities of such specimens.

This study was supported by the Leverhulme Trust (Grant F134BB to LA) and the NERC (grant GR/H616/74 to BW).

5 Use of multimedia in anatomy teaching. Interactive anatomy: computer guided dissection. By C. A. Briggs, N. Eizenberg, P. Barker and C. Adams. *Department of Anatomy and Cell Biology, The University of Melbourne, Parkville, Vic., Australia*

Although dissection is ideally the most valuable means of gaining an understanding of the subject of anatomy, it is difficult technically, complex logistically, time consuming and expensive. The development of 'Interactive anatomy: computer guided dissection' is an attempt to address these problems. This multimedia project is being completed in the context of a new practical anatomy teaching program developed at The University of Melbourne. A text, compatible with this program, has already been designed and piloted. An interactive multimedia CD-ROM is currently under development, using real anatomical specimens incorporating dissections of the entire body and including over 200 layers and 2000 individual anatomical structures. The protocol that has already been constructed enables the student to 'dissect' on computer as a preliminary to, or even as a substitute for, cadaver dissection. A region (e.g. the scalp), layer (e.g. skin), system (e.g. arterial), or an individual structure (e.g. the superficial temporal artery), may be removed or replaced at will. Combinations of regions, layers, systems and structures may be 'constructed' or 'deconstructed', as the student wishes. In addition, selected structures and creative visuals (including exploded 3-dimensional diagrams) may be magnified, enhanced or rotated to further improve understanding. These will enable a simpler conceptualisation of the complex reality. The high degree of interactivity in searching for, uncovering and identifying particular anatomical structures, associated with a given clinical problem, will provide invaluable feedback to students (and teachers). A modular design enables the different subdivisions of the body to be studied in any order, while the student may control both the rate and sequence of revealing its architecture. Since the internal organisation of the project combines both regional and systemic perspectives, it is appropriate for traditional, problem-based and self-directed learning courses. One of many original

features will be the anatomical basis of 'practical procedures' that may be required of a junior doctor (e.g. airway management, tracheostomy) combined with real-time video presentation of the actual procedure. While no computer based approach can substitute for the 3D and kinaesthetic experiences obtained in the dissecting room, this program will more actively engage students in directed learning tasks not available using computer terminals, point and click browsing systems, electronic reference atlases and videos of dissections, all of which are useful, but have the limitation of not being interactive.

6 Anatomical basis for the possible use of the sartorius muscle in the construction of a dynamic neosphincter around an abdominal stoma. By D. A. Shanahan, R. K. Jordan, J. Varma*, I. Nichols* and A. Coulthard*. *Anatomy and Clinical Skills Centre and Department of Surgery**, *The School of Surgical Sciences*, *University of Newcastle Upon Tyne*

Patients with a colostomy lack the normal sensory and motor functions of the intact anorectal mechanism and are usually faecally incontinent. Surgical attempts to restore continence focused on the transposition of a skeletal muscle around the perineal stoma forming a transient barrier to the faecal stream. The adductor longus (Fedorov, *Zentralbl. Chir*. **110**, 1985) and gracilis muscles (Mander et al. *Ann. Surg*. **224**, 1996) have been transposed around the perineal stoma and electrically stimulated to create a dynamic neosphincter able to actively contain intracolonic pressures. Similar transposition and electrical stimulation of a skeletal muscle around an abdominal stoma has not been undertaken. The aim of this study was to determine if a thigh muscle fulfilled the anatomical criteria for it to be used to create a dynamic neosphincter around an abdominal stoma. Following a preliminary study of the neurovascular anatomy and length of the thigh musculature, sartorius was selected for further study. This work is based on the dissection of 80 cadaveric thighs to elucidate the neurovascular anatomy and length of the muscle, and of 20 sartorius muscles to determine the intramuscular arterial anatomy. The tibial attachment of sartorius was cut and any vascular branches that prevented the transposition of the muscle to the epigastric region were divided. The freed sartorius was manipulated to form an alpha configuration around the approximate siting of an abdominal stoma and the freed distal attachment was placed onto the ipsilateral pubic tubercle. The mean entry point of the nerve branches to sartorius (measured from the anterior superior iliac spine) was 16.2 cm (S. D. 4.9) and of its upper major vascular pedicle was 12.7 cm (s.d. 3.7). The mean length of sartorius was 54.7 cm (s.d. 4.5). The transposable section of the muscle (i.e. the part manoeuvred completely out of its anatomical position) was from 3 cm distal to its neurovascular hilum to the lower attachment of the muscle. In this study, the motor point and at least 1 major vascular pedicle entered the nontransposed section of the muscle in 67/80 thighs (83.75%). The sartorius was of sufficient length to be wrapped around the site of a stoma in the ipsilateral hypogastric region and have its freed distal attachment placed onto the pubic tubercle of the cadaver. Intramuscular arterio-arterial anastomoses were found between the major vascular pedicles to the nontransposed and transposed sections of this muscle. Therefore, the majority of sartorius muscles fulfilled the anatomical criteria for them to be used to create a dynamic neosphincter around an abdominal stoma.

7 Arterial anastomoses in sheep latissimus dorsi muscle. By A. Tang, J. C. Jarvis and S. Salmons, *Department of Human Anatomy and Cell Biology*, *University of Liverpool*

There is much interest in the surgical use of a transposed autologous latissimus dorsi muscle graft to assist the failing heart. However, interruption of perforating collateral arteries during surgical mobilisation could cause ischaemic damage to the distal part of the graft, compromising the viability and performance of the muscle. Radiographic and resin injection techniques have indicated the presence of communicating vessels connecting the vascular territories of the thoracodorsal artery proximally and the collateral vessels distally. Such connections would be important, as they could allow the thoracodorsal artery to maintain distal graft viability following surgical mobilisation. We have therefore sought evidence for such arterial anastomoses in the ovine latissimus dorsi muscle under physiological conditions of pressure. The study was performed with a fluorescent microsphere technique for the measurement of blood flow that was established, validated and optimised in our laboratory. An adult Suffolk sheep was anaesthetised and a catheter was passed into the left ventricle via the left carotid artery. Fluorescent microspheres were injected according to the following sequence: (1) blue-green microspheres, to measure collateral flow after cross-clamping the thoracodorsal artery; (2) yellow-green microspheres, to measure thoracodorsal flow after dividing all collaterals and dividing and resuturing the aponeurotic attachments of the muscle. A reference blood sample was withdrawn simultaneously from the femoral artery at a known rate. All measurements were made under conditions of maximum hyperaemia, produced by stimulating the muscle acutely for 2 min. The animal was killed by anaesthetic overdose and the entire muscle was removed and divided systematically into proximal, middle and distal groups of samples. Thick cryostat sections (30 μm) were cut from biopsies of the distal portion of the muscle and stained with a rhodamine-labelled antibody to von Willebrand factor. Using a fluorescence microscope at 2 wavelengths, we were able to observe, and to photograph, instances in which one or more yellow-green microspheres could be found adjacent to one or more blue-green microspheres within a single capillary. Since these differently labelled microspheres were introduced via one arterial supply with the other blocked, this condition could arise only if the 2 arterial territories were in communication. This represents the first definitive evidence of arterial anastomoses in the latissimus dorsi muscle under physiological conditions of pressure. The observation is consistent with blood flow measurements, made by the dye elution technique, which indicated that a substantial fraction of the flow to the distal part of the muscle remained after ligation of the collateral arteries.

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8 Quantitative and reconstructive methods for assessing the development of the talus. By R. Eggers, O. Schmitt and H. Fritsch (introduced by M. Benjamin). *Institute for Anatomy, Medical University of Lübeck, Germany*

Diagnosing and understanding malformations of the talus requires a fundamental knowledge of its normal development and ossification. In children with clubfeet, for example, there are anomalies in the shape of the talus and deviations in its axes. The aim of our study is to apply quantitative and 3D-reconstructive methods to assess malformations of the talus in an attempt to establish reproducible and objective measures suitable for discriminating between normal and abnormal development. The ossification of the talus was studied in plastinated and histological preparations of 8 normal feet and 1 clubfoot from newborn children. Using point counting methods, volumes and volume fractions of the talus and its ossification centre, as well as articular and nonarticular surfaces of the bone were estimated from serial sections. With the aid of surface based 3D-reconstruction and general image analytical procedures, these parameters were calculated. However, surface based reconstructions lead to problems and inaccuracies if concave, ring-shaped, or bifurcate structures are sectioned. Therefore, a voxel based 3D-reconstruction technique was implemented to determine projection axes and their angles comparable to those obtained radiologically. The computed results achieved by point counting methods are in agreement with those calculated from the reconstructed model. In comparison to a normal foot, the clubfoot talus is smaller and its ossification more advanced. The ratio of articular and nonarticulating surface was diminished and the perichondrium abnormally thickened. Transformations of the reconstructed talus and its surrounding structures made it possible to determine the axes of the hindfoot in a manner similar to that using radiographs.

9 Implantation of MyoD-converted skin cells into the muscles of the mdx mouse. By J. B. Relvas, G. N. Okoli, L. Buluwela*, K. E. Wells**, D. J. Wells** and D. J. Watt. *Departments of Anatomy, Biochemistry* and Pharmacology**, Charing Cross & Westminster Medical School, London.*

Duchenne muscular dystrophy (DMD), a severe muscle wasting disease affects 1 in 3500 live male births. The disease is caused by a deletion in a gene on the X-chromosome coding for dystrophin.The exact function of dystrophin is unknown, yet without it muscle fibres degenerate and die. Attempts to develop a therapy for DMD have involved delivering dystrophin to the myopathic muscle, either by implanting normal muscle precursor cells or by using retroviral and adenoviral mediated-gene delivery. Success however has been minimal, and in clinical trials where cells have been implanted, failure has been attributed to an immune reaction to the donor cells. Suggestions of using the patient's own muscle cells to repopulate the muscle have the disadvantage of introducing cells which are already compromised by the disease and may not be the most appropriate candidate to use in cell-mediated therapy. In previous work we reported the formation of high numbers of new-formed dystrophin-positive fibres when skin cells from a normal mouse were implanted into the muscles of the dystrophin-deficient mdx mouse, one of the animal models for DMD. The results indicated that the skin cells participated in fibre formation and suggested that they were doing so by converting to a myogenic lineage. Mouse skin cells can also deliver exogenous genes to muscle fibres with the resultant expression of their gene products within the fibres. These results could have important implications for therapy for primary muscle disorders as the patient's own skin cells could be easily harvested, the gene in question inserted and the cells re-introduced into the myopathic muscle. In the present work we investigate whether converting cells to myogenesis ex vivo, results in a greater number of dystrophin-positive fibres in implanted muscles of the *mdx* mouse as compared with those implanted with cells which had not been converted ex vivo. It is well known that nonmuscle cells convert to the myogenic lineage when transfected with the muscle transcription factor MyoD. Therefore in order to convert skin cells ex vivo we transfected them with MyoD. We produced an amphotropic retroviral vector containing MyoD under control of the b-actin promoter. The retrovirus also contained the neomycin resistance gene under control of the early promoter SV40 which allows selection of the infected cells when grown in a medium containing the neomysin analogue G418. As expected, infection of skin cells with the retrovirus induced the conversion of these cells to myogenesis. G418-selected cells were then implanted into the tibialis anterior muscle of host mdx mice anaesthetised with a cocktail of Hypnorm and Hypnovel. The skin overlying the muscle was incised and the cells delivered in a 10–15 µl volume through the fascia investing the muscle via a PCR pipette pulled to a very fine point and finally the incised skin closed by suture. Untransfected skin cells were used as controls and similarly injected into hosts. At 3 and 6 wk postimplantation, recipient *mdx* mice were killed by cervical dislocation and the injected muscles removed and prepared for cryostat sectioning. Transverse sections cut throughout the muscle were immunocytochemically stained with an antibody directed against dystrophin and the percentage of newly formed dystrophin-positive fibres present in both control and experimental muscles compared.

10 Expression of blood–brain barrier-associated markers in the sciatic nerve of the rat. By J. G. Lawrenson, T. Finn*, A. R. Reid* and G. Allt*. *Department of Optometry and Visual Science, City University and *Reta Lila Weston Institute of Neurological Studies, University College London Medical School*

Endoneurial microvessels of peripheral nerve share many of the barrier characteristics found in vessels of the cerebral cortex, e.g. impermeability to electron-dense tracers and endothelial cell ultrastructural features. The object of this study was to compare the expression of several blood–brain barrier (BBB)-associated molecules (EBA, GLUT-1, OX-47, transferrin receptor (OX-26), gamma-glutamyl transpeptidase (GGT), and alkaline phosphatase (AP)) in the CNS and PNS. Six adult male Sprague–Dawley rats were anaesthetised and killed immediately by cervical dislocation. Brains and sciatic nerves were removed and frozen rapidly

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in OCT mounting medium. Cryostat sections (7 μm) were incubated in the appropriate primary antibody (1:10 to 1:1000 dilution) followed by detection using an ABC kit (Dako Ltd) while AP was localised using the Gomori method and GGT using the technique of Rutenburg et al. (*J. Histochem. Cytochem.* **17**, 1969). Although EBA and OX-47 were expressed strongly by microvessels in the CNS, no immunoreactivity was detected in endoneurial capillaries. In contrast, intense immunolabelling of GLUT-1 and the transferrin receptor was found in microvessels at both sites. Enzyme cytochemistry showed that AP activity was associated with vessels in brain and nerve. In contrast, GGT showed differential expression. In brain the enzyme was localised exclusively to microvessels whereas the endoneurium showed a more diffuse distribution of the enzyme. In conclusion PNS microvessels display only some of the BBB-associated markers found in the cerebral cortex. This incomplete BBB phenotype in peripheral nerve may arise from an absence of astrocyte-derived barrier-induction factors.

11 Topographical increases in striatal pre-proenkephalin-B (PPE-B) expression associated with dopamine agonist-induced dyskinesia in the parkinsonian (MPTP) primate. By B. Henry, A. R. Crossman and J. M. Brotchie. *Division of Neuroscience, School of Biological Sciences, University of Manchester*

Long-term dopamine-replacement therapy in Parkinson's disease elicits many disabling side-effects, most notably uncontrolled involuntary movements i.e. treatment-related dyskinesia. At present little is known about the mechanisms underlying these dyskinesias. However, other forms of dyskinesia are characterised by underactivity of the medial segment of the globus pallidus (GPm), the major output structure of the basal ganglia. Striatal efferent neurons projecting to the GPm utilise GABA and the opioid neuropeptide dynorphin as cotransmitter. Recently, we have shown that dynorphin modulates glutamate release within the basal ganglia via activation of kappa opioid receptors. The striatum, comprising the caudate nucleus and putamen in the primate, is the major input structure of the basal ganglia and displays a complex heterogeneous organisation of neurochemical systems that are related both to neuroanatomical connections and functional organisation. This heterogeneous organisation is termed striosome-matrix compartmentalisation. Following repeated dopamine-replacement in rodent models of Parkinson's disease we, and others, have demonstrated an increase both in dynorphin peptide levels and dynorphin precursor (pre-proenkephalin-B, PPE-B) expression. In this study, we have investigated the expression of PPE-B levels following repeated dopamine-replacement in the MPTP-treated primate model of Parkinson's disease with respect to striosome-matrix compartmentalisation. Following repeated dopamine receptor agonist treatment in the MPTP primate model of Parkinson's disease, animals display dyskinesias that are indistinguishable from those seen in parkinsonian patients. Utilising in situ hybridisation with ^{35}S-labelled oligonucleotide probes, we have studied the expression of the dynorphin precursor PPE-B within the striatum of dyskinetic primates. PPE-B expression within the primate striatum showed a 'patchy' heterogeneous distribution. In adjacent sections striosome-matrix compartmentalisation was determined by acetylcholinesterase staining and calbindin immunocytochemistry. Patches of higher PPE-B signal were confined within the striosomal boundaries. Densitometric analysis of PPE-B expression, relative to the control probe G3PDH, was performed with respect to striosome-matrix boundaries in both the rostral and caudal striatum. In parkinsonian macaques, following repeated therapeutic doses of the direct dopamine receptor agonist apomorphine, peak-dose dyskinesias were observed. In these animals PPE-B, expression was significantly increased in both striatal compartments, when compared to MPTP-treated macaques following apomorphine reversal of parkinsonian symptoms. We propose that increased dynorphinergic transmission, following long-term treatment with dopamine agonists, may act to reduce glutamate-mediated excitation of GPm and thus cause dyskinesia. Opioid receptor antagonists, acting preferentially at kappa opioid receptors, may provide a useful adjunct to dopamine-replacement in the treatment of Parkinson's disease.

12 The murine chorda tympani: pioneering and early morphology. By L. Scott and M. E. Atkinson, *Department of Biomedical Science, University of Sheffield*

Many studies demonstrate that differentiation of certain sensory receptors during development is induced by their nerve supply. Thus the navigational accuracy of pioneering fibres to their targets is crucial to this process. The special gustatory elements of the facial and glossopharyngeal nerves are used extensively as model systems in this field. We have examined the chorda tympani, the gustatory component of the facial nerve, to determine the precise timecourse of its development in mice. It would then be possible to speculate upon the inductive relationship between axons and their sensory receptors and the importance of target-derived chemotropic influences. Pregnant time-mated MF_1 mice were killed by cervical dislocation. Embryos were dissected from the uteri and immediately decapitated. The heads were fixed in a solution of phosphate buffered 4% paraformaldehyde and 0.2% glutaraldehyde (pH 7.2). The transganglionic fluorescent tracer DiI was injected into the anterior aspect of the mandibular arch of fixed embryos aged between 30 and 50 somites (E10 and E12) and permitted to diffuse retrogradely via the geniculate ganglion to the brainstem. After 1–4 wk, the distribution of DiI was determined using confocal laser scanning microscopy. Geniculate ganglion cells were first labelled at the 34 somite stage (E10). Pioneering chorda tympani fibres that arise from these cells passed peripherally and followed an oblique course as they grew toward the mandibular arch. At the 36 somite stage (E10.5), the peripheral component followed an intricate postspiracular course and passed anteriorly to arch over the primitive tympanic cavity, en route to the lingual epithelium. From the 36–50 somite stages (E10.5–E12), it consistently traced in the fashion of a U bend. The central fascicle also traced at the 36 somite stage (E10.5) and just made contact with the brainstem. At the 40 somite stage (E11) the central fibres clearly chose a route of descent into the spinal trigeminal tract and branched into the solitary tract. Pioneering chorda tympani fibres contact the lingual

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epithelium when the target is primordial. The lingual epithelium is possibly a source of a neurotropic factor that attracts peripherally orientated chorda tympani fibres. However, the chorda tympani is probably not a vital influence on the subsequent differentiation of gustatory papillae, since the papillae are elaborated 5 d later at E15 in murine embryos. The earliest morphology of the nerve is true to the mammalian phenotype.

L. Scott is an Anatomical Society Research Student.

13 Differential regenerative capacities and molecular responses of adult rat cerebellar neurons following axonal injury and the implantation of a peripheral nerve graft. By Y. Zhang, E. Vaudano, G. Campbell, P. N. Anderson and A. R. Lieberman. *Department of Anatomy, University College London*

When segments of peripheral nerve are autografted to the dorsal thalamus of adult rats, very few thalamocortical projection neurons (or interneurons) regenerate axons into the graft, whereas large numbers of neurons in the thalamic reticular nucleus (TRN) do so (Benfey et al. *J. Neurocytol.* **141**, 1985; Morrow et al. *Exp. Neurol.* **120**, 1993). These neurons show early and persistent upregulation of the immediate early gene c-jun (Vaudano et al. *Eur. J. Neurosci.* in press), the growth associated protein GAP-43 (Vaudano et al. *J. Neurosci.* **15**, 1995) and the cell adhesion molecule L1 (Zhang et al. *J. Comp. Neurol.* **361**, 1995). We now report a comparable pattern of differential gene expression associated with axonal regeneration after implanting such grafts in the cerebellum. The proximal end of a segment of tibial nerve was pushed through a craniotomy and an incision in the dura mater into the white matter core of the cerebellum in deeply anaesthetised (Halothane) adult albino rats. Following survival periods of a few days to a few weeks the animals were killed by anaesthetic overdose, in some cases 48 h after application of HRP or CT-HRP to the distal end of the graft. Sections through the brain were processed to identify and locate retrogradely labelled cell bodies, or hybridised with 35^s, alkaline phosphatase or digoxygenin labelled oligonucleotide or cRNA probes to identify cells expressing mRNAs for c-jun, GAP-43 and L1. Purkinje cells and other neurons of the cerebellar cortex were never retrogradely labelled with HRP and never showed upregulation of c-jun, GAP-43 or L1 even though the axons of many such neurons must have been interrupted by the grafts. Neurons of the deep cerebellar nuclei (DCN), especially those located within a few hundred μm of the graft tip, were HRP-labelled and hybridised strongly with all 3 mRNA probes. Thus upregulation of several growth related molecules in the DCN appears to be correlated with their ability to regenerate axons into peripheral nerve grafts.

This work was supported by the Wellcome Trust, the MRC and the EC.

14 Cerebral cortical microglia in schizophrenia. By K. Radewicz, L J. Garey and R. Reynolds. *Department of Anatomy, Charing Cross and Westminster Medical School, London*

There is increasing evidence that schizophrenia may be a neurodevelopmental disorder associated with subtle modifications of neuronal processes in the cerebral cortical neuropil. Developmental abnormalities may manifest themselves as alterations of cortical circuitry and imbalances in neurotransmitter systems. Glia play a major role in neuronal migration, synapse formation and maintenance, and control of neurotransmitter metabolism in the developing and mature nervous system. We have therefore studied possible structural and functional changes in glia in schizophrenia, in a cortical region implicated in schizophrenia, the dorsolateral prefrontal cortex (Brodmann's area 9). Frozen sections (20 μm thick) from 9 schizophrenic and 10 control brains taken postmortem from subjects from whom consent had been obtained in life, were immunostained in a blind study with an antibody, LN3, against an HLA-DR antigen. Cell counts were made in individual cortical layers (determined after cresyl violet staining) of both cerebral hemispheres. We found a significant 25% increase ($P < 0.05$) in numerical density of microglia in schizophrenics (648 ± 46 cells/mm^2, n = 9, mean age 78 y) versus controls (517 ± 24 cells/mm^2, n = 10, mean age 69 y) when combining all cortical layers and both hemispheres. Analysis of individual cortical layers showed an increase in numerical density in all layers of schizophrenics, which reached significance in layers II (29%, $P < 0.05$), III (38% $P < 0.01$) and IV (32%, $P < 0.05$). Our results demonstrate widespread increase in numerical density of microglia in the frontal cortex of chronic schizophrenics, not simply related to ageing, which might be implicated in possible changes in microcircuitry and cortical neuronal architecture.

This study was supported by Glaxo/Wellcome.

15 Dynamic arterial adaptation to experimentally induced flow alteration in the canine mesenteric vascular bed. By K. S. Han, G. Pasterkamp*, J. van der Zande* and B. Hillen. *Department of Functional Anatomy, Utrecht University, Utrecht and *The Heart Lung Institute of the Utrecht University Hospital, Utrecht, The Netherlands.*

Previous animal studies on arterial adaptation to alterations in bloodflow have focused on a single arterial segment, although both in normal healthy people and in patients with vascular occlusive diseases, the whole vascular bed, including the collateral circulation, is involved. The present study aims to assess how an 'intact' vascular bed, including a collateral circulation, reacts and adapts to alterations in bloodflow. All procedures involving the use of animals were approved by the Ethical committee on animal experiments at Utrecht University. Interventions and measurements were performed on the mesentery of 10 beagles under general anaesthesia. The mesentery serves as a model in which the influence of arterial occlusion on the redistribution of bloodflow in the vascular bed and consequently on arterial remodelling can be studied. In 3 adjacent principal arteries (PA 1, PA 2 and PA 3), bloodflow, length and diameter were measured. PA 2 was then occluded. Immediately after occlusion, and after 4 and 8 wk, bloodflow and arterial diameter of PA 1 and PA 3 were measured. A mathematical model was designed to predict the consequences of this occlusion and to calculate the wall shear stresses (WSS). The animals were killed with an overdose of pentobarbital. The results are summarised in the following table:

PAs 1 and 3	Pre-Occlusion	Occlusion	4 wk	8 wk
Flow (ml/min)	4.1 ± 2.0	5.1 ± 2.2*	6.1 ± 2.8*	5.0 ± 2.5
Radius (mm)	0.70 ± 0.20	0.73 ± 0.19*	0.62 ± 0.09*	0.70 ± 0.14
WSS (N/m^2)	1.45 ± 0.87	1.56 ± 1.01	2.61 ± 1.15*	1.72 ± 1.11

Immediately and 4 wk after occlusion, PA 1 and PA 3 compensated for the bloodflow of PA 2 (Table, $*P < 0.05$). At 8 wk, bloodflow and wall shear stress decreased to baseline values and no significant arterial radius changes were observed. By contrast, calculations showed a dramatic overestimation of the bloodflow at 8 wk. Surprisingly, this bloodflow deficit appeared to be compensated for by an increased bloodflow and growth of smaller pre-existing collateral arteries with a diameter of 50–100 μm, bridging the occlusion. Mathematical calculations revealed the tremendous WSS increment in the smaller arteries (mean 499 %) compared to the larger PA 1 and PA 3 (mean 34 %), immediately after occlusion. After occlusion in an intact vascular bed in the acute state, large arteries adjacent to the occlusion may initially compensate for the deprived bloodflow. Contrary to our expectations, this temporary bloodflow increase is not followed by chronic vascular remodelling in these larger vessels. However, in the longer term, smaller collaterals take over the compensating effect of the larger arteries by vascular growth. These results showed that the smaller arteries have a greater tendency to remodel than do larger ones, possibly because of the greater increase in WSS.

16 Ablation of keratin 14 expression due to a homozygous premature termination codon mutation causes severe recessive epidermolysis bullosa simplex. By L. D. Corden, J. E. Mellerio*, M. J. Gratian*, R. A. J. Eady*, J. I. Harper**, M. Lacour**, G. Magee, E. B. Lane, J. A. McGrath* and W. H. I. McLean***. *CRC Cell Structure Research Group*, *Department of Anatomy and Physiology*, *University of Dundee*; **St John's Institute of Dermatology*, *St Thomas's Hospital*, *London*; ***Dermatology Department*, *Great Ormond Street Hospital*, *London and* *** *Epithelial Genetics Group*, *Jefferson Medical College*, *Philadelphia*

Keratins are the intermediate filament proteins which form a dense network of 10 nm filaments within the cytoplasm of epithelial cells. This cytoskeletal system enables the cell to withstand mechanical insults and so hereditary defects in these proteins produce diseases characterised by epithelial fragility. One such disease is the congenital skin blistering disorder epidermolysis bullosa simplex (EBS), caused by mutations in either keratins K5 or K14, which are specifically expressed in the basal cells of the epidermis. Here, we have studied a consanguineous family containing 2 children with severe, generalised EBS. Electron microscopy of skin biopsies from the affected individuals showed that basal keratinocytes were devoid of tonofilament bundles, although some single 10 nm intermediate filaments were visible. Genetic linkage analysis with the microsatellite probe D12S96 excluded the type II keratin gene cluster in this family. However, homozygosity by descent was observed with the polymorphic probes KRT9, KRT10 *Ava II* and D17S1787 in both affected children, consistent with a recessive type I keratin defect. Immunoreactivity to keratin K5 and K15 was normal, but monoclonal antibody LL001 against K14 showed no staining, indicating a lack of this keratin in these individuals. mRNA extracted from patients skin biopsy material was amplified by reverse transcription-PCR, to obtain full length K14 cDNA. Direct automated sequencing of this K14 PCR product identified a homozygous nonsense mutation, W305X, which was not seen in a normal control sequence. A recognition site for the restriction enzyme *Hin*f I is created by this nucleotide transition which was used to confirm the presence of the mutation in this kindred and exclude it from 100 normal chromosomes. This is the 4th kindred with severe recessive EBS for whom a mutation has been found in the K14 gene. In this instance, the premature termination codon is the furthest downstream, occurring in the helix 2 domain of the K14 polypeptide. Although this mutation is in a 3′ location, the heterozygous carriers in the family are unaffected by the disease and display no epidermal fragility. Therefore, translation of the potentially dominant-negative truncated K14 must be greatly down-regulated due to instability of the mutant mRNA. Despite the complete lack of a major epidermal structural protein in these patients, the prognosis is fairly good, based on our previous studies of recessive EBS. Identification of the precise genetic lesion in these patients will allow both carrier detection and genetic counselling to be carried out in this extensive inbred family, as well as allowing the option of DNA-based prenatal diagnosis. This study was carried out with the informed consent of the family members.

17 Role of cell adhesion in the pathway of apoptosis in HT-22 cells. By J. P. Bennett, P. J. Robshaw and J. E. Davies. *Department of Anatomy and Cell Biology*, *Imperial College School of Medicine at St Mary's*, *London*

HT-22 cells are an immortalised neuronal cell line deriving from mouse hippocampal cells. They can be triggered to undergo cell death by excessive concentrations (> 1 mM) of the excitatory neurotransmitter glutamate by a pathway which apparently includes an oxidative step since it can be prevented by antioxidants (e.g. cysteine) or specific oxidase inhibitors (e.g. clorgyline). The cell death pathway has many of the morphological characteristics of apoptosis including a period of intense cell membrane blebbing about 4 h after triggering, and an alteration in the appearance of the nucleus. About 10 min prior to the onset of blebbing, cells invariably showed a change in their attachment to the substratum, from a relatively flattened morphology (projected area 754 ± 285 μm^2; mean ± S.D., n = 26) to a rounded one (257 ± 83 μm^2; $P < 0.01$), and contact with neighbouring cells was lost. By the end of blebbing, the nucleus had condensed (to an apparent 35 ± 11 % of its normal volume; mean ± S.D., n = 50) and acquired an irregular outline but cells which remained attached to the microscope slide failed to show the classic apoptotic nuclear fragmentation. When the cells which had detached from the substratum at this stage were harvested 52 % were found to have proceeded to the fragmented nucleus morphology. These results suggest changes in cell adhesion may be permissive to important stages in the apoptosis pathway, and this was supported by the observation that altering cell

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adhesiveness by pre-coating the substratum with defined extracellular matrix molecules including fibronectin and collagen changed the sensitivity of the cells to glutamate-induced death.

18 Over-expression of the myotonic dystrophy protein kinase gene results in inhibition of myotube formation in the murine myogenic C2C12 cell line. By G. N. Okoli, N. Carey* , K. J. Johnson** and D. J. Watt. *Departments of Anatomy and Surgery*, *Charing Cross and Westminster Medical School*, *London and Division of Molecular Genetics*, *University of Glasgow***

Myotonic dystrophy (DM) is the commonest form of adult onset muscular dystrophy. The presentation of the disease is extremely variable ranging from presenile cataracts as the only detectable symptom in the mildest cases to mental retardation and severe neonatal hypotonia in the congenital form of the disease. In adult cases the skeletal muscle classically shows dystrophic changes and specific fibre-type loss, whereas in congenital cases there is a delay in muscle maturation. The mutation in DM is the expansion of a 3 base pair (CTG) repeat. In affected individuals there may be thousands of copies of this repeat compared with the maximum 50 copies in nonaffected individuals. The repeat lies in the 3′ untranslated region of a putative protein kinase gene (DMPK). Although the repeat is transcribed into mRNA it is not translated into protein. Two other genes have been identified very close to the CTG repeat and it is now known that the expression of at least one of them is influenced by the expansion. It is however unclear how the mutation leads to the pathological changes observed in the skeletal muscle of DM patients. We have developed an experimental system to study the effects of DMPK in muscle development in vitro. We cloned a full-length mouse cDNA for DMPK into a mammalian expression vector which was transfected into C2C12 cells. C2C12 cells are mouse myogenic cells which retain the capacity to fuse and form myotubes in culture. We have shown that over-expression of DMPK in these cells results in a marked reduction in myotube formation in comparison with both untransfected C2C12 cells and cells transfected with expression vector alone. As creatine kinase (CK) is a well established marker of muscle differentiation we assayed CK activity in our control and experimental cultures. In untransfected and control transfected cultures CK activity increased with time in culture correlating with substantial myotube formation (355 ± 0 on d 2 in culture rising to 947 ± 32 UL/mg protein on day 5; $n = 3$). In contrast, DMPK transfected cells showed no increase in CK activity over the same time course confirming that over-expression of DMPK resulted in an inhibition of the cells to differentiate to the multinuclear state (488 ± 1.6 on d 2 falling to 39 ± 12 UL/mg protein on d 5; $n = 3$). When myogenic cells enter terminal differentiation there is a reduction in proliferative activity due to cell cycle arrest. In the present work control cultures showed no increase in proliferative rate with the onset of fusion (2.5 ± 0.07 on d 2 and 2.4 ± 0.09 absorbance at 490 nm by d 5; $n = 4$), whereas DMPK transfected cultures continued to proliferate (2.1 ± 0.07 on d 2 and 2.7 ± 0.2 absorbance at 490 nm at d 5; $n = 4$). Our results suggest that the intracellular levels of DMPK have an effect on the process of muscle cell differentiation/maturation. Our preliminary data may be of significance in understanding the failure of muscle cell maturation in the congenital form of DM and will act as a starting point to examine the effects of the mutated gene in human pre- and post-natal material. We are currently using our system to identify the intracellular targets of DMPK in order to elucidate the mechanims by which these effects on muscle cell maturation are mediated.

19 Computer-generated 3-D reconstructions of serially sectioned mouse embryos. By M. H. Kaufman and R. M. Brune. *Department of Anatomy*, *University of Edinburgh*

A wide range of techniques are available to analyse the morphological features of pre- and postimplantation mouse embryos, and similar techniques are equally applicable for the analysis of comparable stages of development of other mammalian species. Preimplantation embryos may be examined within the reproductive tract by conventional histological techniques, and this has the advantage that they may be examined at the appropriate site consistent with their gestational age. More conveniently, they may be isolated and examined in the unfixed state using a dissection microscope, or fixed and then analysed by conventional histology, or by transmission (TEM) or scanning electron microscopy. During the very early postimplantation period, it is usually more convenient to examine embryos in situ by conventional histology or by TEM. Developmentally more advanced embryos are usually isolated, appropriately fixed and their morphological features analysed by conventional histological techniques. Over the last 3 y we have been involved in a project, in collaboration with scientists in Anatomy (J. B. L. Bard) and at the MRC Human Genetics Unit (D. Davidson and R. A. Baldock), that uses a computer-aided approach for making 3D reconstructions of serially sectioned mouse embryos to produce a digital atlas of normal mouse development. Captured images of adjacent sections are aligned using a warping programme with minimal deformation. The reconstructed computer-generated embryos may then be resectioned in any plane in order to match the viewer's own material. Individual anatomical domains may then be delineated and painted in different colours. 3D images of individual anatomically discrete components, or related sets of components, may then be viewed in isolation or, if required, against a ghost like image of the intact embryo, or specific parts of an embryo. Examples will be presented to illustrate how this approach may be used to facilitate the interpretation of the early stages of development of the cardiovascular system, the gut and other organ systems. The configurational changes that take place during the early postimplantation period in the axis of the embryo will also be illustrated during the process of 'turning' when the mouse embryo adopts the so-called 'fetal' position. Our initial aim is to provide 3D reconstructions of mouse embryos from fertilisation up to about 12.5 d postcoitum. A database of mouse developmental anatomy has also been prepared covering all stages from fertilisation to birth, and when completed this project will allow the accurate spatial mapping of gene-expression and cell lineage data onto the digital atlas of normal mouse development.

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20 The digital atlas of mouse development and gene-expression database. By R. A. Baldock, *J. B. L. Bard, *R. M. Brune, C. Dubreuil, J. T. Eppig**, W. Hill, M. H. Kaufman*, J. E. Richardson**, M. Ringwald**, M. Stark and D. Davidson. *MRC Human Genetics Unit, Edinburgh, *Department of Anatomy, Edinburgh University and the **Jackson Laboratory, Bar Harbor, USA*

Genetic information is expressed in complex and ever changing patterns throughout the development of the mammalian embryo. A description of these patterns and how they relate to the emerging tissue structure of the embryo is crucial for our understanding of the network of genetic interactions that underlie the processes of normal development, disease and evolution. Studies of gene expression are rapidly producing a very large amount of information relating to these complex patterns. This creates serious difficulties in publishing, scanning and accessing the information. It also creates difficulties in making comparisons between the expression of different genes in order to assess the possibility of complex networks of genetic interaction. These problems cannot be addressed by conventional means of publication, but will require the development of a widely accessible, electronic database. Moreover, text descriptions of gene expression are often of limited value partly as a consequence of the spatial complexity of the patterns and partly because domains of gene expression do not necessarily correspond to named anatomical structures. To address these problems we are building a 3D computer model or 'Atlas', of successive stages of mouse embryonic development and using this as the standard framework on which to map the expression of different genes. Images of serial histological sections (generally from the same embryos as illustrated in *The Atlas of Mouse Development*, Kaufman, 1992) are being used to construct full grey level, voxel images of mouse embryos at successive stages of development (approximately daily intervals) in which histological detail is visible in any plane of section. Gene expression will be mapped to this model at 2 levels, textual and graphical. The project, the image processing and reconstruction techniques, the database design, visualisation methods, network access, network interface tools, and the current status will be presented. An overview of the project is available from http://www.hgu.mrc.ac.uk/Research/Devgen/MouseAtlas/richdunc.htm.

21 Interactive 3D reconstructions of embryonic human hearts. By S. C. Whiten, J. C. McLachlan, S. D. Smart and J. F. Aiton. *School of Biological and Medical Sciences, University of St Andrews*

Despite the fact that development of the human embryo heart is of considerable clinical importance, there is still disagreement over the process and the timing of events. It is likely that some of the conflicting accounts may have arisen from difficulties in describing and visualising 3D structures from 2D sections. To help comprehend these changes, we have developed techniques for the creation of interactive 3D models reconstructed from histological sections of human embryos. Sophisticated 3D computer reconstruction of sectioned embryo material is being carried out by other groups. However, since such approaches can require sophisticated computing expertise and specialist hardware, a number of problems may arise. For example, advanced computer hardware can be prohibitively expensive and specially written software may only be effective in the hands of its authors. Fast processor and extensive memory requirements can mean that interactive viewing of reconstructed models can only be performed on advanced workstations. In order to address some of these issues, we have devised a relatively simple method for creating 3D computer reconstructions of sectioned embryonic hearts which uses commercial graphics software designed for the creation of virtual reality environments and 3D models (Macromedia FreeHand and Strata StudioPro for the Apple Macintosh). The embryos used in this study are part of the Walmsley Collection, School of Biological and Medical Sciences, University of St Andrews and the Boyd Collection, Department of Anatomy, University of Cambridge (details of these collections are available via the British Universities Human Embryo Database at http://embryos.st-andrews.ac.uk). Four embryos (1.8, 8, 10 and 12.5 mm), serially sectioned at 10 μm intervals, were selected for this initial study. Hand drawn tracings of the hearts were digitised, aligned and rendered to produce virtual reality objects which could be rotated and inspected from any viewpoint. It is possible to reslice these QuickTime VR objects to show different internal features which may also be visualised in varying degrees of transparency. The models can be accessed and downloaded from the World Wide Web (http://www.st-and.ac.uk/~www_sbms/terrapin/Freebies.html) and can be viewed on a range of personal computers (Macintosh and IBM compatible) using appropriate free software. The ability to construct interactive visual images which both illustrate and communicate complex 3D information contributes to our understanding of the complex developmental changes occurring in embryogenesis.

22 3D imaging, registration and visualisation in image guided surgery. By D. Hawkes. *Radiological Sciences, UMDS Guy's Campus*

Modern magnetic resonance (MR) and computed tomography (CT) scanners can provide volume images with spatial resolutions of 1 mm or better. Functional images with a resolution of between 7 and 10 mm are available using positron emission tomography (PET) or single photon emission computed tomography (SPECT). This talk describes methods for accurately aligning these volume data sets in order to provide a single combined representation. Fully automated registration algorithms are now becoming available to achieve this. Most methods assume that the patient's anatomy does not deform between image acquisitions and that the imaging devices do not introduce significant geometric inaccuracies. The performance of current registration algorithms is such that even within the cranial vault soft tissue deformation and image distortion can limit the accuracy of the resulting 3D representation. Work is in progress to compensate for these errors. One of the main applications for highly accurate registration is image guided surgery. This involves additional steps to align the preoperative images with the patient reference in the operating room and then display the results for surgical

navigation. A novel way of approaching this problem using the surgical operating microscope and the technique of 'augmented reality' is presented.

23 The principles of facial reconstruction upon the dry skull. By R. A. H. Neave. *Unit of Art in Medicine, University of Manchester*

Facial reconstruction or 'facial approximation' as it is sometimes referred to on the continent, is based upon the recognition of the fact that there exists a predictable relationship between the skull and its overlying soft tissues. This relationship was noted and studied by the anatomist His in 1860, and a number of further studies were made by other workers during the course of the next 40 y. All were endeavouring to demonstrate that a face built upon a skull would, or would not, be broadly similar to the face that covered such a skull in life. It is a technique that attempts to make recognisable that which is unrecognisable, and today is seen as having a valuable role to play within the field of craniofacial identification. This paper will outline the techniques employed in The Unit to reconstruct the faces of victims of accidental death and murder, examine the accuracy of the method by control studies and forensic cases and discuss the strengths and weaknesses of the technique as a tool in forensic medicine.

24 Modern 3D image processing and visualisation. By Bart ter Haar Romeny. *Image Sciences Institute, Utrecht, The Netherlands*

The use of current 3D rendering software in combination with recent developments in computer vision techniques enables an exciting range of applications for the visualisation, measurement and interactive manipulation of volumetric data. The presentation will be an overview of the current state-of-the-art in this area. Before any visualisation, an essential step is to prepare the data properly; examples will be discussed of image enhancement and denoising techniques, and the complex process of automatic segmentation. A new and promising methodology called 'multiscale image analysis' is based on a mathematical analysis of human (cortical) visual processing. The matching of 3D volumes of different modalities has been refined to such an extent that functional information, like SPECT brain perfusion or functional MR data, can be colourcoded on the rendered 3D anatomy from e.g. MR or CT. In the same way, metric data e.g. skull thickness, or shape data (e.g. Gaussian curvature) can be visualised. The purpose of 3D visualisation in radiology is, to a large extent, the interaction with the surgical speciality. This creates the need for fast and truly interactive displays. Modern and increasingly cheaper workstations ($< \$10{,}000$) allow this to be a reality. Interactive manipulation also helps the perception of 3D by introducing depth cues from motion, occlusion and texture gradients. Other examples include the automatic detection of the optimal viewing angle perpendicular to the neck of an aneurysm, and the simulation of the design and placement procedure of intra-abdominal aortic stents. These developments, together with the availability of high resolution datasets of modern scanners and e.g. the Visible Human data, also have a dramatic impact on interactive 3D anatomical atlases.

25 Monte Carlo modelling of electron scattering processes in calcified tissues. By P. G. T. Howell and A. Boyde*. *Department of Prosthetic Dentistry, Eastman Dental Institute, London and *Department of Anatomy, University College London*

Knowledge of the image forming process is fundamental to understanding and interpreting images derived from any form of microscopy. The present interest lies in the study of the calcified tissues using scanning electron microscopy in the backscattered electron (BSE) emission mode. BSEs carry information about the mean atomic number of the small volume with which they have interacted. In general, it is understood that a substrate of higher mean atomic number gives rise to more BSEs and a brighter image. However, real samples of bone embedded in PMMA are prepared by polishing or micromilling: topography free surfaces are difficult to obtain because of the differential hardnesses between layers with contrasting structural orientation or differering composition. We shall illustrate the use of Monte Carlo techniques to model electron scattering in the calcified tissues to help explain the contrast mechanisms in these composite biological materials. Single electrons are tracked as they travel through the sample where they are scattered by the atoms of the material that they encounter. Each scattering event is considered to be elastic with energy lost between events. The electron's path is traced until it has either lost sufficient energy to be captured by the sample, or for it to have returned to the sample surface where it is counted as a backscattered electron. Each electron may undergo 200–300 scattering events in this process. For statistical significance of the simulation, therefore, up to 1 million electron trajectories need to be studied. This figure corresponds well with routine operating conditions in an SEM. The calcified tissues are modelled by an empirical formula derived from a mixture of a protein (collagen) matrix into which bone salt (hydroxyapatite) is added: water is substituted by PMMA: mineral therefore substitutes PMMA. The model assumes no long range order. The selection of which atomic species is involved at each scattering event is weighted in proportion to the elemental contribution to the Rutherford elastic cross-section. Internal structural arrangements can be modelled, for example, boundaries between tissue phases with differing mineral contents. Surface topography is additionally modelled to elucidate signal variations due to surface finishing artefacts, of particular importance in respect of lamellae. Modelling the compositional and surface topographic signal level and contrast effects using Monte Carlo techniques is pivotal to the correct morphological and numerical evaluation of digital BSE-SEM micrographs.

26 Morphological appearance of the embryonic mouse heart between Theiler stages 12–14 (E8–9) from 3-D computer reconstructions. By R. M. Brune, R. A. Baldock*, J. B. L. Bard, D. R. Davidson* and M. H. Kaufman. *Department of Anatomy, University of Edinburgh and *MRC Human Genetics Unit, Edinburgh*

Morphological studies of mammalian heart development have been limited by a lack of either a 3D overview when analysing serially sectioned material or histological detail

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when using scanning electron microscopy (SEM). 3D computer reconstruction of serially sectioned material in combination with delineation of tissues within the reconstruction allows the investigator to view 3D images of delineated tissues in any combination and orientation. Alternatively, these reconstructions can be 'browsed' at any arbitrary angle with the delineated anatomy displayed as a see-through wash on top of the computer-generated representation of the histology. Histological sections through 3 representative mouse embryos at Theiler stages (TS) 12, 13 and 14 were captured and warped to correct for random processing/sectioning distortions. Individual anatomical domains were then delineated within the reconstruction. Analysis of computer-generated 3D images of the heart and its closely allied structures now allows us to re-evaluate the external and internal morphological features of the embryonic mouse heart as it progresses through the process of 'turning' when the mouse embryo inverts its germ layers in order to adopt the characteristic 'fetal' position. The 3 embryos studied were at an early, intermediate and advanced stage of the turning process and were matched with similar embryos that had previously been analysed using SEM. During the earliest stage studied, when mouse embryos possess about 6–8 pairs of somites, the heart has the appearance of a median mass and is suspended by a wide dorsal mesentery. In the intermediate stage studied when embryos possess 8–13 pairs of somites, the diameter of the primitive heart tube diminishes as 'looping' is initiated. The formation of the transverse pericardial sinus facilitates the looping process. In the most advanced stage studied in an embryo with about 15–18 pairs of somites, when the process of turning is almost completed, the primitive heart is considerably more elongated than previously and is more clearly subdivided into a common atrial chamber (proximally and dorsally), a primitive ventricle (mostly located towards the left of the ventral midline) and a primitive outflow tract (with a bulbus cordis region proximally, and aortic sac distally). Over this relatively short period of time the initially symmetric short tubular heart mass becomes modified to form an asymmetric and elongated tubular structure. The ability to view the external morphology and internal features in any desired orientation complements previous histological and SEM observations on the events that occur during turning as well as providing new insights into cardiac morphogenesis.

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27 Pharmacological bioassay development using rat cardiac myocytes cultured over microfabricated extracellular electrode/FET arrays. By M. C. Denyer, M. Riehle, C. Sproessler*, S. Britland**, A. Offenhaeusser*, W. Monaghan, M. Robertson and W. Knoll* (introduced by R. A. Smith). *Centre for Cell Engineering, Division of Infection and Immunity, University of Glasgow, *MPI Polymerchemie, Mainz, Germany and **School of Pharmacy, University of Bradford*

Vast numbers of cardioactive compound analogues can be synthesised using standard biochemical methods and the process of light-directed spatially addressable parallel chemical synthesis. All these compounds require sorting in relation to their bioactivity, but this is difficult and extremely time consuming using standard electrophysiological techniques. What is required is an assay that can process large numbers of these analogues simultaneously. Such an assay may be developed using electrogenic cells cultured over microfabricated extracellular electrode arrays from which long term electrophysiological recordings can be made. Therefore we are examining the development of systems consisting of neonatal rat cardiac myocytes cultured on microfabricated arrays of metal microelectrodes and field effect transistors (FETs). Cardiac myocytes display coordinated rhythmic contractile and electrogenic activity in vitro, thus we can monitor the contractile behaviour of these cells optically whilst simultaneously studying their electrophysiology. To demonstrate that such systems are suitable for use in assays of novel cardioactive compounds we need to show: (1) that we can reliably make long term extracellular electrophysiological recordings from muscle cells cultured over electrode/FET arrays, (2) that we can modify myocyte electrophysiology using bioactive compounds, and (3) that we can record large potentials suitable for detailed pharmacological studies. Preliminary results show that we can record extracellular potentials of between 20 μV and 3.5 mV from myocytes cultured on arrays of metal electrodes, and these potentials can be reversibly blocked by μM solutions of the sodium channel blocker Lidocaine. We can also record extracellular potentials of between 200 μV and 25 mV from myocytes cultured over arrays of FETs. These results demonstrate that both systems can be used in assays of cardioactive compound analogues.

28 Stem-cell populations in developing and regenerating mammalian liver: emerging perspectives. By D. Brynmor Thomas. *School of Biological and Medical Sciences, University of St Andrews*

When hepatocyte proliferation following partial hepatectomy is abolished by the administration of a carcinogen, regeneration is affected by the proliferation and differentiation of cells which have been termed 'oval cells'. These cells appear to emanate from the portal space, give rise to progeny which form cohesive columns and differentiate into new hepatic plates (Alison et al. *J. Pathol.* **171**, 1993). Remarkably, oval cells closely resemble haematopoietic stem cells, which measure 7–10 μm in diameter, have very high nuclear–cytoplasmic ratios and contain few cytoplasmic organelles (Thomas et al. *J. Anat.* **124**, 1977). Even more remarkably, all known oval cell antigens are expressed by haematopoietic stem cells; several biochemical markers are shared by the 2 cell populations—which are exceedingly difficult to separate (Brill et al. *Proc. Soc. Exp. Biol. Med.* **204**, 1993)—and the accumulation of very early blood cell precursors in the liver during regeneration following partial hepatectomy (Hays et al. *Exp. Hematol.* **6**, 1978), may reflect the susceptibility of haematopoietic stem cells or their immediate progeny to regulatory mechanisms which are implicated in the proliferation of hepatocyte precursors. Haematopoietic stem cells and oval cells—which appear to be the stem cells of hepatocytes—are thus so similar that the possibility of their being the progeny of a single population

cannot be disregarded. The spectra of morphological continuity which link generalised blast cells to hepatocyte precursors on the one hand and on the other to orthochromatic erythroblasts (Thomas & Yoffey, *Brit. J. Haematol.* **10**, 1962), may thus represent divergent differentiation from similar populations of blast cells. A spectrum of morphological continuity (Thomas, *Ciba Found. Symp.* **13** (new series), 1973) is compatible with the derivation of these blast cell populations either from oval cells or from haematopoietic stem cells. Information is accumulating about oval cells and haematopoietic stem cells in developing and in regenerating liver, about haematopoietic stem cells very early in development (Dieterlen-Lievre et al. *J. Anat.* **190**, 1997; Medvinsky et al., *J. Anat.* **190**, 1997) and about the apparent ability of primordial germ cells to differentiate into haematopoietic stem cells in the presence of appropriate growth factors (Rich, *J. Anat.* **190** 1997). Such information can be expected to necessitate a critical reappraisal of hitherto established notions and may contribute to the development of techniques for the in vitro cultivation of haematopoietic stem cells capable of acquiring tolerance to host antigens following transplantation to suitably prepared recipients (Thomas, *Stem Cells*, **11 (Suppl 1)**, 1993).

29 Enhancing human osteoblast culture with autologous serum. By M. G. McALINDEN and *D. J. WILSON. *Orthopaedic Research Unit*, *The Queen's University of Belfast and *School of Biomedical Sciences/Anatomy*, *The Queen's University of Belfast*

It has been suggested that a composite of cultured human osteoblasts and a bone graft substitute may present a solution to the well-recognised complications and limited availability associated with harvest of fresh bone graft. Conventionally, culture medium is supplemented with fetal calf serum (FCS). However, such serum presents potential risks of foreign protein contamination and transmission of viral or prion-related disease if used in culture of osteoblasts intended for human reimplantation. This study aimed to compare the proliferative response of human osteoblasts supplemented with FCS or autologous human serum (AHS) to determine whether AHS is a practical alternative. Explant cultures of human osteoblasts were established using greater trochanter trabecular bone from 10 consented patients (aged 57–84 y) undergoing total hip arthroplasty. At the same time, serum was harvested. The osteoblasts were characterised by alkaline phosphatase expression and by 'in vitro' mineralisation in enhanced medium. At confluence, osteoblasts were aliquotted into multiwell plates and grown for 9 d in medium supplemented with 5, 10, 15 or 20% AHS or 10% FCS. Proliferative response was determined by a crystal violet dye binding assay. There was no significant difference between the proliferation of osteoblasts in 5% AHS and 10% FCS. However, 10, 15 and 20% AHS all produced significantly greater proliferation than 10% FCS. The proliferative response was dose-related. FCS is said to be rich in growth and attachment factors, which is why it is widely used in tissue culture. These results suggest that species specificity, even when using adult serum, outweighs these advantages. It should therefore be considered as a prerequisite for any programme involving reimplantion of cultured human cells. Clinical trials of cultured human osteoblasts have now begun.

30 Limb development in the mouse mutant *Doublefoot*. By C. HAYES, J. M. BROWN, M. F. LYON* and G. M. MORRISS-KAY. *Department of Human Anatomy*, *University of Oxford*, *South Parks Road*, *Oxford. and *MRC Mammalian Genetics Unit*, *Harwell*, *Didcot*, *Oxon*

The mouse mutant *Doublefoot* (*Dbf*) exhibits preaxial polydactyly, with all 4 limbs affected to the same extent. The mutation is inherited in a dominant manner. Gene expression patterns, as revealed by in situ hybridisation, place the mutation downstream of *Sonic hedgehog* (*Shh*), a gene which has been implicated as one of the main signalling molecules controlling limb development. Heterospecific mouse/chicken grafts, together with the gene expression data, revealed that the anterior mesenchyme of *Dbf* limb buds exhibits polarising activity in the absence of *Shh* expression. These results establish the *Dbf* mutation as a gain of function mutation. The demonstration of an ectopic zone of polarising activity (ZPA) that functions independently of *Shh* in the anterior margin of *Dbf* limbs identifies a new genetic component of the limb patterning mechanism. This new component may be the active signalling molecule of the ZPA. Pregnant dams were killed by cervical dislocation; embryos were killed by immersion in fixative for in situ hybridisation, or decapitation for grafting experiments, according to Home Office regulations.

POSTERS

D. 1 3D Anatomical reconstructions of mouse embryos at E7, E8.5 and E9. By R. M. BRUNE*, R. A. BALDOCK*, J. B. L. BARD, D. DAVIDSON* and M. H. KAUFMAN. *Department of Anatomy*, *University of Edinburgh and **MRC Human Genetics Unit*, *Western General Hospital*, *Edinburgh*

The developing mouse embryo is now the prime model system for investigating human development, and a key part of this work is analysing gene-expression patterns as part of the strategy for elucidating the molecular networks that underpin the emergence of anatomical organisation. Such has been the progress of work here that the amount of data now available is too great for an individual to absorb, while, due to space limitations in the literature, only limited amounts of the expression data are publicly available. Our approach to handling this problem is to construct a database in which domains of expression data can be directly and graphically linked to spatial domains in 'digital' reconstructions of mouse embryos (Ringwald et al. *Science* 265, 1994), and the work reported here demonstrates some of their features. Such digital mice are made from haematoxylin-and-eosin-stained serial sections which have been digitised, warped (to remove distortions in the sections) and reconstructed to give what are known as grey-level voxel images. While such reconstructions do show standard morphology, the computer has no means for identifying one domain from another; for this, all the tissue boundaries

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have to be delineated by computer 'painting' the voxel image (and, as part of the work, we have prepared complete lists of all the tissues (> 1000) present at each stage of mouse development). Once the painting has been done (and, for example, the Theiler stage [TS] 14 [E9] has some 50 separate tissues to be delineated), any given organ can be separately highlighted in its own distinct colour. The software now available for the system allows the complete set of tissues in an embryo to be displayed in any combination and orientation in 2D, 3D, or stereo (with special glasses). The 3D display allows interactive turning of the reconstructions for assessing relative size measurements and topological relationships. The approach thus gives considerable insight into the developmental anatomy at these stages, and, in particular, into the process of turning that helps establish lateral asymmetry. Apart from its value in acting as the platform for gene-expression studies, these reconstructions, which will soon be available on CD-ROM, will form a valuable navigation and teaching tool for analysing morphogenesis in normal and mutant embryos. Further information is available at http://genex.hgu.mrc.ac.uk/.

D. 2 Three-dimensional reconstruction of fetal and neonatal rat bone using confocal laser scanning microscopy. By J. Gormally, *J. M. French, * M. M. Parkinson and. C. D. Ockleford. *Department of Pre-Clinical Sciences, Leicester University Medical School and *GlaxoWellcome Research and Development, Ware, Hertfordshire*

The confocal laser scanning microscope (CLSM) has attracted interest as a means of producing 3D images of an object. It collates a series of image slices through a specimen which are then reconstructed using computer software. The requirement to section a specimen physically is avoided, thereby preventing specimen destruction and maintaining structural relationships. Better resolution than that of conventional microscopy is achieved with this system because out-of-focus information from planes above and below a plane of focus can be excluded. Used in immunofluorescence microscopy, CLSM is a popular tool. Could its use be extended to the examination and comparison of relatively large and complicated structures such as fetal rat bone? A CLSM study of the calcification process in alizarin red S stained fetal and neonatal rat humeri is described. Alizarin red S is a fluorescent stain which has previously been used in a CLSM study of calcification on bioprosthetic heart valves (Bernacca et al. *J. Heart Valve Dis*. **3**, 1994). It is commonly used to demonstrate the whole ossified skeletal system, intact within the body, as in embryofetal development studies. There the effects of a drug on the developing fetal skeleton are assessed by the evaluation of the alizarin red S stained specimens using light microscopy. Han Wistar rats from the rodent breeding colony at GlaxoWellcome were mated overnight. Designation of d1 of gestation was made on observation of sperm in a vaginal smear, or the presence of a copulation plug the following morning. Pregnant dams were given rat and mouse diet and water ad libitum. Dams were killed on d 19, 20 or 21 of gestation by exposure to carbon dioxide. Fetuses were cleared and stained with alizarin red S as described by Dawson's method (Dawson, *Stain Tech*. **1**, 1926). Neonates aged 1–4 d were given lethal intraperitoneal injections of sodium phenobarbitone, cleared, and stained with alizarin red S. The humeri of 52 alizarin stained specimens (all from different litters) were measured and the average length of ossified bone for any particular age determined. Humeri correlating to the average length for specimen age were dissected from the soft tissues and slide mounted. Optical sections of the posterior aspect of the mid diaphysis were collected (z series). Sections were taken at 5 μm intervals (z step), from the bone surface to approximately 50 μm in depth. These were then projected linearly with computer software (z projection) to create 3D images of complex calcified trabecular structures. The images show the formation and thickening of trabeculae during a period when ossification of the cartilage skeleton has only just begun. They show cell lacunae and differing textures. Ossification is seen to progress rapidly to create mature, dense calcified bone. There is potential for this method to be used in the analysis of drug effects upon the skeleton in embryofetal development studies. Adverse effects on calcification and subtle changes in bone texture may be seen at this histological level.

D. 3 The effects of extracellular matrix components on mouse pre-Sertoli cells in vitro. By S. Mackay, R. A. Smith, S. H. Booth and S. Logan. *Laboratory of Human Anatomy/IBLS, University of Glasgow*

Sertoli cells in vivo, as most other epithelial cells, rest on a basement membrane. Laminin and reconstituted basement membrane preparations have been shown to promote differentiation of postpartum Sertoli cells in culture with the establishment of cord-like structures (Hadley, *J. Cell Biol*. **101**, 1985). The present study set out to investigate whether extracellular matrix components also affected the maintenance in primary culture of pre-Sertoli cells isolated from 13.5–19 dpc embryos. Embryos, taken from pregnant CBA mice killed by CO_2 inhalation, were decapitated so that testes could be excised. The adjacent mesonephroi were removed and pooled testes were dissociated in Hanks Buffer containing 0.25% collagenase and 0.025% trypsin for 20 min, followed by differential centrifugation and cell retrieval, as a modification of a protocol devised by Chapin (Chapin et al. *J. Androl*. **8**, 1987). Pre-Sertoli cells were cultured on uncoated and coated (fibronectin, laminin, and commercial reconstituted basement membrane [RBM]) Thermanox coverslips in supplemented Dulbecco's modified Eagle's medium for 4 d prior to preparation for light and electron microscopy. Fibronectin and laminin promoted cell adhesion together with signs of cellular differentiation: enhanced cytoskeletal organisation, formation of multilayer associations (as opposed to only monolayer cultures), and the development of cytoplasmic appendages in contrast to flattened phenotypes. Sertoli cells cultured on RBM adopted a striking appearance with the generation of cord-like aggregates. The present results demonstrate that extracellular matrix components influence immature Sertoli cell differentiation, similar to the response of adult cells reported by Hadley and colleagues, and that such cultures provide a model system for the investigation of factors involved with gonadal differentiation.

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D. 4 Age-related changes in mouse olfactory epithelium. By Y. Rosli, R. A. Smith and L. J. Breckenridge. *Laboratory of Human Anatomy/IBLS, University of Glasgow*

The olfactory system shows general decline in function with age but there have been very few studies in this area. Reports exist of changes in aged olfactory epithelium which include a reduction in thickness due to receptor neuron loss, intermingling of respiratory and olfactory epithelium and an increase in pigment granules (Naessen, *Acta Otolaryng.* **75**, 1971). This study aims to study in detail, age-related changes in mouse olfactory epithelium. Male CBA mice, aged 6 (n = 12) and 30 mo (n = 9), were deeply anaesthetised with sodium pentobarbitone followed by perfusion through the left ventricle with fixative, and the olfactory epithelium prepared for characterisation by light microscopy, TEM and SEM. Olfactory receptor neurons were identified by the immunocytochemical localisation of olfactory marker protein (OMP) (Margolis *TINS* **8**, 1985), and evidence of apoptosis assessed by the TUNEL method. Atrophic changes with degeneration were only observed in olfactory epithelium of 30-mo-old mice. Microscopic analysis of toluidine blue stained resin sections showed localised degenerated areas. EM observations showed atrophic areas with irregularly shaped nuclei of both olfactory and supporting cells, loss of regular zonal arrangement with an intermingling of cells of the olfactory and respiratory epithelia. Accumulation of irregularly shaped dense bodies was evident throughout the affected areas. The integrity of the demarcation between olfactory and respiratory epithelia was less well preserved in aged animals as evidenced by SEM. Apoptotic cells were evident in superficial layers of discrete areas of the olfactory epithelium. These results give further support to our previous findings (Breckenridge et al. *J. Anat.* **191**: 151, 1997) of substantial but localised degeneration within the aged olfactory epithelium.

D. 5 The effects of endothelial growth factor on cultured human endothelial cell morphology. By R. Jablenska, J. F. Dye, J. L. Donnelly, P. Clark, *L. Leach and J. A. Firth. *Department of Anatomy and Cell Biology, Imperial College School of Medicine at St Mary's and *Department of Human Anatomy, Queen's Medical Centre, University of Nottingham*

Different models of cultured endothelial cells have differing requirements for growth and survival factors. We are studying the behaviour of human placental microvascular endothelial cells (HPMEC) in an effort to develop an in vitro model of capillary permeability. We have been evaluating the effects of endothelial cell growth factor (ECGS), a form of α-fibroblast growth factor, and fetal bovine serum on the proliferation, morphology and phenotype of HPMEC. These cells have a surprisingly low requirement for serum, and ECGS was not obligatory for their growth. We have also been unable to demonstrate effects of ECGS on the expression on endothelial markers. This contrasts with the behaviour of classical cultured endothelial cells (e.g. human umbilical cord endothelial cells), which require both high serum and fibroblast growth factor. However, ECGS had a clear effect on the morphology of HPMEC, promoting the projection of cellular processes and the extension of one cell over another. In this study we wanted to investigate the ultrastructural basis of these differences. HPMEC were grown to confluence on gelatin-coated membrane inserts in FBS-supplemented medium with or without ECGS. Cells were then fixed in half-strength Karnovsky's solution followed by osmium/ferricyanide and processed for transmission electron microscopy. Two types of cell morphology were apparent. The first type was more common and exhibited a smooth, flattened profile, with the presence of extensive lamellar membranous structures in the cytoplasm, whereas the second showed a rounded profile, with abundant microvilli, mitochondria, lysosomes and endoplasmic reticulum. With ECGS treatment, there was a greater extent of cell overlapping, with relatively long, oblique clefts. Also, the rounded cells were less frequent, and they often contained myelin-like figures and lysosomes. Some of these rounded cells showed apoptotic features. These observations suggest that fibroblast growth factor does promote the morphological differentiation of cultured HPMEC, and contributes to their squamous epitheloid behaviour by increasing the extent of cell–cell contacts.

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D. 6 Reorganisation of epithelial cell junctions by scatter factor/hepatocyte growth factor. By M. J. Williams and P. Clark. *Department of Anatomy and Cell Biology, Imperial College School of Medicine at St Mary's, London*

Scatter factor/hepatocyte growth factor (SF/HGF) is a multifunctional polypeptide growth and motility factor. The MDCK epithelial cell line has been used intensively to study the effects of SF/HGF in vitro. Previous studies have shown that the immediate responses are a flattening and spreading of the cells, followed by a breakdown of cell–cell adhesion and increased motility (scattering) after around 6 h. However, the cellular mechanisms of scattering remain unclear. Our laboratory has previously found that the breakdown of cell–cell contacts is modulated by signals from extracellular matrix (ECM) receptors. Scattering occurs when cells are cultured on fibronectin, but not vitronectin or serum, whereas increased spreading occurs on all surfaces tested. We have investigated the SF/HGF-induced changes in the organisation of cell–cell junctions with time, in both fully polarised confluent MDCK monolayers and nonconfluent cultures. The cells, grown on either glass coverslips or permeable filters, were examined using both confocal microscopy and transmission electron microscopy. Changes in cytoskeletal organisation, alterations in the distribution of components of cell–cell contacts, and detailed morphological changes were examined at various times after exposure to SF/HGF. We observed redistribution of E-cadherin, β-catenin, and actin, the loss of desmosomes, and the acquisition of highly irregular interdigitating basolateral cellular protrusions. These, and other changes, appeared to be dependent on the composition of the substratum, and they provide clues about possible crosstalk between information received from soluble signals and from the ECM.

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

D. 7 Stimulation of in vitro mineralisation in mouse fibroblasts. By M. G. McAlinden, *M. T. Gibson, **D. J. Watt and *D. J. Wilson. *Orthopaedic Research Unit, The Queen's University of Belfast, *School of Biomedical Sciences/Anatomy, The Queen's University of Belfast and **Department of Anatomy, Charing Cross and Westminster Medical School, London*

Mesenchymal stem cells are known to differentiate into osteoblasts, myoblasts, fibroblasts and chondroblasts inter alia. Conversion from a mouse dermal fibroblast lineage to a myogenic one has been reported in vivo (Gibson et al. *J. Cell Sci.* **108**, 1995). This project aimed to determine whether fibroblasts could be induced to express an osteoblastic lineage in vitro. Cultured human osteoblasts and mouse dermal fibroblasts were initially tested for alkaline phosphatase activity. For 21 d they were cultured in medium supplemented with organic phosphate, ascorbic acid and insulin. Every 7 d cultures were tested for mineralisation. Osteoblasts exhibited early mineralisation by 7 d and extensive mineralisation by 21 d. In contrast, fibroblasts showed no mineralisation at 7 or 14 d. However by 21 d, limited mineralisation deposits were seen in fibroblast cultures. These results suggest that, under the correct environmental conditions, fibroblasts may be able to redifferentiate to express an osteoblastic phenotype. This has implications for stimulation of fracture healing, particularly in delayed or nonunions.

D. 8 Evaluation of histological changes in the rat humerus in experimental osteoporosis induced with calciferous drugs. By A. Anasiewicz, *A. Gawron, **J. Wysokinska-Miszczuk, *M. Niedzwiedz and *G. Orfin (introduced by M. Benjamin). *Department of Human Anatomy, Medical Academy, *Department of Comparative Anatomy and Anthropology, University Maria Curie-Sklodovska and **Department of Dentistry, Medical Academy, Lublin, Poland*

The purpose of the present investigation was to study the possibility of reducing the destructive influence of hydrocortisone on the structure of the spongy, proximal part of the humerus through the simultaneous administration of calciferous drugs. Seven groups of Wistar strain rats were used (body weight 250–270 g): (1) untreated controls (2) treated controls—physiological saline i.p. 0.5 ml/kg b.w. daily for 8 wk, (3) hydrocortisone /hydrocortisone hemisuccinate-Polfa (Poland)/, i.p. 22.5 mg/kg b.w. twice daily, (4) 10% solution of calcium i.p./10% Calcium-Polfa (Poland)/, 15.0 mg/kg b.w. twice daily for 8 wk and 200 u. vit.A with 100 u. vit.D3 /Vitamin A + D3-Terpol (Poland) daily for 8 wk, (5) Hydrocortisone, calcium, vit.A + D3 identical dose, time and method of administration, (6) Miacalcic/Miacalcic-Sandoz (Switzerland)/, i.p. 5.u./kg b.w. daily for 8 wk, (7) hydrocortisone and Miacalcic identical dose, time and method of administration. The rats were killed on d 56 and the humerus examined histologically. On the basis of these studies, it is clear that hydrocortisone caused considerable damage. Partial atrophy of bone trabeculae, the appearance of empty cavities and an extremely irregular arrangement of the remaining trabeculae were observed. A substantial reduction in marrow volume and thickness of epiphysial cartilage were also noted. The administration of calcium simultaneously with vitamins A + D3 and hydrocortisone resulted in a decrease in the destructive changes in the humerus. The humerus of rats treated with hydrocortisone simultaneously with Miacalcic had a dense system of thin trabeculae and small intratrabecular spaces. In animals treated with Miacalcic, the marrow volume was decreased compared with controls, but increased compared with the hydrocortisone group and the 'hydrocortisone with calcium' group. The thickness of the epiphysial cartilage was similar to that in controls. The results indicate that the destructive effect of hydrocortisone after prolonged administration can be considerably reduced by simultaneous administration of calciferous drugs.

D. 9 Embryotoxic effects of SM-2 administered intraperitoneally in mouse Swiss albino strain. By A. Anasiewicz (introduced by M. Benjamin). *Department of Human Anatomy, Medical Academy, Lublin, Poland*

The new compound SM-2 (9-methyl-2[-3-(4-m-chlorophenyl-1- piperazinylpropyl)]-1,2,3,4-tetrahydro-β-carbolin-1-one) has an action similar to atypical antidepressant drugs and has been synthesised at the Institute of Pharmacology at the Polish Academy of Sciences in Cracow. Pharmacological studies of SM-2 have shown that it has a potent antiserotonin action. The purpose of the current study was to examine the influence of SM-2 on the development of the mouse fetus. The studies were performed in line with the recommendations of the WHO. Pregnant female Swiss albino mice (10–12 animals per group) were treated with 1/50, 1/100, 1/250, 1/500, 1/1000 of DL50 (750 mg/kg b.w.) of SM-2 i.p. on each of d6–12 of gestation. Three groups of mice were used as controls—UC-untreated controls, TCa-treated with physiological saline i.p. in equal volume, and TCb-treated with carboxymethylcellulose i.p. in equal volume. Pregnant females were killed and caesarean sections were performed on d 18 of gestation. Implantation sites were recorded as having live, dead or resorbed fetuses. The evaluation of birth defects of internal organs was carried out according to Wilson's technique with Barrow & Taylor's modifications. The results show that SM-2 had embryotoxic effects at doses 1/50, 1/100, 1/250 of DL50, but no embryotoxic effects at the two lower doses.

D. 10 A problem-based approach to the teaching of anatomy to speech students. By S. McHanwell. *Department of Neurobiology, The Medical School, Newcastle upon Tyne*

In designing anatomy courses for specialist groups it is important to ensure that the content of the course is relevant to the professional needs of the students. In Newcastle, speech students are currently taught all their anatomy in the first year of their 4 y degree programme. Over a number of years the content of the anatomy speech course has been

carefully tailored to the requirements of the students. This has been achieved by designing the course in consultation with staff in the Department of Speech. An important feature of the course is the integration of anatomy with other parts of the course by the use of relevant clinical examples and in the case of phonetics, in particular, by the use of carefully selected exercises in living anatomy practical classes. This integration is now being taken one stage further by the introduction of some problem-based teaching into the first year of the course and extending it to students in the 2nd and 3rd years. The main objectives are to encourage independent student learning and to stimulate further interest in anatomy by demonstrating its relevance to clinical practice. Cases have been selected by prevalence and treatability criteria and have been written in collaboration with Speech Department staff. At present 6 cases have been prepared covering respiratory, voice and articulatory disorders, cerebral and brainstem stroke and Parkinson's disease. Further cases are planned. These cases are being given to mixed groups of students in their 1st to 3rd years of study, the intention of this being to permit more senior students to pass on the benefit of their increasing clinical experience to junior students while at the same time reinforcing their own basic knowledge acquired in the 1st year of their course. These classes are staffed jointly by an anatomist and members of the Speech Department and their effectiveness is presently under evaluation. Strategies for the assessment of students in this component of the course are yet to be decided. MCQs and SAQs are under active consideration.

D. 11 The face of Robert the Bruce. By R. I. Macleod, B. Wohlgemuth, D. Hunter, *B. Hill and **P. Vanezis (introduced by M. H. Kaufman). *Dental Institute, University of Edinburgh, *Newcastle Dental Hospital and **Department of Forensic Medicine, University of Glasgow*

In the absence of a contemporary facial description of Robert the Bruce, we set about applying 2 modern forensic facial reconstruction techniques to produce a facial likeness. The 2 techniques are, firstly, a standard one based on the onlay of terracotta over a cast of the skull, building up the muscles to skin using standardised soft tissue depth measurements, carried out by Brian Hill; secondly, facial reconstruction using computer technology in which Peter Vanezis laser scanned the cast of the skull into a computer and created a mask using mean anthropological measurements. We compared these modern facial reconstruction techniques with the facial interpretation of Robert the Bruce by the sculptor C. Pilkington Jackson ARSA, FRBS, who was responsible for the statue of Bruce at Bannockburn. Pilkington Jackson, (in conjunction with Professor Romanes, Emeritus Professor of Anatomy in Edinburgh University), based his facial sculpture on the cast of the skull using anatomical data. We scanned photographs of the cast of the skull, terracotta reconstruction and the Pilkington Jackson head into a computer and produced transparent masks which were overlaid so that the face of one image was visible through the face of the others. This was carried out for the terracotta head and the Pilkington Jackson head, then for the computer generated head and the terracotta head. All 3 techniques produced a similar result, and therefore what can confidently be described as a likeness of Robert the Bruce.

D. 12 Intra-articular pressure distribution in simulated ankle malunion. By J. Dowdall, P. Felle and *A. Devitt. *Department of Human Anatomy and Physiology, and *Department of Orthopaedic Surgery, University College Dublin*

Osteoarthritis of the ankle is usually secondary to malunion of ankle fractures. It has been postulated that osteoarthritis following malunion is due to increase in the peak contact stress (Brown et al. *J. Orthop. Res.* **6**, 1988) or altered contact area (Ramsey & Hamilton, *J. Bone Joint Surg.* **58**, 1976) within the joint. The objective of this study was to investigate the contact area and peak contact stress within the tibiotalar joint following simulated malunion of the ankle. Eight legs of formalin-fixed cadavers were amputated at approximately one third down the tibia. The proximal parts of the tibia and fibula were set in a circular polymethacrylate cement mould. The leg was installed in a Lloyd 2 materials testing machine. Pressure sensitive film (Fuji Prescale Film) was inserted between the tibia and talus. Specimens were subjected to 600 N axially directed force from above and the film removed. A fracture was then made through the lateral malleolus. This was set using a tubular AO plate and screws. Each joint was first set in lengthened malunion, a 2 mm gap at the fracture line, and the procedure with pressure sensitive film and force of 600 N repeated. Each joint was then fixed in shortened malunion by removing 2 mm segment of bone at the fracture site and fixing. The force study was repeated as above. Peak contact stress was assessed in each case by analysing the colour and comparing with the Fuji colour chart. The area of contact for each case was measured by scanning the pressures sensitive film into computer and measuring the area of pressure using NIH Image. Lastly, the position of the peak contact stress for each case was noted. One of the specimens was found to have osteoarthritis and was excluded from the study. The mean contact area in the normal joint was 2.61 cm^2 ($\pm$0.4), in lengthened malunion was 2.46 cm^2 ($\pm$0.49) and in shortened malunion was 2.21 cm^2 ($\pm$0.55). There was no significant difference between these groups (Mann–Whitney *U* test). The mean peak contact stress for the normal, lengthened and shortened groups were respectively 22.64 kgf/cm^2 ($\pm$2.93), 24.06 kgf/cm^2 ($\pm$3.78) and 24.69 kgf/cm^2 ($\pm$3.97). There was no significant difference between these groups (Mann–Whitney *U* test). The position of peak contact stress changed following malunion. Lengthening in all cases resulted in the position of peak contact stress moving medially, while shortening in 5 of 7 ankles resulted in posterior shift of the position of peak contact stress. This has not been previously reported. Hyaline articular cartilage varies in thickness in different parts of a joint (Williams & Warwick, *Gray's Anatomy*, 1979). We propose that peak stress on an unsuitable area of cartilage following malunion may be an aetiological factor in posttraumatic osteoarthritis of the ankle joint.

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D. 13 Distribution of hormones and neurotransmitters in the pancreas of the Houbara bustard. By E. P. K. Mensah-Brown, *T. A. Bailey, P. A. Lawrence, *J. H. Samour, D. J. Pallot and **A. Garner. *Departments of Anatomy and **Pharmacology, FMHS, U.A.E. University, Al Ain, United Arab Emirates; *Veterinary Research Department, NARC, Abu Dhabi, United Arab Emirates*

The distribution of the hormones insulin (INS), glucagon (GLU), somatostatin (SOM), pancreatic polypeptide (PP), the neurotransmitter 5-hydroxytryptamine (5-HT), and the neuropeptide substance P (SP), in the pancreas of the Houbara bustard (*Chlamydotis undulata mcqueenii*) has been studied by immunohistochemistry. Specimens were collected from 8 captive birds over a period of 12 mo. All the birds had suffered injuries and had to be destroyed. The whole pancreas was fixed in Zamboni's solution, embedded in paraffin wax and 6 mm sections immunostained by the PAP diaminobenzidene method. The number of immunoreactive cells for each hormone was determined by cell counting and expressed as a percentage of the total number of cells in the islets. Distribution and concentration of immunoreactivity in the islets and around the acinar cells was similar regardless of the time of year the pancreas was obtained. INS (69%) and SP (65%) immunoreactive cells were discernible mainly in the central portion of the islets. Consecutive sections revealed that INS and SP colocalised within the same islet cells. SOM (26%), GLU (18%), PP (10%) and 5-HT (8%) cells were detectable at the periphery of the islets. Immunoreactivity to SOM and 5-HT was detectable in peripherally located nerve fibres. SOM, GLU, PP and 5-HT immunoreactive neurons and nerve fibres were discernible within the pancreatic acinar and in the wall of blood vessels but SP was absent at these sites. We conclude that the distribution of hormones and neurotransmitters within the pancreas of the Houbara bustard is comparable with other avian species with the notable exception that somatostatin immunoreactivity exceeded that of glucagon. Colocalisation of substance P and insulin seems not to have been reported previously in birds. The distribution of these hormones and neurotransmitters does not seem to be affected by seasonal changes. It will be interesting to determine whether serum levels are similarly unaffected.

D. 14 Immunohistochemical localisation of neuropeptide-Y and its effects on insulin and glucagon secretion from normal and diabetic pancreatic tissue fragments in rats. By E. Adeghate, A. S. Ponery and D. J. Pallot. *Department of Human Anatomy, Faculty of Medicine and Health Sciences, United Arab Emirates University*

Neuropeptide-Y (NPY) has been identified in the pancreas of many mammalian species. There have been conflicting reports about its role on insulin and glucagon secretion in normal pancreas. No study has so far been performed on the role of this neuropeptide on insulin and glucagon secretion in diabetic pancreas. The present study investigates the effect of NPY on insulin and glucagon secretion from the in vitro rat pancreas of both normal and streptozotocin-diabetic rats. Diabetes mellitus (DM) was induced in rats by a single intraperitoneal (55 mg kg/body wt) injection of streptozotocin (Sigma). Three weeks after the induction of DM, the diabetic pancreata were removed (after chloral hydrate anaesthesia), and processed for immunohistochemistry using antibodies against NPY and radioimmunoassay for insulin and glucagon. Normal pancreata from age-matched nondiabetic rats were used as controls. NPY-immunoreactivity was observed exclusively in neural tissues of the pancreas. Most of the NPY-immunopositive neurons were grouped into ganglia. NPY was also detected in varicose nerve fibres innervating blood vessels. There was no significant difference ($P > 0.05$, Student's t test) in the number and pattern of distribution of NPY-positive neurons between normal and diabetic pancreata. Basal insulin secretion expressed as mean $\pm$S.E.M. was 8.9 ± 0.9 μUI/ml per 100 mg tissue. Stimulation of normal pancreatic tissue segments in vitro with NPY (10^{-6} M–10^{-12} M) resulted in increased insulin secretion (10^{-12} M NPY: 27.4 μUI/ml per 100 mg tissue). There was inhibition of insulin secretion when diabetic pancreatic tissue fragments were incubated with different concentrations of NPY (10^{-12} M NPY: 6.5 ± 2.2 μUI/ml per 100 mg tissue). Basal and NPY (10^{-12} M)-evoked glucagon secretion were 11.8 ± 4.2 and 18.4 ± 2.1 pmol/ml per 100 mg tissue respectively. A higher increase (139.1 ± 30.0 pmol/ml per 100 mg tissue) in glucagon secretion was observed when diabetic pancreatic tissue fragments were treated with NPY. In conclusion, NPY stimulates glucagon secretion from both normal and diabetic pancreatic tissue fragments in vitro. NPY has stimulatory effect on insulin secretion from normal but an inhibitory effect from diabetic pancreas. These observations show that the signal transduction mechanism in the streptozotocin-diabetic pancreas may be impaired.

D. 15 Effect of *Mormordica charantia* fruit juice on islet morphology in the pancreas of streptozotocin-diabetic rat. By I. Ahmed, E. A. Adeghate, A. K. Sharma, D. J. Pallot and *J. Singh. *Department of Human Anatomy, Faculty of Medicine and Health Sciences, United Arab Emirates University, Al Ain, United Arab Emirates and *Department of Applied Biology, University of Central Lancashire, Preston*

The *Mormordica charantia* fruit juice has been shown to have hypoglycaemic effects and ameliorate the symptoms of diabetes mellitus (Karunanayake et al. *J. Ethnopharmacol.* **11**, 1984). The purpose of this study is to investigate the effect of *M. charantia* juice on the distribution and number of pancreatic alpha, beta and delta cells in the streptozotocin (STZ)-induced diabetic rats by immunohistochemical methods. Normal and untreated diabetic animals served as controls. Diabetes mellitus (DM) was induced in rats by a single intraperitoneal (60 mg kg per body wt) injection of streptozotocin (Sigma). Ten weeks after the induction of DM, the diabetic pancreata were removed (after chloral hydrate anaesthesia), and processed for immunohistochemistry using antibodies against insulin, glucagon and somatostatin. The results indicated that there was a significant increase ($P < 0.005$) in the number of beta cells in *M. charantia*-treated animals (50.2% of total cell number in the islets of Langerhans) when compared with untreated diabetics (27.0% of total cell number in the islets of

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Langerhans). However their number was still significantly less than that obtained for the normal rats (60.0% of total cell number in the islets of Langerhans). On the other hand, there was a significant ($P < 0.001$; Student's t test) increase in the number of alpha cells in STZ–diabetic rats (52.2%) compared to normal (30.1%) which was not influenced by *M. charantia* in the treated group (51.6%). The number of somatostatin-producing delta cells also increased significantly ($P < 0.001$) in STZ-diabetic rats (26%) compared to normal (6.4%). Like the case for glucagon-producing alpha cells, this increase in cell number was not reduced by *M. charantia* treatment (20.8%). Our results suggest that oral feeding of *M. charantia* fruit juice may have a role in the regeneration of insulin-producing beta but has no effect on number and distribution of alpha and delta cells in STZ–diabetic rats.

D. 16 Short term hypoxia induces changes in JNk, c-JUN and D_2 receptors in the rat carotid body. By I. Ahmed, *A. Nur, E. Kamal, D. J. Pallot and *M. S. Lakhani. *Departments of Human Anatomy and *Biochemistry, Faculty of Medicine and Health Sciences, UAE University, Al-Ain, United Arab Emirates*

Hypoxia has been shown to increase the size of the carotid body by unknown processes involving hypertrophy and hyperplasia. In addition hypoxic exposure induces increased mitotic activity in the type I cells of the carotid body within 24 h and this increased activity reaches a peak at 72 h. There is also evidence that the chronically hypoxic carotid body is less sensitive to changes in PaO_2 and this depressed sensitivity may be due to increased activity of the dopamine receptors within the carotid body. We have investigated the relative amounts of the 2 cell division regulators JNk and c-JUN using immunoblot techniques in normal animals and in animals exposed to 10% O_2 for a period of 48 h. In addition the amount of D_2 receptor protein within the same 2 groups of animals was also studied. JNk occurs as 2 species with molecular weights corresponding to 45 and 55 kDa. The amount of the 45 kDa species was greatly increased in the carotid bodies of hypoxic as compared with normal animals. However in the case of the 55 kDa species, hypoxia failed to induce any detectable change. C-JUN was greatly increased in animals exposed to 10% O_2 for 48 h. The amount of D_2 receptor was increased by hypoxic exposure. The relative increase in the amount of JNk and c-JUN provide a possible mechanism for the stimulation of cell division and suggest that hypoxia might be involved in stimulating cell division in the hypoxic carotid body by inducing JNk and c-JUN gene expression. Previous studies have indicated that dopaminergic blockade restores the depressed hypoxic sensitivity of chronically hypoxic animals. Our finding of an increased amount of receptor protein provides a mechanism for this previous observation.

D. 17 $GABA_A$ and NMDA-R1 receptor mRNA in the dendrites of magnocellular neurosecretory neurons of rat. By D. Ma and J. F. Morris. *Department of Human Anatomy, University of Oxford*

There is growing evidence for local protein synthesis in the dendrites of neurons. They may contribute to local regulation of synaptic activities, by synthesis of neurotransmitter receptors near synapses. Magnocellular neurosecretory neurons, which are oxytocin- and vasopressin-secreting neurons in the supraoptic nucleus (SON) and paraventricular nucleus (PVN) of the hypothalamus, have GABAergic and glutamatergic innervation as their main inhibitory and excitatory controls. We have previously demonstrated that their dendrites contain protein synthetic machinery (polyribosomes, rough ER), some of which are located near synaptic sites. Poly(A) mRNA, rRNA and tRNA were found in similar location. In the present study we visualised $GABA_A$ alpha 1 and NMDA-R1 receptor mRNAs in the magnocellular dendrites of Long–Evans rats, by nonradioactive in situ hybridisation using oligonucleotide probes. Usually it is difficult to detect mRNAs in dendrites by standard visualisation methods at the light microscopic level due to the low amount of mRNA, and the radioactive method gives poor resolution, although it is very sensitive. We have therefore used a new method involving signal amplification, based on catalysed reporter deposition to intensify the visualisation. Positive labelling of these mRNAs was found in the magnocellular neurons of both SON and PVN. They were located in cell body cytoplasm, with denser labelling in the peripheral part of cell body where the RER is abundant. Both $GABA_A$ and NMDA-R1 receptor mRNA could also be detected in the dendrites of the magnocellular neurons, mainly in proximal and middle parts and occasionally in distal parts. The presence of mRNAs for $GABA_A$ and NMDA-R1 receptors in the magnocellular dendrites strongly suggests that local protein synthesis of these receptor proteins can occur in the dendrites and may thereby contribute to their synaptic control.

D. 18 Glial cell derived neurotrophic factor as a branch-promoting epithelial morphogen—evidence from urinary collecting ducts developing in culture. By K. Sainioi, P. Swantoi, M. Saarmai, U. Arumae, X. Meng, M. Lindahl, *J. Davies and H. Sariolai (introduced by M. H. Kaufman). *Biocentre IA, University of Helsinki, Finland and *Department of Anatomy, University of Edinburgh*

GDNF (glial cell line derived neurotrophic factor) is a growth factor of the TGF-J family. It was first identified as a protein important to the survival of certain types of neuron in culture and, while it has been studied most intensively in relation to the nervous system, several lines of evidence have recently converged to suggest that GDNF also has important developmental functions elsewhere. There are particularly good reasons for believing that GDNF is of great importance to renal development; (1) GDNF mRNA is expressed by the mesenchyme surrounding the developing urinary collecting duct system in fetal kidneys, (2) the tyrosine kinase receptor for GDNF, c-Ret, is expressed by developing collecting duct epithelia, and (3) GDNF −/− knockout mice and c-Ret −/− knockout mice show severe defects in kidney development, most probably arising from failure of collecting duct devel-

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opment. It has already been established that the controls of collecting duct growth and branching morphogenesis are to some extent separable (Davies et al. *Development* **121**, 1995). We have therefore adapted existing culture systems to determine which of these facets of collecting duct development, if any, are controlled by GDNF. We find that GDNF is a potent initiator of collecting duct branching, which at high concentration can even promote the formation of supernumerary ureteric buds from the wolffian duct.

D. 19 The effect of graft length in the repair of nerves with nerve and muscle autografts in the New Zealand white rabbit. By S. Drew, D. V. Lenihan and M. A. Glasby (introduced by M. H. Kaufman). *Department of Anatomy*, *University of Edinburgh*

The repair of nerves where a length defect has been created by injuryq remains the largest problem in this field of surgery. Neither the absolute useful length of grafts nor their mechanism of failure is currently understood. The present experiments were a preliminary attempt to record the electrophysiological and morphological differences between grafts of a length known to be successful and grafts of a greater length which do not support useful regeneration and recovery of function. Long (8 cm) and short (3 cm) nerve and muscle autografts were used to repair gaps of similar length in the common peroneal component (CPN) of the sciatic nerve in rabbits. Anaesthesia was maintained with 2% Halothane. Experimental groups as models of nerve injury and repair as a primary procedure were: (l) CONTROL: no treatment; (2) CRUSH: the CPN was crushed but not divided; (3) NERVE–NERVE SUTURE: the CPN was divided and repaired by direct epineurial suture; (4) SHORT NERVE GRAFT: the CPN was divided and repaired with a 3 cm nerve graft; (5) LONG NERVE GRAFT: the CPN was and repaired with an 8 cm nerve graft; (6) SHORT MUSCLE GRAFT 1: the CPN was divided in the upper thigh and repaired with a 3 cm freeze-thawed muscle graft; (7) SHORT MUSCLE GRAFT 2: the CPN was divided in the upper thigh and 3×1 cm freeze-thawed muscle grafts sutured between the proximal distal stumps; this meant that there were 4 suture lines in this group as opposed to 2 suture lines in the previous group; (8) LONG MUSCLE GRAFT: the CPN was divided in the upper thigh and repaired with an 8 cm freeze-thawed muscle graft. Eight months later recovery was assessed by measuring nerve conduction velocity, EMG, isometric twitch tension in extensor digitorum longus, nerve blood flow in the regenerated nerve using a laser Doppler blood flow monitor and population distributions of nerve fibre diameter, axon diameter and myelin sheath thickness. Where short (3 cm) grafts were used, there was no discernible difference in outcome between repair using nerve grafts and repair using muscle grafts. Where long (8 cm) grafts were used, the results were all significantly poorer but the nerve grafts performed better than the long muscle grafts. If the 3 cm grafts were fashioned from 3 l cm pieces of nerve or muscle sutured together instead of consisting of a single piece, the results were considerably worse. This confirms the view that the outcome of nerve repair worsens where multiple suture lines are used.

D. 20 The in vivo effects of insulin like growth factor 1 on the development of oligodendrocytes in the anterior medullary velum of the rat. By D. R. Goddard, *M. Berry and A. M. Butt. *Departments of Physiology and *Anatomy*, *UMDS*

Oligodendrocytes are the myelinating cells of the central nervous system. Insulin-like growth factor I (IGF-1) is known to have many trophic effects throughout the body including the central nervous system. In vitro studies have shown that IGF-1 stimulates oligodendrocyte differentiation and expression of myelin-related proteins by up to 6-fold compared with controls (McMorris et al. *PNAS*, **83**, 1986). In vivo, cell death in oligodendrocytes following optic nerve transection in rat pups is reduced by 70% by injecting COS cells transfected with IGF-1 (Barres et al. *Current Biol*. **3**, 1993). Furthermore, axonal myelination is advanced during development in transgenic mice which overexpress IGF-1 (Ye et al. *J. Neurosci*. **15**, 1995). In the present study we have investigated the effects of intrathecally injected IGF-1 on oligodendrocyte development and myelinogensis in the rat anterior medullary velum (AMV) which forms part of the roof of the 4th ventricle. Human recombinant IGF-1 was administered via the lateral ventricle in a volume of 10 ml to give a final concentration of 500 ng/ml of cerebrospinal fluid; controls received 10 ml of sterile saline vehicle. Rats were injected twice daily at both postnatal day (P) 6 and P7, and AMV were analysed on P8 using immunohistochemical labeling and Western blotting with Rip, a mouse monoclonal antibody which is specific for oligodendrocytes and their associated myelin sheaths. For immunohistochemistry, rats were perfused via the heart with 4% paraformaldehyde under terminal anaesthesia. In parallel experiments, injection of horseradish peroxidase (HRP) into the lateral ventricles showed HRP within axons and glia in the AMV 10 min after administration, demonstrating that agents injected into the lateral ventricle have rapid and direct access to the AMV. In this study, we have focused on the rostral area of the AMV in which myelination commences between P6 and P8 and which is populated mainly by immature oligodendrocytes in control rats. Qualitative examination of Rip immunolabelled whole-mount AMV indicated that the AMV contained a greater number of both oligodendrocytes and myelinated fibres following IGF-1 treatment. Quantitative analyses of a 200 mm^2 area in the rostral regions of Rip immunolabelled AMV ($n = 4$ for controls, $n = 5$ for IGF-1, all data expressed as means $\pm$ standard error of the mean) confirmed that in IGF-1 treated rats there were more immature oligodendrocytes (IGF-1 = 17 ± 3, control = 3 ± 1), mature oligodendrocytes (IGF-1 = 18 ± 6, control = 6 ± 1), and myelin sheaths (IGF-1 = 15 ± 3, control = 10 ± 1). Western blotting analysis demonstrated that Rip expression in total AMV was approximately doubled (optical density = 2147 for IGF-1 and 1162 for controls, $n = 5$ in both cases). These results show that IGF-1 promotes oligodendrocyte maturation and myelinogensis in the rat AMV, supporting a role for this cytokine in the control of oligodendrocyte differentiation in vivo.

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D. 21 Combining immunohistochemistry and enzyme histochemistry to observe the effects of age on the peptidergic innervation of the rat hypogastric ganglion. By A. L. Warburton and R. M. Santer. *School of Molecular and Medical Biosciences* (*Anatomy Unit*), *University of Wales Cardiff*

Previous work has demonstrated that a selective, age-related decrease occurs in the postganglionic sympathetic nervous supply to the urinary tract: the parasympathetic nervous supply, however, remains relatively unaffected (Warburton & Santer, *J. Autonom. Nerv. Sys.* **45**, 1993). In the male rat a unique anatomical situation exists whereby both the sympathetic and parasympathetic postganglionic neurons supplying the lower urinary tract arise from a single (hypogastric) ganglion (HG)—the 2 populations identifiable by means of tyrosine hydroxylase (TH) and NADPH-diaphorase staining respectively. Identification of the different neuronal types has formerly only been possible on consecutive sections. Here we report a method that we have developed to demonstrate intraganglionic neuropeptides and simultaneously identify both the sympathetic and parasympathetic neurons on a single section. This method employs dual-labelling immunohistochemistry in combination with NADPH-diaphorase enzyme histochemistry. Using this methodology we have investigated the peptidergic innervation of the HG of both young adult and aged rats to assess the affects of age. Pairs of young adult (4 mo) and aged (> 24 mo) male Wistar rats were killed by overdose of ether and the HG ganglia removed into 4% paraformaldehyde. Cryosections were then stained immunohistochemically with a mixture of antibodies to TH and either met-enkephalin (ENK), calcitonin gene-related peptide (CGRP), substance P (SP) or somatostatin (SOM), followed by incubation for the detection of NADPH-diaphorase activity. Sections were observed using a Leitz fluorescence microscope and a Molecular Dynamics Sarastro 2000 confocal microscope. Neuropeptidergic fibres were observed either traversing the ganglion or forming perineuronal baskets around both sympathetic and parasympathetic postganglionic cell bodies. Such an uneven distribution of immunostained neuropeptidergic fibres within the HG, made even semiquantitation difficult. However we could not detect any differences in the density of fibres or in their distribution in the HG between the 2 ages. The CGRP, SP and SOM fibres are considered to be mainly sensory and these results suggest that the sensory innervation of pelvic viscera is age-defiant. ENK immunoreactivity in the HG is likely to be colocalised in the preganglionic input, and as earlier work has shown (Dering et al. *J. Neurocytol.* **25**, 1996), a considerable decrease in preganglionic input to the HG in aged rats occurs. Thus, the maintenance of ENK immunoreactivity in the HG of aged rats may be due to sprouting of the remaining terminals to retain the status of the preganglionic input.

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D. 22 Protease activities in the brain, spinal cord and muscle tissues of the mutant mouse wobbler. By S. McHanwell, G. Falkous, M. M. Simmons and D. Mantle. *Department of Neurobiology*, *The Medical School*, *Newcastle upon Tyne*

The wobbler mutant mouse is an autosomal recessive mutation characterised by vacuolar degeneration and loss of motor neurons mainly in the cervical spinal cord and brainstem (Pollin et al. *J. Neurocytol.* **19**, 1990). Recent work from this laboratory has suggested a possible role for nitric oxide synthase in motor neuron death in this mutant (Clowry & McHanwell, *Neurosci. Lett.* **215**, 1996). The purpose of this study was to determine whether there may also be an abnormality of intracellular protein processing by undertaking a systematic investigation of the activities of a representative range of proteolytic enzymes in the cytoplasmic and lysosomal pathways. Tissue was taken from the brain, cervical and lumbar spinal cords and muscles of the forelimb and hindlimb. Enzyme activities were examined in mice of 3–5 wk of age before the main period of motoneuron death and in mice older than 3 mo when motoneuron death is largely complete. Unaffected littermates were used as controls. In wobbler mice older than 3 mo, of the various proteases examined, only the activities of the cytoplasmic enzymes pyroglutamyl aminopeptidase and proline endopeptidase were altered. The activities of these 2 enzymes were increased by 88 and 49% respectively. This difference was not seen in the younger animals. The activity of a number of lysosomal proteases was increased in forelimb and not hindlimb muscles in the mutant mice in a manner similar to that observed in denervation atrophy. The results of this study show that there are selective increases in cytoplasmic but lysosomal proteases in the spinal cords of wobbler mice. The changes observed in this wobbler mutant are similar to those observed in human motoneuron disease cases. The selective increase observed in the activity of the 2 cytoplasmic proteases in the spinal cord may be an adaptive response to motoneuron death as the consequence of an increased processing of thyrotropin releasing hormone.