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The Summer Meeting of the Anatomical Society of Great Britain and Ireland was held at University College Dublin, from 10 to 12 July 2001. It included a symposium on 'The respiratory system'. The following are abstracts of communications and posters presented at the meeting.

TALKS

1

Microscopic analysis of experimentally induced tympanosclerosis in the rat

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Otitis media is a common problem in children with approximately 5–10% of cases progressing to chronic otitis media with effusion (OME). Tympanosclerosis, a pathological condition affecting the middle ear and tympanic membrane, is a frequent sequel to persistent OME and/or its treatment. The underlying mechanisms of this disease are still unknown. The aim of this study was to examine the development and progression of tympanosclerosis in the pars tensa of rats using a new model for persistent OME (Russell & Giles, *Laryngoscope* **108**,1998).

56 male specific pathogen free CD Wistar rats underwent unilateral eustachian tube obstruction under general anaesthesia. The contralateral ear served as a control. Only rats with sterile effusions were studied. The pars tensa from rats with effusions ranging from 1 to 12 month was examined by light and electron microscopy.

At 1 month post obstruction focal thickenings on the mucosal side of the membrane were seen. Ultrastructural analysis revealed that a submucosal layer invested with fibroblasts, blood vessels and mucus secreting cells had developed. At 3–6 month calcium plaques were seen in the submucosal, radiate and circular fibrous layers. At 9–12 month calcium plaques were seen throughout the lamina propria and the normal arrangement of the latter structure into circular and fibrous layers was lost. Atrophy was not found in any of the specimens examined.

Tympanosclerosis is a progressive disorder which appears to be the main response of the rat pars tensa to prolonged sterile (OME). The severity of the disease was directly proportional to the duration of the otitis media. In conclusion this model would seem to be excellent for the study of tympanosclerosis.

2

Ethanol induced malformations of the developing nasal capsule and mandibular arch of Gallus

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The frequency of birth defects stemming from alcohol exposure is placed at between 1 and 2 live births per 1000 with the frequency of partial expression at 3–5 live births per 1000. Its abuse during pregnancy result in a wide variety of malformations including craniofacial anomalies and CNS dysfunction. This unique constellation of anomalies is termed FAS (Fetal Alcohol Syndrome).

In this study the fowl embryo was used as an animal model to investigate the FAS and evaluate the teratogenic abnormalities resulting from its exposure to ethyl alcohol in ovo. 120 eggs from a nutritionally and genetically uniform pedigree breeding flock were selected and the air chamber was injected with 0.1 mL of a 40% solution of ethanol in sterile distilled water. A minimum of 5 embryos were removed at 24 h intervals after injection. The embryos were evaluated for gross abnormalities, body mass, total length and developmental stage. Comparisons of these values were used for selection of embryos for microscopical investigations. These embryos were sagittally or transversely sectioned at 10 μm and graphic reconstructions were made.

The visceral arches display various discrepancies in the development and most of these indicate a slower rate of development and chondrification in the various structures. The most conspicuous structural difference is seen in the mandibular arch where Meckel's cartilages are severely affected by dramatic dorsoventral as well as lateral undulations along its course which results in the overall anteroposterior shortening of this structure. In a 17 d embryo Meckel's cartilage bears a third posteriorly directed process situated between processes retroarticularis and angularis on its left side.

The anterior region of the nasal capsules seems to be more severely affected by the delayed growth and degree of chondrification when compared to the posterior region. The planum antorbitale and the sidewall of the posterior region of the nasal capsule are also severely affected This manifested in the lack of continuity between these elements and the roof of the parietotectal in this region. The base of the orbital and nasal septa display a characteristic curve and terminates in the prenasal process situated on the right lateral side of the embryo.

The administration of ethanol results in a definite retardation in the development and chondrification growth in the anterior region of the chondrocranium and visceral arches with a consistant undulation in Meckel's cartilage.

3

Junctional phenotype of human vessels during vasculogenesis – a study of the first trimester placenta

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Vasculogenesis and angiogenesis are regulated by the capacity of endothelial cells to adhere to each other and assemble into new vascular

structures. The presence and role of adhesive receptors, specifically junctional adhesion molecules, during physiological vasculogenesis remain unknown. We have used a combined utrastructural and immunocytochemical approach to establish the molecular phenotype of intercellular junctions in the developing vessels of normal first trimester human placenta (n = 10) and compared this with vessels in the full term placenta (n = 5). Our study is the first to show that the interendothelial junctions of newly formed human placental vessels possess vascular endothelial cadherin, α- and β-catenin and zonula occludens-1 but lack plakoglobin and the tight junctional transmembrane molecules occludin, claudin-1 and claudin-2. This profile is similar to that found in terminal capillaries of the full term placenta. The latter are known to be highly angiogenic and involved in maternofetal exchange of nutrients. Fully formed central vessels of the early placenta do possess occludin but not plakoglobin or claudin-1 which are constituents of junctions in large vessels of full term placenta. The angiogenic growth factor VEGF (and its KDR receptor) was localised to the trophoblast and endothelium of the first trimester placenta and to a lesser extent in the terminal villi at term, whilst angiopoietin-2 appeared to be the predominant growth factor in the last trimester. Endothelial junctions in the human placenta appear to possess different molecular phenotypes, stable or dynamic, depending on the maturity, function and plasticity of vessels and regulated by temporal and site-specific expression of angiogenic factors.

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4

Scanning oblique illumination in scanning electron microscopy and light microscopy

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Fast-electron SEM images carry directional information. They may be enhanced by combining separate recordings made with multiple directional detectors which face the sample obliquely. Firstly, playing back image series in sequence simulates dynamic motion of illumination sources. Secondly, spectral colour encoding enhances the static image. These 2 approaches can be combined.

Each SEM field of view is documented with at least 4 scans with separate detectors for back scattered (BSE) or forward scattered electrons. The number of images in a cycle can be increased by interpolation. Sequential playback continuously sweeps the apparent angle of illumination and increases 3 dimensional (3D) interpretation.

By using spectral colour coding of the apparent direction of illumination, wider sectors of the total data set are used simultaneously and thus enhance the information content of still images. The combined images code surface slope via brightness and direction via hue. Surfaces normal to the electron beam appear grey.

Most anatomical SEM research samples are dry low density insulators and are coated with a heavy metal which returns the high spatial resolution component of the BSE signal. Thus these new SEM methods work with all typical samples having topographic relief.

In transmitted light microscopy direct-view 3D images can be obtained from conventional single objective systems by controlling off-axis aperturing of the illumination. We now study the use of temporal display sequences of images obtained with oblique ray bundles. Changing the direction of incidence of an oblique illuminating cone such that it spins whilst tilted with respect to the mean optic axis of the microscope system creates the illusion of the sample tilting continuously and of the successive layers within the imaged volume moving past each other. The resulting advantages of using motion, rather than stereoscopic, parallax can be seen directly at the microscope and on a video display screen. Using, for example, PowerPoint, single rotation clips played back continuously now provide the means for demonstrating 3D aspects of the information content of the titular 'thin' sections used in all histology.

Supported by the horserace betting levy board.

5

Microcrack growth in compact bone

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Microdamage in bone in the form of microcracks contributes to the loss of bone quality in osteoporosis and is thought to play a major role in the foprmation of both fragility and stress fractures. Despite previous studies the behaviour of microcracks in compact bone remains poorly understood. Using a novel sequential labelling technique this study seeks to look at how microcracks interact with the bone's microstructure and the process by which they initiate, propagate and eventually cause failure in bone.

A series of fluorescent stains have been identified which are as effective as the standard method – basic fuchsin – in detecting microcracks but which are site specific as they chelate to calcium ions lining crack walls. These agents were applied in sequence to label microcracks formed at different intervals during a mechanical fatigue test. Specimens were taken from fresh bovine tibiae, and machined into typical waisted 'dog-bone' type test specimens of circular cross section. 10 specimens were fatigue tested in cyclic compression at a stress range of 80 MPa. The specimens were initially stained with alizarin, which had the greatest affinity for calcium, to label pre-existing damage. They were then fatigue tested in a second agent, xylenol orange, for the first 10 000 cycles. This agent was then replaced with calcein to 50 000 cycles and finally calcein blue between 50 000 cycles and failure. The specimens were then sliced in 2, the upper and lower blocks randomly assigned for either transverse or longitudinal sectioning and labelled microcracks identified using UV epifluorescence microscopy.

Microcracks were found to accumulate early in a specimen's life and the rate of accumulation then plateaued followed by an increased rate of accumulation in the period prior to failure. Bone microstructure greatly influenced microcrack growth with the vast majority of microcracks being found in interstitial bone between Haversian systems. Microcracks grew in length primarily in the longitudinal direction, parallel to Haversian systems and the longitudinal axis of the bone. These results support the concept of a microstructural barrier effect in bone and a decreasing microcrack growth rate with increasing microcrack length. The method has the potential to become a useful tool in fracture mechanics.

6

Radiological anatomical evaluation: the influence of X-ray beam filtration

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The use of visualisation grading of specific anatomical structures, as reproduced in a radiographic image, was recommended by the Commission of European Communities in 1996 (CEC, 1996). This method of evaluating radiographic image quality formed an integral part of this study which investigated the effect of three X-ray beam filters on radiation dose and radiographic image quality. A range of commonly performed X-ray examinations were included as follows: lumbar spine, pelvis, abdomen and chest.

An anthropomorphic phantom study and a systematic patient survey were undertaken. A standard level of X-ray beam filtration was employed for the control cohort of patients and 2 groups of patients were imaged with either of 2 experimental filters. Ethics Committee approval was obtained. The image evaluation criteria were divided into 2 sections. Firstly the radiographic images were assessed for good radiographic technique to reduce bias during the visual grading of the anatomical structures. Secondly a panel of 3 experts assessed all images. One of the experimental X-ray filters recorded the highest evaluation scores for 6 out of 8 radiographic projections, whilst also recording statistically significant reductions in patient radiation dose of 34%–50%. No significant differences in radiation dose between the experimental groups were shown. Consistently lower image quality scores were however, recorded for the second experimental filter compared to the control group.

This study demonstrated the application of X-ray beam filters capable of reducing patient radiation dose whilst maintaining image quality. Recognised test objects routinely used to measure radiographic image contrast and spatial resolution were also exposed using the 3 X-ray beam filters and findings showed limited correlation with the anthropomorphic and patient study results. Trends in image quality scores also varied between the anthropomorphic and patient studies for chest examinations.

The authors recommend the use of the CEC (1996) visual grading criteria as a necessary method of evaluating image quality between different imaging techniques. Further research is needed to determine the suitability of use of phantom objects during radiographic image quality evaluation.

7

Potential for communication between the superficial and deep cervical spaces in the neck

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In the course of anatomical dissection of cadavers we observed a tendency for methylene blue dye injected just below the investing fascia of the neck to appear in the deep cervical space, deep to the prevertebral fascia. Traditionally it is considered that the deep and superficial cervical spaces do not communicate (Bowden, *Proc. Roy. Soc. Medical* **59**, 1966) and the prevertebral fascia is thought to be impenetrable by infection, tumour or local anaesthetic (Granite & Wilmington *J. Oral Surg*. **34**, 1976; Lindner, *Annals Surg*. **204**, 1986; Guntamukkala & Hardy, *Brit. J. Anaes*. **66**, 1991). We therefore proceeded to investigate this finding more formally.

Six preserved human cadavers were used (3 male, 3 female; age approximately 60–75 y at death). Injections were made using a standard 23G needle and 30 mL syringe. In 2 cadavers (called cadavers 1 and 2) we performed an injection of 30 mL 0.01% methylene blue dye into the intact neck, just deep to the investing fascia. The fascia was identified by appropriate feel of the needle (as if performing a superficial cervical plexus block in clinical practice). In a further 2 cadavers (cadavers 3 and 4) we first dissected and reflected away the skin to expose the layer of investing fascia. We then injected 30 mL of 0.01% methylene blue dye just deep to this now visible investing fascia. In all these 4 cadavers we then performed careful neck dissections to ascertain the extent of the spread of dye. Finally we performed 2 'control' injections. In cadaver 5 we performed an injection of 30 mL dye very superficially into skin only (subcutaneous injection). In cadaver 6 we performed an injection deep to the prevertebral fascia at the level of the vertebral body (as if performing a deep cervical plexus block in clinical practice).

In all 4 cadavers 1–4 dye was found in the superficial space just below the investing fascia and also deep to the prevertebral fascia, coating the scalene muscles and also the phrenic nerve. In cadavers 1 and 3 dye was also found to be tracking down to the axillary sheath, suggesting communication between the superficial cervical space and the brachial plexus. In control cadaver 5 dye remained within the skin where it was injected and did not spread further. In control cadaver 6 dye remained confined to the deep cervical space and there was no retrograde spread to the superficial space beneath the investing fascia.

With this large volume of injectate, we have demonstrated the potential for communication between the superficial and deep spaces of the neck. While this result might explain why it is impossible to distinguish the efficacy of the superficial and deep cervical plexus blocks in anaesthetic practice (Pandit et al. *Anesthesia Analgesia* **91**, 2000), it is unclear why the result differs from observations on the behaviour of infections and tumours in the neck (Granite & Wilmington *J. Oral Surg*. **34**, 19xx; Lindner, *Annals Surg*. **204**, 1986). One possibility is that the large volume of injectate in the normal neck opens up channels or pores in the prevertebral fascia, these channels being closed by the abnormal inflammatory processes associated with disease. Microscopic spread of tumour along the perineurium from superficial to the deep roots of the cervical nerves has been demonstrated, suggesting that routes may exist between the 2 spaces of the neck (Esclamado & Carroll, *Arch. Otolaryngol. Head Neck Surg*. **118**, 1992). It is also possible that fixation of the cadavers has produced artefacts though this is relatively unlikely in view of the results of our control injections.

8

Comparative morphology of the gas exchangers

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Gas exchangers provide molecular oxygen, a necessary resource for development and survival. The respiratory medium utilised,

phylogenetic levels of development attained, habitat occupied and lifestyles pursued are the main determinants of the various morphologies of the gas exchangers. The remarkable differences in the physicochemical properties of water and air have prescribed 2 primary groups of rather discordant gas exchangers, respectively, gills and lungs. Water is a more viscous medium and is hence more costly to breathe, contains less oxygen per unit volume and the diffusivity of oxygen is lower. The gills develop by evagination and the lungs by invagination. The highly specialised transitional (bimodal) breathers extract oxygen from water and air. Amidst the remarkable morphological differences, prescriptively, gas exchangers have certain common features. These include large surface area, thin partitioning between the respiratory media and high degree of vascularisation. The refinements that set the respiratory efficiency of gas exchangers are achieved through permutative processes that entail certain trade-offs and compromises. The structure of the respiratory organs of a wide range of invertebrate and vertebrate animals, some living in water and air, some displaying different metabolic capacities and some occupying different environments will be presented to illustrate the stratagems and exceptional morphological adaptations that animals have adopted to acquire molecular oxygen.

9

Molecular biology of lung development

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The emergence of proximal and distal cell phenotypes during lung branching morphogenesis results from a highly coordinated cross talk between epithelial and mesenchymal cells. These interactions are mediated by signalling molecules differentially expressed at specific sites and times in the developing lung. Local gene networks are activated that in turn control cellular activities such as proliferation, migration and differentiation. We investigated the role of retinoic acid (RA) and fibroblast growth factor (FGF) signalling in these processes. Using models that reproduce budding and branching morphogenesis *in vitro*, we show that activation of FGF signalling by mesenchymal FGF-10 plays a role on induction and guidance of lung epithelial buds. In turn, RA signalling activation opposes the FGF effects during branching. By assessing sites of RA synthesis, utilisation and metabolism during lung morphogenesis we show that at the onset of lung development RA signalling is ubiquitously activated in primary buds. Airway branching, however, appears to require down regulation of RA pathways by decreased synthesis, increased RA degradation in the epithelium and inhibition of RA signalling in the mesenchyme. These mechanisms controlling local RA signalling may be critical for normal branching since we show that manipulating RA levels in organ culture, to maintain RA signalling activated as in the initial stage, leads to an immature lung phenotype characterised by failure to form typical distal buds. We show that this phenotype likely results from RA interfering with the establishment of a distal signalling center, altering levels and distribution of FGF-10, sonic hedgehog (Shh) and bone morphogenetic protein BMP-4, genes whose expression is required for distal lung formation.

10

Novel putative G protein-coupled receptors cloned from lung

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In order to identify potential therapeutic targets and markers of disease progression in primary pulmonary hypertension, differential gene expression between primary pulmonary hypertensive and control lung peripheral tissue was examined by suppression subtractive hybridisation. A novel clone was identified in a subtracted primary pulmonary hypertensive cDNA library which had sequence identity to a chromosome 9 clone (AC006241) located in the teleomeric region at 9q34.2–3. The gene spans 86.4 kb and contains 18 exons. Exon prediction programs were used to generate a tentative transcript sequence and PCR primers designed to the 5′- and 3′ ends were used to amplify a full-length cDNA from a lung library. The 2690 bp cDNA (GenBank AF376725) encodes a 552 residue ORF with a theoretical mass of 62 kDa and an isoelectric point 6.72. The protein is predicted to have 7 transmembrane domains and has been named LUng Seven Transmembrane Receptor-1 (LUSTR1) since it is the first of 4 related genes which are widely dispersed in the genome. The genes 2–4 are located on 19p13.3 (18 exons), 15q14–15 (20 exons) and chromosome 2 (not yet defined), respectively. We also have cloned the mouse LUSTR2 cDNA of 1836 bp (GenBank AF376726) (18 exons) which encodes a 562 residue ORF with a theoretical mass of 63.3 kDa and an isoelectric point 7.26. They have 49% identity and 78% similarity. The NT of the proteins have predicted hydrophobic signal peptide sequences and long extracellular domains which are not as highly conserved as the CT 7 transmembrane domains which contain a putative G protein-binding domain in the 3rd intracellular loop with a signature of N-[LIT]-[AV]-[KR]-[LF]-[KS]-L-[FY]-R-H-[FY]. We aim to examine the expression of this gene in primary pulmonary hypertensive and normal lung by *in situ* hybridisation.

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11

Elevated oxygen and ciliary abundance of rat trachea *in vitro*

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Many intensive care patients require an increased FiO_2 which may have significant adverse effects upon the lungs (Bonikos et al. *Am. J. Path*. **85**, 1976). Qualitative reports describe ciliary loss (Konradova et al. *Respiration* **54**,1988) and reduced ciliary beat frequency (Staneck et al. *Brit. J. Anaesth*. **80**, 1998) in the upper airways. This study quantitatively assessed damage that increased levels of oxygen may have on the airways main defence mechanism, the mucociliary apparatus.

230 sections of rat trachea (male, Sprague-Dawley) were cultured for 12 d in 21% or 100% oxygen with only partial submersion, allowing the ciliary border to maintain an air interface similar to natural physiological conditions. Samples were collected daily, prepared and blinded before being imaged with a scanning electron microscope. Contiguous micrographs (500×) were taken of the total mucosal surface. A standard computer and software analysed the images, differentiating between ciliated and nonciliated cells, providing a novel highly quantitative measure of percentage ciliary abundance. Exponential curves were fitted to the plotted data from the two groups (air and oxygen), to model the decreasing percentage of ciliary abundance with time. Comparing this model with one which does not allow for a difference between groups, a statistically significant difference was found (F statistic = 63, $P = 0.0001$). We estimate a 10% (95% CI 8–12%) decrease in percentage ciliary abundance per day in the 21% oxygen group, and a 23% (95% CI 18–28%) decrease per day in the 100% oxygen group.

We have found that 100% oxygen increases ciliary loss in cultured rat trachea. Additional work is needed to evaluate any dose dependant relationships or threshold effects.

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12

Central mechanisms regulating eupnea and the upper airway

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The network model of respiratory rhythmogenesis that drives normal breathing (i.e. eupnea) is dependent upon reciprocal inhibitory synaptic connections (e.g. Richter, In *Comprehensive human physiology* ed. Greger & Windhurst, 1996). Richter has proposed that eupnea is a 3-phase rhythm comprising inspiration, postinspiration (stage I expiration) and expiration (stage II; Richter, 1996). This is reflected in the upper airway: during inspiration the vocal folds dilate to ease air into the lungs whereas they narrow during postinspiration to steady airflow out of the lungs and maintain functional residual capacity. We addressed the role of glycinergic synaptic inhibition within the pontomedullary respiratory network for the maintenance of eupnea, including its modulation of the upper airway, in neonatal and mature rats.

Experiments were performed on arterially perfused *in situ* working heart-brainstem preparations of both neonatal and mature rats (1 h to 50 d old) at 31 °C. This preparation is decerebrated at the precollicular level and unanaesthetised. We recorded phrenic, hypoglossal and recurrent laryngeal motor activity as well as the intracellular activity of single respiratory neurones with sharp microelectrodes. We also measured airway resistance by recording changes in subglottal pressure (SGP) during constant airflow perfusion of the upper airway in the expiratory direction (see Dutschmann et al. *Autonomic Neurosci*. **84**, 2000).

In both neonatal and mature rats there was rhythmic inspiratory motor activity in phrenic, hypoglossal and recurrent laryngeal nerves. Additionally there was postinspiratory activity in the recurrent laryngeal nerve that was associated with transient increases in SGP. Application of strychnine (0.1–0.5 μM) into the perfusate resulted in a severe reduction of postinspiratory motor activity. Strychnine abolished the inspiratory inhibition in postinspiratory neurones and revealed an underlying synaptic excitatory drive that resulted in an unprecedented inspiratory related burst discharge. Loss of glycine receptor integrity reduced the postinspiratory laryngeal adduction. Paradoxically the glottis started to constrict during the phrenic nerve burst. Since hypoxia is known to depress inhibitory synaptic transmission within the respiratory network (Schmidt et al. *J. Physiol*. **483**, 1995), we gassed the perfusate with isocapnic hypoxia (5% O_2, 5% CO_2 and 90% nitrogen). This produced a similar effect to that described for strychnine: loss of postinspiratory motor activity and a paradoxical laryngeal adduction during neural inspiration.

Our studies demonstrate the importance of inhibitory glycinergic transmission within the pontomedullary network for eupnoea and the normal respiratory modulation of the laryngeal muscles. We suggest that prolonged hypoxia could result in upper airway obstruction due to a central re-organisation of the respiratory network.

Studies supported by the British Heart Foundation and the Deutsche Forschungsgemeinschaft.

13

The diaphragm, two physiological muscles in one

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To the respiratory physiologist or anatomist the diaphragm muscle is of course a prime mover of tidal air. However gastrointestinal physiologists are becoming increasingly aware of the value of this muscle in helping to stop gastric acid from refluxing into the oesophagus. The diaphragm should be viewed as 2 distinct muscles, crural and costal, which act in synchrony throughout respiration. However, the activities of these 2 muscular regions diverge during certain events such as swallowing and emesis. In addition transient inappropriate crural relaxations are associated with reflux episodes. Studying the motor control of this muscular barrier may help elucidate the mechanism of these episodes. In mammals the phrenic nerve divides into three branches before entering the diaphragm, and it is possible to sample single neuronal activity from the crural and costal branches. This review will discuss our recent findings with regard to the activity patterns displayed by axons running in the phrenic nerve of the rat. In addition we will outline our ongoing search for homologous structures in basal vertebrate groups. In particular the pipid frogs possess a muscular band around the oesophagus that appears to be homologous to the mammalian crural diaphragm. This structure does not appear to interact directly with the respiratory apparatus, and could suggest a role for the diaphragm which was originally nonrespiratory.

We wish to acknowledge the generous support of the Health Research Board (Ireland).

14

A reassessment of the respiratory function of upper airway muscles

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The mammalian pharynx is a musculomembranous tube ideally adapted to ingesting a varied diet or modulating and modifying the acoustic output of the larynx. However, as an element of the respiratory tract, the pharynx has little bony or cartilaginous support, leaving the airway susceptible to collapse.

Narrowing or obstruction of the pharyngeal airway is normally prevented by the action of skeletal muscles located within or attached to the pharyngeal wall. It has been assumed that the muscles responsible for maintaining airway patency are those which act to widen the airspace, such as the genioglossus and palatoglossus, and there has been considerable interest in the neural control of these airway dilator muscles.

Muscles which act to narrow the airway, such as tongue retractor and pharyngeal constrictor muscles, may play a role in regulating expiratory airflow but otherwise were not thought to have a major respiratory function. However recent findings suggest that these muscles, by stiffening the wall of the pharynx and immobilising the tongue, may be important in stabilising the airway and preventing obstruction. The effect of these muscles on pharyngeal collapsibility may depend upon the activity of other pharyngeal muscles and the initial resting size of the airspace. Furthermore it remains to be seen whether there are differences in respiratory function between different components of the pharyngeal constrictor apparatus. Developing a detailed understanding of the motor and reflex control of pharyngeal muscles and the biomechanical result of their activation promises to be an important, complex and challenging problem.

15

Nicotine-evoked ventilatory reflexes in cats: sites of origin and afferents

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Systemic administration of nicotine has been reported to induce acute enhancement and/or depression of ventilation. We restudied this problem in pentobarbitone-anaesthetised adult cats. Iv injections of nicotine bitartrate 1–100 μg/kg evoked dose-dependent transient increases in tidal volume (VT); this response was preceded by 1–3 cycles of reduced VT when testing with 200 μg/kg of the drug. After bilateral section of the aortic (depressor) and carotid (sinus) nerves, brief apnoea followed by cycles of reduced VT was observed in response to nicotine 50–200 μg/kg injections. These depressant ventilatory responses disappeared after subsequent bilateral section of cervical vagi. In other series of experiments, cats were first subjected to bilateral vagotomy, in which case only hyperventilatory responses were observed in response to nicotine 1–200 μg/kg; such responses disappeared after subsequent bilateral section of the aortic and carotid nerves. Furthermore, in neurologically intact cats, topical application of nicotine to the exposed surface of the carotid bodies evoked transient increase in VT; such response was suppressed by ipsilateral section of the carotid nerve and not elicited by nicotine application to the central stump of the carotid nerve or the petrosal ganglion. Animals were euthanised by an overdose of pentobarbitone at the end of experiments. It is concluded that ventilatory responses to systemic application of nicotine are entirely reflex, that hyperventilation results from stimulation of arterial chemoreceptors (carotid and aortic bodies), while hypoventilation results from stimulation of vagally innervated lung receptors.

16

The neurogenesis of gasping is independent of inhibitory synaptic transmission within the brainstem of the juvenile rat

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'Automatic' breathing movements are generated within pons and medulla. There are several patterns of 'automatic' ventilation. Eupnea is 'normal' breathing. If eupnea ceases, metabolic demands will not be met and hypoxia will develop. Severe hypoxia recruits a second pattern, gasping, which serves as a powerful defensive mechanism for autoresuscitation.

Gasping can be generated by mechanisms inherent to the medulla whereas both pons and medulla are required for the expression of eupnea. We hypothesised that different neurophysiological mechanisms underlie the neurogenesis of eupnea and gasping. Eupnea requires a ponto-medullary neuronal circuit for its genesis and/or expression that is dependent on synaptic inhibition. In contrast gasping is generated by the discharge of 'pacemaker' neurons in the rostral medulla. Hence we evaluated the hypothesis that gasping is not dependent upon inhibitory synaptic transmission.

Activity of the phrenic nerve was recorded in a decerebrate and paralysed juvenile rat *in situ* preparation which was maintained viable by an extracorporeal perfusion system. The pattern of phrenic activity was altered from eupnea to gasping by equilibrating the perfusate with a hypoxic gas mixture or by a temporary cessation of perfusion. In eupnea phrenic activity had an 'incrementing' discharge pattern. Phrenic activity was 'decrementing' in gasping with peak activity being reached immediately after onset.

To block $GABA_A$ receptors bicuculline or picrotoxin was add to the perfusate. Strychnine was used to block transmission by glycine. Following administrations of either bicuculline, picrotoxin or strychnine, the augmenting rise of eupnea was replaced by rapidly rising bursts of low and high amplitude. In contrast none of the blockers caused alterations of the decrementing pattern of the gasp.

We conclude that the neurogenesis of gasping is not dependent upon inhibitory synaptic transmission. Such transmission is important for the expression of eupnea. These results support the overall concept that the discharge of 'pacemaker neurons' in the medulla underlies the neurogenesis of gasping.

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17

Structure and composition of airway surface liquid

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The airways are lined with a film of liquid. In healthy airways, this is usually just deep enough to cover the cilia (i.e. about 5 μm). Irritation, inflammation or disease cause mucus to be secreted by submucosal glands and goblet cells in the surface epithelium, resulting in the formation of a mucous gel of variable thickness that lies over the 'periciliary sol'. Inhaled particles are trapped in the gel. Cilia beating in the low viscosity sol contact the underside of the mucous gel and propel it towards the mouth. In this way the airway surface is kept clean. To investigate how the depth of sol and gel are regulated we studied rapidly frozen specimens of bovine trachea by low temperature scanning electron microscopy. In specimens fractured perpendicular to the epithelium the baseline depth of the airway liquid layers was ~20 μm. Stimulation of active Cl^- secretion in submucosal glands induced transient liquid secretion that increased the depth of the airway surface liquid by ~50 μm within 2 min. After that active Na^+ absorption by the surface epithelium resulted in the depth being brought back to baseline at ~1 μm per min. We believe that Na^+ absorption cannot reduce the depth beyond a certain minimum (5–20 μm) at which forces of surface tension effectively oppose the transepithelial osmotic forces generated by active solute transport. By freeze etching the fracture surface we could distinguish putative sol and gel, and at the height of gland secretion the sol was considerably deeper than the length of the cilia. We speculate that at this time mucociliary clearance ceases, and is not resumed until active Na^+ absorption has brought the depth of the sol back down to ~5 μm. Finally we are applying X-ray microanalysis to rapidly frozen cultures of human tracheal epithelium to determine the elemental composition of sol and gel, and how they are altered in cystic fibrosis. We expect the sulphur signal (which comes predominantly from sulphate on mucus) to be increased in cultures from cystic fibrosis patients. X-ray microanalysis can also be used to test the conclusion obtained with other methods that the NaCl concentration of airway liquid is increased in cystic fibrosis.

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Pulmonary vascular remodelling in chronic lung disease

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Chronic lung disease in humans is frequently complicated by the development of secondary pulmonary hypertension, which is associated with increased morbidity and mortality. Hypoxia and inflammation are the primary stimuli although the exact pathways through which these initiating events lead to pulmonary hypertension remain to be completely elucidated. The increase in pulmonary vascular resistance is attributed in part to remodelling of the walls of resistance vessels. This consists of intimal, medial and adventitial hypertrophy leading to encroachment into and reduction of the vascular lumen. In addition it has been reported that there is a reduction in the number of blood vessels in the chronically hypoxic lung which would also contribute to increased vascular resistance.

These structural alterations in the pulmonary vasculature contrast sharply with the responses of the systemic vasculature to the same stimuli. In systemic organs both hypoxia and inflammation cause angiogenesis and the pathways underlying these responses are under intensive investigation. Furthermore, remodelling of the walls of resistance vessels is not observed in these conditions.

Thus it is generally stated that in the adult pulmonary circulation angiogenesis does not occur. However a number of more recent reports suggest that new vessel formation may be seen in this circulation. Prompted by the previous observations that chronic airway inflammation can lead to pulmonary vascular remodelling without hypertension we have shown, using quantitative stereological techniques, that angiogenesis can occur in the adult pulmonary circulation (Hopkins et al. *J. Appl. Physiol*. in press, 2001). We suggest that the mechanisms underlying this angiogenesis are different from those that operate in the systemic circulation.

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Airway and blood vessel interaction during lung growth and postnatal adaptation

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In the adult lung the pulmonary arteries run alongside the airways and branch with them. The pulmonary veins lie between the arterial/airway bundles; however, they have a similar number of branches. There is a close association of airway and blood vessel development.

The airways lined by epithelium develop from the primitive foregut at 4 week of gestation. A series of dichotomous branching into the undifferentiated mesenchyme form the airways until the end of the pseudoglandular period (about 16–17 week of gestation) and by this time all prospective adult airways to the level of the terminal bronchioli are present. As each airway divides it is surrounded by a network of capillaries which arise by vasculogenesis. The epithelial cells produce vascular endothelial growth factor which may stimulate this. The capillaries coalesce to form either arterial tubules which line up alongside the airways or venous tubules found between airways. The airway branches act as a template for vascular development.

During the canalicular phase, as the respiratory airways form, the arteries and veins also multiply. The capillaries move closer to the airway epithelium in the peripheral airways and initiate the thinning of the epithelial cells to form the blood gas barrier. An interaction between the airway and blood vessels. alveoli appear from 30 week of gestation and continue to increase in number up to 2–4 y of age. During the development of alveoli the surface area of capillaries and small blood vessels increases. Formation of new alveolar septa depends upon the presence of a double capillary network.

For the arteries there are 3 origins of smooth muscle cells: from fibroblasts, endothelial cells and, more surprisingly, the bronchial smooth muscle cells in the peripheral airways. Systemic vessels, bronchial arteries, appear by 8 week gestation and extend in the walls of airways as they increase in size.

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NG2 glia (oligodendrocyte progenitor cells) and sodium channel clustering at developing nodes of Ranvier in the rat brain

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NG2 glia have the antigenic phenotype of oligodendrocyte progenitors (OPC) in the adult and developing brain. However adult NG2 glia (OPC) are highly complex cells, with multiple long branching processes that form discrete contacts with the axolemma at nodes of Ranvier, the sites of axonal action potential conduction. This led us to suggest that NG2 glia may be a functionally specialised cell with unresolved functions related to nodes. The present study addresses the possibility that NG2 glia may play a role in sodium channel clustering at nodes of Ranvier, which previous studies showed to be dependent on axoglial contact. Rat pups aged postnatal day (P) 9–12 were humanely killed by overdose of sodium pentobarbitone and perfused via the left cardiac ventricle with 4% paraformaldehyde. Vela were dissected free and processed for double and triple immunofluorescence labelling and confocal microscopy using antibodies to NG2, myelin basic protein, neurofilament, sodium channels (NaCh) and ankyrin-3/G (ank3/G). The results showed that in the neonatal velum complex NG2 glia with an adult morphological phenotype extended processes that formed discrete contacts with axonal ank3/G and sodium channel clusters. Foci of axonal ank3/G labelling always had associated NG2 glial cell processes which formed equivalent axonal contacts on premyelinated axons and at heminodes. Ank3/G is a cytoskeletal protein that is expressed at immature and presumptive nodes of Ranvier before NaCh and is implicated in their clustering. These results support a possible role for NG2 glia in inducing sodium channel aggregation and in the localisation and induction of nodes of Ranvier, functions previously attributed to both astrocytes and oligodendrocytes. Also, their perinodal processes make NG2 glia (OPC) ideally situated to detect and respond to axonal signals, which may be important in development and in their response to injury.

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Response of NG2 glia (oligodendrocyte progenitor cells) to platelet derived growth factor in the rat anterior medullary velum

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Oligodendrocytes differentiate into the myelin-forming cells of the central nervous system (CNS) from oligodendrocyte progenitor cells (OPC) in response to environmental cues. The AA isoform of platelet derived growth factor (PDGF-AA) is considered a key factor in the control of oligodendrocyte development, and OPC are identified *in vivo* by their expression of PDGF-alpha receptors (PDGFαR). In the developing brain 'perinatal' OPC are numerous and give rise to oligodendrocytes or 'adult' OPC which persist in the mature CNS. Both perinatal and adult NG2 glia (OPC) have been shown to express PDGFαR and the aim of the present study was to investigate their response to PDGF-AA in the anterior medullary velum (AMV) of rats aged postnatal day (P) 6 to P9. Rat pups were anaesthetised using 5% isofluorothane and PDGF-AA was delivered into the cerebrospinal fluid (CSF) via the lateral ventricle twice daily between P6 and P9. Rats were humanely killed by overdose of sodium pentobarbitone and perfused via the left cardiac ventricle with 4% paraformaldehyde. AMV were dissected free and processed for either immunohistochemistry or nonradioactive *in situ* hybridisation. At P6-P9 the caudal velum was myelinated and populated by mature myelin forming oligodendrocytes and 'adult' NG2 glia (OPC), whereas the rostral velum was premyelinated and populated by immature oligodendrocytes and 'perinatal' NG2 glia (OPC). The main actions of PDGF-AA were to decrease the number of mature myelin producing oligodendrocytes and markedly increase the number of immature oligodendrocytes, in both the rostral and caudal AMV. Significantly, the number of OPCs was unaltered. Since oligodendrocytes do not express PDGFαR, we conclude that PDGF-AA had similar actions on PDGFαR expressing 'perinatal' and 'adult' NG2 glia (OPC), promoting the proliferation of newly formed oligodendrocytes and inhibiting their differentiation into mature myelin producing cells. The results indicate that 'adult' NG2 glia (OPC) have the capacity to give rise to oligodendrocytes in response to PDGF-AA, which has implications for oligodendrocyte regeneration in Multiple Sclerosis.

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NG2 glia (oligodendrocyte progenitor cells) do not give rise to oligodendrocytes in the absence of axons *in vivo* but do *in vitro*

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NG2 glia (oligodendrocyte progenitor cells, OPC) have an OPC antigenic phenotype in the developing and adult central nervous system and give rise to oligodendrocytes *in vitro*. During development 'perinatal' NG2 glia (OPC) give rise to oligodendrocytes and 'adult' NG2 glia (OPC), and axonal signals are believed to be important in OPC differentiation and survival. The aim of the present study was to investigate the response of 'perinatal' and 'adult' NG2 glia (OPC) to axonal degeneration. Rats aged postnatal day (P) 1 or 21 were deeply anaesthetised by inhalation of 5% isofluorane and enucleation was performed by retinal ablation at P1 or by transecting the nerve behind the eyeball at P21. At 15 d post enucleation, rats were humanely killed by overdose of sodium pentobarbitone and either perfused via the left cardiac ventricle with 4% paraformaldehyde and optic nerves processed for immunohistochemistry or explants of optic nerves were placed in culture. The major findings were that: (1) following enucleation at P1 or P21, NG2 glia (OPC) persisted in large numbers and had a 'reactive' phenotype, with short thick branching processes; (2) myelin-forming oligodendrocytes were not lost following enucleation at P21, but did not develop in the absence of axons following enucleation at P1; (3) in cultures of explants from enucleated and control P1

optic nerves, NG2 glia (OPC) were observed to differentiate into oligodendrocytes expressing myelin basic protein and galactocerebroside. The results indicate that neither 'perinatal' nor 'adult' NG2 glia (OPC) depend on axons for their survival. Significantly, the development of oligodendrocytes is dependent on axons *in vivo*, although NG2 glia (OPC) can differentiate into oligodendrocytes *in vitro*. We conclude that either negative signals in the enucleated optic nerve inhibit differentiation of NG2 glia (OPC) into oligodendrocytes, and/or that oligodendrocyte differentiation *in vitro* is promoted by positive factors, which are normally produced by axons *in vivo*.

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Glutamate and ATP mediate glial calcium signalling in isolated intact rat optic nerves

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Glial cells are traditionally regarded as passive structural elements of the central nervous system (CNS), but respond to a range of extracellular signals that mediate an increase in intracellular calcium ($[Ca^{2+}]_i$). The present study identifies glutamate and ATP as important mediators of Ca^{2+} signalling in isolated intact optic nerves from developing rats. The rat optic nerve is a highly organised CNS white matter tract in which glia are arranged in long interfascicular rows which lie in parallel with the axon bundles but it does not contain neuronal cell bodies or synapses. Optic nerves were studied between postnatal day (P) 2 (neonatal) and 29 (juvenile). In neonatal nerves approximately 50% of the cells in the nerve are NG2 glia (oligodendrocyte progenitor cells, OPC) and immature oligodendrocytes, which are distributed in rows of 5–10 cells within which are interposed solitary astrocytes, which comprise the remainder of cells. In juvenile nerves a small population (approximately 7%) of NG2 glia (OPC) persists and these are slightly offset from the rows of oligodendrocytes and astrocytes. Rats were humanely killed by CO_2 narcosis (in accord with Home Office regulations) and optic nerves removed to a brain slice chamber where they were continually superfused with oxygenated artificial cerebrospinal fluid. Glial cells were visualised using the Ca^{2+}-sensitive dyes fluo-3 and fura-2. The response to superfused glutamate or ATP (1 mM) was a widespread increase in fluorescence within which individual glial cell somata were distinguished. In neonatal nerves the response to glutamate and ATP was equivalent in cells identified as astrocytes and OPC/immature oligodendrocytes. However the glutamate response was lost with development, whereas the ATP response was equivalent at all ages. Thus glutamate mediated Ca^{2+} signalling in immature but not mature NG2 glia (OPC), oligodendrocytes and astrocytes, indicating an unresolved role for glutamate in glial development. The results indicate ATP is an important signalling molecule in the developing and mature optic nerve, consistent with its proposed roles in the glial response to CNS injury.

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The dorsal root transitional zone model of CNS axon regeneration: morphometric findings

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The dorsal root transitional zone (DRTZ) was used to investigate axon degeneration and regeneration along the DR and into the CNS.

Three protocols were used regeneration in the presence of neurotrophin-3 (NT3) (protocol A); regeneration in the absence of neurotrophin (protocol B). Protocol C provided background data on tissue changes in experimental controls in which axons were absent following dr ganglionectomy. Unbiased stereological methods (Mayhew, *Exp. Physiol*. **6**, 1991) were used to study the associated CNS and PNS tissue changes. Myelin degradation products were more prominent in cns than in PNS tissues. Schwann cell degenerative/regenerative features were prominent in all protocols. In the PNS few axons were found in protocol C but were frequent in the others. In the CNS compartment axons were few in protocol B, but were prominent in protocol A. In protocol A axon calibre distribution and mean values were similar in CNS and PNS compartments; regression data showed regenerating myelin sheaths to be thin relative to axon calibre; and regenerative indices suggest that on average as many as 40% of axons passed between the PNS and CNS compartments. In untreated tissue (protocol B) few did so. Interstitium was generally markedly increased (4- to 7-fold) following axonal interruption, sometimes containing flocculent material. Phagocytes comprised a small proportion of the tissue (4–5%). The rate of debris removal was slower centrally than peripherally. In all 3 protocols blood vessel density in the PNS compartment showed a 5-fold increase over normal tissue and the proportion of cytoplasmic reticulum (Fraher and O'Leary *J. Anat*. **184**, 1994) increased 5- to 10-fold over normal tissue. In the CNS interstitial space was very limited. Astrocytic tissue changes were similar in all 3 protocols: the proportion was increased 3- to 4-fold over controls. The cytoplasm contained disintegrating myelin and lipid material. A prominent fringe grew distally into the root.

25

Ultrastructural abnormalities of unmyelinated fibres in the peripheral nerves of genetically diabetic (db/db) mutant mice

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Human diabetic neuropathy is characterised by decreased nerve conduction velocity associated with a variety of morphological

changes (Robertson and Sima, *Diabetes* **29**, 1980). The genetically diabetic mouse C57BL/Ks (db/db) is a model of type II diabetes. Alterations in patterning of microtubules in unmyelinated axons in the tibial nerve of these animals have been reported suggesting that the pathological insult of diabetes may have some effect on the axonal cytoskeleton (Dockery et al. *J. Anat*. **173**, 1990). We explored this finding further to test the hypothesis that the reported disturbance in axonal transport has a structural correlate.

In the present study transmission electron microscopy and morphometric methods were used to assess the subcellular composition of unmyelinated tibial nerve axons of genetically diabetic (db/db) and nondiabetic (m/m) control mice at 6 and 15 month of age ($n = 6$ for each group). All animals were anaesthetised and the tibial nerve was exposed and removed. The animals were then killed using humane procedures.

A 2-way analysis of variance (2AOV) of axon area data revealed a significant effect of condition and interaction but no effect of age, the 6 month diabetic group being larger than age matched controls and 15 month diabetic ($P < 0.05$). A 2AOV of index of circularity revealed a significant effect of age but no effect of condition or interaction. 6 month diabetic animal values were larger than both 15 month control and 15 month diabetic animals ($P < 0.05$). A 2AOV of the percentage ratio of organelle area to axonal area revealed a significant effect of condition and interaction but no effect of age. 15 month diabetic animal values were larger than age matched controls ($P < 0.05$).

This study has documented structural alterations in axonal architecture that may correlate with reported transport disturbances in this model. A decreased rate of axonal organelle transport associated with diabetic neuropathy is strongly indicated. These changes may provide an insight into complex underlying mechanisms in early human diabetic neuropathy.

POSTERS

P1

Anatomical image quality criteria for thoracic radiological quality control

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The ability of radiographic images to answer clinical queries relates to the ability of an image to demonstrate disease and delineate anatomical structures. Anatomical structures can be used to assess the performance of aspects of radiographic imaging technique. Once the anatomical structures have been specified and the level of visualisation quantified, observers can mark the quality of an image. Chest radiographs were acquired ($n = 120$) using 2 image acquisition techniques, under the same radiographic conditions and using patients paired by body mass index, age and sex. The quality of chest images produced has been evaluated in a side by side viewing session using anatomical image criteria.

The level of visualisation of specified anatomy was assessed and quantified by observers awarding a mark for each anatomical structure. Anatomical structures whose radiographic visualisation were most affected by differences in image acquisition technique were established. Significant differences between the mean visualisation scores for the 2 sets of images were noted particularly for low inherent contrast structures such as the peripheral lung vessels ($P < 0.001$), the thoracic spine through mediastinal structures ($P < 0.001$), retrocardiac lung vessels ($P < 0.001$) and the trachea and proximal lung vessels ($P < 0.001$). No significant visualisation differences were found for high contrast features, namely costophrenic angles, borders of the heart or aorta.

Anatomical image quality criteria provide information on a variety of anatomical structures along with quantitative data for statistical analysis. Anatomical structures with low inherent radiographic contrast provide useful comparative data when comparing alternative acquisition methods. High contrast anatomical structures should usually be visible with any reasonable modality. Specification of anatomical image criteria is an effective and efficient method of image quality evaluation.

P2

Distribution of T and B cells in the pig lung

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Respiratory pathology is of great importance to the pig industry. To date, several features of respiratory immunity and in particular the bronchus-associated lymphoid tissue (BALT) have been described in pigs, such as its presence in healthy and infected pigs and the distribution and cellular components of BALT. However data on the distribution of T and B cells in the pig lung are still scarce. This study examines the presence of these lymphocyte subsets in different anatomical compartments of the pig lung: the epithelium, mucosal connective tissue, BALT and alveolar tissue.

The lungs of 5 slaughtered pigs from an abbattoir, which showed no macroscopic signs of lung pathology, were fixed in 4% paraformaldehyde and rinsed in a buffer solution. The left lung was cut into 2 cm thick slices. In each slice, tissue blocks of the main and a secondary bronchus, as well as a smaller bronchiolus along with its surrounding alveolar tissue, were taken. The snap frozen tissue blocks were sectioned and the presence of T and B cells was investigated using immunohistochemical staining techniques.

Preliminary results showed the presence of lymphocytes in all compartments examined. In the epithelium T cells were seen almost exclusively. The solitary mucosal lymphocytes, scattered throughout the airways, were shown to be both T and B cells. In the interglandular tissue associated with the larger bronchi, only B cells could be seen. In BALT which was clearly composed of a follicular and a parafollicular compartment, the former was mainly populated by B cells while the latter harboured both T and B cells. In the alveolar tissue mainly T cells were encountered.

The distribution of both T and B cells is indicative of adaptations of the respiratory immune response in the different anatomical compartments of the airways and the lung parenchyme.

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P3

NADPH-diaphorase neurons in the airways of developing pigs

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Recent reports on the distribution of nNOS in the airways have focused on the neuronal cell bodies in intrinsic ganglia. Adult human and porcine lungs show a similar pattern of NOS-containing neurons while small laboratory animals such as the guinea pig are devoid of NOS-immunoreactivity in the peripheral airways. The present study reports on the occurrence of NOS-containing neurons in porcine airway intrinsic ganglia during development using NADPH-diaphorase staining.

14 fetal and 3 neonatal pigs were used in this study. Whole mount preparations of the trachea and the 2nd lateral segmental bronchus were incubated in the NADPH-diaphorase medium containing 10 mg NADPH, 5 mL 0.01 M PBS with 0.3% Triton-X-100, 1.25 mg nitro blue tetrazolium for 15 min at 37 °C. After rinsing in PBS the tissue was mounted in a 3 : 1 glycerine/0.01 M PBS mixture.

The trachea shows a nerve plexus where nerve bundles interconnect different ganglia. During development the density of this network decreased indicating that ganglia become more separated when the trachea grows in diameter and length. These ganglia show NADPH-d positive neurons with different degree of staining intensity. With age more neurons show the NADPH-d staining with a maximum intensity and presence just before birth.

In comparison with the trachea the innervation of the lateral segmental bronchus is characterised by nerve bundles interconnecting smaller ganglia. These ganglia also show NADPH-d positive neurons. Preliminary quantitative observations demonstrated the increase in numerical density in NADPH-d positive neurons in the ganglia with fetal age. For the same age group it was observed that the density of NADPH-d stained neurons is higher in trachea than in the segmental bronchus. In neonatal animals the number of positive cells still seems to increase in the peripheral airway ganglia while the tracheal ganglia shows some saturation.

The distribution of NADPH-d neurons in the airways of developing pigs confirms the importance of their neuromodulating capacities both in trachea and peripheral airways in the perinatal period.

P4

Endothelial ultrastructure in human endometrium following exposure to levonorgestrel intrauterine system

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The Levonorgestrel Intrauterine System (LNG-IUS) is an effective contraceptive device, which also brings about a profound reduction in menstrual blood loss. Use of the LNG-IUS is a recognised treatment for menorrhagia. A common cause of discontinuation of treatment is the side-effect of intermenstrual bleeding, which is particularly troublesome during the first 6 month of use. We postulate that intermenstrual bleeding is related to transient changes in vascular development after exposure to high dose levonorgestrel. In the present study we have employed stereological methods to document the ultrastructure of the endothelial cells in LNG-IUS treated and a control group of women. 33 women opting for hysterectomy as a treatment for menorrhagia were randomised into two groups- LNG-IUS (n = 12) and untreated control (n = 21) and their uteruses collected. This data is also compared with published data from normal women taken at various stages of the menstrual cycle (Dockery et al. in *Disorders of the Menstrual Cycle, ed. O'Brien* et al. 2000). This study had full ethics committee approval.

The maximum vessel diameter was larger in the LNG-IUS group. The mean (± SE) percentage volume fraction Vv of mitochondria per cell increased from d LH + 6 (2.5 ± 0.5) to d LH + 12 (4.7 ± 0.8) in the normal cycle. The value for the LNG-IUS was 5.7 ± 0.6. The Vv rough endoplasmic reticulum and secretory apparatus per cell increased from d LH + 6 to d LH + 12, this correlates with an increased thickness of the basal lamina. However in the LNG-IUS group the Vv of rough endoplasmic reticulum and secretory apparatus were not different from those of day LH + 6. There was a thinner vessel basal lamina, which may be associated with changes in vessel morphology and may predispose the patient to intermittent bleeding.

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P5

The effects of a xeno-oestrogen on human endometrial cell structure: a physiological and morphological study

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The endometrium undergoes dynamic morphological and functional changes during the menstrual cycle, and oestrogen plays an essential role in the regulation of these changes. Xenoestrogens are common environmental pollutants which are structurally different to oestrogen but can mimic its endocrine effects. The ability to mimic oestrogen may allow xenoestrogens to interfere with oestrogen dependent reproductive processes. Calcium is important in the transduction of many signal cascades which bring about change be it functional or morphological in many cell systems. Rapid nongenomic variations in intracellular calcium concentrations upon exposure to oestradiol have been reported in both endometrial tissue and other tissues (Dockery et al. in *Disorders of the Menstrual Cycle, ed. O'Brien* et al. 2000). Changes in the concentration of free calcium ions have also been implicated in the signalling cascades, which trigger rearrangements of cytoskeletal elements. Here we describe the effects of 17β oestradiol and the xenoestrogen bisphenol A on microtubules in the human endometrial cell line RL95-2 using immunocytochemistry and confocal microscopy.

Our physiological studies using confocal microscopy studies show that 17β oestradiol and the xenoestrogen bisphenol. A both cause rapid alterations in intracellular calcium. Immunocytochemical studies show alterations in the appearance of the microtubule arrays within the cells the may be linked to the fluctuations in intracellular calcium.

These findings may have important implications in the understanding of normal endometrial cell dialogue and in the dysfunctional responses to xeno-oestrogens.

P6

Development of human placental villi from 10 week to term: interpretation in the context of trophoblast growth, renewal and repair

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Villous trophoblast is a continuously expanding epithelium exhibiting phases of proliferation, recruitment, maturational differentiation, terminal differentiation (apoptosis) and extrusion. These occur in distinct regions: cytotrophoblast (CT), fusion zones, nonsyncytial knots (nonSK), syncytial knots (SK) and syncytial fragments. We have examined human placentas in order to study growth in different regions and relate this to events in villous morphogenesis: sprouting, branching, bridge formation and abruption, denudation, deposition of perivillous fibrin-type fibrinoid (pFTF) and re-epithelialisation. Fields on trichrome stained wax sections were selected by hierarchical uniform random sampling and trophoblast volumes and surfaces estimated stereologically by test point and intersection counting. Apparent differences over time were tested by analysis of variance and relationships by regression and contingency table analyses. In all cases the null hypothesis was rejected at *P*-values of < 0.05. All trophoblast regions expanded but at different rates: the volume and surface of nonSK grew commensurately with trophoblast as a whole whilst CT volume was relatively greater at earlier stages (10–20 week) and SK regions (both true knots and syncytial bridges) occupied greater fractions of total volume and surface near term (37–41 week). The findings are consistent with reported declines in CT proliferation indices, changes in trophoblast thickness and increases in incidences of knots, bridges and branchpoints. Denudation sites also expanded faster than overall villous surface area but continued to occupy a small fraction (2–5%) of that surface. Since sites result from various forms of trauma (e.g. fragment extrusion and abruption of larger intervillous bridges), this suggests that damage is not cumulative or long-term. Sites are first plugged by pFTF and then repaired by proliferation and re-epithelialisation. The increase in volume of pFTF was commensurate with changes in villous surface area, supporting the idea that the trophoblast surface is influential in regulating the local coagulation-fibrinolysis steady state. Repair events offer a plausible mechanism for formation of new bridges, viz. shared re-epithelialisation and syncytial fusion on contiguous villi. These and other aspects of villous development can be interpreted in terms of a coherent concept of trophoblast turnover in which early proliferation is mainly for growth whilst that at later stages is for renewal and repair. The concept may prove useful in studying villous morphogenesis in complicated pregnancies.

P7

Enterocyte cell loss and its association with intraepithelial lymphocytes in porcine small intestine

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Alternative types of epithelial cell loss operate concurrently in vertebrate small intestine and are partly species dependent. Where functional and physical integrity of the epithelium are maintained during extrusion (by preserving intercellular tight junctions) subtypes of cell loss have been recognised and enterocyte apoptosis has been implicated. Where tight-junctional adhesion is compromised, necrosis like processes operate and epithelial integrity is disrupted. Both processes are associated with the activity of intraepithelial lymphocytes (IELs) and lamina propria mononuclear phagocytes (LPMPs). The small intestine of the domestic pig has an unusually rich content of IELs and, using glutaraldehyde fixed and resin embedded material from juveniles and adults, we have examined cell loss and regional contents of IELs on villi by transmission electron microscopy. Animals were killed humanely under anaesthesia. In piglets and adults IELs occupied 10–20% of epithelial volume and showed base-apex differences along the villus and within the epithelium. In piglets no discernible differences between upper and lower halves of villi were detected, but in adults the upper portions of villi showed higher volume proportions. In piglets and adults, however, IEL volume proportions were higher in basal than apical regions of the epithelium. IELs were associated with necrosis (defined by cell swelling, decreased cytoplasmic density, organelle and tight junction disruption) and with *in situ* cell shrinkage (increased cytoplasmic density, organelle disruption, tight-junctional integrity). In the latter, enterocytes separated into anucleated apical fragments (bearing intact tight junctions) and nucleated basal portions. Eventually apical fragments were extruded into the lumen. In a novel mechanism not described so far in other species, groups of IELs often formed bridges or domes separating the apical and basal cell portions. At least in piglets, LPMPs were found at the bases of these bridges from where they may sequester basal cell fragments. In necrotic loss the resulting epithelial discontinuities were often plugged by IELs. The findings provide further evidence that mechanisms of cell loss are to some degree species dependent, and show that the contents and roles of IELs and LPMPs are species and site dependent. Base-apex differences in IEL content within the epithelium probably reflect the pathway of migration from lamina propria. However there is an additional gradient along the villus axis which appears to be influenced by ontogenetic factors.

P8

The use of Field Effect Transistors (FETs) in pharmacological research

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In vitro studies have been extensively used in physiological and pharmacological research. Measurements of the effects of drugs

on the heart are commonly performed using the isolated whole organ or electrophysiologically on cultured cardiac myocytes (CM). Over the past few years, FET arrays have been used as a tool for monitoring electrochemical signals from neuronal cultures. However, a quantitative use of the FET array as a tool for pharmacological measurements has not been fully validated. In this study we aim to demonstrate that FET arrays may be useful for future long-term pharmacological bioassays by showing that FET based systems can be used to detect the changes of myocyte responses in the presence of some well established cardioactive agents: isoproterenol (ISO), verapamil (VP) and carbamylcholine (CARB). In this study the rat CM cells were isolated from 8 to 10 embryonic Sprague Dawley rats (gestation d 15–18) and were plated at concentrations of $1–2 \times 10^4$ cells per recording device. In our experiments a simple drug cycle regime was employed. Basal beat frequency in beats/min (bpm) of the cells on each device was recorded for 60 s prior to drug administration (Control, C). ISO (0.1 μM), VP (10 μM) or CARB (10 μM) was added and remained in contact with the cell layer for 60 s. The recording chambers were then washed 5 times with prewarmed fresh culture media, re-equilibrated for a further 5 min and then another 60 s control recording was taken to ensure complete recovery. All readings were taken at 33–35 °C. We found that ISO (a β-adrenoceptor agonist which stimulates G-proteins and enhances L-type calcium channel activity) caused significant increased in beat frequency (29 ± 3 bpm, $n = 24$, vs. 215 ± 6 bpm, $n = 13$, mean ± SD, $P < 0.001$) with no significant change in membrane Na^+ current. Although VP (a L-type calcium channel antagonist) did not cause a reduction in beat frequency, it completely diminished the Ca^{2+} current and reduced the Na^+ current by 25% ($n = 4$). CARB (receptor-coupled, reduces cAMP, inhibits Ca^{2+} current and opens K^+ channels) caused 95% reduction of beat frequency (214 ± 12 bpm, $n = 7$, vs. 11 ± 7 bpm, $n = 7$, $P < 0.001$). All statistical comparisons were made using a Student's unpaired *t*-test. This study clearly shows that FET systems can be used in the detection of responses of some well established agents. In our experiments we also found that one single culture could yield enough cells to prepare between 50 and 100 encapsulated devices with potentials to perform multichannels and multiFETs recordings simultaneously. Furthermore the FET system is sensitive enough to discriminate efficiently different ionic signatures. The experiment itself is straightforward and will be particularly advantageous when only a small quantity of drug is available or tissue availability is limited. More refinements are currently being considered in terms of the operating systems, FET fabrication and drug delivery. The current FET based bioassay system shows definite promise for drug testing on variety of cells in the near future.

P9

Pituitary Adenylate Cyclase Activating Polypeptide in the rat urinary bladder: effects of age

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Previous studies have shown that the innervation of the rat urinary bladder undergoes different changes with age with the sympathetic but not the parasympathetic innervation subject to age associated attrition. The sensory innervation of the bladder is a vital part of the neural circuitry responsible for proper filling of the bladder and for the initiation of micturition. Pituitary Adenylate Cyclase Activating Polypeptide (PACAP) is a neuropeptide that has been found in sensory nerves of many different tissues including a widespread distribution in the lower urogenital tract. As part of an extensive investigation into the effects of ageing on the sensory innervation of the bladder we have examined the effects of age upon PACAP content and distribution in different layers of the urinary bladder by means of immunohistochemistry and also in whole bladders by radioimmunoassay. Two age groups of male Wistar rats, 3-month-old-and 24 + mo old, were used in this study. Urinary bladders from terminally anaesthetised animals were fixed in 4% paraformaldehyde prior to cryosectioning for immunohistochemistry. PACAP immunoreactivity was revealed by tyramide signal amplification of immunofluorescence. For radioimmunoassay, the bladders were removed, weighed and kept at – 80 °C before being processed for radioimmunoassay. The PACAP innervation density in the aged bladder base showed a marked decrease in the bladder base subepithelium and to a lesser extent in the muscle layers. Other regions of the bladder revealed no significance differences in the distribution of PACAP immunoreactive nerves between young and aged rats. On the other hand, the mean concentration of PACAP determined by radioimmunoassay showed that the concentration in young rat urinary bladder of 3.68 ± 0.5 pmol/g (mean ± SD) was not significantly different ($P > 0.05$) from that (2.8 ± 0.3 pmol/g) in the aged rat urinary bladder. Thus the subepithelial and muscular decreases of PACAP immunostaining with age raise the possibility that the perturbation of the sensation or the transmission of sensory information from the aged bladder reported previously in electrophysiological studies (Hotta et al. *Jap. J. Physiol*. **45**, 1995) may involve changes in PACAP content.

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P10

Transfection of trigeminal sensory and motor neurons by adenoviral vectors containing GFP and Bcl-2 constructs in neonatal mice

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After axotomy, nerves in the PNS and CNS die because of a loss of trophic support by the target tissue. Overexpression of the anti-apoptotic protein Bcl-2 has been found to prevent neuronal cell death after neurotrophin deprivation. Our goal was to use this strategy to examine regeneration and reconnection of the trigeminal system in neonatal mice. We have used an adenovirus containing a human Bcl-2 construct to transfect neurons *in vivo*. A control adenovirus containing a GFP construct and the tracer, Fluorogold, were used to test the success of the injections and adenoviral transfections.

In P0 mice, GFP and Bcl-2 adenoviruses (Titre – 1×10^{10} pfu per ml, 3–4 μL injections) and Fluorogold (2% as 1–2 μL injections) were injected into the jaw and whisker pad (trigeminal) and tongue (as a control to label hypoglossal neurons). The Bcl-2 protein was detected using a immunohistochemistry and GFP and Fluorogold by direct immunofluorescence.

Fluorogold labelled successfully the trigeminal ganglion and mesencephalic sensory neurons. It also labelled the trigeminal, facial and hypoglossal motor nuclei. The GFP adenovirus transfected the 3 motor nuclei but not the sensory neurons. However Bcl-2 was found in hypoglossal neurons and not the trigeminal system, sensory or motor.

To test the ability to transfect sensory neurons we isolated trigeminal ganglion cells *in vitro*. Addition of either GFP or Bcl-2 adenoviruses did successfully transfect neurons, but at a very low efficiency (approximately 1–2%).

This suggests that the trigeminal sensory neurons do not express the appropriate receptors required for adenoviral transfection. Thus there seems to be limited success and usefulness of adenoviral mediated Bcl-2 transfection and over-expression in promoting cell survival to aid regeneration of trigeminal nerves.

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P11

Antimitotic treatment to achieve neurone enriched ventral mesencephalic cultures for studies of neurotrophins

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Parkinson's disease is characterised by the progressive degeneration of midbrain dopaminergic neurones. Neurotrophins, such as GDF-5 and GDNF, have been proven to have potent effects on dopaminergic neurones both *in vitro* and *in vivo*. However the mechanism of action of these proteins is as yet unknown and the possibility exists that it may be dependent on the presence of glia. GDF-5 has been shown to increase astroglial numbers in culture (Krieglstein et al. *J. Neurosci. Res*. **42**, 1995). Thus increases in the numbers of dopaminergic neurones observed in mesencephalic cultures after the addition of GDF-5 may be dependent on the presence of glia. This possibility will be investigated by examining the effects of GDF-5 on glial-depleted ventral mesencephalic cultures. Cells were removed from ventral mesencephalon of E14 embryos removed under terminal anaesthesia, and treated with 5-fluoro-2′-deoxyuridine (an antimitotic agent). Glial cell numbers were assessed using antibody to GFAP in both glia-depleted and mixed cell cultures to assess the effects of the antimitotic. Then the effect of glial cell depletion on dopaminergic neuronal survival was assessed. The effects of GDF-5 on dopaminergic neurone survival in glia-depleted neuronal cultures will be compared with those in mixed cell cultures to investigate whether or not a glial presence is necessary for the neurotrophic action of GDF-5.

P12

Investigation of the expression of Growth/Differentiation Factor-5 in the developing rat brain

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Growth/Differentiation Factor-5 (GDF-5) is a member of the Transforming Growth Factor-β superfamily (Storm et al. *Nature* **368**, 1994). It has been shown to be a potent neurotrophic factor *in vitro* (Krieglstein et al. *J. Neurosci. Res*. **42**, 1995) and *in vivo* (Sullivan et al. *Neurosci. Lett*. **233**, 1997) for rat dopaminergic midbrain neurones, those that degenerate in Parkinson's Disease. However there is no information on the role of GDF-5 in the normal brain. We have examined the expression of GDF-5 protein in the developing rat brain using embryos of E11 to E21 removed under terminal anaesthesia.

Western blotting of monoclonal antibody for GDF-5 revealed 2 immunoreactive bands at 56.8 kDa and 54.8 kDa. This agrees with the predicted size of the mouse monomeric GDF-5 precursor protein (preproGDF-5). In the rat dopaminergic neurones differentiate at around E12 and proliferate up to E15. We have found GDF-5 levels to be maximal at E15. The GDF-5 immunoreactive bands remain detectable throughout the developmental period. They are present in homogenates of rat ventral mesencephalon at E14 and E15, which contains developing midbrain dopaminergic neurones. Intense immunoreactivity was also found in the adult striatum and midbrain suggesting a possible role for this molecule in the maintenance of the adult nigrostriatal system. This is the first report detailing the expression of GDF-5 protein in the developing embryonic and adult rat brain. Its expression pattern matches the differentiation of dopaminergic neurones and the presence in the adult suggests that GDF-5 possibly provides support for dopaminergic neurones *in vivo*, lending support to the notion that GDF-5 could be used as a potential treatment for Parkinson's Disease.

P13

Growth/Differentiation Factor 5 enhances dopaminergic graft survival and reverses motor asymmetry in a rat model of Parkinson's disease

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Parkinson's disease is a primary neurodegenerative disorder, the cardinal feature of which is selective degeneration of the nigrostriatal dopaminergic system. Resultant clinical symptoms and deficits are alleviated by exogenous dopamine replacement. Unilateral injection of 6-hydroxydopamine into the medial forebrain bundle (under anaesthesia using a stereotaxic frame) results in near total ablation of the nigrostriatal neural pathway on that side with consequent motor asymmetry due to ipsilateral dopamine deficiency. In rodents this represents a robust model of Parkinson's disease. Endogenous dopamine replacement by dopaminergic grafts derived from ~E14 rat embryos has proven effective in diminishing motor deficits though is compromised by poor graft survival. Neurotrophic growth factors aid graft survival and functional integration. Growth/Differentiation Factor 5 (GDF-5) is a recently discovered growth factor with neuroprotective properties *in vitro* and *in vivo*. We demonstrate the beneficial effects of GDF-5 on dopaminergic graft survival and consequent reversal of motor asymmetry in the 6-OHDA rat model of Parkinson's disease.

P14

Neuroprotective effects of Growth/Differentiation Factor-5 in a rat model of Parkinson's disease

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Growth/Differentiation Factor-5 (GDF-5) is a member of the transforming growth factor β superfamily, which has protective effects on nigrostriatal dopaminergic neurones *in vitro* (Krieglstein et al. *J. Neurosci. Res*. **42**, 1995) and *in vivo* (Sullivan et al. *Neurosci. Lett*. **233**, 1997). We have compared the neuroprotective effects of GDF-5 with those of GDNF, a well-established dopaminergic neurotrophin. We administered GDF-5 or GDNF into the adult rat striatum and substantia nigra (SN) at the same time as a complete 6-hydroxydopmaine (6-OHDA) lesion of the medial forebrain bundle (MFB) using a ketamine/xylazinium anaesthetic. We found that 50 μg GDF-5 is at least as effective as 50 μg GDNF in preventing amphetamine induced rotations, a measure of striatal dopamine levels. We also found that this dose of GDF-5 is as effective as GDNF in promoting dopaminergic cell survival in the SN. At a dose of 10 μg, GDF-5 showed significantly greater protective effects than 10 μg GDNF. Application of both GDF-5 and GDNF in combination did not significantly increase the effects above those induced by either factor alone. This study demonstrates that GDF-5 can protect nigrostriatal dopaminergic neurones against 6-OHDA toxicity. Its capacity to induce neuroprotective effects at a lower dose than that of GDNF highlights the potential for therapeutic use of GDF-5 in Parkinson's disease.

P15

Further observations on neuronal somata and dendrites in chronic schizophrenia

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Clinical, imaging and immunocytochemical studies implicate prefrontal dysfunction in schizophrenia. Although the primary cause remains unknown, it is clear that the glutamatergic neurotransmitter system is compromised (Hirsch et al. *Pharm. Bioch. Behav.* **56**, 1997). Pyramidal neurons in layer III of the cerebral cortex are glutamatergic and project within and between cortical areas. Their dendrites are covered in spines, which are the main sites of excitatory input and are reduced in schizophrenia (Garey et al. *JNNP* **65**, 1998). In our study, the Rapid Golgi technique was used to impregnate neurons in prefrontal cortex (area 9) of 9 chronic schizophrenics and 7 controls from the Charing Cross Prospective Schizophrenia Study, with Ethical Committee approval and consent of patients, family and/or legal representatives. Morphological parameters (spine numerical density, soma area, total dendritic length, number of basal dendrites and branching complexity) were measured in 10 layer III pyramidal cells from both cerebral hemispheres. Using ANOVA analysis for each parameter, control and schizophrenic groups, apical and basal dendrites, and the two cerebral hemispheres were compared. Significant differences were found in spine density on apical and basal dendrites between groups, which occurred in a lateralised manner ($P < 0.016$). Apparent differences were also noted in cell body area and dendritic parameters though the results were not significant. There were significant lateralised differences in the number of dendritic segments between controls and schizophrenics ($P < 0.035$). Analysis of Nissl stained sections revealed significant lateralised changes in neuronal numerical density between diagnostic groups ($P < 0.015$) and cortical thickness was significantly increased in the schizophrenic group ($P < 0.05$). These differences reflect the subtle nature of the pathology associated with schizophrenia.

P16

NG2 glia (oligodendrocyte progenitor cells) in the adult rat spinal cord

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Injury to the central nervous system (CNS) results in the laying down of a glial scar which is a potential 'no-go' zone for regenerating axons. The glial scar may not only be a physical barrier for neurite growth but it also contains numerous molecules inhibitory to axon growth, one of which is NG2, a chondroitin sulphate proteoglycan. NG2 is expressed in the CNS by a novel type of glial cell that has an oligodendrocyte progenitor cell (OPC) antigenic phenotype and does not express any of the phenotypic markers for mature astrocytes, oligodendrocytes or microglia. Adult NG2 glia (OPC) have been shown to become reactive in response to a range of CNS insults and are hypothesised to inhibit axon regeneration in the CNS. The aim of this study was to characterise NG2 glia (OPC) in the normal spinal cord and investigate their response to a crush lesion in male rats, weighing 180–200 g For crush lesions, rats were anaesthetised by a single intraperitoneal injection of combined Hypnorm/Hypnovel anaesthetic (0.2133 $mgkg^{-1}$ fentanyl citrate, 6.75 $mgkg^{-1}$ fluanisone, 3.375 $mgkg^{-1}$ midazolam), and the dorsal funiculus of the spinal cord was exposed at the level of T12. The dorsal columns were crushed using fine forceps, and spinal cords analysed at 7 d post lesion. Untreated ($n = 6$) and lesioned ($n = 3$) rats were humanely killed by overdose of sodium pentobarbitone and perfused via the left cardiac ventricle with 4% paraformaldehyde. Spinal cords were dissected free and fixed overnight in the same fixative, prior to embedding in either polyethylene wax, for thin (7 μm) sections, or gelatin, for thick (30–50 μm) sections. Sections were single (ABC) or double (fluorescence) immunolabelled using antibodies for NG2 and glial fibrillary acidic protein (GFAP, for astrocytes). Results show that adult NG2 glia (OPC) are numerous in both the white and grey matter of the spinal cord, suggesting they may have a function other than being a pool of stem cells that can generate oligodendrocytes throughout life, since grey matter is devoid of myelin-forming oligodendrocytes. In the crush lesioned spinal cords, NG2 glia (OPC) and astrocytes became reactive at the lesion site to form the glial scar. Reactive NG2 glia (OPC) were characterised by increased expression of NG2 and the elaboration of a dense mat of intertwined processes. The astrocyte and NG2 glial (OPC) injury response decreased with distance proximal and distal to the lesion site. Adult NG2 glia (OPC), with astrocytes,

were also a significant component of the dorsal root entry zone (DREZ), consistent with the possibility that the NG2 CSPG has a inhibitory role at the CNS–PNS interface, blocking the growth of regenerating fibres into the CNS. These results form the necessary baseline for investigating the cellular and trophic pathology of NG2 glia (OPC) in response to a crush lesion of the dorsal columns of the rat spinal cord.

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P17

The dorsal root transitional zone model of CNS axon regeneration: morphological findings

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The dorsal root transitional zone (DRTZ) model was used to investigate axon degeneration and regeneration along the DR and into the CNS. Three protocols were used regeneration in the presence of neurotrophin-3 (NT3) (protocol A); regeneration in the absence of neurotrophin (protocol B). Protocol C provided background data on tissue changes in experimental controls in which axons were absent following DR ganglionectomy. In all protocols glial fringe complexes occurred at PNS levels. These showed extensive apposition of Schwann cells and astrocytic processes in common sleeves of basal lamina. In the normal TZ such apposition is very limited, being confined to the transitional node (Fraher and Kaar, *J. Anat*. **139**, 1984). Complex formation involves astrocyte process outgrowth under the basal lamina at the erstwhile node gap. It indicates lessening of any repulsion between the cell types. A fine endoneurial cytoplasmic reticulum resembling that in cranial nerve rootlets (Fraher and O'Leary, *J. Anat*. **184**, 1994) became increasingly prominent with time. In most axons at PNS levels of protocols A and B, myelination was absent. In a small number it was at an early stage. In protocol C, axons were absent, apart from a few small and occasional large myelinated axons in the PNS compartment. The latter were also seen in protocols A and B. The small axons may be autonomic, derived from perivascular plexuses. Some could represent distal outgrowths from the CNS (Carlstedt *J. Anat*. **190**, 1997). The large axons were probably derived from vagrant plexiform connections with undamaged roots. That they were not regenerating is clear from known growth rates (Fraher, *Brain Res*. **105**, 1976). In protocol A, axons traversed the CNS–PNS interface in a distinctive manner: they were commonly enfolded by a Schwann cell perikaryon immediately distal to it. This and its ensheathing processes were closely apposed to TZ astrocyte processes without intervening basal lamina. Within the CNS, axons frequently ran in groups separated by astrocyte processes from the persistent myelin debris.

P18

Body composition of the wobbler mutant mouse

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The Wobbler mouse is a widely used model for the investigation of human inherited motoneuron diseases, including amyotrophic lateral sclerosis (ALS).

In this presentation we document dramatic changes in the body composition of this mutant. In this study 6 wobblers (3-month-old-stage 4) and age matched controls were examined. All animals were anaesthetized and sacrificed by cardiac perfusion. There was found to be a 49% reduction in the total body weight of the wobblers. A number of organs were weighed and measured. The percentage reduction found in the wobblers was as follows; liver weight 45%, spleen 44%, kidney 37%, Intestinal length 26%. Qualitatively the suprarenal gland appeared to be much larger in the wobbler group.

The tissues were processed for electron microscopy. Stereological studies were performed on semithin sections of the small intestine taken from a proximal, middle and distal sites. In this presentation we document alterations in the cellular architecture of the gut wall. A 2-way analysis of variance (2AOV) of the Vv of villi showed a significant effect of site ($P < 0.01$) but no effect of disease or interaction. The Volume and surface area of villi and volume of muscle revealed a significant effect of site ($P < 0.01$) and disease ($P < 0.01$) but no interaction.

Stereological studies were also performed on the optic nerve and eye of these animals. The number of nonmyelinated axons in the optic nerve was increased in the wobblers, although there was no difference in the myelinated axon number or degree of myelination nor in the volume or numerical density of the glial cells.

There were changes in the composition of the eye in the mutants: a 2AOV revealed a significant effect of layer ($P < 0.01$), no effect of condition, but a significant interaction ($P < 0.01$) suggesting that the disease had a differential effect on different layers of the eye. The choroid layer was 65% thicker in the wobbler than in the control, and the outer nuclear layer was found to be thinner in the wobbler.

This study clearly demonstrates the complex nature of this mutant and it highlights the need for caution when using this or any other animal model.

P19

The ultrastructure of the ventral horn of the wobbler mutant mouse: a stereological study

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The homozygous Wobbler mouse represents a useful model for the investigation of human inherited motoneuron diseases,

including amyotrophic lateral sclerosis (ALS). The clinicopathological features of ALS differ from the Wobbler MND because of the lack of involvement of the human corticospinal tract. Nevertheless the pathogenesis of the mouse and human diseases may have some important common features.

Previous pathological reports have indicated that swollen and vacuolated motoneuron cell bodies are the most predominant feature characterising the wobbler mouse motoneuron disease. Here we extend our earlier stereological study (Dockery et al. *J. Anat*. **191**, 1997) which documented the light microscopical changes in neuronal volumes in the wobbler mutant.

In this study we document the ultrastructure of the ventral horn in wobbler mutants and control animals at 3 week (young, stage 1) and 3 month of age (old, stage 4) using stereological methods ($n = 6$ for each group). All animals were anaesthetised and sacrificed by cardiac perfusion.

A 2-way analysis (2AOV) of variance of the volume of rough endoplasmic reticulum data revealed a significant effect of condition ($P < 0.01$) for the volume of RER but no significant effect of age or interaction. The wobbler group was significantly smaller than age matched controls. A 2AOV of the volume of vacuoles showed a significant effect of condition ($P < 0.01$), age ($P < 0.05$) and interaction ($P < 0.05$). The 9 month wobbler had the greatest volume (representing 26% of the volume of the cell) reflecting the early involvement of vacuolation on the disease process. A 2AOV of mitochondrial volumes did not reveal any significant differences, although the volume of the 15 month wobbler group was almost half that of controls. Glial volume showed a significant effect (decrease) of age ($P < 0.01$) but no effect of condition or interaction.

This study combines new and traditional stereological methods to provide an insight into changes in the cellular composition of the ventral horn of the wobbler mutant mouse.

P20

Preparing for patients

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The effective clinical training of medical students has been challenged in recent years by the trend towards shorter hospital admissions, with reduced patient availability. Student-patient contact needs to be optimised so that the student gains maximally from each encounter with the patient.

In 1999, following a pilot programme, we introduced a clinical skills training programme for second year (preclinical) medical students. Students are taught clinical examination of a region/system in conjunction with the surface anatomy and clinical anatomy of that region. Each student practices that examination on a volunteer prior to visiting hospitals. The course also includes training in communication skills and minor procedures such as venepuncture. Students performed an evaluation of the new course on its completion. One year later that group of students have a practical exam on history taking and clinical examination. They will perform a further evaluation of the 'preclinical' course following that exam.

Early feedback from students and clinical teachers suggests that the course is helpful in preparing students for patient contact and further studies in clinical examination.