Proceedings of the Anatomical Society of Great Britain and Ireland, the Nederlandse Anatomen Vereniging and the Anatomische Gesellschaft

A Tripartite Meeting of the Anatomical Society of Great Britain and Ireland, the Nederlandse Anatomen Vereniging and the Anatomische Gesellschaft was held at St John's College, University of Cambridge, from 24th – 26th July 2000. It included symposia on 'The neuroanatomical basis of the emergence of behaviour' and on 'Anatomy, the challenges ahead'. An Anatomical Society Review Lecture was given by Professor Martin Johnson of the University of Cambridge, and a European Federation for Experimental Morphology (EFEM) Lecture by Professor Karl Zilles of the University of Düsseldorf. The following are abstracts of communications and posters presented at the meeting.

TALKS

1 Reproduction in the 'Noughties'—will the scientists have all the fun? By M. H. JOHNSON. *Department of Anatomy, University of Cambridge, UK*

The past 20 years have seen major advances in our ability to manipulate human and animal reproduction, as well as major social changes in human reproductive and sexual attitudes and behaviour. Many if not most of these advances and changes developed out of the first fully successful invitro fertilisation of the human oocyte which was achieved here in Cambridge 22 y ago. In this lecture, I will try to convey, through examples, something of the flavour of what is currently happening in Assisted Reproduction Clinics around the world and what is being talked about for the future in respect of emergent clinical needs and patient demands. I will use this clinically driven approach to discuss some underlying scientific aspects of reproductive research, touch on some of the ethico-legal issues that may arise, and comment on how this anticipated future might influence our current approaches to medical education. The future will be considered not simply in terms of the New Reproduction itself but also in its interaction with the opportunities and challenges presented by the New Genetics. It is perhaps in the interaction between these 2 fields of endeavour that the most difficult challenges ahead lie.

2 Nutrition of the human fetus during the first trimester: more histiotrophic than haemotrophic? By G. J. BURTON, E. JAUNIAUX, J. HEMPSTOCK and Y. -P. BAO. *Department of Anatomy, University of Cambridge, and Academic Department of Obstetrics and Gynaecology, University College London, UK*

It is generally believed that the invasive form of interstitial implantation displayed by the human blastocyst is associated with early onset of maternal blood flow within the developing placenta. Hence haemotrophic nutrition is thought to predominate once implantation is complete. Recent Doppler ultrasonographic evidence has cast doubt upon this however, as moving echoes indicative of significant blood flow cannot be detected prior to 12 wk of pregnancy. We have recently measured the oxygen tension within the intervillous space in vivo with informed written consent at various stages of gestation using a multiparameter probe. Values rose from below 20 mm Hg during the 8th week to over 60 mm Hg by the 15th week, with most of the increase occurring between wk 10–12. Increases in mRNA concentration and activity of the antioxidant enzymes catalase and glutathione peroxidase were also observed with gestational age ($r = 0.544$, $n = 20$, $P = 0.012$ and $r = 0.763$, $n = 19$, $P < 0.001$ for activity respectively). These findings confirm that there is little maternal blood flow to the placenta during the first trimester, and correlate with morphological observations that the openings of the maternal spiral arteries are plugged by aggregates of cytotrophoblast cells during this period. Reviewing the histological slides of placenta-in-situ specimens contained within the Boyd collection we have observed discharge of copious secretions from the uterine glands into the intervillous space at 6 wk of pregnancy and uptake of specific endometrial proteins, glycodelin A and MUC-1, into the syncytiotrophoblast. As pregnancy advances the uterine glands regress, but connections with the intervillous space can be observed up to 9 wk. We conclude therefore that the opportunity for haemotrophic nutrition is limited during the first trimester and that histiotrophic nutrition may represent an important pathway. We speculate that this may facilitate embryogenesis by reducing the risk of oxygen free radical associated teratogenesis.

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3 Placental defects in mice associated with imprinting on chromosome 12: implications for human placentopathy. By P. GEORGIADES, M. WATKINS, G. J. BURTON and A. C. FERGUSON-SMITH. *Department of Anatomy, University of Cambridge, UK*

Mammalian genomic imprinting is necessary for the correct development of both the embryo and extraembryonic tissues. Mouse chromosome 12 is imprinted because its 2 homologous copies are functionally different in a parental origin dependent fashion. These functional differences manifest as expression level inequalities between the 2 alleles of chromosome 12 linked imprinted genes. To investigate the role of these genes we have misexpresed them by generating conceptuses that inherited both copies of chromosome 12 from one parent (uniparental disomy 12; UPD12). Paternal UPD12 (pUPD12) conceptuses die late in gestation and have a variety of defects including pla-

centomegaly. Our aim was to investigate pUPD12 placental development in more detail.

We found that pUPD12 placentae have a variety of anatomical defects, as judged by immunohistochemistry, RNA in situ hybridisation and morphometric analysis. The earliest detectable anomaly was defective invasion of maternal spiral arteries (branches of the central maternal artery supplying the placenta) by placental invasive trophoblast. This was followed by abnormalities in trophoblast and fetal blood vessels of the labyrinthine zone that were likely to disrupt maternofetal exchange. Moreover the physiological changes that normally occur in the maternal spiral artery appeared to be absent.

We conclude that the correct expression of as yet unknown chromosome 12-linked imprinted gene(s) are necessary for normal mouse placental development. Intriguingly these defects are reminiscent of clinically important human placentopathies of unknown genetic basis. Understanding the molecular basis of pUPD12 placental defects may therefore have clinical implications.

4 Plasminogen activators and inhibitors are transcribed during early macaque implantation. By Q. FENG[1,2], K. LIU[3,4], Y.-X. LIU[2], S. BYRNE[1] and C. OCKLEFORD[1]. [1]*Department of Pre-Clinical Sciences, University of Leicester, UK;* [2]*State Key Laboratory of Reproductive Biology Chinese Academy of Sciences, Beijing, China;* [3]*Department of Medical Chemistry and Biophysics, Umeå University, Sweden;* [4]*The Center for Blood Research, Harvard Medical School, USA*

Plasminogen activators and inhibitors may be important early in primate implantation but evidence for this is sparse in nonhuman primates. We defined the expression of urokinase type plasminogen activator (uPA), tissue-type plasminogen activator (tPA), plasminogen activator inhibitor type1 (PAI-1) and type 2 (PAI-2), receptor for uPA (uPAR) and fibrin/fibrinogen in primate implantation sites. In situ hybridisation and immunohistochemical localisation of rhesus implantation sites (15–16 d postovulatory) indicated: (1) uPA mRNA was localised to placental trophoblast, epithelial plaque, and endometrial stroma; (2) tPA mRNA, was mainly expressed in glandular cells of endometrium; (3) PAI-1 expression was linked with a specific population of trophoblast that confront maternal cells, adding support to our view that it has a regulatory role in trophoblast invasion; (4) localisation of tPA antigen confirmed that uterine glands are the major source of tPA and that it is also closely associated with fibrin(ogen) suggesting its possible function during implantation is fibrinolysis; (5) Unlike uPA mRNA however the distribution of uPA protein and its cell surface receptor uPAR suggested that it mediates trophoblast invasion and plays a significant role in angiogenesis; (6) PAI-2, the inhibitor associated with pregnancy in humans, was found in unidentified cells located specifically along the maternofetal junction. This localisation adjacent to areas of cell death at the maternofetal junction implies that it may have a role as a protective curtain with anti-apoptotic function. In conclusion our results suggest that gene expression of PAs and PAIs in early implantation sites are tissue specific, location sensitive and function related.

5 Perivillous fibrin-type fibrinoid in human placenta is deposited preferentially at sites of syncytiotrophoblast de-epithelialisation. By T. M. MAYHEW, C. BOWLES and G. ORME. *School of Biomedical Sciences, University of Nottingham, UK*

Perivillous fibrin-type fibrinoid (pFTF), a blood coagulation product, is found in the maternal intervillous space of normal and other human placentas and associated with the surfaces of villi. It is suggested that pFTF preferentially forms in regions of trophoblast degeneration/damage but there have been no rigorous tests of whether deposits are non random or differ in alternative types of pregnancy. We have devised a stereological and statistical method for testing these predictions. Random samples of microscopical fields were stained with connective tissue stains and lattices of test lines were superimposed so as to be random in location and orientation. At least 3 subdomains of the villous surface may be distinguished: nonsyncytial knots (nonSK), syncytial knots (SK) and sites of trophoblast denudation (DEN). If necessary SK can be subdivided to account for syncytial 'bridges and branchpoints'. The relative numbers of test line intersections with subdomains provided a convenient expected distribution (the pattern to be seen if pFTF is deposited randomly). Subsequently, this was compared with an observed distribution which was calculated from the actual numbers of intersections with subdomains associated with pFTF (e.g. nonSK + pFTF, SK + pFTF and DEN + pFTF). Expected and observed distributions were compared by χ^2 analysis. If the null hypothesis (no difference between expected and observed distributions) is rejected, χ^2 values for individual subdomains help to identify sites of preferential deposition. Comparisons could be drawn for individual placentas within a group, for groups of placentas (to assess interplacental variability) or between groups of placentas (e.g. to test whether deposition patterns differ in control and other pregnancies). So far results from analyses of normal and complicated (high altitude and maternal cigarette smoking) term pregnancies have shown that pFTF deposition is nonrandom and preferentially located at sites of epithelial denudation. This finding is highly reproducible (detected in every placenta examined). Results also indicate that despite being nonrandom at low and high altitudes pFTF deposition patterns differ between altitudes. Denudation may occur via syncytial degeneration but could also, at least in part, be a consequence of normal trophoblast turnover. In this process, SK fragments rich in (pre-)apoptotic nuclei detach and are deported via the maternal intervillous space. Nascent detachment sites are covered by pFTF prior to re-epithelialisation. The methods could be used to test predictions about relationships between pFTF, syncytial knot formation, apoptosis, the phosphatidylserine flip, annexin-V binding and antiphospholipid antibodies in normal and complicated pregnancies.

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6 Antibodies to human placental endothelium can be selected from chicken-derived phage display antibody libraries by panning on paraffin sections. By A. VERSMOLD, U. SCHMITZ, P. KAUFMANN and H.-G. FRANK. *Department of Anatomy, University of Technology, Aachen, Germany*

For the study of placental angiogenesis, it is important to obtain antibodies which (a) react against endothelial cells at various stages of angiogenesis, (b) are applicable on paraffin sections. Antibodies with these characteristics are rare. In order to generate such antibodies we have immunised chickens with placenta preparations and prepared phage display antibody libraries from the splenocytes of the immunised animals. The libraries were built in pComb3HSS with scFv-coding DNA-constructs. With panning strategies against human adult blood and human cord blood, we have generated a phage display library with scFv phage clones recognising epitopes on blood cells and endothelial cells. In a second selection procedure we have panned on paraffin sections of 1st trimester and term human placenta. Phages and the endothelial cells to which they were bound were isolated by microdissection of the paraffin sections. By this method, we have selected 5 clones which specifically react with endothelial cells and/or thrombocytes in paraffin sections. Blots revealed that the antigens comprised Factor VIII related antigen as well as other endothelium specific proteins. In summary, panning on paraffin sections is a useful tool for the selection of anti-endothelial antibodies. It is a way to include both, the effects of tissue pretreatment and embedding and the morphological expertise of the investigator into the selection of monoclonal antibodies. Moreover we now use this approach for selection of other clones of interest, which mark specific cell populations in paraffin sections of the human and mouse placenta.

7 Liver stem cells. By M. R. ALISON[1], R. POULSOM[2], C. SARRAF[1] and N. A. WRIGHT[1,2]. [1]*Department of Histopathology, Imperial College School of Medicine; and* [2]*Histopathology Unit, Imperial Cancer Research Fund, London, UK*

There are at least 3 possible tissue sources of hepatocyte progenitor cells. The hepatocytes themselves can be considered as the functional stem cells since these are the cells that normally divide in response to parenchymal damage. As they need only to traverse the cell cycle 2–3 times to restore preoperative cell mass in response to a two-thirds partial hepatectomy, it is often mistakenly believed that hepatocytes have only limited division potential. On the contrary, transplanting enzyme-positive (e.g. dipeptidyl peptidase IV+ or fumaryl acetoacetate hydrolase+) hepatocytes into enzyme-deficient rodents has demonstrated the massive division capacity of at least some hepatocytes. A potential stem cell compartment resides in the intrahepatic biliary tree, but it is only activated when either hepatocyte regeneration is compromised or when parenchymal damage is extensive. The progeny of these stem cells are known as oval cells; during their amplification phase they display biliary phenotypic markers, but biliary traits disappear with hepatocyte differentiation. In rodents this stem cell compartment resides in the small interlobular ducts and/or canals of Hering that abut the limiting plate, but in humans the canal of Hering that extends deep into the lobule is the likely location for these potential stem cells. The plasticity of murine haemopoietic stem cells is widely appreciated, and mouse and rat haemopoietic stem cells are apparently capable of differentiating into hepatocytes in vivo. Human haemopoietic stem cells are also able to differentiate into hepatocytes: Y chromosome-positive hepatocytes can be found in the livers of female patients after they have been transplanted with male bone marrow. Combined with the relative ease by which haemopoietic stem cells can be harvested from umbilical cord blood, there is every prospect that soon it will be possible to propagate hepatocytes from cultures of human haemopoietic stem cells.

8 Hepatocyte precursors and haematopoietic stem cells. By D. BRYNMOR THOMAS. *School of Biomedical Sciences, University of St Andrews, UK*

When hepatocyte proliferation in response to partial hepatectomy is inhibited, regeneration of the liver is effected by the proliferation and differentiation of oval cells, which are similar in appearance to haematopoietic stem cells. The 2 cell populations are accommodated in the liver before and after birth, increase in size following partial hepatectomy, express the same antigens, share several biochemical markers and are exceedingly difficult to separate. These similarities have prompted the suggestion that haematopoietic stem cells and oval cells are the closely related progeny of a single cell population. This interpretation is consistent with the occurrence of spectra of morphological continuity that link both haematopoietic stem cells and oval cells to generalised blast cells, which are in turn linked to hepatocyte precursors on the one hand and on the other to orthochromatic erythroblasts. It has now been endorsed by the observation of host hepatocytes in liver allografts and of donor oval cells and hepatocytes in irradiated rodents following bone marrow transplantation. The presence of only occasional transplanted oval cells and hepatocytes in the liver contrasts with the replacement of host cells by donor cells in the blood, the medullary cavities and the lymphoid tissues, suggesting that hepatocyte precursors as well as hepatocytes are less radiosensitive than haematopoietic stem cells. A more substantial increase in the proportion of donor hepatocytes, following the availability throughout development of stem cells exchanged between dizygotic twins or introduced by prenatal transfusion, would confirm the ability of cells derived from extrahepatic sources to infiltrate the liver and differentiate into hepatocytes but it would not clarify the relationship between extra hepatic precursors of hepatocytes and the earliest precursors of blood cells. Precise information about the properties of isolated, sorted and adequately characterised stem cells, from each of the populations under consideration, will be required to elucidate the relationship between oval cells and haematopoietic stem cells and the no less unexpected relationships which have been described between haematopoietic stem cells and muscle stem cells, neural stem

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cells and primordial germ cells. For this purpose assays of proliferation and differentiation comparable to those which are being used to study haematopoietic stem cells will be required. At the same time it will be necessary to characterise the micro-environments which stem cells occupy. Meanwhile there must be serious doubt about the validity of many currently accepted notions relating to the ontogeny of cell populations but great excitement about the possibility —however remote—that suspensions of blood cell precursors may include cells capable of contributing to the growth or regeneration of the liver, muscle, nervous tissue or the reproductive system, in view of the ease with which blood cell precursors can be transplanted.

9 Do stem cells exist that are capable of repopulating skeletal muscle? By K. GOLDRING[1], G. E. JONES[2] and D. J WATT[1]. [1]*Department of Neuromuscular Diseases, Division of Neuroscience & Psychological Medicine, Imperial College School of Medicine; and* [2]*Department of Anatomy, The Randall Institute, King's College London, UK*

Skeletal muscle forms by fusion of mononuclear precursor cells. In mature muscle, a reserve population of precursors remain between the muscle cell sarcolemma and the basal lamina of each fibre. Such satellite cells are recruited to form new muscle fibres when injured muscle regenerates. Evidence exists for a myogenic stem cell within this satellite cell population. However is this the only stem cell that can repopulate regenerating muscle? Recent evidence shows a cell within bone marrow also acts in such a role. Our work suggests a cell in the dermis of skin can also contribute to fibre formation. High numbers of dystrophin positive fibres were formed when normal mouse dermal fibroblasts were implanted into dystrophin negative *mdx* mouse muscles. Dermal fibroblasts which fused with host muscle cells also expressed endogenous genes within fibres, suggesting these cells converted to a myogenic lineage and participated in new fibre formation. Our in vitro studies showed only 10% of cells converted and only when grown in myoblast conditioned-medium. Is there therefore a subpopulation of dermal cells more capable of converting than the general population? Dermal fibroblasts were cloned and grown in myoblast conditioned-medium. 40% of cells converted in clones compared with a 10% conversion rate with uncloned cells. Is there also a factor within conditioned-medium that converts the cells? A number of factors act on muscle tissue, but one that attracted our attention was the lectin Galectin-1 shown to be secreted by myoblasts and myotubes and to be a cell growth regulator of fibroblasts. Transfection of COS-1 cells with a plasmid containing the Galectin-1 construct CDM8 resulted in Galectin-1 being secreted into the COS cell medium. 30% of noncloned dermal fibroblasts converted in Galectin-1 conditioned medium compared with 100% of cloned cells. It appears there is a cell in the dermis with greater converting capabilities than others, conversion being greatly enhanced when cloned cells are grown in medium enriched with Galectin-1.

10 Analysis of cranial neural crest migratory pathways in axolotl using cell markers, homotopic and heterotopic transplantation. By H,-H. EPPERLEIN[1], D. MEULEMANS[2], H. STEINBEISSER[3], M. BRONNER-FRASER[2] and M. SELLECK[4]. [1]*Institut für Anatomie, Technische Universität, Dresden, Germany;* [2]*Division of Biology and Beckman Institute, California Institute of Technology, USA;* [3]*Max-Planck-Institut für Entwicklungsbiologie, Tübingen, Germany; and* [4]*Department of Cell and Neurobiology, University of Southern California School of Medicine, Los Angeles, USA*

We have examined the ability of normal and heterotopically transplanted neural crest cells to migrate along cranial neural crest pathways in the axolotl using focal DiI injections and in situ hybridisation with the neural crest marker AP-2. DiI labelling demonstrated that cranial neural crest cells migrate along prescribed pathways to populate the maxillary and mandibular processes of the first branchial arch, the hyoid arch and gill arches 1–4, following migratory pathways similar to those observed in other vertebrates. This pattern was confirmed using the transcription factor AP-2 as a neural crest marker. It was expressed within premigratory neural crest cells of the neural folds, and on neural crest cells as they migrate to and populate the branchial arches. Rotations of the cranial neural folds suggested that premigratory neural crest cells are not committed to a specific branchial arch fate, but can compensate when displaced short distances from their targets by migrating to a new target tissue. In contrast, when cells were displaced far from their original location they appeared unable to respond appropriately to their new milieu such that they failed to migrate or attempt to return to their former target region. Most dramatically, when trunk neural folds were heterotopically grafted into the head, trunk crest cells migrated in a highly disorganised fashion and failed to follow normal cranial neural crest pathways. Despite the random nature of their migration, we found incorporation of some trunk cells into branchial arch cartilage. This is the first demonstration that trunk neural crest cells can form cartilage when transplanted to the head. These results indicate that although cranial and trunk neural crest cells have inherent differences in ability to recognise migratory pathways, trunk neural crest can differentiate into cranial cartilage when given proper instructive cues.

11 Migration of cerebellar external granule cells is controlled by meningeal cells via the SDF-1/CXCR 4 signalling pathway. By K. REISS[1], R. MENTLEIN[1], J. SIEVERS[1] and D. HARTMANN[2]. [1]*Department of Anatomy, University of Kiel, Germany; and* [2]*Centre for Human Genetics, Catholic University of Leuwen, Belgium*

The cerebellar external granular layer (EGL) is a highly unusual secondary proliferation zone established by neuronal and glial stem cells migrating out of the ventricular zone at the caudal margin of the rhombic lip to attain a position immediately beneath the pial surface. Meningeal fibroblasts play a decisive role in the attraction of neural stem cells to the cerebellar surface and thus in the formation and maintenance of the EGL (Sievers, *J. Neurocytol.* **23**, 1994; Hartmann, *J. Neurocytol.* **27**, 1998) as their selective

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destruction leads to the evacuation of the EGL and the formation of widespread cortical ectopia. Similar ectopia have recently been shown to occur as a side effect of the genetic knockout of stroma-derived factor 1 (SDF-1), a chemokine normally discussed as an important regulator of hematopoietic precursor cell chemotaxis (Ma, *Proc. Natl. Acad. Sci. USA* **95**, 1998). We have thus set out to investigate the possibility whether this chemokine and its specific receptor CXCR4 could be the molecular mediators of the meningeal-neuroepithelial interaction governing cerebellar development.

Our immunohistochemical data indicated that SDF-1 is present in meningeal cells both in vivo and in vitro, where it is rapidly secreted into the medium. Correspondingly, CXCR4 immunoreactivity could be shown on stem cells of the EGL, but was absent on still undifferentiated cells migrating away from it towards the IGL as well as from maturing internal granule neurons themselves. Fitting into data that SDF-1 in vivo is concentrated by heparan sulphate proteoglycans, we demonstrated the presence of perlecan immunoreactivity exclusively within the EGL, but not in other regions of the cerebellar anlage. In vitro, SDF-1 could be shown to attract undifferentiated neuronal cells isolated from the EGL chemotactically in a Boyden chamber assay.

Our findings indicate that SDF-1 is employed as a short-range chemotactic factor secreted from meningeal cells and concentrated by perlecan within the EGL to attract proliferating stem cells to the surface, and that its action is limited by modulation of receptor expression.

12 The effect of p75 on developing cholinergic neurons of the mouse medial septum is modulated by the genetic background. By T. NAUMANN[1], E. CASADEMUNT[2], E. HOLLERBACH[1], M. FROTSCHER[1], G. DECHANT[2], and Y.-A. BARDE[2]. [1]*Institute of Anatomy, University of Freiburg; and* [2]*Department of Neurobiochemistry, Max Planck Institute of Neurobiology, Martinsried, Germany*

The function of the neurotrophin receptor p75 in regulating the survival of central basal forebrain neurons during postnatal development is unclear. In the rat medial septum/diagonal band (MSDB) cholinergic neurons express both trkA and p75, and it has been reported that both receptors are required for NGF signalling. p75 seems to be involved in the induction of apoptosis of MSDB neurons, though the data reported in the literature are somewhat controversial. One reason might be the use of genetically different mouse strains.

In the present study we investigated whether the effects of p75 on the number of cholinergic MS neurons are influenced by the genetic background. Two different deletions of the p75 locus (Lee et al. 1992; Dechant et al. unpublished), which were initially generated in mixed Sv129, BalbC and C57B16 (B6) backgrounds, were transferred into a congenic B6 background by consecutive backcrossing into this inbred strain. For statistical analysis cell counts were performed for each genotype ($n = 10$) at P15 and at 3 months of age using ChAT immunolabelling of cholinergic MS neurons. In addition, based on studies combining retrograde tracing and fluorescence immunocytochemistry for ChAT, the boundaries of the MS were determined for each mutant line.

We found that: (1) an increase in the genomic B6 component of a mouse is accompanied by a lower number of cholinergic MS neurons at P15, (2) the p75 mutation generated by Lee et al. leads to a significant increase in the number of cholinergic neurons only in the B6 background, whereas (3) the effect of the Dechant et al. mutation seems to be less background dependent and (4) the phenotypic effect of the Dechant et al. deletion is more pronounced than that of the Lee mutation. We are currently investigating whether the effects observed in P15 mice persist into adulthood.

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13 Postnatal development of the astroglial and microglial cells in the paraclaustral reservoir. By O. NARKIEWICZ, B. LUDKIEWICZ, B. DOMARADZKA-PYTEL and J. MORYŚ. *Department of Anatomy and Neurobiology, Medical University of Gdańsk, Poland*

In the rat brain at birth the distinct group of small cells lying in the ventral part of external capsule (paraclaustral reservoir) is clearly visible. It contains cells generated in the neocortical neuroepithelium which migrate in the lateral cortical stream and accumulate in this area. Our previous morphometric investigations have shown that in Nissl stained sections the paraclaustral reservoir disappears at the end of the first postnatal week.

28 rat brains of various ages sampled in the day of birth (P0) and postnatal days P4, P7, P10, P14, P21 and P30 were studied. Animal care and use followed National Institute of Health guidelines. After perfusion fixation the brains were frozen, and cut on the cryostat in the coronal plane and stained either with cresyl violet or with immunohistochemical methods using the antibodies OX-42, ED1 (for microglia) and GFAP (for astroglia).

From birth to P4 most of the GFAP positive cells in the paraclaustral reservoir resembled transitional astroglia. Starting from the end of first postnatal week they showed morphological features of the astrocytes present in adult animals. However during the next week their density was somewhat higher than in neighbouring areas. On P21 the shape and density of astroglial cells did not differ from that in surrounding regions. ED1/OX-42 positive microglial cells were present in the paraclaustral reservoir during the first postnatal week as amoeboid structures. During the second week they began to transform into the ramified microglia and after P21 only OX-42 positive resting microglial cells were observed in this area.

These results and our previous investigations suggest that paraclaustral reservoir is a site of transient accumulation for migrating neurons as well as astrocytes and microglia. The neuronal population disappears about P7, whereas accumulation of glial cells occurred over a week later. Microglia and astroglia of the paraclaustral reservoir may serve as the source of glial cells for other areas well as performing local developmental functions.

14 Immunohistochemical studies on the development, distribution and innervation of neuroendocrine cells in the human female urogenital system. By S. LICHTE, N. OMLOR, E. WEIHE and G. AUMÜLLER. *Department of Anatomy and Cell Biology, Philipps-University, Marburg, Germany*

The presence of so-called 'clear' or paracrine cells has been described by Feyrter (*Z. mikr.-anat. Forsch.* **S7**, 1951) in the human urethra, urinary bladder and the endometrium of the uterus, whereas they have not been found in the ovary, fallopian tube and vagina. As 'clear cell' or neuroendocrine carcinomas have been observed, e.g. in the cervical glands, we studied the distribution, development and presumptive innervation of the human female urogenital system. Paraffin serial sections from 9 human fetuses (archival material, 7–14 wk gestation), a 7 y old girl and a 32 y old woman were used for immunohistochemistry. Monoclonal and polyclonal antibodies (both commercial and prepared in house) were used against chromogranin A (CgA), protein gene product 9.5 (PGP 9.5), tyrosine hydroxylase (TH), vesicular monoamine transporter 2 (VMAT-2), vesicular acetylcholine transporter (V-AChT), bombesin, calcitonin and serotonin.

No CgA-immunoreactivity could be detected in any genital organ anlage of a 9 wk embryo. In a 10 wk embryo, a few CgA-immunoreactive cells were present in the epithelium and several more in the stroma of the urogenital sinus. Also CgA-immunoreactive ganglionic cells, some of which also contained bombesin, TH, and serotonin/VAChT immunoreactivity, were present in the parametrial ganglia, containing several VAChT-ir nerves. Both the epithelium and the stroma of the Mullerian ducts were free of NE cells. The number of NE cells in the epithelium of the urogenital sinus and especially of its stroma increased up to the 12 wk and then decreased. In the infant and adult female urogenital system neuroendocrine cells were restricted to the epithelium of the urethra. In no case were the cells innervated.

The distribution pattern of NE cells in the female urogenital tract is clearly different from that in the terminal rectum of the same specimens, where their number was highest in the transition zone, consisting mostly in the 'open' type, whereas in the colorectal zone, their number was lower and consisted of the 'closed' type. In contrast to the fetal urogenital stroma, no neuroendocrine cells were present in gut stroma. We conclude there are significant differences in the development and distribution of the neuroendocrine cells of the gut and urogenital system.

15 Simulation of angiogenesis, vascular remodelling and haemodynamics in normal and neoplastic microcirculatory networks. By R. GOEDDE[1], W. DÜCHTING[1] and H. KURZ[2]. [1]*Institute of Regulatory and Controlling Technology, University of Siegen; and* [2]*Institute of Anatomy II, University of Freiburg, Germany*

We introduce a new computer model for the simulation of microvascular growth and remodelling into arteries and veins that imitates angiogenesis and blood flow in real vascular plexuses. The simulation was based on geometric and biophysical initial and boundary conditions. Geometry was defined on a hexagonal grid using defined sources and drains and elementary bifurcations that were able to proliferate or to regress under the influence of random and deterministic processes. Biophysics was defined by pressure, flow and velocity distributions in the network using the nodal-admittance-matrix method and accounting for haemodynamic peculiarities such as the Fahraeus-Lindqvist effect and exchange with extravascular tissue. The simulated vascular systems accounted for geometric limitations due to finite microvessel thickness, and were mapped on spheroidal surfaces. The proposed model is the first to simulate interdigitation between the terminal branches of arterial and venous trees. This was achieved by inclusion of vessel regression and anastomosis in the capillary plexus, and by remodelling according to haemodynamics. The choice of regulatory properties influenced the resulting vascular patterns. The model predicts homogeneous haemodynamic patterning, if shear stress-, but not if pressure-dependent remodelling was applied. By closely approximating details of natural vascular patterns we can calculate homogeneity of transport, spatial distribution of haemodynamic properties and biomass allocation to the vascular wall or blood. The model can be applied to characterise transport characteristics in tumours and may provide a rationale for anti- or pro-angiogenic therapeutic approaches.

16 Basic information to define quality standards for anatomical internet sites. By T. J. FILLER, F. DIEDERICH, F. PERA and E. T. PEUKER. *Institute of Anatomy, Clinical Anatomy Division, Westfalian Wilhelms-University Münster, Germany*

The purpose of this study was the determination of possibilities for anatomical Internet sites in content, technical realisation and features. Thus the basis for defining requirements and standards for websites of anatomists can be provided. Regarding our previous surveys we decided to take the most advanced sites and focused our investigation on US and Canadian homepages. First, US and Canadian websites were localised by means of search engines and indices. Next, our former criteria to investigate anatomical websites were defined more specifically. Evaluation was focused on technical parameters (editors, scripts, programming languages, dynamic generation, implementation of multimedia, and compatibility with important browser software and plug-ins), design and user guidance, and realisation of contents (actuality, amount, presentation, education, body donor system, additional and commercial links).

The most important data out of 163 websites from anatomical institutes are presented. The amount of multimedia content was 12% in education, 6% in self presentation, and 3% in research. In 4% no consideration was given to browser compatibility. HTML 3 was used in 56% and HTML 4 in 44%. 17% of the websites were generated dynamically. The following scripts and programming languages were applied: JAVA (37), CGI (27), CSS (18), Perl (9), Active X (7), and DHTML (2). Among these 7 insecure applications were found. Both self presentation and information were offered from all institutes, however, different priorities were given. User orientated realisation predominated over presentation orientation ones. A uniform design and structure, easy to navigate, was found in most sites. This was maintained by use of frames, sitemaps, indices, and search routines. Lists of scientific literature

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were present in 64% (17% abstracts, 3% full text). Other main topics were annotated educational content (40%), with 52% having additional links such as teaching aids. 11% of the anatomical institutes give information on the body donor system.

These findings show a broad spectrum of contents exceeding a pure self presentation by far. Almost the complete range of innovative techniques is applied without overtaxing the users' possibilities. With regard to content students appear to be the main target group. Obviously subject matter and technique are mainly reconciled with the users' needs. However, a certification or specific declaration of responsibility for content is not provided. These results may serve as indicators for the maintainers of German and European anatomical internet sites especially for increase and preservation of user-oriented quality.

17 The incidence of false probability estimates in biological and medical research. By N. T. JAMES. *SigmaMetrics Statistical Consulting Services, Sheffield, UK*

In a detailed analysis of a systematic random sample of 1000 papers in biological and medical journals in the copyright libraries of Oxford and Cambridge Universities the majority were found to contain incorrectly calculated statistical values. Typical errors included the inadequate use of assumption testing for the valid use of both normal and nonparametric (distribution free) statistics, e.g., for distributional properties, (symmetry and homogeneity of variance), autocorrelation and multiple comparison effects. The application of appropriate levels of data (nominal and interval) to specific tests were often ignored and replaced by incorrect ordinal or hedonic levels of data. Many authors seriously misused ANOVA (analysis of variance) techniques and interaction effects were usually ignored. Inappropriate significance testing was common. In general, independent calculations (e.g., using QBasic algorithms in all MSDOS issues post 5.0) were rarely used and results presented to preclude independent statistical calculations from being performed. Causes of statistical error appear to be multifactorial and include the use of inadequate contemporary practices of evidence based medicine, sequestration of real data on transient journal web sites, availability of recipe based statistical packages, poor supervision of higher degree candidates and the practice of replacing primary data by secondarily derived data which prevents subsequent independent data analysis. The retention of papers on BIDS database sites for only limited periods exacerbates poor statistical practice by example often reflected in some standard textbooks. Statistical assumptions should never to be assumed but are always specifically testable entities. The use of unbiased methodology never offers protection against biased results. Many published studies (including anatomy) failed to extract all possible statistical information. Implications for medical and biological curricula are that considerable improvement in the teaching of experimental design and analysis is required.

18 Motoneurons and their synaptic targets are involved in triggering spontaneous episodes of activity in the developing spinal cord of the chick embryo. By M. J. O'DONOVAN and P. WENNER. *Laboratory of Neural Control, NINDS, National Institutes of Health, Bethesda, USA*

Spontaneous activity is a characteristic property of developing networks that may play an important role in their formation and function. In the developing chick embryo, spinal networks are spontaneously active and generate recurrent episodes of limb movements that are critical for the normal development of muscles, bones and joints. We have been studying the mechanisms of this activity in the isolated lumbosacral cord of the embryonic chick (E7–E12) maintained in vitro. We have argued that spontaneous episodes of activity are a general property of hyperexcitable developing networks that exhibit activity dependent synaptic depression (Tabak et al. *J. Neurosci.* **20**, 2000). Little is known however about the mechanisms controlling the initiation of spontaneous episodes. To address this question, we recorded electrically from the ventral roots and optically from spinal interneurons labeled with calcium-green dextran. The experiments were performed on single isolated segments of the lumbosacral cord. We found that motoneurons began to discharge up to 500 ms before the start of a spontaneous episode and before activity was detected in interneurons. We hypothesised that the pre-episode motoneuron discharge would excite the spinal targets of motoneurons—designated R-interneurons—and that this activity would then spread to the rest of the interneuronal network. R-interneurons receive monosynaptic, cholinergic inputs from motoneurons and project depolarising GABAergic synapses to motoneurons and to other spinal interneurons (Wenner & O'Donovan, *J. Neurosci.* **19**, 1999) and are probably the avian equivalent of the mammalian Renshaw cell. They are located in a nucleus dorsomedial to the lateral motor column. To establish if motoneurons excite R-interneurons, and thereby the spinal network, we compared the timing of motoneuron and interneuron activity before and after blockade of the cholinergic synaptic connection between motoneurons and R-interneurons. Under control conditions, activity began on one side of the cord and then spread to the other side. On the initiating side ventral root slow potentials and discharge preceded the activity of interneurons recorded either optically or electrically from the ventrolateral funiculus. Amongst the labeled interneuronal population activity began within and around the R-interneuronal region and then spread medially and dorsally to encompass all of the labeled interneurons. On the contralateral side the discharge of motoneurons was delayed with respect to the initiating side but still preceded that of interneurons. Following cholinergic blockade the recruitment pattern of the network was altered so that motoneurons were recruited synchronously on each side of the cord and interneuronal activity began bilaterally within a region medial to the motoneurons. Consistent with a role for R-interneurons in mediating the effects of motoneuron activity at episode onset, we found that stimulation of motor nerves could trigger spontaneous episodes and that this effect was blocked by cholinergic and GABAergic antagonists.

A role for motoneurons in triggering spontaneous

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episodes in the spinal cord is surprising, because motoneurons are traditionally thought of as output elements, but is consistent with recent evidence that these cells play a more active role in spinal motor activity than previously suspected.

19 Swimming in embryos and young tadpoles of a frog: *Xenopus*. By A. ROBERTS. *School of Biological Sciences, University of Bristol, UK*

The hatchling *Xenopus* tadpole is a useful preparation for the study of basic features of the organisation and function of the vertebrate nervous system. Once it has hatched the tadpole spends most of its time hanging from a mucus strand secreted by a cement gland on the head. However if it becomes detached, it can swim, either spontaneously or when touched, and it makes violent struggling movements when grasped. I will outline the anatomy and function of the neuronal circuits that control swimming behaviour, compare the *Xenopus* tadpole spinal cord organisation with that of other lower vertebrates, and present new results from calcium imaging on the role of the pineal eye in controlling the direction of swimming.

20 Activity-dependent emergence of receptive fields in the developing retina. By E. SERNAGOR. *Department of Neurobiology, School of Neurosciences, The Medical School, University of Newcastle upon Tyne, UK*

Long before they become exposed to sensory experience at birth, embryonic neurons are electrically active while they wire up together to form the precise connections ultimately required in the adult nervous system to perform in an immense repertoire of sophisticated functions. In the developing vertebrate retina, this early electrical activity takes the form of spontaneous bursts of action potentials synchronised between neighbouring retinal ganglion cells (RGCs), resulting in waves sweeping across the retina. Both at retinal and extraretinal levels, these embryonic retinal waves are believed to play a fundamental role in wiring of the visual system using Hebbian mechanisms of synaptic strengthening. In the first part of this talk, I will present evidence for a role of these embryonic spontaneous bursts of activity in shaping developing complex receptive field properties of RGCs in the turtle embryonic retina. I will also discuss the role of visual experience at birth in establishing visual functions of these cells, and how spontaneous activity and visual experience interact with each other to bring developing receptive fields to maturation. When turtles are reared in normal light and dark cycles the spontaneous bursts disappear within a month post-hatching, coinciding with the completion of the expansion of receptive field areas. However when turtles are reared in the dark during that same initial period post-hatching, the spontaneous bursting activity persists, indicating that visual experience normally controls its disappearance. At the same time receptive field areas expand far beyond normal adult dimensions. On the other hand when the bursts are chronically abolished in ovo or at hatching with curare, a cholinergic nicotinic blocker, receptive field areas remain smaller, indicating that spontaneous bursting activity blockade freezes the expansion of receptive fields. We have hypothesised that these physiological changes reflect modifications in the dendritic arbours of RGCs, the anatomical substrate of their receptive fields. Intracellular staining of embryonic RGCs with lucifer yellow indeed reveals that the layout of their small, polarised dendritic tree often overlaps with their immature, non-homogenous receptive fields (measured by responses to edges of light moving at different directions or bars flashing at different orientations). When RGCs are chronically exposed to curare, their dendritic trees remain small and polarised, while in dark reared turtles dendrites proliferate, resulting in larger and more branched trees, suggesting that immature spontaneous bursting activity contributes to dendritic growth in RGCs. To understand how the spatiotemporal patterns encoded in retinal waves might precisely contribute to the establishment of neural circuitry involved in the expression of RGC receptive field properties, first we must clarify the mechanisms mediating the generation and propagation of these waves. In the second part of this talk, I will show how pharmacological manipulations modify chick embryo retinal waves visualised by calcium imaging. Although both acetylcholine and glutamate are required to generate the waves, acetylcholine contributes mainly to the spatial extent of the waves whereas glutamate controls their speed. Future chronic studies using specific spatial or temporal alterations of the waves will shed a new light on how the wave dynamics help carving retinal receptive fields.

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21 Developmental conductive hearing loss in humans and ferrets: anatomical and behavioural consequences. By D. R. MOORE. *Laboratory of Physiology, University of Oxford, UK*

Conductive hearing loss produced by middle ear disease (MED) is the most common reason both for children to visit their GP and for childhood surgery. Our own research has shown that MED occurs in almost all children. In about 10–15% of children, MED is present in one or both ears for at least half of the first 5 y of life. There has been debate for many years concerning the effect of MED on language development. Many children with recurrent MED lack any obvious impairment. However, a history of recurrent MED appears to be a feature of many children with language, learning or social difficulties. This debate has typically lacked detailed data concerning either the history of MED or the psychoacoustic performance of the children. Research on animals first alerted us to the possibility that unilateral or asymmetric MED may produce long-term changes in the structure and function of the central auditory system. Conductive hearing loss, unlike sensorineural loss, does not appear to affect auditory processing in the cochlea or the cochlear nucleus. However, we found that unilateral ear plugging in infant ferrets changed the symmetry of neural connections between each ear and the auditory midbrain. These changes could then persist after the peripheral hearing loss had resolved.

Several experiments have examined the functional consequences of a conductive loss. In humans we have shown that a history of MED leads to impairments in 2 central auditory processing tasks, binaural unmasking and backward masking. For binaural unmasking, a measure of the

ability to segregate sounds spatially, the impairment is present for at least some months *after* the most recent episode of MED. Gradually unmasking improves to normal. In ferrets reared with a unilateral earplug impairments of both binaural unmasking and sound localisation have been demonstrated, showing the link between the earlier hearing loss and the central processing impairments. Recent psychoacoustic studies have shown that many language impaired children have difficulty with auditory backward masking, a measure of the ability to segregate sounds in time. Our studies have shown that children with a history of MED also have difficulty with backward masking. These data have lead us to the hypothesis that there is a causal link between recurrent MED in early childhood, impaired central auditory processing, and language based learning impairments. We are currently exploring this hypothesis further. We have also begun to examine whether systematic auditory training can more rapidly improve recovery from the hearing impairments produced by conductive hearing loss than does passive listening.

22 Prenatal emergence of human behaviour. By H. F. R. PRECHTL. *Department of Physiology, Karl-Franzens-University Graz, Austria*

Modern neurobiology has provided extensive experimental evidence for the existence of endogenously generated activity (central pattern generators—for recent review: see *Current Opinion in Neurobiology* **9**, 1999). An excellent example of endogenously generated motor activity are human fetal movements. Our systematic ultrasound studies have altered many of the existing ideas about fetal movements, which had been based on previous reflex studies on aborted fetuses. The new findings on healthy fetuses studied with ultrasound in real time demonstrate the first fetal movements as lateral head bending at 8 wk postmenstrual age. 1 to 2 wk later complex movements involving the whole body (general movements, and startles) can be observed. Interestingly, isolated arm or leg movements only follow a few days later. During the following weeks a variety of other specific movements emerge: breathing movements, stretches, yawns, head rotations, trunk rotations, alternating leg movements, sucking and swallowing, slow and rapid eye movements and at about 36 wk organised behavioural states of sleep and waking. Right from the very first beginning, all fetal movements are distinct and specific. They continue to be present after birth without any remarkable change in form.

The functional aspect of fetal movements is at least twofold: Fetal movements anticipate postnatal patterns. As endogenously generated neural activity, fetal movements are important for the fine tuning of the structural development of the nervous system.

23 The molecular anatomy of the human genome. By I. DUNHAM. *The Sanger Centre, Wellcome Trust Genome Campus, Hinxton, Cambridge, UK*

The human genome project aims to provide a new anatomy of humans by establishing a molecular description of our genetic components. It is intended that a complete catalogue of our genes will be obtained through sequencing and analysis of the 3 billion base pairs of DNA that constitute our haploid genome. The past year has seen dramatic progress in the project with completion of the sequences of the 2 smallest chromosomes, and the recent announcement of the working draft version of the rest of the genome. Analyses of these sequences have highlighted the great variety in the structures possible for human genes, and in the way that genomic DNA is utilised. However we are still some way from having the definitive catalogue of the human genetic material. I will summarise the progress so far, and reflect on what we have learnt and what remains to be found.

24 Neurogenomics: high throughput determination and analysis of steady state and dynamic gene expression patterns in the mammalian brain. By G. EICHELE. *Max Planck Institute, Hannover, Germany*

Genomes of animals contain between 15000 (e.g. Drosophila) and 100000 (human, mouse) genes, many of which encode proteins involved in regulatory processes. Sequence data opens up ways to study complex genetic interactions that underlie biological regulation.

There are numerous examples demonstrating that an understanding of regulatory networks consisting of multiple components is significantly advanced by a detailed knowledge of the spatiotemporal expression pattern of each of the components. For example, during embryonic development genes that belong to a certain signalling pathway are frequently expressed at specific times and locations and in specific cell types. Knowing the expression pattern of each component greatly helps in formulating specific hypotheses about the developmental function of the genes in question. Expression patterns can readily be determined by RNA in situ hybridisation.

The challenge emerging from the knowledge of the sequence of entire genomes is that assignment of biological functions to genes needs to be carried out on an appropriately large scale. In terms of gene expression analysis by RNA in situ hybridisation, efficient technologies need to be developed that permit determination and representation of expression patterns of thousands of genes within an acceptable time scale.

We set out to determine the spatial expression pattern of several thousand genes encoding putative regulatory proteins in the mouse CNS. To achieve this goal we have developed high throughput technologies that allow the determination and visualisation of gene expression patterns by RNA in situ hybridisation on tissue sections at cellular resolution. In particular we have invented instrumentation for robotic in situ hybridisation capable of carrying out in a fully automated fashion, all steps required for detecting sites of gene expression in tissue sections. In addition,we have put together hard- and software for automated microscopic scanning of gene expression data that are produced by RNA in situ hybridisation. The potential and limitation of these techniques and our efforts to build a Web-based database of gene expression patterns will be discussed.

25 Imaging and the future. By A. K. DIXON. *Department of Radiology, University of Cambridge, UK*

Those working in Radiology over the last 2 decades have witnessed an extraordinary revolution. Gone are the

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complex and scarcely credible images of the past, viewed in darkened rooms and subject to wide differences in interpretation. Now the images are instantaneous and non-invasive and of a quality that rivals (and sometimes surpasses) the information provided by cadaveric dissection. The images currently available provide a ready means of teaching anatomy and the in vivo anatomical data have opened up new avenues for research. The next generation of equipment will provide even faster data acquisition at an even lower (or zero radiation dose). Even wider fields of view will provide whole body data sets which will be stored and then retrospectively interrogated according to different clinical possibilities. The combination of anatomical and physiological information will get even simpler. Nevertheless the basic techniques will remain similar for the foreseeable future, namely: ultrasound (US), X-Ray Computed Tomography (CT) and Magnetic Resonance Imaging (MRI). Radionuclide techniques (e.g. Positron Emission Tomography, PET) will continue to develop and provide the functional data which can be coregistered with the more anatomical data provided by the more standard techniques.

Ultrasound machines are already poised to become the stethoscope of the 21st century. They are already briefcase size and, in many parts of the world, are no longer the restricted property of radiologists. Training continues to be a big issue; the number of people who can exploit the full capabilities of this remarkable tool remains limited. No other form of imaging requires so much skill, not only in acquiring good images but also in analysing them. Because US images are produced without recognisable landmarks, the only person who can really interpret the images is the person who acquired them. Although many Departments are working towards 3D US data sets, they are unlikely ever to provide the spatial resolution of a perfectly targeted localised examination. Doppler and other forms of flow sensitive imaging have given US access to physiological information. Recently small field high resolution probes (using 15–20 MHz, rather than the more conventional 3.5–5 MHz) have been introduced.

CT has moved up a gear with multidetector arrays which can now provide isometric voxels for whole body applications. Certainly the instantaneous switching from axial to sagittal and coronal imaging planes is a huge advance. Data acquisition has become much faster. This brings real possibilities for a whole body anatomical data set being available for interrogation by any interested party (radiology, orthopaedics, anatomical research, etc). In many ways CT still remains the most easily understood imaging technique for the purposes of anatomical display. This attribute is likely to continue for the foreseeable future.

MRI has become the primary imaging investigation for a wide range of anatomical sites and clinical conditions—brain, spine, knee, etc. However, in order to obtain optimal images of certain anatomical structures (e.g. the contents of the lumbar canal), it is advantageous to purposely suppress signal returning from some neighbouring structures (e.g. flowing blood in the aorta). Thus the images will often appear to 'fade off' peripherally according to saturation bands and other technical manoeuvres. Such steps, allied with a much wider potential range of tissue contrast, make some MR images less easy to understand than CT. It is probable that future technology will overcome these problems. Improvements in software will also allow much easier image fusion and coregistration. Ultimately artificial intelligence will reconstruct images to allow automatic display of certain anatomical structures. Whether such aids will ever replace all diagnostic processes is open to question!

26 Prospects for structural bone analogues. By W. BONFIELD. *Department of Materials Science and Metallurgy, University of Cambridge, UK*

Biomaterials are either modified or natural materials, with an appropriate response in the host tissue, which find application in a wide spectrum of medical implants and prostheses. Bone and joint replacement, with clinical procedures ranging from bone grafting to the total replacement of arthritic joints, has to date been largely achieved with available engineering materials with adequate mechanical properties which are tolerated by the body (first generation biomaterials). However, considerable progress has been made recently with custom made biomaterials that mimic the biological template and combine favourable bioactivity in a skeletal site with mechanical compatibility (second generation biomaterials). Notable examples are synthetic hydroxyapatite, which resembles bone mineral, and hydroxyapatite reinforced polyethylene composite, which is a first approximation analogue of cortical bone. It has been demonstrated that such materials produce bone apposition at an implant surface, rather than the inevitable bone resorption produced by first generation biomaterials. As a consequence of providing favourable and stable sites for the recruitment of cells from the surrounding biological environment, the subsequent cellular processes lead to the expression of matrix new bone formation.

For hydroxyapatite osteoconduction can be accelerated by precise control of surface chemistry, and specific ion substitution, the establishment of an interconnecting porosity and/or the incorporation of cells (tissue engineering) and biologics. By combining hydroxyapatite with a polymer it is possible to produce comparable stiffness to that of cortical bone with superior fracture toughness. These research developments are reviewed, and the prospects for refined bone analogues are considered.

27 Brain mapping: the contribution of anatomy to functional imaging. By K. ZILLES. *C.&O. Vogt Institute for Brain Research, University of Düsseldorf and Institute of Medicine, Research Center Jülich, Germany*

The analysis of the association of structure and function is the most fundamental research concept in biomedicine, and represents the beginning of modern anatomy as demonstrated by the anatomical drawings of Leonardo da Vinci. In the case of human neuroanatomy, 2 basic questions can be derived: (1) which functions are localised in which anatomically definable parts of the brain, and (2) how are the neural mechanisms structurally organised. In the past preliminary answers to these questions were provided by clinical observations of diseases and inference from animal experiments. However a lesioned brain is not an experimental model of a normal brain, because it shows a complex situation of postlesional compensation and plas-

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ticity. Moreover the brains of animals differ in many respects from those of humans. Thus for a long time human neuroanatomy was restricted to histological and histochemical analyses of postmortem brains. Although these pioneering efforts provided rather detailed brain maps, e.g. the cytoarchitectonic maps at the beginning of the last century, the function was inferred by comparisons with data from brain lesion studies or animal experiments. Cell and molecular biology are clearly powerful approaches to study basic mechanisms, but they do not allow the analysis of complex functions at a higher systems level (cognition, language, memory, etc.) because of the regionally heterogeneous architecture of the brain.

Functional imaging of the brain is a revolutionary approach to study the organisation and mechanisms of such higher functions in living human subjects by using positron emission tomography (PET), functional magnetic tomography (fMRI) or magnetic encephalography (MEG). However the spatial resolution of these techniques is poor and only indirectly accessible (MEG) or in the range of several millimeters (PET, MRI). Thus postmortem data provided by modern histological and histochemical techniques must be combined by bridging several orders of magnitude with in vivo functional imaging data in the same spatial reference system. The aim of the present talk is to demonstrate such an integrative structural-functional approach.

It will be shown how (1) observer-independent architectonic mapping of cortical areas was realised using a quantitative analysis of histological cell-body-stained, serial sections through complete human postmortem brains, and how comparisons of cytoarchitecture with transmitter receptor distributions (receptor architecture) were performed; (2) these microstructural and molecular maps were mapped to image sequences of a selected living human brain (reference brain) by a novel warping technique; (3) intersubject variability of the position and extent of cortical areas was studied and represented by probability maps in the reference brain; and how (4) functional imaging data were mapped to the reference brain, and a direct microstructural-functional correlation was realised.

Results will be demonstrated, which show that this approach provides novel insights into: (1) the structure of the human sensorimotor cortex, the intersubject variability of the human cortical areas as well as the anatomical counterpart of Broca's speech region; and (2) the molecular specificity (receptor fingerprints) of cortical areas comprising the same or different functional systems, and the complex functional organisation of the architectonically defined posterior part (area 4p) of the primary motor cortex, which is not a simple motor control region but an attention-modified unit.

28 Analyses of the connectional organisation of primate prefrontal cortex. By R. KÖTTER[1,2] and K. E. STEPHAN[1]. [1]*C. & O. Vogt Brain Research Institute and* [2]*Department of Morphological Endocrinology and Histochemistry, Heinrich Heine University, Düsseldorf, Germany*

The principles governing prefrontal cortical organisation are controversial, despite extensive structural and functional investigations. Computational analyses of traced fibre connections provide powerful tools for interpretation but require systematic and innovative data collation methods to yield reliable insights. Within the framework of the connectivity database CoCoMac (http://www.cocomac.org) we performed a systematic collation of published tracing studies in the prefrontal cortex of macaque monkeys.

Data from 72 tracer injections were mapped into Walker's parcellation scheme (W) using objective relational transformation (ORT, *Phil Trans R Soc Lond B* **1393**:37). The resulting connectivity matrix provided a nearly complete ($> 90\%$) record of connectivity between prefrontal cortical areas. The matrix was analysed using several complementary statistical methods including multidimensional scaling, optimal set analysis and novel connectivity indices that classify areas according to the density, reciprocity and predominance of afferent and efferent projections.

The obvious division of prefrontal cortical areas into 2 large groups comprising lateral areas W8a, W8b, W9, W45 and W46, on the one hand, and orbito-medial areas W10, W11, W12, W13, W14, W25, on the other hand, was confirmed by a χ^2 test ($P < 10^{-5}$). Multidimensional scaling analysis shows in addition their convergence onto anterior cingulate area W24. The connectivity indices differentiate within the prefrontal cortex densely connected areas (W24, W12, W9) from weakly connected areas (W8a), and afferent-dominated areas ('receivers': W24, W11) from efferent-dominated areas ('speaker': W45). Area W24 is both densely connected and receives afferents from all prefrontal cortical areas. Afferents and efferents of area W14 are well balanced but show low reciprocity.

These anatomical features match the functional segregation of prefrontal cortical areas into medial and lateral groups, and the frequency of anterior cingulate activation in cognitive imaging paradigms involving prefrontal cortex. Reciprocal connections between complementary areas ('receiver' and 'speaker') constitute a prime system for functional integration. Thus we provide links between connectional features of individual areas and global processing properties. It will be interesting to see to what extent the role of individual cortical areas varies in the context of different functional systems.

29 Ambiguous figures and neural correlates of perceptual awareness in human visual cortex. By T. J. ANDREWS, D. SCHLUPPECK and C. BLAKEMORE. *University Laboratory of Physiology, Oxford, UK*

Ambiguous figures provide a powerful tool for studying the neural basis of visual perception. In the absence of any extrinsic changes in the stimulus, perceptual changes must reflect selective processes in the brain. We used functional magnetic resonance imaging (3T) to monitor stimulus-selective responses in the human visual cortex while subjects viewed objects and faces. Areas in the right fusiform gyrus were significantly more active when subjects viewed faces than when they viewed an assortment of common objects (resel corrected $P < 0.01$). Conversely more medial and bilateral areas in the lingual/parahippocampal gyri showed increased activity when subjects viewed non-face objects. These specific areas of activation were used to define particular regions of interest for each of 4 subjects. We then

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monitored activity in these areas when subjects viewed Rubin's ambiguous vase-face stimulus, and reported perceptual switches by pressing one of 2 buttons. Local contrast gradients could be added to the vase-faces stimulus to trigger the perception of either the vase or the face. Using an event related design we compared the activity during periods when this change in the image caused a perceptual switch to periods when the percept remained unchanged. Perceptual alternations were accompanied by changes in the activity of some of the previously defined regions of interest, suggesting that these areas reflect the perceived rather than the retinal stimulus.

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30 Loss of normal distribution of entorhinal and associational fibres in entorhino-hippocampal cocultures from Nex* Beta2/Neuro D double mutant mice. By B. HEIMRICH[1], S. ZHAO[2], A. DRAKEW[2], K.-A. NAVE[3], and M. FROTSCHER[2]. [1]*Institut für Anatomie, Universität Rostock;* [2]*Anatomisches Institut I, Universität Freiburg; and* [3]*Max Planck Institut für Experimentelle Medizin, Göttingen, Germany*

The neuronal transcription factors NEX and Beta2/Neuro D are involved in the determination of neuronal cell fate. Mice lacking the Beta2/Neuro D gene reveal structural defects of cerebellar and hippocampal development. This loss of function of the Beta2/Neuro D gene also affects pancreas development which causes early postnatal death of mutant mice. To overcome this early lethality we used organotypic hippocampal slice cultures which allows a prolonged analysis of hippocampal development. In the present study we compared the differentiation of dentate granule cells and the formation of axonal projections in cultured slices derived from various genotypes.

Single hippocampal slice cultures and entorhinohippocampal cocultures were prepared from newborn mice and cultivated for up to 14 d. Anterograde biocytin tracing and Timm stain were carried out to label the entorhinohippocampal projection and the hippocampal mossy fibre projection, respectively. Immunocytochemistry with antibodies against reelin and calretinin as well as Nissl staining were performed to identify cellular elements and the cytoarchitectural organisation of the developing fascia dentata.

In slice cultures from Beta2/Neuro D mutant mice we never observed a granule cell layer or a mossy fibre projection. Calretinin immunostaining revealed the typical supragranular axon plexus of hilar mossy cells in slice cultures from wildtype and heterozygous animals. In contrast calretinin-immunpositive fibres spread throughout the entire molecular layer of the fascia dentata in cultures from double mutant mice. Reelin-immunopositive Cajal-Retzius cells were normally aligned along the hippocampal fissure, but in double mutant tissue these neurons were clustered and the distribution of entorhinal fibres was altered.

Our data demonstrate that the granule cell development arrests in Nex* Beta2/Neuro D slice cultures and that the distribution of Cajal-Retzius cells regulates the innervation pattern of entorhinal afferents in the dentate gyrus.

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31 Regulation of oestrogen receptor expression by oestrogens in the rat and marmoset hippocampus: evidence for an autocrine loop. By G. M. RUNE[1], J. PRANGE[1], U. WEHRENBERG[1], G. BRÜNING[2], G. ADELMANN[3] and M. FROTSCHER[3]. [1]*Institute of Anatomy, University of Greifswald;* [2]*Institute of Anatomy, Free University of Berlin; and* [3]*Institute of Anatomy, University of Freiburg, Germany*

Previous studies have shown that oestrogens influence synaptic plasticity by inducing dendritic spine formation of hippocampal neurons. Oestrogens are synthesised in the hippocampus and both genomic oestrogen receptor (ER) subtypes (α and β) are highly expressed in this brain region.

In sexually mature, female and male rats and marmosets, we found ERα and ERβ in pyramidal cells of the CA1 and CA3 region and in granule cells of the fascia dentata, as revealed by immunohistochemistry (light microscopy, post-embedding EM immunogold labeling) and in situ hybridisation with no differences between gender. Double labelling experiments (in situ hybridisation and immunohistochemistry) showed that both receptors are colocalised up to 50% in the cells of the pyramidal layer. Using confocal microscopy, we found that the mRNA for aromatase, the enzyme responsible for the conversion of testosterone into oestradiol, is colocalised with ERs in hippocampal neurons.

Both ER subtypes were also expressed in dispersed adult rat hippocampal cultures. Supplementation of the medium with letrozol, an inhibitor of aromatase, led to a down-regulation of ERα and to an upregulation of ERβ. This was seen at the protein as well as mRNA level. Our findings suggest that oestrogens, synthesised in the hippocampus, regulate their receptor expression in an autocrine manner.

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32 Neurotransmitter plasticity of human sympathetic ganglia. By V. ROUDENOK. *Department of Human Anatomy, Minsk State Medical Institute, Belarus; and Department of Anatomy, Lübeck Medical University, Germany*

The functional significance and studies of different classes of neuropeptide in developing and mature neurons of central and peripheral nervous system has recently become an area for intensive investigation. Besides classical neurotransmitters noradrenaline and acetylcholine, neuropeptides are involved in autonomic chemical transmission and induce different physiological effects on the target organs. Neuropeptide Y (NPY), vasoactive intestinal polypeptide (VIP) and calcitonin gene-related peptide (CGRP) can be counted among such peptides. NPY colocalises with noradrenaline in sympathetic nerve cells and fibres and is involved in the regulation of cardiac activity and vasoconstriction. VIP and CGRP coexist with acetylcholine and cause the different biological effects including long lasting vasodilatation, relaxation of smooth muscle and increased cardiac contractility. The existence of these substances in the mammalian autonomic ganglia has been widely documented. However there are no reports on the developmental aspects

of neuropeptide expression in the human sympathetic ganglia.

Developmental patterns of NPY-, CGRP- and VIP-immunoreactivities (IR) were investigated in the stellate, thoracic and lumbar sympathetic ganglia of postmortem human premature and mature neonates, children and adults. The postmortem delays varied between 2 and 6 h. Fixation was carried out using Zamboni's solution for 1–5 d at 4 °C. For quantitative analysis the number N of IR neurons was calculated from measurements of 5 randomly selected fields on each of 10 sections per ganglion.

In ganglia of premature neonates few NPY-IR neurons were found (under 7%), and these increased with gestational age and during childhood to adult levels (up to 89%). In contrast VIP-IR neuronal number was maximal in ganglia of premature neonates (up to 31%) and decreased during maturation to a constant adult level of 0–1.5%. A similar dynamic was found for CGRP-IR neurons. There were also regional differences in NPY-, VIP- and CGRP-IR within the sympathetic trunk.

The results showed age related changes of neuropeptide Y-, VIP- and CGRP-IR in human sympathetic ganglia during maturation and ageing and suggest these neuropeptides have different functional roles in sympathetic ganglion neurons according to age. The regional neurochemical differences among ganglia are conditioned by peripheral projections of their neurons as well as the functional activity and morphological differentiation of their target organs. The developmental pattern of NPY-, VIP-, and CGRP-IR in the principal sympathetic ganglion nerve cells defines not only a new qualitative level in regulation of differentiation of autonomic neurons and function of target organs, but also serves as an index of neuronal maturity.

33 Regional differences in the neurochemical coding of primary sensory neurons supplying the porcine vas deferens. By J. KALECZYC[1], D. W. SCHEUERMANN[2], Z. PIDSUDKO[1], M. MAJEWSKI[1], M. LAKOMY[1] and J. -P. TIMMERMANS[2]. [1]*Department of Animal Anatomy, Warmia and Masuria University in Olsztyn, Poland; and* [2]*Laboratory of Cell Biology and Histology, University of Antwerp (RUCA), Belgium*

This study was aimed at determining which dorsal root ganglia contribute to the innervation of the porcine vas deferens and to investigate whether these primary sensory neurons represent one or more neurochemically distinct populations. Retrograde tracer Fast Blue (FB) was injected into the wall of the left vas deferens (n = 4) during laparotomy performed under pentobarbital anaesthesia. 5 wk later, the animals were reanaesthetised and perfused transcardially with buffered paraformaldehyde. Serial cryostat sections from the thoracic, lumbar, sacral and caudal spinal ganglia were viewed under a fluorescence microscope to count FB-positive (FB+) neurons and then processed for immunofluorescence using antibodies against substance P. (SP), calcitonin gene-related peptide (CGRP), galanin (GAL), somatostatin, Leu5-enkephalin, vasoactive intestinal polypeptide, nitric oxide synthase and choline acetyltransferase. FB+ neurons were distributed mainly in the lumbar (L) and sacral (S) ganglia. L2, L3 and S2, S3 pairs of DRG were found to contain the vast majority (approx. 90%) of all the FB+ neurons (~250 per animal). ~80% of these nerve cells occurred in the ipsilateral ganglia. Immunohistochemistry revealed that most of FB+ neurons contained CGRP and/or SP. However, a distinct difference in the occurrence of these peptides was found between the neurons located within the lumbar and sacral ganglia. In the lumbar ganglia, virtually all the FB+ neurons (~97%) contained CGRP and/or SP while in the sacral ganglia only ~55% of the nerve cells expressed immunoreactivity to these peptides. Some FB+ neurons found within both the lumbar and sacral ganglia expressed also GAL often in combination with SP and/or CGRP. Thus SP- and/or CGRP-positive nerve fibres supplying the porcine vas deferens found in the earlier study (Kaleczyc et al., *Histochem. Cell Biol.* **107**, 1997) appeared to originate from the dorsal root ganglia. In the present study, it was also revealed that a small but distinct proportion of the SP-positive nerve terminals contained GAL (the occurrence of this peptide in the wall of the porcine organ was not previously studied).

This study reveals for the first time that primary sensory neurons projecting to the mammalian vas deferens comprise different regionally confined populations with respect to their neuropeptide content.

34 The cavernous sinus ganglia are sources for parasympathetic innervation of cerebral arteries in rat. By R. L. A. W. BLEYS[1], R. E. DAALDER[1], C. THRASIVOULOU[2] and T. COWEN[2]. [1]*Department of Functional Anatomy, Rudolf Magnus Institute for Neurosciences, University Medical Centre, Utrecht, The Netherlands; and* [2]*Department of Anatomy and Developmental Biology, Royal Free Hospital School of Medicine, London, UK*

The pterygoplatine and otic ganglia are peripheral sources for parasympathetic innervation of the basal cerebral arteries. Additional parasympathetic innervation may derive from ganglia in the cavernous sinus that reach the internal carotid artery retrogradely via the abducens nerve (Bleys et al. *J. Comp. Neurol.* **396**, 1996). These ganglia include a large cell mass along the vidian nerve, which is continuous with the 'classical' pterygopalatine ganglion and may be considered as the cavernous part of the pterygopalatine ganglion, and scattered small groups of ganglion cells. To find out whether the cavernous sinus ganglia contribute to cerebrovascular innervation and to investigate the nature of the ganglion cells, retrograde tracing and immunohistochemical staining were performed. In 8 anaesthetised (2.5% halothane) Sprague Dawley rats (male, 12 wk of age) the tracers fluorogold and fast blue were applied to a branch of the middle cerebral artery. After 1–4 d the ipsilateral and contralateral cavernous sinuses were studied as whole mount preparations and sections. In selected sections additional staining for vesicular acetylcholine transporter (VAChT) was performed. Sections of the cavernous sinus region of 3 adult male Wistar rats (anaesthetised by 0.1 ml Nembutal/100 g body weight i.p.) were immunohistochemically stained for protein gene product (PGP) 9.5 as a general nerve stain, tyrosine hydroxylase (TH) for sympathetic neurons, VAChT for parasympathetic neurons and cal-

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citonin gene-related peptide (CGRP) to demonstrate sensory neurons. The results demonstrated that after application of the tracer, numerous labelled neurons were visible in both the cavernous part of the pterygopalatine ganglion and in small cavernous sinus ganglia. Furthermore fibres in the abducens nerve and the cavernous nerve plexus were labelled, all on the ipsilateral side. In some specimens positive neurons were seen in cavernous sinus ganglia of the contralateral side. The ganglion cells showed staining for PGP 9.5 and VAChT, with a few CGRP-positive cells in small ganglia. In double labelled sections some of the VAChT-positive cells also contained tracer. The results indicate that the cavernous sinus ganglia contribute considerably to the innervation of cerebral arteries and that the abducens nerve is involved in the pathway from the cavernous sinus ganglia to the cerebral arteries. Furthermore the majority of the ganglion cells are parasympathetic with some sensory cells as well. Therefore it is concluded that the cavernous sinus ganglia are peripheral sources for parasympathetic innervation of cerebral arteries.

35 Cerebral amyloid induces aberrant axonal sprouting and ectopic terminal formation in amyloid precursor protein transgenic mice. By T. DELLER[1], A. L. PHINNEY[2], M. STALDER[2], M. E. CALHOUN[2], B. SOMMER[3], M. STAUFENBIEL[3], M. FROTSCHER[1] and M. JUCKER[2]. [1]*Institute of Anatomy*, *University of Freiburg*, *Germany*; [2]*Department of Neuropathology*, *Institute of Pathology*, *University of Basel*; *and* [3]*Central Nervous System Research*, *Novartis*, *Basel*, *Switzerland*

A characteristic feature of Alzheimers disease (AD) is the formation of amyloid plaques in the brain. To investigate the effect of amyloid deposition on axons and neuronal connectivity, we studied the axonal projections from the entorhinal cortex to the hippocampus in APP23 mice that overexpress mutated human amyloid precursor protein (APP) and form amyloid plaques progressively with age (Sturchler-Pierrat et al. *Proc. Natl. Acad. Sci. USA* **94**, 1997). To visualise the entorhinal axons, the sensitive anterograde tracer *Phaseolus vulgaris* leucoagglutinin (PHAL) was employed, which allows for the analysis of projections at the level of single axons. The entorhinal projection to the hippocampus was investigated in young and aged APP23 mice as well as in nontransgenic control animals. Tracing of entorhinal fibres revealed that entorhinal fibres form dystrophic boutons around dense core amyloid plaques in the entorhinal termination zone of the hippocampus. Moreover entorhinal boutons were found associated with amyloid in ectopic locations within the hippocampus, the thalamus, white matter tracts, as well as surrounding vascular amyloid. Many of these ectopic entorhinal boutons were immunopositive for the growth associated protein GAP-43 and showed light and electron microscopic characteristics of axonal terminals. These findings suggest that amyloid plaques lead to aberrant sprouting and the formation of ectopic axon terminals. Thus cerebral amyloid leads to the disruption of neuronal connectivity, which in turn may significantly contribute to Alzheimer's disease.

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36 Morphological structures of the human tibiofemoral joint and the development of a new knee endoprosthesis. By H. NÄGERL[1], B. MIEHE[2], D. KUBEIN-MEESENBURG[3] and J. FANGHÄNEL[2]. [1]*Physics Institute IV*, *University of Göttingen*; [2]*Institute of Anatomy*, *University of Greifswald*; *and* [3]*Department of Orthodontics*, *University of Göttingen*, *Germany*

In the constructive design of total knee arthroplasty (TKA), the functions of a whole series of morphological structures in the human knee were not taken into consideration. These structures include the proprioreceptive apparatus and the curvature of the articular surfaces.

The morphological examinations were conducted on the knee joint ligaments of *Sus scrofa domestica*. These ligaments were either frozen in liquid nitrogen or fixed in Bouin's Solution or 4 % formalin, and embedded in paraffin. From the tissue embedded in paraffin, 6 μm thick slices were taken 150 μm apart. In the HE sections, the number of superficial and interfascicular lamellar Pacini corpuscles and Ruffini corpuscles was counted for each section. From the frozen tissue blocks, 10 μm thick cryostat slices were cut 150 μm apart for use in HE staining, an enzymatic histochemical acetylcholinesterase assay, an immunohistochemical detection of Protein S 100 (PAP technique) and neurofilaments (APAAP technique), and a gold chloride representation. The Corpuscula nervosa terminalia were also counted. Pacini and Ruffini corpuscles are present in the entire ligamentary apparatus of the knee joint. We succeeded in showing that the healthy knee possesses a 3-D registration system (in 6 degrees of freedom) of the knee position, in which the menisci control the axial rotation.

Parallel to the functional plane in the human joint, the tibial articular surfaces exhibit laterally a convex curvature and medially a concave curvature, the radii of which were measured on a replica. Based on model trials and calculations, we demonstrated that (1) this difference in curvature causes the articular surfaces in the human knee to roll off of one another during up to about 30° of flexion, and (2) the form of curvature enables the knee to be mechanically stably or unstably positioned in the functional direction through the synergetic functioning of the musculature. This explains how the problems of (1) friction and (2) stable body posture and its antithesis, fast movement, were solved in the natural joint. The curvature morphology of the tibial and femoral articular surfaces also determines the maximal extension of the knee under compressive articular force.

37 Fibrocartilage in the transverse ligament of the human acetabulum. By S.MILZ[1], G.VALASSIS[1], A.BÜTTNER[2], M. MAIER[3], R. PUTZ[1], J. R. RALPHS[4] and M. BENJAMIN[4]. [1]*Anatomische Anstalt*, [2]*Institut für Rechtsmedizin*, *Orthopädische Klinik und* [3]*Poliklinik*, *Ludwig-Maximilians-Universität*, *München*, *Germany*; *and* [4]*School of Biosciences*, *University of Wales Cardiff*, *UK*

Biomechanical experiments on isolated hip joints have suggested that the transverse ligament acts as a bridle for the lunate articular surface of the acetabulum during load bearing. However such studies suffer from inherent limitations because the specimens are fixed artificially to testing

devices and there are no modifying influences of muscle pull. Further evidence is thus needed in cases with contradictory results. Here we argue that if the horns of the lunate surface are forced apart under load, the ligament would straighten and become compressed against the femoral head. It would thus be expected to share some of the features of tendons and ligaments that wrap around bony pulleys yet previous work has suggested that the transverse ligament is purely fibrous. Transverse ligaments were removed from 8 cadavers (aged 17–39 y) and fixed in 90% methanol. Cryosections were immunolabelled with antibodies against collagens (types I,II,III,VI), glycosaminoglycans (chondroitins-4 and -6-sulphate, dermatan sulphate, keratan sulphate) and proteoglycans (aggrecan, link protein, versican, tenascin).

A small sesamoid fibrocartilage, was consistently present in the centre of each transverse ligament, near its inner surface at the site where it faced the femoral head. Additionally, a more prominent enthesis fibrocartilage was found at both bony attachments. All fibrocartilage regions, in at least some specimens, labelled for type II collagen, chondroitin 6 sulphate, aggrecan and link protein, molecules more typically associated with articular cartilage. The results suggest that the ligaments contain a 'moderately cartilaginous' sesamoid fibrocartilage, adapted to withstanding compression. This supports the inferences that can be drawn from previous biomechanical studies. We cannot give any quantitative estimate of the levels of compression experienced. All that can be said is that the ligament occupies an intermediate position in the spectrum of fibrocartilaginous tissues. It is more cartilaginous than some wrap-around tendons at the wrist, but less cartilaginous than certain other wrap-around ligaments e.g. the transverse ligament of the atlas.

38 Fibrocartilage in the suprascapular ligament of man. By B. MORIGGL[1], P. JAX[2], A. BÜTTNER[3], M. BENJAMIN[4] and S. MILZ[1]. [1]*Anatomische Anstalt, Ludwig-Maximilians-Universität, München, Germany;* [2]*Institut für Anatomie der Universität Innsbruck, Austria;* [3]*Institut für Rechtsmedizin, Ludwig-Maximilians-Universität, München, Germany; and* [4]*School of Biosciences, University of Wales Cardiff, UK*

The suprascapular (superior transverse scapular) ligament converts the suprascapular notch into a foramen that separates the suprascapular nerve and vessels. No biomechanical function has properly been attributed to it, for the ligament connects 2 regions of the same bone and does not cross any joint. Nevertheless variations in its thickness and length and its tendency to ossify are well known, and both suggest that the ligament must be responsive to changes in mechanical load. Here we seek to determine the molecular composition of its ECM in order to comment on the type of loading that the ligament is most likely to experience.

The complete ligament (including both entheses) was removed from 7 cadavers (aged 32–75, mean 45 y) within 48 h of death and fixed in 90% methanol. The material was decalcified in 5% EDTA and cryosectioned at 12 µm. Sections were stained with toluidine blue and with a panel of monoclonal antibodies against collagens (types I,II,III,VI), glycosaminoglycans (chondroitins 4 and 6 sulphate, dermatan sulphate, keratan sulphate) and proteoglycans (aggrecan, link protein, versican). Antibody binding was detected with a Vectastain ABC Elite avidin/biotin/peroxidase kit.

Both entheses were strongly metachromatic and distinctly fibrocartilaginous. There was an additional, small sesamoid fibrocartilage in the centre of the ligament. The ECM of all these fibrocartilages labelled extensively for type II collagen, chondroitin 6 sulphate, aggrecan, and link protein.

The fibrocartilaginous nature of the ligament probably accounts for the tendency of the ligament to ossify and suggests that the mechanism of ossification is endochondral. It also shows that the suprascapular ligament must be subject to both compressive and tensile loading despite its lack of any obvious mechanical function. Finally our study demonstrates that enthesis fibrocartilage is not restricted to ligaments and tendons at sites where the insertional angle of these structures varies with neighbouring joint movement, as in tendons and ligaments of the knee and shoulder.

39 Macroscopic anatomy of the "rotator interval". By I. KOLTS[1], L.-C. BUSCH[3], H. TOMUSK[1], E. RAJAVEE[1], A. ELLER[2], M. MERILA[2], M. RUSSLIES[4] and W. KÜHNEL[3]. [1]*Institute of Anatomy, and* [2]*Clinic of Traumatology and Orthopaedics, University of Tartu, Estonia; and* [3]*Institute of Anatomy, and* [4]*Clinic of Traumatology and Orthopaedics, Medical University of Lübeck, Germany*

The clinical term 'rotator interval' denotes the capsular gap between the subscapularis and the supraspinatus muscles. The revision of the anatomy of the origin, course and insertion of the coracohumeral ligament and discovery of the capsular band between the Tuberculum minus et majus, called 'Lig. semicirculare humeri' (Clark & Harryman, *J. Bone Joint Surg*. **74-A**, 1992; Kolts et al. *Anat. Anz*. in press, 2000) influenced the understanding of the anatomy of the 'rotator interval'. The aim of the present investigation was to bring a new insight into the anatomical composition of the 'rotator interval' in correlation with the new anatomical findings.

16 alcohol-formalin-glycerol fixed cadaver shoulder joints (56–68 y old) were investigated. The soft tissues, acromion, acromioclavicular ligament and subacromial bursa were removed. Rotator cuff tendons were separated from the joint capsule. The joint capsule, coracohumeral and coracoglenoidal ligaments, 'Lig. semicirculare humeri' and glenohumeral ligaments were prepared. The coracoid process was cut at its base and placed posteriorly. The capsule under the coracohumeral ligament was separated from the underlying structures and moved to the side. The long head of biceps brachii with its insertion and the superior and middle glenohumeral ligaments at their origin were visualised.

The 'Lig. semicirculare humeri' strengthened the lateral part of the capsule between the subscapularis and supraspinatus muscles. The coracohumeral ligament built up the mediosuperior part of the 'rotator interval' and inserted into the 'Lig. semicirculare humeri'. The fibres of the superior glenohumeral ligament coursed in 2 directions. Inferior fibres proceeded towards the lesser tubercle and under the long head of biceps brachii. Superior fibres coursed over the long head of biceps brachii and inserted into the 'Lig. semicirculare humeri'.

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The 'rotator interval' is not a weak capsular gap strengthened only by the coracohumeral ligament, but a complex framework of ligamentous structures.

40 The trapezoid form of the trochlea tali in man. By E. BRENNER, J. PIEGGER and W. PLATZER. *Institute for Anatomy and Histology*, *Leopold-Franzens-University of Innsbruck*, *Austria*

Morphometric data gain great importance when an attempt is made to replace a destroyed talus by an artificial one. Talar arthroplasty may be performed for the treatment of various entities. The aim of this study is to deal with the morphology of the trochlea, especially its wedge shape. From measured data the angle between the medial and lateral border was calculated as an absolute and therefore more reliable parameter.

Two sets of specimens were used. The 1st set consisted of 99 matched pairs of macerated tali. The 2nd set covered 67 single tali, which were measured twice, first as wet specimens with the articular cartilage in situ and secondly macerated with the articular cartilage removed. Wet specimens were taken from isolated embalmed leg specimens.

The wedge shaped superior surface of the trochlea may be seen as a trapezoid. The angle of this trapezoid was calculated with 16.04° for the left tali and 12.48° for the right tali, respectively ($P < 0.005$). Comparing wet with macerated specimens in set 2, all measured parameters were significantly higher in wet specimens except the length of the trochlea.

An increase of the angle of the trochlea also intensifies the incongruency of the ankle in plantarflexion. Since the angle is larger in left tali, the incongruity is also larger on the left side. Thus increased internal rotation or 'wobbling' movements become possible. Therefore the talus, and with it the whole foot, can be tilted around a sagittal axis. When the left foot is taken as the one that provides for stabilising support it makes sense that the trochlea shows a wider angle, which will allow for an increase in rotatory movements. On the other hand the right ankle should be more stable to ensure better guidance for the intended movement. Therefore, a strong connection can be suspected between footedness and the form of the trochlea.

When constructing a prosthesis one has to consider that the trochlea cannot be simply mirrored from one side to the other but should be recalculated. Also the fact that footedness seems to correlate with the angle of the trochlea can additionally be helpful.

41 Acoustic biomicroscopy of the porcine and bovine intervertebral disc: a 50 MHz study. By S. JOHNSON, M. HALLIWELL and D. McNALLY. *Department of Anatomy*, *School of Veterinary Science*, *University of Bristol*, *UK*

The intervertebral disc (IVD) is a major source of low back pain, and its internal structure is key to its mechanical function. So far it has only been possible to investigate the internal structure of the IVD in normal and pathological states using dissection and histological techniques, both of which lead to the destruction of the IVD. We present the results of an ongoing study to image the internal structure of IVDs using an acoustic microscope operating at 50 MHz, with a resolution of 30 μm. Two porcine tails and lumbar spines, and 2 bovine tails were all obtained fresh from an abattoir. Soft tissue was dissected free from all specimens and the posterior elements removed to leave only the vertebral bodies and intervening IVDs. IVDs from porcine tails, bovine tails and porcine lumbar spine were approximately 5 mm, 20 mm and 35 mm in diameter respectively. The acoustic microscope consists of a 50 MHz transducer that operates in pulse-echo mode and all images are displayed as a brightness-modulated timebase (B-scan) in which the amplitude of echoes reflected from tissue boundaries determines the brightness of the display. The time difference between the propagation of the initial pulse and the return echo gives a measure of the depth of the reflecting surface. B-scan images of the IVDs along different scanning axes were acquired to produce images of transverse and longitudinal sections through the IVDs. Spatial compounding was performed on the smaller porcine tail IVDs to produce a complete transverse section through the disc. All images showed distinct repetitive banding indicative of the lamellar pattern within the annulus fibrosus (AF); lamellar discontinuities were also apparent. Lamellar thickness varied: 48–65 μm, 120–162 μm and 81–129 μm for porcine and bovine caudal discs and porcine lumbar IVDs respectively. The latter showed regional variation with lamellae being much more compact and narrow at the posterolateral regions compared with the anterior and posterior regions. Where tissue penetration permitted there was a clear distinction between the AF, nucleus pulposus, vertebral body, cartilage and IVD. A pathological abnormality was also noted in one of the porcine tail IVDs.

42 MR dynamic anatomy of the cervicothoracobrachial outlet in asymptomatic subjects. By C. FONTAINE, X. DEMONDION, C. PAUL, A. COTTEN, A. DRIZENKO and J.-P. FRANCKE. *Institute of Anatomy*, *Medical Faculty "Henri Warembourg"*, *Lille*, *France*

Cadaveric studies have already showed the behaviour of the cervicothoracobrachial outlet (CTBO) during shoulder abduction. Nowadays MRI allows its dynamic study in living subjects. The interpretation of the modifications observed on MRI at the CTBO in symptomatic patients needs the knowledge of the usual behaviour of this CTBO in the asymptomatic subjects. The aim of this study was to determine the modifications of the CTBO during shoulder abduction in a reference population of 20 asymptomatic subjects (15 women, 5 men) whose mean age was 35 y (22–49). We used a 1.5 Tesla MRI with a body coil; acquisitions were performed in 2 positions: the upper limb along the body, then at 130° of abduction, lateral rotation of the arm, flexion of the elbow, head in neutral position. 2 sequences were used: spin echo T1 and angioMR. Measurements were carried out by 2 independent examiners. Statistical analysis used Student and Mann–Whitney tests. The following parameters were measured and observed: (1) on the slice showing the limits of the interscalenic triangle: the interscalenic angle (ISA) and the maximum thickness of the muscular belly of the scalenius anterior muscle (AST);

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(2) on the slice where the 1st rib shows its greater length: the angle between the 1st rib and horizontal (AFRH), the minimum costoclavicular distance (CCD) between the posterior border of the clavicle and the 1st rib, the thickness of the subclavius muscle (TSC), the position of the subclavius related to the clavicle and subclavian vessels, and the distances between the posterior border of the clavicle and the thoracic wall in front of the subclavian vein (DSCV) and the subclavian artery (DSCA); (3) on the slice showing the clavicular insertion of subclavius, the distance between the posterior border of subclavius and the thoracic wall (DSCM) and the thickness of subclavius (TSCM); and (4) on the slice showing the insertion of pectoralis minor on the coracoid process, the distance between the posterior border of pectoralis minor and the thoracic wall (DSPC) and the thickness of pectoralis minor (TPMM) measured in front of the axillary vessels.

We noted the following statistically significant modifications between both positions: (1) horizontalisation of the 1st rib (AFRH decrease of 14%) and retroposition of the clavicle; (2) narrowing of the costoclavicular passage (CCD decrease of 37%, and DSCM of 44%), compression of the subclavian vein (DSCV decrease of 57%) and compression of the subclavian a. (DSCA decrease of 56%); (3) close contact between the subclavius m. and the subclavian vein in 17 subjects; (4) narrowing of the subpectoral passage (DSPC decrease of 44%), thickening of pectoralis minor, closer relationships between subclavian vessels and the thoracic wall in 19 cases out of 20; and (5) venous compression in 20 prescalenic canals, 22 costoclavicular passages and 11 subpectoral passages (among 36 sides), mild arterial compression in 2 costoclavicular passages.

43 Anomalies in the deciduous and permanent dentitions following the removal of deciduous canine tooth buds in the Maasai children. By J. HASSANALI. *Department of Human Anatomy*, *University of Nairobi*, *Kenya*

Amongst the Maasai and other pastoralist communities in Kenya, deciduous canine tooth buds (dcb) have been removed by elder women since about 1960 in infants aged 6 mo to 2 y as a cure for febrile illnesses. The Maasai also remove the mandibular deciduous and permanent central incisors as traditional practice at 6 mo and 6 y respectively to create a space for feeding in case of lockjaw. Dental plaster casts (250) of rural Kenyan Maasai school children aged 3–16 y were prepared over the period 1985–1990. In 60 dental casts of 6–8 y old Maasai children, 64% were lacking deciduous canine (DC) with loss of canine space. A missing DC was more frequent in the mandibular (52%) than maxillary (27%.) arch. Biometric analysis of 23 dental casts of children aged 3–5 y (with missing mandibular central incisors) was carried out to assess the effect of dcb removal in the deciduous dentition. All DC were present in 10 casts and missing in mandibular, maxillary or both arches in 13 casts. There was a marked reduction in the arc circumference and in the canine space in the casts with DC missing. In the dental casts of 78 male and 44 female 12 y olds, 28 males and 20 females showed anomalies of permanent teeth. In the casts of 14 male and 5 female 16 y olds, 5 males had dental anomalies. The anomalies included mainly missing, rotated or ectopic permanent canine and crowding in premolar segment of the dental arch. There is a high prevalence of gastrointestinal and other febrile diseases with high child mortality in the children of nomadic communities. Due to the sociocultural aspects, harsh environment, poor transport and health facilities, the community carries out dcb removal as cure for illness. In the deciduous dentition, mesial drift of the deciduous molars results in reduced canine space. With the additional removal of mandibular incisors, deciduous dentition is mutilated. The permanent teeth germs are damaged during the removal of dcb resulting in anomalies and crowding of the permanent dentition.

44 Detection of splice variants VEGF121 and VEGF165 of vascular endothelial growth factor in human Achilles tendon. By T. PUFE, W. PETERSEN, R. MENTLEIN and B. TILLMANN. *Department of Anatomy*, *Christian-Albrechts-University*, *Kiel*, *Germany*

Large aspects of angiogenesis within human tendons are unknown. The aim of this study was to investigate whether vascular endothelial growth factor (VEGF) and which of its splice variants are expressed by fetal and adult tenocytes.

Biopsies were obtained from fetal and adult Achilles tendons. Adult Achilles tendons were obtained from patients with degenerative tendinosis and from human cadavers without any signs or history of tendinosis. VEGF splice variants were detected using RT-PCR. Extra- and intracellular deposition of VEGF and its receptor FLT 1 was analysed immunohistochemically. Quantitative analysis of VEGF was performed by ELISA. To investigate the influence of growth factors or hypocia we established a rat tenocyte model.

Increased VEGF levels were measured in fetal and degenerative tendons in comparison to VEGF levels of normal adult tendons which did not differ significantly from baseline. Immunostaining for VEGF was positive within the intracellular and pericellular matrix of tenocytes in the fetal and degenerative adult Achilles tendons, respectively. In all tissues examined vascular endothelial cells labeled positively for FLT 1. RT-PCR revealed mRNA of VEGF splice variants 121 and 165 but not 189 within the fetal and degenerative tendons of adults. RT-PCR for VEGF mRNA of normal adult tendons remained negative. When we incubated 10^6 tenocytes for 72 h in the presence of epidermal growth factor (EGF), or under hypoxia, the concentration of VEGF rose in the supernatant compared to controls. However EGF and hypoxia in combination yielded a much higher increase indicating a synergistic effect on VEGF synthesis.

Our findings indicate that splice variants VEGF121 and 165 are expresssed by tendon cells in the fetus and tendinosis. VEGF 121 and VEGF 165 which do not bind to heparan sulphate proteoglycans of the cell surface and the extracellular matrix reach the endothelial cells directly via diffusion. Increased levels of the potent endothelial mitogen VEGF within tendinosis and fetal tendons suggest that this molecule plays a role in angiogenesis within those tissues. The function of neovascularisation, induced by hypoxia and EGF, in tendinosis is not exactly known. One function might be the repair of microdamage. However ingrowth of vessels within dense connective tissue is combined with

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tissue degradation and may therefore reduce the tensile strength of the tissue.

45 The microarchitecture of rat liver vessels as studied with SEM and EDX. By U. M. SPORNITZ, I. BARTUSKOVA and G. MORSON. *Department of Electron Microscopy, Institute of Anatomy, University of Basel, Switzerland*

The nature of the dual afferent blood supply of the liver is well understood. The microcirculation, and in particular the morphological features of the terminal junctions between the arterial and portal blood stream and the sinusoids however, have so far not been satisfactorily elucidated. Scanning electron microscopy of corrosion casts does not distinguish the terminal branches of the arterial and portal vessels. The terminating segments which connect to the sinusoids can practically not be distinguished from one another on corrosion casts. For the purpose of a clear distinction between portal and arterial vessels we combined corrosion cast and EDX (energy dispersive x-ray analysis). Methacrylate resins, which were used for the preparation of corrosion casts, were mixed with chromium and cobalt salts. The liver was then perfused with methacrylate resins from 2 sides, namely the hepatic artery and the portal vein. The final corrosion casts were then examined with the aid of an energy dispersive x-ray detector (EDAX). The marker substances (cobalt and chromium) could thus easily be detected and their origin traced to the portal or arterial branches.

The major supply is achieved through individual draining of the terminal hepatic arteries and the terminal hepatic veins into the sinusoids. There are however various other patterns of vascular drainage into the sinusoids. Particularly interesting is the arterial drainage with larger branches reaching far into the liver lobule. Other types of vascular drainage are anastomoses of terminal hepatic veins and terminal hepatic arteries into the same sinusoid. The most intriguing type of supply of the liver lobules is that of the terminal hepatic arteries forming some sort of an arterial wall at the periphery of the liver lobules. This can best be observed in corrosion casts which have only been injected through the hepatic artery, but can also perfectly be verified in casts injected from both sides namely arterial and portal with different markers added to the casting resin. The 'arterial wall' consists of a fine network of capillaries supplied from the terminal arterial vessel. It appears logical that this network must have a higher hydrostatic pressure than the sinusoids supplied solely through the terminal portal vessels.

46 The angioarchitecture in human primary tumours and precancerous states. By M. A. KONERDING, E. FAIT, and A. GAUMANN. *Institut für Anatomie, Johannes Gutenberg-Universität Mainz, Germany*

According to current tumour biology concepts solid tumours grow in an avascular phase to a limited size and induce angiogenesis for further growth. However these concepts do not take into account the significance of the changes which have already occurred in vascularity of precancerous states. In addition there has been no comparative data of the microvascular unit in precancerous states and only a little quantitative morphometric information on the tumour type specificity of the vascularity in human primary tumours.

We have recently shown using a simple and accurate technique for 3D-measurements in microcorrosion casts that at least experimental tumours develop a tumour type characteristic vascular network. The aim of this study was to compare the vascularity in human colorectal carcinomas with that of precancerous stares by means of vascular corrosion casting and immunostaining. For this, parameters defining the microvascular unit were analysed on 3D reconstructed images.

Comparisons of the quantified parameters within a given segment of the colon as well as group comparisons of all segments did not reveal any significant interindividual differences. The average value for the intercapillary distance was 107.2 ± 27.6 μm, for the interbranching distances 51.3 ± 28.5 μm, and for the branching angles $87.4 \pm 29.2°$. In colorectal adenocarcinomas the highest vascular densities were generally found in the the peripheral tumour surface (invasion front) with intercapillary distances of 52.2 ± 32 μm, whereas in the tumour centre the intercapillary distances were 175.8 ± 149.9 μm. The smallest vascular diameters were seen in the controls with a mean of 11.9 ± 1.8 μm, whereas those of the tumour periphery were 19.4 ± 6.7 μm and in the tumour centre 30.8 ± 23.9 μm, significantly wider. Again comparisons of the different tumour localisations within the large intestine showed no significant differences.

Comparisons with the vascularity of solitary adenomas and polyposis coli specimens showed that despite a high interindividual variability the average intercapillary distance is 53.9 μm, very close to that of the tumour periphery. Comparable results are found for the interbranch distances and vascular diameters. Interestingly we have also observed a very close correlation ($r = 0.88$ and 0.98 respectively) between intervessel distance and interbranch distance and vascular diameter. The smaller the interbranch distance, the smaller the vascular diameter and intervessel distance. This correlation was not seen in the tumours.

From these data it may be hypothesised that in these precancerous states at least angiogenesis sets in long before the onset of tumour growth. We can however not yet comment on a possible value of the vascular densities in adenomas as a predictive assay, since the numbers studied are not large enough, but it is tempting to ascertain whether microvascular density counts could be of predictive value in adenomas.

47 Turnover of brain perivascular cells of mice and rats. By I. BECHMANN[1], E. KWIDZINSKI[1], A. KOVAC[1], E. SIMBÜRGER[1], U. GIMSA[1], J. PRILLER[2] AND R. NITSCH[1]. [1]*Department of Cell and Neurobiology, Institute of Anatomy, and* [2]*Department of Neurology, Humboldt-University Hospital Charité, Berlin, Germany*

Virchow-Robin's perivascular spaces comprise a region between the basement membrane around pericytes and the basement membrane at the surface of the glia limitans. They are directly connected with the subpial space and harbour a population of cells which are distinct from pericytes and

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perivascular microglia. Due to their high phagocytic capacity they can be labelled by intraventricular injection of tracers. The morphology, function, and cell surface proteins of these perivascular cells suggest their origin from the monocyte/macrophage lineage. Currently, it is unclear whether perivascular cells represent a resident population or undergo continuous turnover.

Using transplants of Green-Fluorescent-Protein (GFP)-transfected bone marrow, we investigated the turnover of these cells in adult mice. GFP-positive cells in the perivascular spaces appeared at 2 wk and increased in number until 12 wk post transplantation. At this time, about one third of perivascular cells (labelled by intraventricular injection of rhodamine-conjugated dextran amine) were also GFP-positive and thus derived from the transplanted bone marrow. To exclude the possibility that this migration observed in chimeric mice was a consequence of bone marrow transplantation rather than a physiological occurence, we evaluated invasion of macrophages of normal adult rats applying techniques different from bone marrow transplantation: (1) consecutive injections of different tracers; and (2) ex vivo isolation of macrophages from the blood, cell labelling, and injection into the same animal to avoid MHC mismatch. Both approaches revealed vivid de novo invasion of macrophages, rendering untenable the concept that perivascular cells form a stable population of macrophages in the brain. Thus brain perivascular spaces are under permanent immune surveillance by blood-born macrophages.

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48 Invasive pericytes in a MDA-MB231 tumour angiogenesis assay. By D. LAUER, M. PAPOUTSI, J. WILTING, B. CHRIST and H. KURZ. *Institute of Anatomy II, University of Freiburg, Germany*

The involvement of pericytes in physiological or tumour angiogenesis is a matter of debate. We studied the expression of pericyte, smooth muscle cell and matrix markers in experimental tumours of the mammary ductal adenoma MDA-MB231 cell line that were grown on chick or quail chorioallantoic membrane (CAM) for 7 d (Papoutsi et al. *Histochem. Cell Biol*. **113**, 2000). The expression pattern and colocalisation of fibronectin and laminin, of β1 integrin and endothelial markers HT7 and QH1, and of smooth muscle actin (αSMA) and desmin were analysed with conventional and confocal laser scanning microscopy.

The CAM arterial wall showed strong αSMA signal in all smooth muscle cell layers, but only the innermost layer was desmin-positive. Ramified cells with delicate desmin staining accompanied most minor vessels and were also seen basal to the capillary plexus indicating the presence of pericytes. Distribution of matrix components and β1 integrin was well differentiated outside, but not inside tumour spheroids. In the tumours, a diffuse αSMA signal without definite relationship to vascular structures was detected. Strongly desmin positive cells were frequent in the basal region of small tumour nodules and were scattered everywhere in larger tumour masses. They were occasionally associated with vascular structures, but frequently appeared as single migrating cells. We conclude that (1) pericytes stabilise the normal capillary network and microvessels of the differentiated CAM; (2) pericyte like cells may be attracted by MDA-MB231 cells during tumour angiogenesis, but fail to interact properly with endothelial cells in the tumour environment.

49 Analysis of surfactant storing lamellar bodies in type II pneumocytes of human donor lungs—a design-based stereological approach. By M. OCHS[1], J. R. NYENGAARD[2], T. WAHLERS[3], H. J. G. GUNDERSEN[2] and J. RICHTER[1]. [1]*Department of Anatomy, Division of Electron Microscopy, University of Göttingen, Germany;* [2]*Stereological Research Laboratory, University of Århus, Denmark; and* [3]*Division of Thoracic and Cardiovascular Surgery, Hannover Medical School, Germany*

The surfactant system of the lung prevents alveolar collapse by a surface area dependant reduction of the alveolar surface tension. Surfactant consists of about 90% lipid, mainly saturated phosphatidylcholine, and about 10% protein. It is synthesised and secreted by type II pneumocytes of the alveolar epithelium. The intracellular surfactant pool is represented by characteristic storage organelles termed lamellar bodies. Alterations in intra-alveolar surfactant composition and function have been reported to be associated with ischaemia/reperfusion injury during experimental and clinical lung transplantation. It is unknown whether these alterations are due to ischaemic damage to surfactant producing type II pneumocytes. In the present study, we therefore characterised the lamellar bodies in type II pneumocytes of human donor lungs by means of design based stereological methods.

In 5 cases of single lung transplantation, the contralateral human donor lung was used for stereological analysis, provided it could not be matched to another suitable recipient. Systematic uniform random samples were obtained for light (LM) and transmission electron microscopy (TEM) after ischaemic storage of 228–303 min. The following parameters were estimated. (1) The mean volume of type II pneumocytes (by applying the planar rotator on cells sampled with a physical disector at the LM level). (2) The mean number of lamellar bodies per type II pneumocyte (by applying the physical disector at the TEM level). (3) The mean volume of the lamellar bodies (by use of the global estimator Vv/Nv). (4) The volume-weighted mean volume of the lamellar bodies (by applying point-sampled intercepts) and, by combining (3) and (4), the standard deviation of the size distribution of the lamellar bodies. (5) The mean diameter (height) of the lamellar bodies (by profile counting in a physical disector).

The mean volume of type II pneumocytes was found to be in the range of 600–1000 μm^3 (mean 780 μm^3). The volume fraction of lamellar bodies (mean 11.36%; CV 13%; range 9.5–13.3%) as well as the total volume of lamellar bodies per type II pneumocyte (mean 91 μm^3; CV 13%; range 76–106 μm^3) remained fairly constant. The mean number of lamellar bodies per type II pneumocyte was 400 (CV 31%; range 250–590), their mean individual volume was 0.24 μm^3 (CV 30%; range: 0.16–0.33 μm^3), their mean volume-weighted mean volume was 0.33 μm^3 (CV 43%; range 0.17–0.55 μm^3), and their mean diameter was 1.1 μm (CV 21%; range 0.9–1.5 μm). In the 5 lungs investigated a

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smaller lamellar body size was related to a higher number of lamellar bodies and vice versa.

The same amount of intracellular surfactant can either be stored in more but smaller or in fewer but larger lamellar bodies. Although the volume fraction of lamellar bodies within type II pneumocytes might give an indication of coarse alterations of the intracellular surfactant pool, provided the volume of the type II pneumocytes remains constant, this parameter alone is not suitable for a detailed quantitative characterisation since it does not reflect differences in the way type II pneumocytes store their surfactant material prior to secretion. To elucidate the functional significance of these findings, data concerning the number and the size of lamellar bodies can be correlated with preoperative (donor-related) and postoperative (recipient-related) clinical data.

50 Renal excretory sectors. By M. BURYKH. *Department of Operative Surgery and Topographic Anatomy, Kharkiv State Medical University, Ukraine*

The most vulnerable aspect in endourological operations and partial resections is anatomy. There are still such complications as haemorrhage and renal infarctions as well as an extravasation of urine through a nephrostomy and urine fistulas.

1094 normal human kidneys and 18 abnormal (with duplication of ureter) were studied by the corrosion method and pyelography with the following topometric and mathematics analyses.

It has been stated that in fact the renal pelvis is a calicopelvic complex (CPC) built up of renal calyces (RC), urine ducts (UD) and renal pelvis (RP). To open into the RP, RC join together forming UD (superior and inferior or superior, middle and inferior; or superior, middle anterior, middle posterior and inferior) which transport urine to its container, the RP. One can see that groups of RC with pyramids and surrounding them cortical substance form renal excretory sectors (RES) of the kidneys where processes of urine formation and transportation through elements of nephrons and CPC take place. These are 2 (superior and inferior) or 3 (superior, middle and inferior), or 4 (superior, middle anterior, middle posterior and inferior) RES. The existence of RES is proved by such congenital anomaly of CPC as duplication of ureter where UD of superior and inferior RES do not form RP but run to the urinary bladder separately. On the basis of obtained anatomical data, RES may be distinguished as analogous to bronchopulmonary segments in lungs. The suggested data about RES will contribute to further improvement in operative technique of partial renal resections as well as to anatomical nomenclature.

51 Muscarinic acetylcholine receptors in naevi, primary melanomas, and metastases: possible function of muscarinic receptors in infiltrative growth. By M. OPPITZ, A. BOSS, E. STEINER, U. GARBE and U. DREWS. *Institute of Anatomy, University of Tübingen, Germany*

In an earlier study we identified muscarinic receptors in atypical cells of malignant melanomas and naevi, in cryostat sections with the antibody M35 (Lammerding-Koppel, *J. Cutan. Pathol.* **24**, 1997). Pharmacology of Ca^{2+}-mobilisation on muscarinic stimulation of the cell line SK-mel 28 indicated the presence of the M3 subtype identical to the muscarinic receptors expressed in embryonic development (Noda, *Cancer Lett.* **133**, 1998). We now present data from immunohistochemistry using subtype specific antibodies in paraffin sections. Melanoma cells reacted with the m3 subtype, whereas the m2 subtype specific reaction was found in blood vessels. Muscarinic receptors were present in solid strands and nests of melanoma cells. Strength of immunoreaction was increased in peripheral zones of tumours or naevus cell nests. In addition, infiltrating white blood cells—in particular neutrophils—showed strong immunoreaction for muscarinic receptors.

To clarify this observation, neutrophils from peripheral blood were compared with SK-mel 28 human melanoma cells. In contrast to melanoma cells neutrophils showed no Ca^{2+}-mobilisation. In western blots SK-mel 28 cells exhibited a regular m3 subtype pattern, whereas with neutrophils the respective bands were slightly shifted to lower molecular weights.

Re-expression of muscarinic receptors was correlated with immunoreactivity to a monoclonal antibody specific for melanosomes. In analogy to above referenced data from comparative pharmacology, immunohistochemistry gives evidence for an m3 muscarinic receptor subtype on melanoma and naevus cells. In conclusion we suggest that muscarinic acetylcholine receptors mediate active infiltrative growth of transformed skin melanocytes.

Acetylcholine acts as a mediator of embryonic growth in morphogenetically active cells. We assume that embryonic neurotransmitter pathways are reactivated during malignant transformation of adult cells and enable malignant cells to undergo invasive growth. Muscarinic acetylcholine receptors thus may function as proto-oncogenes.

52 Pharmacological treatment of isolated goldfish retinae influences the morphology of PKC-immunoreactive Mb bipolar cell axon terminals and their staining with FM1-43. By U. D. BEHRENS, A. F. MACK, B. WALLENFELS-THILO and H.-J. WAGNER. *Anatomisches Institut, Tübingen, Germany*

We investigated the morphology of Mb bipolar cell axon terminals, specifically the curvature and the number of terminal swellings, by pharmacological manipulation of the activity of the Mb cells in isolated retinae. Our aim was to create controlled conditions for a link between the state of activity and the morphology of the terminals.

Isolated dark-adapted goldfish retinae were incubated for 30 min in K^+-Ringer (50 mM), Ringer containing bicuculline (100 μM), a specific GABA-A receptor antagonist, or Ringer containing TPMPA (100 μM), a specific GABA-C receptor antagonist. Some experiments were done in the presence of nifedipine (50 μM) to block L-type Ca^{2+} channels. Aldehyde fixed tissue was then prepared for PKC-immunohistochemistry and analysed at the light microscopical and at the EM level. The activity of Mb terminals was directly monitored in slice preparations treated with TPMPA (100 μM) and the styryl dye FM1-43 (5 μM) and analysed using confocal microscopy.

The number of terminal swellings of Mb terminal processes was significantly increased after incubation with K^+-Ringer (205%), bicuculline (254%) and TPMPA

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(265%) compared with dark controls (100%). With K^+-Ringer, bicuculline and TPMPA treated retinae the number of convex sides of the terminal was significantly enhanced compared with dark controls. Treatment of retinal slices with FM1-43 and TPMPA leads to the specific labelling of Mb terminals and their processes, where GABA-C receptors are preferentially localised. The effects of K^+-stimulation on the morphology were not observed in the absence of external Ca^{2+} or in the presence of the L-type Ca^{2+}-channel blocker nifedipine.

In vitro experiments with dark adapted retinae reveal that depolarisation with K^+ or pharmacological disinhibition of Mb terminals leads to morphological changes which are typical for light adapted terminals. Our results indicate that the increased activity of the Mb terminals results in a convex synaptic curvature. Therefore the Mb terminals can be used as a model to analyse the molecular mechanisms of activity dependent changes of neuronal structures.

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53 Protective effects of endogenous leukaemia inhibitory factor on cholinergic basal forebrain neurons. By O. SCHNELL[1], T. NAUMANN[1], Q. ZHI[1], M. KIRSCH[1], O. SCHUBERT[1], M. SENDTNER[2] and H.-D. HOFMANN[1]. [1]*Department of Anatomy, University of Freiburg; and* [2]*Department of Neurology, University of Würzburg, Germany*

Cholinergic neurons of the medial septum/diagonal band complex (MS/DB) project to the hippocampal formation via the fimbria-fornix system. Transection of this system leads to degenerative changes in these neurons. Application of exogenous neurotrophic proteins including NGF and the gp130-associated neurokines ciliary neurotrophic factor (CNTF) and leukemia inhibitory factor (LIF) attenuates degenerative changes in axotomised cholinergic MS neurons. Interestingly, the neuroprotective effect of LIF includes the maintenance of choline acetyltransferase (ChAT), while the closely related CNTF does not prevent the axotomy induced loss of ChAT-immunoreactivity but improves postlesional survival and maintains p75 expression. A corresponding endogenous role of these proteins has not been demonstrated so far.

To investigate the potential role of endogenous CNTF and LIF in the lesioned brain, we have compared changes in alternate 5 μm in the number of cholinergic MS neurons following bilateral transection of the fimbria-fornix in wildtype, CNTF−/− and CNTF−/−/LIF−/− mice. In addition, we have analysed the postlesional regulation of CNTF, LIF and CNTF/LIF receptor components. Unexpectedly we found that during the first 2 wk after lesion, the number of ChAT-immunoreactive neurons remained significantly higher in CNTF-deficient (60% of unlesioned controls) as compared to wildtype animals (34%). Three weeks postlesion cell numbers had decreased to 23% of the controls both in wildtype and CNTF knockout mice. When in addition to CNTF, one or both alleles of the LIF gene were inactivated, axotomy induced degeneration was enhanced: 16%, 30%, 52% of the cholinergic MS were lost during the first postlesional week in CNTF−/−/LIF+/+, CNTF−/−/LIF+/− and CNTF−/−/LIF−/− mice respectively. As shown by in situ hybridisation, CNTF receptor α mRNA was transiently upregulated in many MS neurons following lesion. Upregulation of LIF receptor β mRNA was stronger and was preferentially located in ChAT-immunoreactive neurons. LIF expression was markedly upregulated in the MS neurons and in astrocytes at the lesion site.

Our results indicate that endogenous gp130-associated cytokines, and LIF in particular, exert neuroprotective effects on axotomised cholinergic basal forbrain neurons in the early postlesional period.

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54 Evaluation of the number of nitric oxide-synthesising neurons in the spinal cord of chronically spinalised rats. By P. TRUDRUNG, U. WIRTH and S. MENSE. *Department of Anatomy and Cell Biology III, University of Heidelberg, Germany*

It has been hypothesised that chronic pain close to the level of the lesion and hyperreflexia in the lower urinary tract (LUT) after spinal cord injury (SCI) develop due to a reduction in the activity of inhibitory mechanisms in the spinal cord itself. Previous work from our laboratory has shown that the local release of nitric oxide (NO) exerts a tonic inhibitory influence on nociceptive neurons in the spinal cord dorsal horn. The aim of the present study was to analyse if there are alterations in the number of NO synthesising neurons in the vicinity of a complete chronic SCI and in lumbosacral segments related to the innervation of the LUT which, if there is a reduction in number, might contribute to the generation of pain close to the level of the lesion and hyperreflexia in the LUT, the latter being almost regularly observed after SCI.

Female Sprague Dawley rats (6 per group) were deeply anaesthetised and under aseptic conditions either completely spinalised at the level T9–T10 or sham-operated (laminectomy only). Postoperatively animals were closely monitored for any signs of distress, they received antibiotics and analgetics. After 6 wk survival time, horizontal serial sections of the spinal cord rostral and caudal to the lesion site in lumbosacral segments and corresponding segments of sham-operated controls were analysed for the presence of NO synthesising neurons histochemically (NADPH-dependent diaphorase reaction) and immunohistochemically (antibody against nNOS). Cell numbers were counted and statistically evaluated (Mann–Whitney U-test, 2-tailed).

The number of NO synthesising neurons was reduced on both sides of the lesion, the reduction being statistically more significant for nNOS and rostral to the lesion site. In lumbosacral spinal cord segments related to the innervation of the LUT the number of NO synthesising neurons was also significantly decreased in comparison to sham-operated controls.

These results support the hypothesis that in the vicinity of, and especially rostral to, a chronic lesion of the spinal cord NO synthesising neurons are strongly reduced. This reduction of NO synthesis might contribute to a considerable decrease in tonic inhibitory influence on neurons processing nociceptive information in the dorsal horn and therefore to an increased perception of pain in segments in the vicinity of the chronic lesion of the spinal cord. The decrease in number of NO synthesising neurons in lumbosacral segments of the spinal cord in a similar way might

contribute to a hyperactivity in reflex pathways involved in the innervation of the LUT.

55 The role of CNTF in glial responses following optic nerve lesion. By M. KIRSCH, A. MARTIN and H.-D. HOFMANN. *Department of Anatomy I, University of Freiburg, Germany*

Ciliary neurotrophic factor (CNTF) is a member of the IL-6 family of cytokines with pleiotropic effects in the nervous system. When injected directly into the CNS it can induce multiple aspects of gliosis, suggesting an involvement in regulating postlesional responses. We have shown previously that expression of CNTF and its receptor components is upregulated in activated astrocytes in a variety of lesion models. To more fully understand the role of CNTF in postlesional processes in the CNS we studied glial reactions in mice with mutations of the CNTF gene following optic nerve lesions.

The optic nerve of anaesthetised mice was crushed intraorbitally and cryosections of the superior colliculus (SC) were stained with immunocytochemical markers for astrocytes and microglial cells (5 animals of each genotype). In addition, expression of mRNAs for GFAP, vimentin, CNTF, its receptor components, LIF, IL-6 and TGFβ-1 was studied by quantitative RT/PCR.

In the SC of unoperated wildtype animals CNTF was found to be expressed at low levels. Its expression was strongly upregulated following lesioning. This was accompanied by activation of astrocytes as shown by their increased GFAP expression and hypertrophy. Interestingly, basal levels of GFAP were significantly reduced in control CNTF knockout animals to 30% of wildtype levels, which would suggest that CNTF is involved in normal astrocyte function. However following lesioning GFAP expression (mRNA and protein) was upregulated more strongly and more rapidly in CNTF knockout mice (10 fold, cf. 3 fold in wildtype), arguing against a direct and dominant role for CNTF in regulating GFAP expression. Of the other growth factors studied, only TGFβ-1 was more strongly upregulated in knockout animals, which could account for the observed increase in astrocyte activation. Expression of LIFR and gp130 mRNA increased more strongly in knockout as compared to wildtype animals. Strength and time course of microglial activation was similar in wildtype and in knockout animals.

Our results suggest that CNTF plays a role in regulating astrocyte function in the normal brain and that in the absence of this factor astroglial responses to lesion are exaggerated, probably because other gliosis-inducing factors like TGFβ-1 are upregulated more strongly.

56 Switching off the gene in mice overexpressing peripheral myelin protein 22 results in a reversal of phenotype. By P. K. THOMAS[1], A. M. ROBERTSON[1,2], R. H. M. KING[1] and C. HUXLEY[2]. [1]*Department of Clinical Neurosciences, Royal Free and University College Medical School; and* [2]*Division of Biomedical Sciences, Imperial College School of Medicine, London, UK*

Charcot-Marie-Tooth disease type 1a (CMT1A) usually results from a duplication on chromosome 17p11.2 leading to overexpression of the PMP22 gene. This causes a progressive demyelinating neuropathy. To determine whether the phenotype is reversible we have made a conditional mouse model where overexpression of the PMP22 gene can be switched off by introducing a tetracycline-responsive promoter. mRNA studies have confirmed that the tTa inducer can be turned off within 7 d by the addition of tetracycline to the drinking water.

Animals fed tetracycline from before birth showed minimal pathological changes. They were compared with mice that had been overexpressing PMP22 for 2 mo, resulting in a pathological phenotype, followed by a 2 mo period where the gene was turned off. Within 2 mo myelination had improved considerably, to nearly the same level as the animals that were corrected for life. In a second set of animals that had been corrected for the first 2 mo and then had the tetracycline withdrawn for a further 2 mo, demyelination occurred but less extensively than in those not initially treated.

57 Molecular basis of protective effects of Th2 cells in CNS inflammation in murine organotypic cultures. By U. GIMSA, S. PETER, I. BECHMANN, D. HAAS and R. NITSCH. *Department of Cell and Neurobiology, Institute of Anatomy, University Hospital Charité, Berlin, Germany*

Autoreactive T cells are involved in demyelination, neurodegeneration and recruitment of peripheral macrophages and unspecific activated T cells in autoimmune diseases of the central nervous system such as multiple sclerosis (MS). The role of Th2 cells in the pathogenesis of or recovery from these diseases remains to be elucidated. Also, it is still unclear whether Th2 cells are able to cross the blood-brain barrier since studies searching for Th2 cytokines within the brain of experimental autoimmune encephalomyelitis (EAE) models and MS patients have been controversial. We studied the activation of microglial cells (MG) by antigen-specifically activated Th1 and Th2 cells of transgenic mice whose T cells express a receptor specific for myelin basic protein. We utilised organotypic entorhinal-hippocampal slice cultures from wildtype mice which gives us the advantage to study cell-cell interactions without influences of the blood-brain barrier. The activation state of MG was judged by morphology and expression of ICAM-1 and CD86 (B7-2). ICAM-1 is an adhesion molecule expressed on activated MG which has costimulatory functions. CD86 is a costimulatory molecule necessary for induction of T cell proliferation. We regard it as the main results of our study that MG were highly activated in slices with Th1 cells but displayed a resting phenotype in slices cocultured with Th2 cells. Resting MG show a ramified morphology in slices with invaded Th2 cells whereas activated MG become amoeboid when Th1 cells have entered brain tissue. Moreover ICAM-1 was highly expressed at MG in slices cocultured with Th1 cells. In slices cocultured with Th2 cells ICAM-1 was not detectable at all. The failure of Th2 cells to elicit MG activation could be the reason why Th2 cells have protective effects in EAE models. Interestingly CD86 is highly expressed on MG in control cultures and slice cultures with Th2 cells but not detectable in slices with Th1 cells. Other reports suggest a role of CD86 in preferentially stimulating Th2 cells. We hypothesise that Th1 cells suppress the inherent anti-inflammatory micro-environment of the

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brain by downregulating CD86 expression and upregulating ICAM-1 expression while Th2 cells support intrinsic anti-inflammatory properties.

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58 Arterial variations of the upper limb in human embryos. By M. RODRÍGUEZ-NIEDENFÜHR[1], G. J. BURTON[2], J. DEU[1] and J. R. SAÑUDO[1]. [1]*Unit of Anatomy and Embryology, School of Medicine, Autonomous University of Barcelona, Spain; and* [2]*Department of Anatomy, University of Cambridge, UK*

Variations of the brachial, radial, ulnar and median arteries have been reported in almost 20% of adults. To explain them several hypotheses have been advanced but none of them has been confirmed by embryological studies. Furthermore in the available literature there is no previous report which analyses the development of arterial patterns of the upper limb in all human embryonic stages and on a large sample including several embryos from each stage.

The present work is based on a sample of 112 serially sectioned human embryos, comprising several embryos from each of the stages 12 to stage 23. The embryos belong to the Bellaterra Collection (Spain) and the Boyd Collection (U.K.).

Our results show that from stage 18, a well developed arterial pattern could be observed as far as the hand, therefore we present the cases of arterial variations observed in stages 18 to 23 (75 embryos-150 upper limbs). The variations observed were as follows. (1) Origin of the radial artery above the elbow: 21 cases (14%). (2) Origin of the ulnar artery above the elbow: 7 cases (5%). (3) Superficial brachial artery dividing into the radial and ulnar artery and the deep brachial continuing as the interosseous trunk: 1 case (1%). (4) Superficial course of the brachial artery in front of the median nerve: 13 cases (9%). (5) Two brachial arteries rejoining before reaching the elbow: 1 case (1%). (6) Median artery reaching the palm: 28 cases (19%). (7) Superficial course of a median artery: 1 case (1%).

Each of those variations has been observed in different stages, therefore it can not be concluded that the variations observed in the adult are the persistence of a transitory embryonic stage as has been proposed. We present the development of the arterial patterns of the upper limb as an initial capillary network that, following a proximo-distal sequence, remodels to get to its definitive morphology as it has been proposed in experimental studies, the definitive adult pattern being established at stage 20/21.

59 A morphometric study of the extra-coronary collaterals. By N. LACHMAN[1], R. D. ACLAND[2], E. A. VANKER[5], E. H. AUSTIN[3], B. A. ARRONSON and K. S. SATYAPAL[5]. [1]*Department of Human Biology, Technikon Natal, South Africa; Departments of* [2]*Surgery,* [3]*Cardiology and* [4]*Radiology, University of Louisville, Kentucky, USA; and* [5]*Department of Anatomy, University of Durban Westville, South Africa*

Collateral vessels from bronchial, diaphragmatic and mediastinal regions and the vasa vasorum of the great vessels are known to form anastomoses with the branches of the coronary arteries. These extra-coronary collaterals (ECCs), first described by Hudson (1921), are today thought to be the source of unwanted bleeding in trying to maintain a dry operative field during cardiac surgery. In addition they are now being seen as potential supplementary vessels that may be therapeutically enhanced to sustain a compromised myocardium. Although Hudson's classic presentation provided a basis for the localisation of ECCs, its value is limited by the use of extensively invasive exploration techniques coupled with the technological limitations at the time. For clinicians to be able to manipulate ECCs, their detailed morphometry needs to be documented. This has not been described in the literature reviewed. This study employs a minimally invasive technique that allows cannulation of the coronary ostia endoscopically. In pilot investigations the ostia were visualised using an endoscope passed through the right subclavian artery. The coronary arteries were cannulated with 4 mm through-lumen Fogarty catheters. Under fluroscopic control a PbO-microfil solution was injected to produce complete filling of the coronary tree. After allowing the injection mass to solidify a detailed microdissection was carried out. ECCs were identified and measured by direct optical comparison using calibrated metric rods. In pilot investigations it was possible to trace ECCs to their points of origin, count the number of connections, and measure the length and external diameter of the main branches. Results of our findings will be presented.

60 Shape transformation of the parasellar internal carotid artery and its relationship to intimal hyperplasia. By W. J. WENINGER[2], S. MENG[1], S. U. WENINGER[1], G. B. MÜLLER[1] and C. REITER[3]. [1]*Department of Anatomy, University of Vienna, Austria;* [2]*National Institute for Medical Research, London, UK; and* [3]*Department of Forensic Medicine, University of Vienna, Austria*

In 84% of the population a transformation from a straight running fetal parasellar internal carotid artery (pICA) to a heavily bent adult "carotid siphon" starts in newborns and continues during childhood. We analysed the right pICAs of 30 human infants (aged 0–9 mo) in order to find a relationship between intimal hyperplasia and the shape of the pICA. In 22 specimens the pICA showed the characteristic features of a siphon, although the angle of the occipital knee of the siphon was less acute than in adults. One intimal cushion was related to each, the C3-, the C4-, and the C5-segment of the pICA. In 8 specimens however the pICA took a straight course through the parasellar region. It could be demonstrated that cushions within the C4- and the C5-segment occur significantly less often in these straight blood vessels than in the curved ones. In 4 of the straight pICAs neither a cushion within the C4-segment, nor a cushion in the C5-segment occurred. We therefore conclude that intimal hyperplasia, and subsequently the occurrence of atherosclerotic plaques, is closely linked to the shape transformation process of the pICA.

61 Observations on the orbital groove in South African blacks. By G. T. LEBONA and A. I. EJORH. *Department of Human Anatomy, Medical University of Southern Africa, Medunsa, South Africa*

Low (*Anat. Rec.* **95**, 1946), perhaps for the first time, referred to a groove in the lateral wall of the human orbit in a skull with bilateral absence of the foramen spinosum. He attributed it to the route of an anomalous middle meningeal artery, which probably originated from the infraorbital artery. Years later, Royle (*J. Anat.* **115**, 1973) demonstrated that the groove was a relatively common variant in normal skulls, and concluded that it housed an anastomosis between the middle meningeal and the infraorbital arteries. Diamond (*J. Anat.* **173**, 1990) however, showed it to be an artefact produced by an abrupt thinning of bone. Incidences of 8.5%–45% have been reported in various racial groups. This study set out to establish a database for South African blacks. A total of 340 dried adult skulls were examined for the presence of the orbital groove and its character. The groove was found in 216 of the 680 orbits (31.8%) representing 156 individuals (45.9%). 60 skulls (38.5%) displayed a bilateral groove, 72 (46.1%) showed it on the right side, and 24 (15.4%) on the left side. A vertical cleft crossed the orbital plate of the greater wing of the sphenoid in varying degrees of length, width, and depth from the lateral extremity of the superior orbital fissure to the posterior end of the inferior orbital fissure. Each groove was continuous with that in the middle cranial fossa made by the orbital branch of the middle meningeal artery. In 2 cases (1.8%) the groove projected to a foramen meningo-orbitale. Our overall incidence of 31.8% accords well with 34.4% reported in the UK (Royle, *J. Anat.* **115**, 1973), but is considerably higher than 8.5% cited for the USA (Diamond, *J. Anat.* **173**, 1990). While the morphological results presented here correlate in part with those previously reported, the groove's rare origin from a foramen meningo-orbitale does not allow population comparisons to be drawn. The finding of a continuous groove in the orbit and in the middle cranial fossa supports the hypothesis linking it with a vascular shunt. Verification by cadaveric dissection is, however, essential.

62 The left vagus nerve: a wax anatomical model made by Tramond (late 19th century) representing the 10th cranial nerve dissected at the levels of the head, neck and trunk. By P. LE FLOCH-PRIGENT. *Institut d'Anatomie, U.F.R. de Medecine Paris-Ouest, U.F.R Biomidicale des Saints Pères, Paris V, France*

A real size wax model represents the left vagus from the base of the cranium to the stomach with its pathway and its branches exactly represented. It includes the head and the left anterolateral part of the neck. The pathway through the middle and the posterior mediastinum with the aorta and its upper large vessels behind the left root of the lung (medially reclined and posteriorly dissected) is shown in front of the left costal wall in endothoracic view with the sympathetic chain, the intercostal vessels and nerves. The passage through the diaphragm by the oesophagus accompanied by the left vagus (then below its terminal branches) is presented in the upper part of the abdomen with the inferior aspect reflected by 2 hooks, exposing its inferior aspect with the biliary, venous, arterial and nervous elements. The spleen and the 2 arterial circles of the lesser and greater curvature are presented around a full stomach. The sample (number 263 in the Surgical and Radiological Anatomy catalogue of 1995) lies flat on a whitish cloth, the nape pushed up by a semicylindrical log in wax, laid horizontally on a wooden base ($78 \times 48 \times 5$ cm) with a groove hollowed inside its upper perimeter in order to accept a glass lid (now replaced by a more substantial one in glass with a metallic part of 5 cm in height). A paper label "TRAMOND" is stuck on the upper face of the base authenticating is origin. Some aspects of the representation, particularly cutaneous ones approach a mannered style. This sample is made on a real skeleton explaining its actual size. Tramond was a maker of anatomical models in wax in Paris, very active in the second part of the 19th century; he worked closely with medical anatomists.

63 Respect and dignity in the dissecting room: learning from Thailand? By A. WINKELMANN[1] and F. H. GÜLDNER[2]. [1]*Institute of Anatomy, Freie Universität, Berlin, Germany; and* [2]*Department of Human Anatomy, University of Natal, Durban, South Africa*

The 'dissecting room experience' has been described as disturbing for medical students. It may lead to undue detachment from future patients who are seen as mere objects. Although these views are controversial, they remain an issue for most (Western) students facing their first dissection session. Based on our experience from anthropological fieldwork (A.W.) and several years of teaching anatomy (F.H.G.) in Thailand we present a fresh view on these issues by means of a cross-cultural comparison.

Since at least the 1920s, anatomical dissection by students has been an integral part of medical education in Thailand. More recently, a well functioning body donation programme has been established although it partly contravenes cultural tradition, particularly individuals' concerns about rebirth. Overall a remarkable way of dealing with issues of respect and dignity in the dissecting room has been developed. At the beginning of the dissection course students are 'introduced' to the cadavers in a Dedication Ceremony, a Buddhist mortuary ritual adapted for the purposes of anatomical dissection. As an anatomical cadaver the donor is honoured as 'ajarn yai', Great Teacher, which signifies a high social position in Thai society. Students know the cadaver's name and history and enter into a well defined and familiar social relationship with them, similar to a relationship of respect and gratitude for a teacher. This is facilitated by Thai-Buddhist attitudes towards death which differ markedly from Western concepts and blur the boundary between 'donor' (the living) and 'cadaver' (the dead). At the end of the course students and teachers express their respect and gratitude through another ceremony, in which the students carry 'their teacher' to the cremation ground.

In this way, we observe a different approach to handling the inevitable ambiguities of anatomical dissection which produces a less stressful and more dignified atmosphere in the dissecting room. This may not easily be transferable to a Western context, but offers valuable 'food for thought' for those dealing with cadaver dissection.

64 Micro-injection of small GTPases Rac, Rho and Cdc42 into neutrophils in culture. By M. M. BIRD[1], A. RIDLEY[2] and A. W. SEGAL[3]. [1]*Division of Biomedical Sciences, Queen Mary and Westfield College, London; and* [2]*Ludwig Institute for Cancer Research and* [3]*Department of Medicine, University College London, UK*

The neutrophil is a specialised phagocytic cell capable of engulfing micro-organisms into membranous vacuoles within which they are destroyed. In order to investigate the effect of various proteins on the cell biology of these cells it would be valuable to be able to micro-inject them with proteins known to be contained within their intracellular vacuolar stores and which are released and incorporated into the plasma membrane to meet the demands of cell adhesion, migration and phagocytosis. This is difficult to do on small cells since the volume of the micro-injection fluid must allow sufficient protein to be injected into the cell whilst maintaining cell viability and limiting damage. In recent years pressure injection experiments have been successfully used on increasingly smaller cells and the purpose of this study was to micro-inject neutrophils and observe subsequent changes to the F-actin cytoskeleton. Neutrophils were purified from fresh blood by dextran sedimentation followed by centrifugation through a ficoll/Hypaque gradient and hypotonic lysis of erythrocytes, re-suspended (1×10^6/ml) in a HEPES-buffered Ham's F-12 culture medium and then plated onto 13 mm glass coverslips coated with fibronectin and collagen and maintained at 37 °C for 60 min. Fine glass needles were loaded with 5 µl injection buffer containing 100 mM KCl and 5 mM $MgCl_2$ buffered with HEPES (1 mM) to which FITC-labelled rat IgG was added as a marker protein to detect injected cells. Neutrophils were injected with V12Rac, V14Rho and Cdc42 (1 mg/ml) at a working pressure ranging between 100–300 hPa. The cells were returned to the incubator and maintained at 37 °C for a further 60 min before fixation and processing with antirat IgG and TRITC-labelled phalloidin.

The micro-injected cells immediately lost their actin stress fibres and rounded up. The F-actin was redistributed as a thick ring immediately below the cell membrane and diffusely distributed elsewhere. With V12Rac the cells became polarised and the actin more evenly distributed. In V14Rho injected cells focal adhesions appeared in areas close to the substrate and with Cdc42 microspikes were formed. The results show that it is possible to micro-inject neutrophils with small GTPases and observe changes in the f-actin cytoskeleton.

65 Facilitated migration of human epidermal keratinocytes and dermal fibroblasts in primary culture. By J. SUTHERLAND[1], M. ROBERTSON[2], W. MONAGHAN[2], M. RIEHLE[2] and S. BRITLAND[1]. [1]*School of Pharmacy, University of Bradford; and* [2]*Department of Electronics and Electrical Engineering, University of Glasgow, UK*

Oriented cell growth, division and motility are vital processes for successful development and are believed to be orchestrated in part by a system of morphogenetic guidance cues. Experimental studies have shown that model morphogenetic guidance cues incorporated into microengineered cell culture substrata are indeed capable of influencing these aspects of cell behaviour. Developing the ability to direct cell growth and steer cell motility has implications within the area of Cell and Tissue Engineering, for example in the seeding of biomimetic tissue scaffolds and in the construction of bioprostheses. The present study has investigated the necessary microtopographic parameters and adhesive characteristics required for microengineered surfaces to facilitate the migration of 2 types of human skin cell in primary culture.

Microtopographic and micropatterned collagen adhesive culture substrates were fabricated using techniques adapted from those employed in the microelectronics industry. Tissues were kindly offered by patients undergoing elective surgery as approved by the Local Research Ethics Committee. Primary human keratinocytes and dermal fibroblasts were isolated, amplified and stored according to methods established in our laboratory. Cells were cultured for 24–48 h in hepes-buffered media at 37 °C prior to quantitative semi-automated phase contrast video microscopical image analysis of cell motility, including parameters such as average velocity and persistence.

On planar surfaces fibroblast (serum-treated) and keratinocyte (collagen-treated) migration was multidirectional with velocities of 3.55 ± 0.46 µm/h and 5.13 ± 0.51 µm/h respectively (mean ± S.E.M). Fibroblasts were exquisitely sensitive to microtopography being aligned and bidirectionally guided even by sub-100 nm deep features at 1–10 µm groove widths (3.6 ± 0.64 µm/h at 2 µm pitch), but were mostly unresponsive to micropatterned adhesiveness. In contrast, keratinocytes were guided by topography alone only at depths in excess of 1 µm at 1–10 µm groove widths with a velocity of 7.93 ± 1.3 µm/h at 7×10 µm grating dimensions. The velocity of keratinocyte migration accelerated to 10.47 ± 1.49 µm/h on 7×10 µm microtopography with superimposed parallel micropatterned adhesiveness of matching width. Interestingly keratinocytes could be encouraged to migrate within grooves or along ridges depending on the relative positioning of the micropatterned adhesive tracks.

This study has confirmed that cellular engineering techniques may be useful in potentiating directed migration of human skin cells.

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66 Human keratinocytes in primary culture display 3 distinct phenotypes with differential motility. By J. SUTHERLAND[1], M. ROBERTSON[2], W. MONAGHAN[2], M. RIEHLE[2] and S. BRITLAND[1]. [1]*School of Pharmacy, University of Bradford; and* [2]*Department of Electronics and Electrical Engineering, University of Glasgow, UK*

The term 'contact guidance' has been used frequently to define the effects of substrata on the morphology, oriented growth and directed motility mainly for cultured cells. A number of reports have also described how model morphogenetic guidance cues can be used to harness the phenomenon of contact guidance and induce alignment and facilitated migration in a variety of cell types. However, it has become clear that cells do not all respond in the same way to the same cue. In the preceeding abstract we reported that primary human dermal fibroblasts and keratinocytes exhibit different behavioural responses on the same model

culture substrata. In the present study we describe how 'homogenous' keratinocytes within the same culture resolve into 3 distinct phenotypes, each having a distinct morphology and exhibiting differential motility.

Microtopographic and micropatterned collagen adhesive culture substrates were fabricated using techniques adapted from those employed in the microelectronics industry. Tissues were kindly offered by patients undergoing elective surgery as approved by the Local Research Ethics Committee. Primary human keratinocytes were cultured according to methods established in our laboratory. Cells were plated and maintained for 24–48 h in hepes-buffered media at 37 °C prior to quantitative semi-automated phase contrast videomicroscopical image analysis of cell motility, including parameters such as average velocity and persistence.

Three distinct keratinocyte phenotypes were observed; clusters of spread cells, single spread cells or single rounded cells. Videomicroscopical analysis revealed the 3 phenotypes to be related. Single spread cells intermittently detached from colonies and some of these went on to assume the rounded phenotype. Rounded cells were the most motile with a mean velocity of 14.61 ± 1.63 μm/h (mean ± SEM) compared to single spread cells and clusters of spread cells at 7.47 ± 0.45 μm/h and 3.62 ± 0.84 μm/h respectively. Differences in velocity were reinforced by measures of total distance travelled and it was apparent that the motility of all 3 phenotypes was guided effectively on the microengineered surfaces. Immunocytochemical observations suggested that changes in keratinocyte phenotype may have been accompanied by variation in the pattern of cytokeratin expression.

The results of the present study suggest that motility in primary human keratinocytes is dependent on cell phenotype and whether cells are isolated or members of colonies.

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67 β1 integrin deletion and destruction of the cytoskeleton causes abolished G protein-mediated signalling and altered G_i distribution in cardiomyocytes. By W. BLOCH[1], B. FLEISCHMANN[2], HAN-JIE[1], M. WALTER[1], Y. FAN, S. XUE, R. FÄSSLER[3], J. HESCHELER[2] and K. ADDICKS[1]. *Institutes of [1]Anatomy I, and [2]Neurophysiology, University of Cologne, Germany; and [3]Department of Experimental Pathology, Lund, Sweden*

The functional relevance of β1-integrin deletion for cellular signalling and the subcellular distribution of G-proteins was investigated in embryonic stem (ES) cell derived cardiomyocytes.

When signalling was investigated using the patch clamp technique, the muscarinic modulation of the L-type Ca^{2+} channel (VDCC) was found to be absent in β1 integrin deficient cardiomyocytes, whereas it was restored in the rescue line (G201N). Conversely the G_B dependent β-adrenoceptor as well as atrial natriuretic peptide mediated modulation of VDCC remained intact. Further experiments provided evidence that the muscarinic signalling defect was at the G_i protein level. This was supported by using immunocytochemistry and deconvolution techniques for high spatial resolution, where G_i was detected but without the network-like, focal adhesion protein (vinculin/talin) associated distribution in the β1 integrin (−/−) cardiomyocytes, which was found in wildtype cardiomyocytes. A direct protein-protein interaction between the β1-integrin and G_i-proteins was excluded by performing double immunogold labelling on wildtype ES cell and murine embryonic heart derived cardiomyocytes. However the fundamental role of the cytoskeleton in regulating G_i-subcellular localisation was further proven by the use of cytocholasin D, known to disrupt the cytoskeleton, which resulted also in loss of muscarinic signalling and an altered distribution pattern of G_i.

We demonstrate for the first time, that β1-integrin deficient cardiomyocytes lack muscarinic signalling, while β-adrenoceptor mediated stimulation of I_{Ca} was found to be intact. This signalling defect appears to be caused by a disturbed cellular distribution of G_i since in addition to our functional data, immuncytochemistry combined with deconvolution techniques as well as ultrastructural studies showed a defect in the localisation of G_i proteins as well as cytoskeletal components.

Therefore it can be suggested that an alteration of cell membrane anchorage as well as actin cytoskeleton organisation are necessary for the localisation and function of G_i-protein in embryonic cardiomyocytes.

68 The human lacrimal drainage system contains lymphoid tissue and is a component of the secretory immune system. By E. KNOP and N. KNOP. *Department for Cell Biology in Anatomy, Medical School Hannover, Germany*

Mucosa-associated lymphoid tissue (MALT) is a component of the immune system located at mucosal surfaces of the body. Knowledge about MALT at the ocular surface and especially inside the lacrimal drainage system is limited. After we identified lymphoid tissue as a regular constituent of the normal human conjunctiva (termed CALT) we were interested to study its continuation in the drainage system, since antigens and foreign materials are probably washed away from the ocular surface and carried there by the tear flow. Therefore the total lacrimal drainage systems from cadaveric human eyes that appeared normal at macroscopic inspection were excised, embedded in paraffin (n = 13), epoxy resin (n = 5), or frozen in OCT compound (n = 6) and investigated histologically, immunohistochemically and ultrastructurally. We found a diffuse lymphoid tissue of lymphocytes and plasma cells in all specimens, as a thin and occasionally discontinuous lining along the lacrimal canaliculi with increasing thickness in the common lacrimal duct and lacrimal sac, showing some reduction in the nasolacrimal duct. In the lamina propria, CD3 positive T-cells dominated over CD20 positive B-cells. Plasma cells were frequent, predominantly positive for IgA (fewer for IgM) and the overlying epithelium expressed their transepithelial transporter molecule (secretory component). Intraepithelial lymphocytes were mainly CD8 positive T-cells and also carried the human mucosa lymphocyte antigen (HML-1). Embedded into the lymphoid layer were follicular accumulations which consisted of central areas densely occupied by B-cells and peripheral T-cell zones connected to high endothelial venules (HEV). Follicles were mainly restricted to the common duct, lacrimal sac and nasolacrimal duct but could occur, as an exception, also in the distal lacrimal canaliculi. An overlying follicle-associated epithelium was not always observed. The lacrimal drainage system contains

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lymphoid tissue of the diffuse and organised type that appears integrated into the MALT system by presence of HML-1 positive lymphocytes and HEV. It can perform an immune response by cells and by the secretion of antibodies, the latter characterising it as a component of the secretory immune system.

69 Defence strategies of the human efferent lacrimal pathways against infectious agents. By F. PAULSEN[1], A. THALE[2], U. SCHAUDIG[3] and B. TILLMANN[1]. *Departments of* [1]*Anatomy and* [2]*Ophthalmology, Christian Albrecht University of Kiel, Germany; and* [3]*Department of Ophthalmology, University Hospital Eppendorf, Hamburg, Germany*

Dacryocystitis is the most frequent disease of the efferent lacrimal system. The present study aimed to identify antimicrobial defence mechanisms at work in the human efferent tear ducts.

The human lacrimal sac and nasolacrimal duct of 98 lacrimal systems were examined histochemically to identify glycoproteins, and immunohistochemically to identify mucins, antimicrobial peptides, plasma cells, secretory immunoglobulins, lymphocytes and dendritic cells.

Glycoproteins produced by goblet cells of the lacrimal passage were found to contain carbohydrates including fucose and sialic acid. Muc 2 was detected in the mucus, whereas Muc 1 was absent. The presence of lysozyme, lactoferrin, phospholipase A_2 and secretory IgA was demonstrated in the cytoplasm of apical epithelial cells. Many PGP 9.5-positive structures were visible. In more than one-third of cases, organised lymphoid tissue was found with the cytomorphological and immunophenotypic features of mucosa-associated lymphoid tissue (MALT). The other two-thirds of the cases showed a diffuse infiltrate of defence cells within the lamina propria.

Synthesised mucins from goblet cells form a specialised protective layer on the epithelium of the lacrimal ducts. Together with antimicrobial peptides and immunocompetent cells, this protective layer plays a role in antigen defence and prevents invasion by pathogenic agents. This system is subject to neuroimmune control. In a terminological analogy to other regions of the human body, organised MALT of the efferent tear ducts is designated tear duct-associated lymphoid tissue (TALT). It is not present in the lacrimal passages of all human individuals and it is suggested that this lymphoid tissue may be acquired either in reaction to immunological changes of the lymphatic tissue inside the body or in reaction to specific infections.

POSTERS

P1 Types of neurons of the lateral geniculate nucleus in the guinea pig: Golgi and Klüver-Barrera studies. By S. SZTEYN, K. BOGUS-NOWAKOWSKA, A. ROBAK and M. RÓWNIAK. *Department of Comparative Anatomy, University of Warmia and Mazury in Olsztyn, Poland*

Many studies indicate that analogous nerve centres in mammals often show different types of neurons. This differentiation of neurons is related to their varied functions and connection patterns (Heifer & Schwrtz, *J. Comp. Neurol*. **244**, 1986).

The aim of our studies was full morphological characterisation of the neuronal types in the guinea pig lateral geniculate nucleus. The studies were carried out on 6 diencephalons of adult guinea pigs. The preparations were impregnated using the Golgi procedure or stained with cresyl violet and luxol fast blue (Klüver-Barrera method). Computerised reconstructions were made from selected impregnated neurons. The different populations of nerve cells were categorised on the basis of features concerning their soma (size and shape, form and distribution of tigroid substance), number and arborisation of the dendritic trunks as well as location of the axon.

The following types of neurons were distinguished in the lateral geniculate nucleus of the guinea pig. (1) Fusiform neurons (perikaryons 32–40 μm maximum diameter). From each pole of the soma arise 1–3 thick dendritic trunks. Their dendrites bifurcate twice, once at a short and again at a larger distance from the soma. The dendritic branches sometimes show varicosities. An axon emerges directly from the cell body. (2) Pear-shaped cells (16–20 μm maximum diameter). From one side of perikaryon emanate 1 or 2 thick dendritic trunks. They divide dichotomically close to the soma. The dendrites branch after a distance of 20–30 μm and divide in a tuft like manner with thin ramifications (characteristic to the interneurons). A short axon arises from the opposite side of the cell body. The tigroid substance penetrates deeply into initial segments of the dendritic trunks. (3) Rounded neurons (15–20 μm). From the soma arise 4–7 dendritic trunks without conus. The dendritic trunks divide once or twice dichotomously. They give finally 2–3 thin ramifications which show a varicose course and have knob like protuberances. The thin axon emanates directly from the soma. The tigroid substance does not penetrate into dendritic trunks. (4) Triangular cells (22–30 μm) with 3 thick conically arising dendritic trunks. They bifurcate dichotomously at a distance of 10–20 μm from the cell body. Most dendritic branches divide once again after a long course. Dendritic trunks and their branches are smooth. The final segments of the dendritic tree have a varicose course. A thin axon emerges near to the one of the dendritic coni.

P2 The ramification of retinal fibres in layer 7 of the optic tectum. By T. SEBESTÉNY, N. ZAYATS and T. TÖMBÖL. *Department of Anatomy, Histology and Embryology, Semmelweiss University, Budapest, Hungary*

The study was made using Golgi stain and light and electron microscopy immunotracer methods in the adult chicken. The Golgi method showed the arborisation pattern of optic fibres in layer 7. In addition special types of radial neurons crossed the layer. These neurons (radial neurons 1, 2, 3) gave off characteristic side branches which extend within layer 7. The dendritic side branches interdigitate with the arborisations of optic fibres in layer 7, and contact them. The arborisations of optic fibres in layer 7 were also labelled using a BDA tracer and the results compared with the Golgi impregnated sections. When the BDA labelled material was

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examined with the electron microscope asymmetric synapses of large optic terminals on small dendritic profiles and dendritic processes were observed. Often the optic terminals established 2 asymmetric synapses with a dendritic profile.

The dendritic profiles are proposed to be the dendritic side branches of radial neurons 1, 2, 3. No complex synaptic arrangement or glomerular like organisation around the optic terminals has been observed in layer 7. The input of radial neurons 2, 3 is supposed to be the optic fibres, and this connection may produce reflex like information processing giving a modulatory effect on visual transmission.

P3 Selective striatal connections of brainstem dopaminergic nuclei in the chick (*Gallus domesticus*). By S. MEZEY and A. CSILLAG. *Department of Anatomy, Histology and Embryology, Semmelweis University, Budapest, Hungary*

One day old chicks peck spontaneously at small objects within their field of view. If the object is coated with an unpleasant tasting substance, the chicks peck once, exhibit a disgust response and subsequently refuse to peck at similar objects. This early learning ability, one trial passive avoidance learning (PAL) has been used as a model to study the cellular correlates of learning and memory formation.

The basal ganglia have an important role in PAL in initiating and coordinating movements. The medial part of the avian striatum the lobus parolfactorius (LPO) is also involved in the formation and storage of long term memory for PAL. The LPO appears histologically uniform but probably consists of more distinct subregions. One such region could be the nucleus accumbens implicated in mammals in appetite or aversive behaviour. Striatonigral and striatotegmental pathways are involved in the motor and limbic control of PAL. Their function is probably the modelling of movements and predicting the consequences of behaviour.

We investigated which subregions of the striatum, in particular LPO, project specifically to the substantia nigra (SN) and the ventral tegmental area (AVT) by injecting the fluorescent retrograde tracer Fast Blue into the AVT or nucleus pedunculopontinus pars compacta (the avian equivalent of the mammalian SN).

Our results show that the LPO is made up of hodologically distinct subdivisions. The source regions of striatotegmental and striatonigral pathways partially overlap in the medial and ventrobasal LPO, tuberculum olfactorium, nucleus accumbens and ventral paleostriatum. Striatonigral afferents also arise from the lateral edge of the LPO, the paleostriatum augmentatum and the nucleus septalis medialis. Selective striatotegmental afferents originate solely from the nucleus septalis lateralis. The central and rostral parts of the LPO contained fewer labelled cells indicating that these subregions are involved in some other circuits of the medial striatum.

In mammals the AVT receives most of its striatal afferents from the nucleus accumbens. Given the position of the cells retrogradely labelled from AVT, it is likely that in chicks the nucleus accumbens is not a separate anatomical region but extends to a part of the LPO adjoining the lateral ventricle.

P4 Distribution pattern of smi-32 in visual cortical areas of the marmoset monkey (*Callithrix penicillata*). By Zs. A. BOROSTYANKÖI[1], T. J. GÖRCS[1], C. TOMAZ[2] and K. ZILLES[1]. [1]*C. & O. Vogt Institut für Hirnforschung, Heinrich-Heine-Universität Düsseldorf, Germany; and* [2]*Department of Physiological Sciences, Primate Center, University of Brasília, Brazil*

While the morpho-functional parcellation of various visual cortical areas in Old World monkeys is well described, there are relatively few morphological data concerning New World monkeys. The neurofilament protein smi-32 has been proved a useful marker for architectonic parcellation not only in visual, but also in other cortical areas. Since smi-32 shows a different distribution pattern in various visual cortical areas of Old World monkeys, the immunohistochemical distribution of smi-32 was investigated in visual occipital (V1, V2, dorsomedial DM, dorsolateral DL), temporal (mediotemporal MT, inferotemporal IT), and parietal cortical areas (PP) of the New World monkey *Callithrix penicillata*. Smi-32 immunoreactivity was located mostly in layer III and V pyramidal neurons. Moreover some multipolar cells were found. Areas V1, V2, and PP presented more supra- than infragranular neurons, whereas in MT and DM similar numbers of infra- and supragranular pyramidal cells were found. Furthermore, dorsally and ventrally from MT, almost no smi-32 immunopositive perikarya were observed infragranularly. These regions correspond to area DL (dorsolateral visual area) and IT respectively. In the primary visual cortex the lamina IVC was void of smi-32 immunolabelling, and many more neurons were observed in the higher tiers of lamina IV. The smi-32 immunostaining clearly delineated the IVA and the IVB sublaminae with a stronger labelling in the upper tier, the smi-32-immunoreactive perikarya also being localised to layer IVA. The characteristical distribution pattern of smi-32 in various cortical visual areas of the New World monkey brain, closely resembles that described for Old World monkeys. These data provide further confirmation, that cortical areas with different functions have also different architectonics.

(Zs. B. was supported by a DAAD scholarship).

P5 Colocalisation of GABA and the GluR1 subunit of AMPA receptors in the CA1 region of the mouse hippocampus. By G. ADELMANN, K. MEWS and M. FROTSCHER. *Institute of Anatomy, University of Freiburg, Germany*

Hippocampal interneurons have in the past attracted much attention because of their morphological heterogeneity, their different content of calcium binding proteins and peptides, and their role in the neuronal network. These mostly GABAergic cells are not only influenced by inhibitory but also by excitatory transmission as revealed by electrophysiological and immunocytochemical investigations. However, it is often difficult to show the colocalisation of GABA and ionotropic glutamate receptors of the AMPA-type in tissues that have been fixed using a high glutaraldehyde concentration. We demonstrate here the colocalisation of GABA and the GluR1 subunit of the AMPA

receptor after using a low glutaraldehyde concentration. These investigations were carried out in adult male mice (B6C3Fe, stock of the Institute of Anatomy, Freiburg). Environmental conditions for housing of the animals and procedures that were performed on them were in accordance with the German law on the use of laboratory animals. Mice were deeply anaesthetised with an overdose of Narkodorm-n (250 mg/kg body weight) and were transcardially perfused with a fixative containing 4% paraformaldehyde, 0.1% glutaraldehyde, and 0.2% picric acid in 0.1 M phosphate buffer (pH 7.4). After removing the brains, vibratome sections of the hippocampus were cryoprotected in glycerol, cryofixed in nitrogen cooled propane, substituted in methanol containing 1.5% uranyl acetate, and embedded in Lowicryl HM20. Ultrathin sections were processed for postembedding immunocytochemistry, using a double immunogold labelling protocol in which GluR1 and GABA were visualised by 5 nm and 15 nm gold-coupled antibodies, respectively. Control sections (double labelling in which one of the secondary antibodies was omitted, or single labelling for GluR1 or GABA) were included in the same incubation procedure. On the processed sections interneurons show a strong immunoreactivity for GABA in their dendrites, soma and axon, especially the mitochondria are heavily marked. All large GABAergic dendrites and the majority of small ones exhibit asymmetric synapses which contain, even if located in the same dendrite, different levels of the GluR1 labelling; in most cases the gold particles were arranged in clusters. Only a small number of these asymmetric contacts were not labelled. This double labelling procedure could provide the basis for a more detailed analysis of the synaptic stoichiometry of interneurons, thereby determining morphological aspects of synaptic transmission in this class of hippocampal neurons.

P6 Axon origin in various classes of neurons in the rat central nervous system. By M. RETHELYI. *Department of Anatomy*, *Semmelweis University*, *Budapest*, *Hungary*

It is generally assumed that the axon takes its origin from the perikaryon of the neurons. Systematic observations of Golgi specimens revealed however that in many neurons the axon originates from one of the primary or secondary dendrites.

Golgi-Kopsch impregnated specimens of young adult rats (body weight 120–140 g) were studied and the axon origin was mapped in 5 classes of neurons: large size neurons in the ventral horn of the spinal cord (presumably motoneurons, Class A); small to medium size neurons in the intermediate zone of the spinal cord (Class B); medium to large size neurons in the spinal dorsal horn with dendrites invading the most superficial part of the grey matter (Class C); medium size neurons in the thalamus with dendritic arborisation characteristic to the thalamocortical projection neurones (Class D); and small size neurons in the medial-basal hypothalamus (Class E).

The neurons in each class were grouped based on the site of axon origin: perikaryon (Group P), adjacent to one of the main dendritic trunks (Group DT), or dendrite (Group D). The table shows the proportions of neurons in each class.

Neuron classes	Group P (%)	Group DT (%)	Group D (%)
Class A (n = 63)	67	13	20
Class B (n = 135)	32	23	45
Class C (n = 22)	32	9	59
Class D (n = 103)	65	10	25
Class E (n = 77)	36	25	39

A one third–two thirds rule seems to emerge from this study indicating that in certain neuron classes only one third of the neurons have the classical arborisation pattern. In two thirds of them the axon origin is either intimately connected to one of the dendritic trunks, or is 'displaced' along one of the dendrites. The ratio is reversed in other classes of neurons.

The present findings invite speculations concerning the development of the neuronal arborisation (how did the axon loose its perikaryal position?), the dendritic integration of the synaptic inputs (does the axon-bearing dendrite have a privileged position to initiate the action potential?), axoplasmic transport (how does the soma know the locus of the axon origin?) and neuronal modelling (where should be the axon positioned?)

(Supported by a grant from OTKA T 23166).

P7 Benefits of using electrophysiological techniques for neuroanatomical tracing in the rat. By K. SAEB-PARSY and R. E. J. DYBALL. *Department of Anatomy*, *University of Cambridge*, *UK*

To understand the nervous system it is important to characterise the connections of particular groups of neurons. We sought to determine whether the characteristics of the inputs to the suprachiasmatic nucleus (SCN) from the arcuate nucleus (ARC), the supraoptic nucleus (SON) and the optic nerve change during the light-dark cycle. We used electrophysiological techniques since rapid changes in neural projections are not amenable to study using anatomical tracing methods.

Single unit extracellular recordings were made in vivo from cells in the SCN region of anaesthetised (urethane, 1.1 g kg^{-1} I.P.) male Wistar rats. Animals were kept in a 12 h light–12 h dark environment (lights on at ZT 0) prior to the experiments, and the experiments were carried out under normal laboratory lighting. The response of SCN cells to electrical stimulation of the ARC region, SON region or the optic nerve were investigated by creating peri-stimulus time histograms (PSTHs), which show the probability of occurrence of an action potential after a stimulus pulse. A peak in the PSTH occurring shortly after application of the stimulus pulse reflects an excitatory projection, whereas a trough reflects an inhibitory projection. Complex responses (with both excitatory and inhibitory components) also occur. Recordings were obtained from cells for as long as possible, and their response to stimulation was tested once every hour.

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The response of 78 cells in the SCN region to stimulation of the ARC region were tested on at least 2 occasions with an interval between tests of 1 h or more. The response of 30 of these cells changed with time (e.g., from 'inhibition' to 'complex'). The type of response of the population of cells was also significantly related to the time of recording ($P = 0.024$, χ^2 test), with 15/35 (42.9%) cells excited at ZT 4–8 compared with 8/49 (16.3%) at ZT 20–24 ($P = 0.013$, test for differences between proportions).

The response of 13 cells (of 74 cells tested more than once) in the SCN region to stimulation of the SON region also changed with time, but the corresponding change in the response of the population was not statistically significant. The response of only 2 cells (of 54 cells tested more than once) to optic nerve stimulation changed with time.

Our results suggest that some projections in the rat hypothalamus are not hard wired but change with the light-dark cycle. Electrophysiological techniques are thus suitable for the investigation of short-term changes in connectivity and complement the information provided by anatomical tracers.

(Supported by the MRC and James Baird Trust).

P8 Traumatic optic neuropathy—an anatomical study. By K. JUNGMANN[1], F. PAULSEN[1], A. THALE[2] and R. ROCHELS[2]. *Departments of [1]Anatomy and [2]Ophthalmology, Christian Albrecht University of Kiel, Germany*

In some cases of closed head injuries after minor blunt trauma an amaurosis can occur. Our knowledge concerning the pathophysiological mechanisms of traumatic optic neuropathy is limited. The aim of this study is to analyse the morphology of the optic canal to understand the effect of mechanical forces in the optic canal and the consecutive injuries.

41 optic canals from body donors were analysed by light microscopic, polarisation microscopic, immunohistochemical and scanning electron microscopic techniques. In the optic canal collagen fibrils of the dural sheath were organised in a scissor like pattern. Within this collagen network multiple vessels were integrated. The main component of the extracellular matrix was collagen type I. The dural sheath and pial sheath were connected by collagen bundles. Parallel to these bundles small vessels formed anastomoses between dural and pial vessel systems. In the arachnoid no blood vessels could be detected by immunohistochemical techniques.

In the case of blunt closed head injury with consecutive amaurosis the following pathophysiological mechanism can be discussed based on our morphological findings: (1) squeezing and rupture of nutritive vessels by transduction of shearing forces via the collagen scissor like network; (2) atrophy by pressure of the optic nerve after injury of microvascularisation followed by formation of micro-haematomas and reactive oedema; and (3) direct injury of axons of the optic nerve by shearing forces within the optic canal.

P9 Growth hormone receptor expression in the central nervous system: does growth hormone promote growth and development of the brain? By D. T. LINCOLN and P. WEST. *Department of Medical Laboratory Sciences, Faculty of Allied Health Sciences, Kuwait University, Kuwait*

Neonatal hypophysectomy does not affect the final brain size and autoradiography shows no uptake of labelled growth hormone (GH) to the brain. Since pituitary derived GH does not pass the placenta and cannot cross the blood-brain barrier, brain growth and function are regarded as being independent of GH. However GH mRNA has been demonstrated in different brain tissue and intracerebro-ventricular injection of GH into adult hypophysectomised rats revealed that both IGF-1 and IGF-2 mRNAs show GH dependence. Such a local response to GH requires the presence of appropriately located GH receptors. We report here the location of the GH receptor in the brain of the rat and rabbit to define potential targets for endogenous GH action in the brain. Receptor distribution was determined by immunohistochemistry with GH receptor/binding protein (BP) specific monoclonal antibodies, obtained by hybridoma technology from Balb/c mice immunised with purified rabbit and rat liver GH receptor. We found widespread distribution of this receptor in the neonatal rat and rabbit brain, with a decline in receptor expression during maturation. GH receptor expression in the rat was most prominent in the neonate and declined with postnatal age. Receptor immunoreactivity was generalised with variation in immunoreactivity in regional areas. In the rat the strongest immunoreactivity was seen in layers 2, 3, 5 and especially layer 6 of the cerebral cortex, in neurons of the thalamus and hypothalamus, in Purkinje cells of the cerebellum, in cells of the cerebellar nuclei, in neurons of the trapezoid body of the brainstem, and in retinal ganglion cells. Glial cells, notably astrocytes, were also strongly reactive, along with ependyma of the choroid plexus, ventricular lining and pia mater. In the neonatal rabbit, the strongest immunoreactivity was evident in layers 2 and 3 of the cerebral cortex, in pyramidal cells of the hippocampus, and in neurons of the inferior and superior colliculi, brainstem reticular formation, dorsal thalamus and hypothalamus. The cytoplasmic presence of this putatively plasma membrane located GH-receptor is accounted for by the high receptor content of endoplasmic reticulum and the existence of a soluble form of the GH-receptor, namely the GH binding protein derived from the membrane receptor by cleavage. Receptor location corresponded to areas of insulin-like growth factor (IGF) 1 expression in published studies. This mitogen is the intermediate in the somatogenic actions of GH, and increases in the brain in response to intracerebro-ventricular GH administration. GH endogenous to the brain may promote both generalised growth and maturation of specific pathways. Since it is now known that GH is produced within the brain, we can postulate an endogenous GH-IGF-1 axis regulating brain growth, development and maturation. The reported retardation of

visuo-motor and intellectual function in Laron dwarfs, who display a specific GH-receptor defect, provides clinical support for this hypothesis.

P10 Nitric oxide synthase and calbindin expression in brainstem motor neurons of the mutant mouse wobbler. By G. J. CLOWRY[1] and S. McHANWELL[2]. *Departments of [1]Child Health and [2]Neuroscience, Medical School, University of Newcastle, UK*

Somatic motor neurons do not normally express nitric oxide synthase (NOS) or calbindin. However, both molecules can be expressed in response to axonal injury particularly when this is accompanied by cell death. The wobbler mouse is an autosomal recessive mutant characterised by vacuolar degeneration, NOS expression (Clowry & McHanwell *Neurosci. Lett*. **215**, 1996.) and loss of motor neurons (Pollin et al. *J. Neurocytol*. **19**, 1990.) predominantly in the cervical spinal cord. In somatic motor neurons in the brainstem we have investigated the expression of neuronal NOS, by NADPH diaphorase histochemistry and calbindin, by immunocytochemistry. Studies were made on wobbler mice aged from 3 to 8 wk, when motor neurone death is actively occurring. Unaffected littermates were used as controls. Animals were transcardially perfused with fixative under pentobarbitone-induced anaesthesia, 50 μm brainstem sections were cut and processed for histology. NOS positive swollen neurons with eccentric nuclei were found in large numbers in the trigeminal motor nucleus and in smaller numbers in the facial motor nucleus. Such neuronal profiles were not found in the hypoglossal motor nucleus or in control animals. However at least 50% of hypoglossal motor neurons in wobbler mice expressed calbindin. This protein was not expressed by any other somatic brainstem motor neurons. These results show that motor neurones in this mutant model exhibit differential responses to the disease which may provide further clues for understanding the mechanisms of motor neuron death in this and other models.

P11 Colocalisation of NOS-II and HNK-1 during early neuronal development of mouse cortex and retina. By C. ANDRESSEN[1], S. WENISCH[2], R. LEISER[2], S. ARNHOLD[1] and K. ADDICKS[1]. *[1]Department of Anatomy I, University of Cologne, Germany; and [2]Department of Veterinary Anatomy, Giessen, Germany*

Nitric oxide is a cell derived highly diffusible messenger involved in neuronal development and plasticity. In this study the expression of the inducible isoform NOS II is shown to appear in early postmitotic neurones of the central nervous system as shown for embryonic d 12 cortex and embryonic d 17 retina. In these selected compartments the expression pattern correlates with that of HNK-1, a marker of early neuronal differentiation. In contrast, the constitutive isoforms NOS I and NOS III along with the guanylate cyclase/cGMP pathway are detectable at later stages during development (approximately 3 to 4 d after first NOS-II expression), when NOS II immunoreaction starts to fade. Furthermore respective primary cultures exposed to a specific inhibitor of the NOS-II isoform reveal reduced neurite outgrowth formation in differentiating neurones. The transient expression of NOS II together with the in vitro findings support the view of this NOS isoform necessary for early differentiation within a narrow time window, independent of the guanylate cyclase/cGMP pathway.

P12 Ciliary ganglion in chinchilla (*Chinchilla laniger*, *Molina*). By J. KUCHINKA. *Department of Comparative Anatomy, Institute of Biology, Pedagogical University, Poland*

Investigations were carried out on 15 adult chinchillas (*Chinchilla laniger*, Molina) of either sex. The animals were killed by decapitation under ether anaesthesia. Then the oculomotor and ophthalmic nerves were exposed. Further procedures followed the thiocholine method (Koelle & Friedenwald, *Proc. Exp. Biol. Med*. **70**, 1949) adapted (Gienc, *Zool. Pol*. **26**, 1977) for macromorphological investigations. From the remaining animals, tissue was taken for routine histological H & E staining.

The ciliary ganglion of chinchilla is topographically connected with oculomotor nerve. Neurocytes form here 2 or more different agglomerations of nerve cells. They are elongated ellipse shaped structures 0.3–0.5 mm long and 0.1 mm wide. They are located at dorsal branch of oculomotor nerve and in the neighbourhood of optic nerve. The cross sections consist of about 20 nerve cells. Neurocytes are equally dispersed on the whole surface of the cross section. The smallest aggregation had only a few nerve cells around the numerous nerve fibres in the middle of cross section. Intensively stained postganglionic fibres connect agglomerations with each other.

P13 Distribution and chemical coding of neurons in the porcine inferior mesenteric ganglion projecting to the urinary bladder trigone. By Z. PIDSUDKO. *Department of Animal Anatomy, Warmia and Masuria University, Olsztyn, Poland*

This study investigated the distribution and chemical coding of neurons in the porcine left and right inferior mesenteric ganglion projecting to the urinary bladder trigone using combined retrograde tracing and double labelling immunohistochemistry. Retrograde fluorescent tracer Fast Blue (total volume 50 μl) was injected into the wall of both the left and the right side of the bladder trigone during laparotomy performed under pentobarbital anaesthesia. The ganglion was found to contain many neurons supplying urogenital regions. The urinary bladder trigone-projecting neurons (UBT-PN) formed a long stripe distributed along the entire length of the lateral ganglionic border, and additionally a small cluster of these nerve cells was always encountered located close to the caudal colonic nerve output. 10 μm thick cryostat sections were processed for double-labelling immunofluorescence using antibodies against tyrosine hydroxylase (TH), dopamine β-hydroxylase (DβH), neuropeptide Y (NPY), somatostatin (SOM), galanin (GAL), vasoactive intestinal polypeptide (VIP),

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nitric oxide synthase (NOS), calcitonin gene-related peptide (CGRP), substance P (SP), Leu5-enkephalin (LENK) and choline acetyltransferase (ChAT). Immunohistochemistry revealed that the vast majority of UBT-PN neurons were non-adrenergic (TH-positive). Many non-adrenergic neurons contained NPY or, less frequently, SOM and/or GAL. A very small number of the UBT-PN non-adrenergic neurons were cholinergic (choline acetyltransferase positive) and a slightly larger subpopulation of these nerve cells were nonadrenergic and noncholinergic. Some cholinergic neurons contained neuropeptide Y. The non-adrenergic non-cholinergic neurons expressed mostly SOM or NPY and some of them contained NOS or VIP. Many of the UBT-PN were supplied with varicose nerve fibres exhibiting LENK-, CGRP-, ChAT-, GAL-, VIP-, SP- or NOS- immunoreactivity. This study has revealed a relatively large population of differently coded inferior mesenteric ganglion neurons projecting to the urinary bladder. As judged from their somatotopic and neurochemical organisation these nerve cells constitute an important element of the complex neuroendocrine system involved in the regulation of the porcine urogenital organ function.

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P14 Distribution of calcium-binding proteins and vasoactive intestinal polypeptide in the superior cervical and stellate ganglia of young adult and aged rats. By L. M. SPRAGUE, R. A. CORNS and R. M. SANTER. *School of Biosciences, Cardiff University, UK*

The localisation of the calcium binding proteins calbindin-D28k, calretinin and parvalbumin was investigated in young (3 mo) and aged (24+ mo) superior cervical (SCG) and stellate (STG) ganglia by indirect immunofluorescence on cryosections of ganglia removed from male Wistar rats under terminal anaesthesia and fixed in 4% phosphate buffered paraformaldehyde. Sections were also double immunostained for calbindin and vasoactive intestinal polypeptide (VIP) as this neuropeptide has previously been colocalised with choline acetyltransferase in secretomotor neurons (Landis et al. *Brain Res.* **377**, 1986) and serves as a good marker of cholinergic ganglionic neurons. Calbindin and VIP colocalisation was also investigated in the submandibular ganglion of young rats to assess the extent of colocalisation in this parasympathetic ganglion. Calbindin and calretinin were localised in a population of post-ganglionic neuron somata in both young SCG and STG but were more common in the STG. Calretinin immunoreactive intraganglionic fibres were also found in the STG. Parvalbumin immunoreactivity was not detectable in either ganglion. VIP was found in a population of neurons in the young STG which are putative cholinergic sudomotor neurons projecting to sweat glands in the forepaw, but VIP neurons were very scarce in the SCG. In the STG of aged rats there was a decrease in the number of calbindin-positive neurons but the numbers increased in the SCG. In the aged STG the number of VIP-positive neurons declined significantly and there was a slight increase of VIP neurons in the SCG. In the aged SCG, colocalisation of VIP and calbindin was seen in 20% of calbindin-positive neurons. In the young STG, 15% of the VIP neurons contained calbindin and this figure increased to 30% in the aged ganglia. VIP was also colocalised in a proportion of submandibular ganglion neurons but no colocalisation of VIP and calbindin was observed. Colocalisation of calbindin and VIP in secretomotor neurons is clearly variable. The reduction of VIP in the STG is consistent with previous findings of a loss of VIP in nerve terminals innervating sweat glands in both aged rat and human skin (Abdel-Rahman & Cowen, *J. Autonom. Nerv. Syst.* **46**, 1993). The increase in calbindin immunoreactivity in the aged STG may reflect a neuroprotective requirement to ensure that sudomotor activity and particularly the blood flow to sweat glands is maintained.

P15 The distribution and chemical coding of primary sensory neurons supplying the rectum in the pig. By Z. PIDSUDKO[1], J. KALECZYC[1], M. MAJEWSKI[1], M. LAKOMY[1], D. W. SCHEUERMANN[2] and J.-P. TIMMERMANS[2]. [1]*Department of Animal Anatomy, Warmia and Masuria University, Olsztyn, Poland; and* [2]*Laboratory of Cell Biology and Histology, University of Antwerp (RUCA), Belgium*

Combined retrograde tracing and double labelling immunofluorescence were used to investigate the distribution and chemical coding of neurons in dorsal root ganglia (DRG) supplying the rectum in the pig. The study was performed in 3 juvenile pigs (10 kg body weight) of the Large White Polish breed. Retrograde fluorescent tracer Fast Blue (FB; total volume 80 µl) was injected into the wall of both the left and the right side of the rectum during laparatomy performed under pentobarbital anaesthesia. After a survival period of 3 wk the animals were reanaesthetised and perfused transcardially with 4% buffered paraformaldehyde (pH 7.4). Collected DRG including thoracic, lumbar, sacral and caudal (C1 and C2) ganglia were cut into 10 µm thick cryostat serial sections. FB labelled cell counts were done prior to immunohistochemistry. All the sections containing retrogradely labelled neurons from the left L2, L3, L4 and from both the left and right S2, S3 ganglia, that appeared to provide the greatest contribution to the innervation of the rectum, were processed for double labelling immunofluorescence for substance P (SP), calcitonin gene related peptide (CGRP), choline acetyltransferase (ChAT), nitric oxide synthase (NOS), somatostatin (SOM), galanin (GAL), vasoactive intestinal polypeptide (VIP) and Leu5-enkephalin (LENK). Consecutive sets of 5 sections were stained for following combinations of the antigens (one section stained for one combination): SP/CGRP, ChAT/NOS, SOM/GAL and VIP/LENK, respectively.

The porcine DRGs contained altogether approximately 322 FB^+ neurons. The retrogradely labelled neurons were distributed within both the left and right Th14-S4 ganglia. However, the vast majority of them (approx. 85%) were located within the L2, L3, L4 and in the S2 and S3 ganglia. Immunohistochemistry revealed that most of FB^+ neurons expressed CGRP and/or SP. However, a distinct difference in the occurrence of these peptides was found between the neurons located within the lumbar and sacral ganglia. In the lumbar ganglia, the vast majority of FB^+ neurons ($\sim$ 82%) contained CGRP and/or SP while in the sacral ganglia only $\sim$ 46% of the nerve cells expressed immunoreactivity to these peptides. Some FB^+ neurons found within both the lumbar and sacral ganglia expressed also NOS ($\sim$ 18%) or GAL ($\sim$ 6%) often in combination with SP and/or CGRP.

P16 Adenoviral infection of astroglial cell lines as a strategy towards delivery of neurotherapeutic agents. By S. ARNHOLD[1], S. KANDIRALI[1], F. KREPPEL[2], S. KOCHANEK[2], C. ANDRESSEN[1] and K. ADDICKS[1]. [1]*Department of Anatomy I and* [2]*ZMMK, University of Cologne, Germany*

The loss of dopaminergic neurons in Parkinson's Disease is associated with progressive motor disorders leading to the clinical triad of rigor, tremor and akinesia. Although the reasons for this neurodegeneration is still under debate, recent in vitro studies have shown the capacity of some cytokines such as BDNF and GDNF to protect dopaminergic neurons. Another approach is to overcome dopamine depletion within the substantia nigra by transplantation of dopamine producing cells.

In this respect gene therapy seems to be a potentially powerful approach for both cytokine and dopamine supplementation treatment. Of the possible vectors, third generation adenoviruses are currently the most efficient for the genetic modification of cells in vitro that can thereafter be used for transplantation in order to deliver continuously appropriate neuroprotective/neurotransmitter substances in vivo.

For the establishment of such approaches, as a first step 3 different astrocytic cell lines were adenovirally infected with high efficiency, yielding in a large population of green fluorescence protein (GFP) expressing cells. The ability of these cells for integration along with continuous GFP production was demonstrated using organotypic slice cultures of various brain regions. Additional long term transplantation experiments into adult rat brains clearly demonstrate the ability of the astrocytic cells to be used as a nontumorigenic carrier with compartment specific integration resulting in spatially restricted delivery of appropriated drugs.

P17 Microglia activation and migration towards sites of excitotoxic neuronal injury requires active poly-ADP-ribose-polymerase. By O. ULLRICH, A. DIESTEL, I. EYÜPOGLU and R. NITSCH. *Department of Cell and Neurobiology, Institute of Anatomy, Medical Faculty (Charité), Humboldt-University Berlin, Germany*

Many neurodegenerative disorders have been related to excitotoxic brain damage, that induces accumulation of activated microglial cells at sites of neuronal injury and results in the production and release of large amounts of free radicals. During these conditions the nuclear enzyme poly-ADP-ribose-polymerase (PARP) is highly activated in microglial cells. The induction of poly-ADP-ribose synthesis from NAD^+ involves the activation of the PARP by DNA strand breaks and is supposed to exhibit several regulatory functions by covalent attachment of the polymers to the PARP itself as well as to other nuclear proteins. To investigate the functionality of PARP in several characteristics of activated microglia, we transfected BV-2 microglia cells with either a GFP-vector containing an antisense-PARP-sequence (asPARP) or the GFP-vector alone. Whereas control BV-2 cells migrated distinctly towards the sites of neuronal injury after supercultivation on NMDA-damaged organotypic hippocampal slice cultures (OHSCs), asPARP-BV-2 cells were not capable of migration to the injured neurons and were distributed diffusely throughout in the slice tissue. These asPARP-BV-2 cells showed a reduced baseline and activation dependent level of CD11a-expression, whereas expression of CD11b, CD18 and ICAM-1 were not altered after activation. BV-2 cells with inhibited or depleted PARP revealed a reduced activation response following exogenous stimuli like lipopolysaccharide, which is supposed to be dependent on the reduced interaction of the nuclear enzyme poly-ADP-ribose-polymerase (PARP) with the translocated NF-κB under participation of HMG1(Y), as shown by co-immunoprecipitation experiments. Therefore we suggest that PARP could act as an important trigger of microglia activation and migration.

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P18 Efficient generation of tyrosine hydroxylase-positive neurons from murine embryonic stem cells in vitro by an inhibitor of phosphatidylinositol-3-kinase. By F.-J. KLINZ[1], S. ARNHOLD[1], K. KRUTTWIG[1], J. SIODLACZEK[1], E. KOLOSSOV[2], J. HESCHELER[2], C. ANDRESSEN[1] and K. ADDICKS[1]. *Departments of* [1]*Anatomy I and* [2]*Neurophysiology, University of Cologne, Germany*

Embryonic stem (ES) cells derived from the murine D3 cell line were cultured feeder-free in the presence of 1000 U/ml leukaemia inhibitory factor and aggregated to form embryoid bodies (EBs). Treatment of EBs with retinoic acid for 4 d followed by plating on gelatin coated culture dishes resulted in the appearance of highly differentiated neuronal networks, as was shown by immunocytochemistry using anti-MAP2 antibody. This protocol yielded a small subpopulation of tyrosine hydroxylase-positive neurons (about 1 % of total neurons). Treatment of embryoid bodies for 4 d with an inhibitor of phosphatidylinositol-3-kinase, LY294002, resulted in a strong increase in the number of tyrosine hydroxylase positive neurons (about 20 % of total neurons). We conclude that differentiation of embryonic stem cells into tyrosine hydroxylase positive neurons is under negative control of the phosphatidylinositol-3-kinase pathway. Our finding that tyrosine hydroxylase positive neurons can be efficiently generated in vitro from embryonic stem cells may be useful for future cell therapy of Parkinson's disease.

P19 Analysis of perineurial basement membrane collagen IV and laminin in diabetic and control peripheral nerve. By R. E. HILL and P. E. WILLIAMS. *Department of Biological Sciences, The University of Hull, UK*

Diabetic neuropathy is associated with potentially deleterious changes to the structure of the perineurium including thickening of the perineurial basement membrane. As yet the cause of this thickening remains unknown, despite much speculation suggesting that increased deposition of the 2 major basement membrane components, collagen IV and laminin, are likely to be responsible. The aim of this work was therefore to compare the quantities of these 2 major basement membrane components, at the light level, in both human diabetic and control sural nerve tissue.

Diabetic nerve tissue (n = 5) was freshly obtained following lower limb amputation performed because of foot ulceration. Control tissue (n = 5) was obtained via post-mortem, within 24 h of death, from individuals free of both diabetes and peripheral neuropathy. In all cases tissue was taken from a site posterior to the lateral malleolus and immediately immersed into buffered formalin. Ethical permission for tissue collection was obtained from Hull and East Yorkshire Ethical and Clinical Trials Committee. Formalin fixed paraffin wax sections were used for immunohistochemistry to identify perineurial basement membrane collagen IV and laminin. Image analysis was employed to semi-quantitate the amounts of perineurial collagen IV and laminin in relation to fascicle size. In total 68 fascicles from the diabetic group and 53 fascicles from the control group were used in the assessment of either perineurial collagen IV or laminin content.

A linear relationship was demonstrated for both control and diabetic nerve samples between fascicle size and collagen IV content per unit of fascicle perimeter. This same relationship was also found, for both groups, in the case of perineurial laminin content per unit of fascicle perimeter. Comparison of control and diabetic data for both collagen IV and laminin produced no significant differences in overall quantities per unit of fascicle perimeter. These results suggest that thickening of the perineurial basement membrane in the diabetic state cannot be attributed to increased deposition of either collagen IV and/or laminin.

P20 Ultrastructural changes of the neuromuscular junction in reperfusion: an electron microscopic study. By T. TÖMBÖL, J. HAMAR and G. PATAKI. *Department of Anatomy Histology and Embryology*, *Semmelweis University*, *Budapest*, *Hungary*

Skeletal muscle can frequently become ischaemic in clinical practice. Blood supply to an extremity can be disrupted by trauma or during various surgical interventions such as arthroscopy. Reperfusion injury always develops following restoration of blood supply if ischaemia lasts long enough. Most studies have focused on the changes of the muscle fibres or vessels. In our present studies we investigated the changes of the neuromuscular junction (NMJ) in a rat model of the hind limb reperfusion injury. A 2 h ischaemia was applied to one of the hind legs and ultrastructural changes of NMJ both in the fast and slow twitch muscles were studied at early and late reperfusion periods up to 4 wk.

The alterations were confined to the nerve terminals on the presynaptic side of the NMJ. No marked change was seen on the postsynaptic side. The changes affected the mitochondria primarily, the synaptic vesicles, the presynaptic membrane, and finally a large number of terminals degenerated. The Schwann cells are activated, as well the macrophages.

The degeneration was larger and more terminals were affected in the extensor muscles, and their recovery took longer than in the soleus muscles. The degeneration was significant after 2 h following reperfusion in both muscles, and it could still be found after 4 wk in extensor muscles. Recovery started 1 d after reperfusion in both muscles and free postsynaptic surfaces were found 4 wk following reperfusion in extensor muscles.

P21 The myenteric plexus in the pigeon. By T. KUDER, E. NOWAK, A. SZCZURKOWSKI and J. KUCHINKA. *Department of Comparative Anatomy*, *Institute of Biology*, *Pedagogical University*, *Kielce*, *Poland*

The aim of this study was analysis of the myenteric plexus in the pigeon. Studies were performed on 7 adult animals either sex. The animals were deeply anaesthetised with ether and intraperitoneal injection of Nembutal. Then the small intestine was studied by the thiocholine method of Koelle-Friedenwald, modified and adapted for whole-mount preparations by Gienc, and by histological examination. Histochemical investigations showed presence of a characteristic nerve network consisting of many nerve fibres crossing each other and creating openings in a variety of shapes. The density of this network is different according to the particular part of intestine: in ileum there were 137 openings per cm^2, but in other parts of the small intestine only 37 openings per cm^2. In this way the density of the nerve network in the ileum is nearly 4 times greater then in remaining part of small intestine. In the places where the nerve fibres cross each other, there were aggregations of cells. Their length ranged from 300 μm to 1350 μm, bright from 45 μm to 100 μm. The ganglionic neurocytes, usually 17 μm in diameter, had a typical large clear nucleus. Cells were situated among the nerve fibres without a connective tissue capsule.

P22 Neurochemical identification of intrinsic oesophageal neurons projecting to the tracheal muscle in the domestic pig. By H. HAYASHI, J. HENS, D. ADRIAENSEN, T. GOMI, L. VAN NASSAUW and J.-P. TIMMERMANS. *Laboratory of Cell Biology and Histology*, *University of Antwerp* (*RUCA*), *Belgium*

Physiological and pharmacological studies of autonomic nerve mediated responses in lungs and airways have indicated that VIP and/or NO mediate relaxation of airway smooth muscle. Immunohistochemical studies showing the absence of VIP-IR and NOS-IR cell bodies in the intrinsic tracheal plexus of guinea pig (Kummer et al., *Neuroscience* **49**, 1992; Bowden & Gibbins, *J. Auton. Nerv. Syst.* **38**, 1992), together with the observation that NANC relaxations to vagal stimulation can be elicited in vitro provided neural connections between oesophagus and trachea are not disrupted (Canning & Undem, *J. Physiol.* **460**, 1993), suggest that the NANC relaxation pathway to the trachea may be associated with the oesophagus. Further evidence for this hypothesis was produced by retrograde tracing studies showing an oesophageal origin of VIP-IR and NOS-IR nerve fibres innervating the guinea-pig tracheal muscle (Fischer et al., *J. Comp. Neurol.* **394**, 1998; Moffatt et al., *Neurosci. Lett.* **248**, 1998). In view of possible species differences, the present study aimed at investigating these pathways in a large mammal model. To this end, either in vivo or in vitro retrograde DiI tracing was performed in the domestic pig. DiI was injected in the tracheal muscle of organotypic tissue cultures containing both trachea and osophagus, or in the wall of a surgically exposed trachea of an anaesthesised pig. After 5 d in culture, or a postoperative period of 1 mo, tissues were fixed, checked for DiI staining and further processed for immunocytochemistry. All experiments were approved by the local ethical committee of the

University of Antwerp. This study revealed a population of neurons within the myenteric and submucous plexuses of the porcine oesophagus projecting to the tracheal muscle. Combined immunohistochemistry also showed the presence of VIP and NOS immunoreactivity in these neurons, providing further support for the hypothesis that reflexes initiated in the oesophagus may affect airway smooth muscle tone (Canning & Undem, *J. Physiol.* **460**, 1993; *Am. J. Physiol.* **271**, 1994).

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P23 The coeliac and intermesenteric plexuses in the Egyptian spiny mouse. By T. KUDER, E. NOWAK, J. KUCHINKA and A. SZCZURKOWSKI**.** *Department of Comparative Anatomy*, *Institute of Biology*, *Pedagogical University*, *Kielce*, *Poland*

The aim of this investigation was the morphology and topography of the cooeliac and intermesenteric plexus in the Egyptian spiny mouse (*Acomys cahirynus*). Our studies were performed on 8 adult animals, of either sex. The animals were killed by decapitation under ether anaesthesia. For histochemical examinations the material was prepared and studied in situ by the thiocholine method of Koelle-Friedenwald, modified and adapted for use in whole-mount preparations by Gienc.

The celiac plexus in *Acomys cahirynus* is situated on the inferior and lateral surfaces of splanchnic artery. The central part of this plexus is occupied by the large coeliac ganglion. It looks like a croissant, surrounding the beginning of the coeliac artery. Significant callosities are visible at the ends of the ganglion. Numerous of postganglionic fibres were seen to emerge from these callosites and from the central narrowed part of the ganglion, going to the splanchnic artery and cranial mesenteric artery. Occasionally very small accessory ganglia were observed along their course. In the most cases 3–4 thick fascicles of fibres passed along the ventral surface of aorta to the caudal mesenteric artery. On their course, in the vicinity of the cranial mesenteric artery, a single small ganglion appeared. Other aggregations of cells, forming typical ganglia (3–4) were noticed at the level of the caudal mesenteric artery. They formed a plexoganglionic structure, which corresponds to the caudal mesenteric plexus of the other species. Thus in the Egyptian spiny mouse the intermesenteric plexus consists only of a few nerve fibres and the caudal mesenteric plexus.

P24 Distribution of neurons supplying the mesentery fat in the pig. By K. CZAJA[1], R. R. KRAELING[2], M. KLIMCZUK[1], A. FRANKE[1] and M. ŁAKOMY[1]**.** [1]*Department of Animal Anatomy*, *Warmia and Masuria University*, *Olsztyn*, *Poland; and* [2]*Animal Physiology Research Unit*, *R. B. Russell Agricultural Research Center*, *Athens*, *USA*

It is well known that lipolytic activity of mammalian adipose tissue is under adrenergic control (Alexander et al. *J. Dev. Physiol.* **2**, 1980; Bray, *Brain Res. Bull.* **32**, 1993; Havel, *ed. American Physiological Society*, 1965; Himms-Hagen et al. *Neurochem. Int.* **17**, 1990). Furthermore it has been found that the adipose tissue is supplied with adrenergic nerve fibres (Bernard et al. *Anat. Rec.* **191**, 1978; Himms-Hagen et al. *Neurochem. Int.* **17**, 1990). However there are no data dealing with the distribution of neurons projecting to the adipose tissue including mesentery fat in the pig.

The experiment was performed on 3 male and 3 female pigs (50 kg b.w.). The animals were deeply anaesthetised with sodium pentobarbital (Vetbutal Biowet, Poland). During the operation the neuronal retrograde tracer Fast Blue (FB, 50 µl) was injected into mesenteric adipose tissue.

After a survival period of 3 wk the animals were deeply reanaesthetised and transcardially perfused with preperfusion solution followed by ice cold buffered paraformaldehyde (pH 7.4). Sympathetic chain ganglia (SChG) and prevertebral ganglia were removed from each animal and cut into 10 µm thick cryostat sections. FB-positive (FB+) neurons were counted in every third section from the ganglia.

Mesenteric fat FB+ projecting neurons were located in the SChG (Th-13, Th-14, L-1, L-2, L-3), coeliac/superior mesenteric ganglion (CSMG), caudal mesenteric ganglion (CaMG), adrenal ganglion (ADG), aorticorenal ganglion (ARG) and ovarian ganglion (OG) or testicular ganglion (TG). No distinct differences were found in the distribution of the FB+ nerve cell bodies between the male and female pigs.

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P25 Cardiac ganglia in the Egyptian spiny mouse. By T. KUDER, E. NOWAK and A. SZCZURKOWSKI**.** *Department of Comparative Anatomy*, *Institute of Biology*, *Pedagogical University*, *Kielce*, *Poland*

Using the histochemical method of Koelle-Friedenwald (modified by Gienc for use in macromorphological species) and paraffin sections the autonomic cardiac ganglia in 5 Egyptian spiny mice (*Acomys cahirynus*) were detected. In all investigated animals the presence of 2 plexuses in the fat tissue of epicardium were observed. One of these was located on the dorsal surface of the left cardiac ventricle in the vicinity of the dorsal interventricular sulcus. It usually consisted of 4–5 thick nerve fibre fascicles and 2–3 small ganglia near the coronary sulcus.

The other plexus was located in the fat tissue of the epicardium on the right atrium. It consisted of 3–4 larger ganglia (10–15 cells on cross sections) and a number of smaller ganglia consisting of a few cells. All the components were connected by nerve fibres and fascicles forming a plexoganglionic structure. Single nerve cells could be observed along these fibres. In 2 cases a small ganglion at the left atrium was observed as well and a delicate complex of nerve fibres and fascicles on the right ventricle was also noticed.

P26 MR dynamic anatomy of the cervicothoracobrachial outlet in symptomatic subjects. By C. FONTAINE, X. DEMONDION, C. PAUL, A. COTTEN, A. DRIZENKO and J.-P. FRANCKE**.** *Institute of Anatomy*, *Medical Faculty* "*Henri Warembourg*", *Lille*, *France*

Our previous study investigated the usual behaviour of the cervicothoracobrachial outlet (CTBO) in asymptomatic subjects. It now allows the interpretation of the modifications observed by MRI at the CTBO in symptomatic

patients. The aim of this study was to determine the modifications of the CTBO during shoulder abduction in a series of 54 asymptomatic patients (40 women, 14 men) whose mean age was 40 y (range 20–61). We used a 1.5 tesla MRI with a body coil; acquisitions were performed in 2 positions: the upper limb along the body, then at 130° of abduction, lateral rotation of the arm, flexion of the elbow, head in neutral position. 2 sequences were used: spin echo T1 and angioMR. Measurements were carried out by 2 independent examiners. Statistical analysis used Student and Mann–Whitney tests. Measured parameters were the same as in the previous study on asymptomatic subjects. The following parameters were measured and observed: (1) on the slice showing the limits of the interscalene triangle: the interscalene angle (ISA) and the maximum thickness of the belly of scalenus anterior (AST); (2) on the slice where the 1st rib shows its greater length: the angle between the 1st rib and horizontal (AFRH), the minimum costoclavicular distance (CCD) between the posterior border of the clavicle and the 1st rib, the thickness of subclavius (TSC), the position of subclavius related to the clavicle and subclavian vessels, and the distances between the posterior border of the clavicle and the thoracic wall in front of the subclavian vein (DSCV) and the subclavian artery (DSCA); (3) on the slice showing the clavicular insertion of subclavius: the distance between the posterior border of subclavius and the thoracic wall (DSCM) and the thickness of subclavius (TSCM); and (4) on the slice showing the insertion of pectoralis minor on the coracoid process: the distance between the posterior border of pectoralis minor and the thoracic wall (DSPC) and the thickness of pectoralis minor (TPMM) measured in front of the axillary vessels.

The following statistically significant modifications were observed between both positions: (1) horizontalisation of the 1st rib (AFRH decrease of 18%); (2) narrowing of the costoclavicular passage (CCD decrease of 37% and DSCM of 46%), compression of the subclavian vein (DSCV decreased of 51%) and compression of the subclavian a. (DSCA decrease of 55%); (3) retroposition of the clavicles; (4) narrowing of the subpectoral passage (DSPC decrease of 42%), thickening of pectoralis minor, closer relationships between subclavian vessels and the thoracic wall.

However vascular and nervous compressions were observed more often than in the asymptomatic subjects: (1) arterial compression in the interscalene canal in 7 patients (1 bilateral case), out of which 1 complete thrombosis, in the costoclavicular passage in 8 patients (15%); (2) venous compression in the prescalene canal in 27 patients (9 bilateral cases), in the costoclavicular passage in 21 patients (39%, 5 bilateral cases), out of which 2 complete thromboses, and in the subpectoral passage in 10 patients (1 bilateral case); (3) nervous compression with disappearance of the fat tissue surrounding the brachial plexus in the costoclavicular passage in 3 patients (5%).

Finally, our measurements do not allow us to distinguish asymptomatic and symptomatic populations. Venous compressions are very frequent in the asymptomatic population and we must be very cautious when interpreting such images. Arterial and nervous compressions occur more frequently in the symptomatic group than in the asymptomatic group; because modifications observed in the CTBO are quite similar in both groups, these compressions are very likely due to fibrous structures, some of which were shown on MRI.

P27 Thoracic outlet syndrome: arterial compression. By J. M. KALIDEEN[1], P. PARTAB[1], L. RAMSAROOP[1], N. LACHMAN[2] and K. S. SATYAPAL[1]. [1]*Department of Anatomy, University of Durban-Westville; and* [2]*Department of Human Biology, Technikon Natal, Durban, South Africa*

Thoracic outlet syndrome (TOS) embraces a constellation of symptoms emanating from compression of the neurovascular bundle at the thoracic outlet. Compressive disorders of the thoracic outlet represent a diverse spectrum of symptomatology including arterial, venous or neurological manifestations. For clarity these disorders may be classified as arterial occlusion, venous occlusion, and mechanical compression of the brachial plexus.

This study specifically addresses arterial occlusive lesions at the thoracic outlet. The subclavian artery normally exits the thorax in a smooth curve over the broad surface of the 1st rib between the insertions of scalenus anterior and medius muscles.

Arterial lesions are typically but not invariably associated with bony, fibrous or musculoskeletal anomalies that contribute to compression. The effect of cervical rib or anomalies at thoracic outlet is to elevate the subclavian artery in the neck, sharpening the curve and angulating and constricting the vessel. Vessel constriction may lead to a variety of structural changes: arterial stenosis and occlusive lesions, periarterial fibrosis, post-stenotic dilatation and aneurysm formation and intimal plaques with distal embolisation. Very significantly, but rarely, proximal propogation of the thrombus may progress to involve the anterior and posterior cerebral circulations.

Subclavian artery compression is rare, found in less than 5% of TOS and may be accompanied by symptoms and signs of C8, T1 root compression. The intimate relationship of the subclavian artery and C8, T1 roots make the latter an ideal surrogate marker for subclavian artery compression. Commoner in females in 3rd and 4th decades, bilaterality is a feature.

Arteriography remains the test par excellence for showing unassailable evidence of compression. It finds use in diagnostic confirmation, surgical planning, localisation and characterisation of arterial damage, collateral circulation. With arterial lesions any surgical strategy must not only provide relief from vascular compression but also include repair of the arterial lesion.

Case studies presented are angiographic demonstrations of variation in site and degree of stenosis, with collateral circulation when present.

P28 Thoracic sympathetic contribution to the cardiac plexus. By N. PATHER[1], P. PARTAB[1], B. SINGH[2], and K. S. SATYAPAL[1]. [1]*Department of Anatomy, University of Durban-Westville; and* [2]*Department of Surgery, Natal Medical School, University of Natal, South Africa*

Cardiac sympathetic denervation for intractable angina pectoris in patients not suitable for conventional revascularisation is currently gaining popularity since this

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procedure may be performed in a minimally invasive fashion. A thorough understanding of cardiac innervation is crucial to successfully effect cardiac denervation. Cardiac sympathetic pathways are highly variable in their topography accounting for morphological contradictions in the literature. Variations in the sympathetic innervation of the heart have therefore acquired renewed clinical significance. This study aimed to demonstrate the cervical and thoracic sympathetic contributions to the cardiac plexus.

The cervical and upper thoracic portions of the sympathetic chain in 6 fetuses and 4 adults were microdissected bilaterally and documented (n = 20 sides). The origin of cardiac rami from the sympathetic chain was found to be asymmetric and highly variable. The superior cervical cardiac ramus originated from the superior cervical ganglion (present in all specimens) in 25% of cases and from the interganglionic chain in 75% of cases. The middle cervical ganglion (incidence of 68.8%: fetal 31.3%, adult 37.5%) gave rise to the middle cervical cardiac ramus. The stellate ganglion (incidence 93.8%: fetal 50%, adult 43.8%) consistently gave rise to 2 cardiac rami. In the thoracic region, 4 cardiac rami arose from the T2–T5 segment of the thoracic sympathetic chain with the T2 cardiac ramus being the largest. All cervical and thoracic cardiac rami were traced consistently to the deep cardiac plexus.

Ellison & Williams (*Am J Anat* **124**, 1969) in their dissection of 24 sides reported a similar bilateral asymmetry to this study. Khogali et al. (*Eur J Cardiothorac Surg*, 1999) reported success of limited T2–T4 sympathectomy in relieving the pain at rest, of patients with intractable angina pectoris, apparently indicating that a significant afferent pain pathway from the heart is selectively interrupted. The variability in pattern of the cervical ganglia, cardiac rami and cervical contributions to the cardiac plexus does not appear to impact on the outcome of limited sympathectomy. The complexity of cardiac pain pathways is not fully understood. This study is ongoing and attempts to contribute to defining the cardiac pathways, particularly the cervical contributions to the cardiac plexus.

P29 Ileal flange. By B. SINGH[1], J. MOODLEY[1] and K. S. SATYAPAL[1,2]. [1]*Department of Surgery*, *University of Natal*, *Congella; and* [2]*Department of Anatomy*, *University of Durban-Westville*. *Durban*, *South Africa*

The abdominal surgeon is largely unappreciative of the peritoneal reflections of the ileocaecal region. This is not surprising since literature on this subject is sparse. The available descriptions in standard texts outline the appendicular mesentry as developing from the lowest aspect of the ileal mesentry. The inferior ileocaecal recess develops between the mesentry of the appendix and the ileocaecal fold; the superior ileocaecal recess develops between the ileal mesentry and the vascular fold of the caecum. The clinical significance of these recesses is minimal as they neither predispose to entrapment of bowel nor serve as useful landmarks. In this context, the peritoneal flange on the antimesenteric aspect of the terminal ileum warrants consideration. This extension of the appendicular mesentry along a variable distance on the antimesenteric terminal ileum is largely underappreciated as it may hold value as a useful landmark.

At 50 consecutive first time laparotomies, the ileocaecal and peritoneal reflections and recesses were assessed via a questionaire. There were 40 females and 10 males with a mean age of 24.4 y (range 16–64).

The results were documented on a standardised diagram of the ileocaecal region. A peritoneal flange on the antimesenteric terminal ileum was noted in 48 patients. The inferior ileocaecal recess was noted in 43 patients; the superior recess was less consistent, being noted in 18 patients. The average length of the peritoneal ileal flange was 4.1 cm (range 1.8–4.5), with a mean measurement of 1.0 cm (0.8–2.0) at its highest point.

The benefits of a landmark along the distal ileum are many. When surgical access is limited by the size of the incision (including at laparoscopy), the orientation of the small bowel may be facilitated by the recognition of an anatomical landmark that may obviate the potential morbidity of a larger abdominal incision or bowel mobilisation. The location of an appendix, which may prove elusive through a small incision, may be determined by the ileal flange. Furthermore, the distal ileum is the site where an ileostomy (either end or loop) is performed. The early and easy identification of this segment of the small bowel by the ileal fin may be accomplished by an incision at the site of the proposed ileostomy, thereby obviating a second abdominal midline incision.

The ileocaecal region is arguably the commonest site of surgical endeavour for the abdominal surgeon. Greater appreciation of the ileal flange merits a wider disclosure.

P30 Morphological and functional aspects of the Papilla Vateri. By T. BOBKA, F. P. PAULSEN and B. N. TILLMANN. *Department of Anatomy*, *Christian-Albrecht University of Kiel*, *Germany*

Pancreatitis caused by reflux of bile, pancreatic juice, or duodenal content is extremely rare. Even after papillotomy, an intervention that destroys the muscular sphincter of the greater duodenal papilla, pancreatitis is observed only in 5%.

35 human greater duodenal papillae including the distal part of the bile and pancreatic duct were examined by light and scanning electron microscopy to analyse the 3 dimensional architecture of the greater duodenal papilla from functional and clinical points of view. Histologically the wall of the ampulla Vateri was built up of 3 layers: lamina epithelialis, lamina propria and lamina muscularis. Finger like mucosal folds projected extensively into the lumen of the ampulla Vateri. The projections consisted of dense connective tissue rich in collagen fibrils and were lined by a columnar epithelium. Serous glands were located in the lamina propria near the lamina muscularis of the ampulla Vateri. Their excretory ducts opened close to the basis of the mucosal folds. Scanning electron microscopy revealed large mucosal folds inside the ampulla which continued into the pancreatic and bile duct. They were comparable to valves which were connected in series. Near the origin but also in the collagenous network of the valves spaces were visible corresponding to dissolved serous glands. Inside the valves collagen fibrils were arranged in a circular manner.

It can be concluded that the mucosal folds form valves in the lumen of the ampulla Vateri. They allow a unidirectional

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flow of secretions into the duodenum. On the other hand they prevent reflux from the duodenum into the ampulla Vateri. In cases of papillotomy the muscular sphincter will be destroyed but not the valvular system. This mechanism prevents reflux from the duodenum into the ampulla hepatopancreatica or the bile and pancreatic duct by obturating the lumen. Secretions of subepithelial serous glands functionally rinse away aggressive contents from the duodenum to protect the epithelium of the papilla Vateri.

P31 Comparative anatomy of the efferent tear ducts. By M. FÖGE[1], F. PAULSEN[1], A. THALE[2] and B. TILLMANN[1]. *Departments of [1]Anatomy and [2]Ophthalmology, Christian Albrecht University of Kiel, Germany*

Investigations of the comparative anatomy of the epithelium and subepithelial structures of the efferent lacrimal passage are not available. Such investigations would be helpful in evaluating an animal which could serve for tear absorption experiments in the efferent tear ducts. Therefore efferent lacrimal pathways from human, cat, rabbit, pig, deer, and rat were compared by means of gross anatomy and light microscopy.

With the exception of the human lacrimal system which was composed of the canaliculi, the lacrimal sac, and the nasolacrimal duct, all lacrimal systems of animals consisted only of the upper and lower canaliculus which led directly into the nasolacrimal duct. At the light microscopical level the rat showed a multilayer epithelium. Human, cat, rabbit, pig, and deer revealed a stratified epithelium consisting of 2 layers, a basal cell layer and a superficial columnar layer. Goblet cells were integrated in the epithelium of human, rat, and rabbit as solitary cells or as intraepithelial mucous glands. In contrast, the epithelium of the pig contained no goblet cells, and that of the cat just a few. Subepithelially the lamina propria of the human lacrimal passage was composed of 2 strata: (1) loose connective tissue containing elastic fibres and lymphatic cells as well as (2) a rich venous plexus comparable to a cavernous body. A surrounding cavernous system of blood vessels also was found in deer, rabbit and pig, but it was absent in rat and cat. Small seromucous glands opening their excretory ducts into the lacrimal passage were integrated in the lamina propria of human and pig. In pig seromucous glands were distributed along the whole nasolacrimal duct whereas in human the glands only were present in the lacrimal sac. All other animals did not possess seromucous glands.

It was concluded that the morphology of the rabbit lacrimal system is the most comparable to that one of the human. Based on these findings the rabbit could serve for experiments in tear absorption.

P32 A case report: double parotid ducts in a cheek. By Z. A. AKTAN, O. BILGE, Y. A. PINAR and O. F. DEMIR. *Department of Anatomy, Ege University Medicine Faculty, Izmir, Turkey*

During the macrodissection of a 63 y old male cadaver fixed in a solution of 10% formalin for medical students in Ege University Medical Faculty Anatomy Department, 2 main Stensen's ducts in the right cheek were observed. This unknown anomaly, double Stensen's duct in a cheek, has not been reported previously in the literature to our knowledge. Structures over the Stensen's duct were dissected away. The lengths and positions of the ducts were noted. The upper came from the superior part of the gland and the lower came from the inferior part. After this examination the ducts were removed from the cheek for histological examination. Both of the ducts had the ductal columnar epithelium under light microscope examination. This report of an unusual case maybe helpful for surgeons during endoscopy and lithotropsy.

P33 Factors influencing the pneumatisation pattern of the maxillary sinus floor. By T. KOPPE[1], M. NAKATSUKASA[2] and A. YAMANAKA[2]. *[1]Institute of Anatomy, Ernst Moritz Arndt University, Greifswald, Germany and [2]Laboratory of Physical Anthropology, Kyoto University, Japan*

According to the functional matrix theory (Moss, *Am. J. Orthod. Dentofac. Orthop.* **112**, 1997), the maxillary bone can be thought of as composed of several skeletal units such as nasal, orbital, alveolar, palatal, and basal. In contrast to these skeletal units, little is known about the so called pneumatic skeletal unit (Moss & Greenberg, *Angle Orthod.* **37**, 1967), as well as its relationship to the former mentioned skeletal units. This study investigated the pneumatisation pattern of the alveolar process of the maxillary bone in a prehistoric population of Japan. 36 adult skulls of the Jomon population of both sexes from Kyoto University, Japan, were CT scanned at the coronal plane at defined positions: P^1, P^2, M^1, and M^2. Only skulls from skeletons with preserved femora were included in this study. These CT scans first served to define the deepest position of the maxillary sinus floor. To obtain the degree of pneumatisation of the alveolar process a tangent was laid at the floor of the nasal cavity. The distance AB of a perpendicular line from this tangent to the deepest point of the maxillary sinus floor served as a measure for obtaining the height of the maxillary sinus floor. Although in most skulls the deepest point of the maxillary sinus was identified in the region of M^1, there was great variation in the degree of pneumatisation of the alveolar process beneath the floor of the nasal cavity. Whereas the relationship between AB and several linear measurements of the palate was relatively weak, a regression analysis between AB and the largest diameter of the left femur (a reasonable indicator of body size) suggested that the degree of pneumatisation of the alveolar process seems to be a function of body size. This study indicates that the pneumatisation pattern of the maxillary bone cannot be explained exclusively by skull architecture. Ongoing studies of anthropoid primates (e.g., Rae & Koppe, *J. Hum. Evol.* **38**, 2000) suggest that epigenetic factors are likely to influence the final size and shape of the paranasal sinuses.

P34 Nerve supply of the posterior crico-arytenoid muscle. By E. MARANILLO, X. LEON, M. QUER and J. R. SAÑUDO. *Unit of Anatomy and Department of ENT, Autonomous University of Barcelona, Spain*

Vocal cord adduction after recurrent laryngeal palsy is in most cases a serious problem requiring surgery to avoid obstruction of the airway. At present microsurgery tech-

niques in the larynx focus on the possibility of selective anastomosis in the different nerve pedicles. Selective neurosurgery relies on an accurate knowledge of the distribution of the motor nerve supply for each individual intrinsic laryngeal muscle, especially the posterior cricoarytenoid muscle (PCA), the only abductor muscle. Classically, the PCA has been considered as receiving only one muscular branch, from the dorsal or ventral branches of the recurrent nerve (RN). However recently it has been proposed as possibly receiving 3 or between 1 and 9 independent muscular branches.

A total of 75 human larynges obtained from necropsies (47 males and 28 females, age range from 41 to 95 y) were examined by careful dissection using a surgical microscope. Our results showed that the nerve supply of the PCA (right and left) was not symmetric. The ventral branch of the RN gave off from 1 to 6 main muscular trunks to the PCA: 1 (7.3%), 2 (42.7%), 3 (34%), 4 (10.7%), 5 (4.7%) and 6 (0.6%). The uppermost trunk arose from a common trunk with the muscular branch to the arytenoid muscle in 89% of cases. When 2 or more main trunks were present, they anastomosed under the deep surface of the PCA in 64% of cases. The average number of branches that sink into the deep surface of the muscle were 7, independent of the number of main trunks. They came from subdivisions of the main trunks and/or from the anastomosis. The number of branches for territories of the PCA were: superior (average 2.4), middle (average 2.5) and inferior (average 2.1).

The complexity of nerve supply to the PCA muscle demonstrates the difficulty of planning selective surgery thereupon.

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P35 Visualisation of nerves of the tongue in rabbits: preliminary microscopic findings. By T. PEKER[1], H. B. TURGUT[1], A. ANIL[1] and C. PELIN[2]. [1]*Department of Anatomy, Gazi University Faculty of Medicine, Beşeveler, Ankara; and* [2]*Department of Anatomy, Başkent University Faculty of Medicine, Bağlica, Ankara, Turkey*

This study was designed to investigate the distribution of the nerve supply in the tongue of adult rabbits. Twelve tongues were carefully dissected and stained by a modification of Sihler's staining technique. The nerve fibres were observed lying straight at the root of the tongue while they gradually became convoluted towards the tip of the tongue. No significant symmetry was observed between the 2 sides of the tongue for the distribution of nerve fibres. In addition no anastomoses were observed between the nerve branches of the 2 sides of the tongue. Such a staining technique is quite useful not only to determine the detailed anatomical structure, but also to evaluate postoperative nerve recovery in surgical researches.

P36 Physiological, morphological and electronmicroscopic lesions of remnant kidneys after low-dose radiation. By M. AUNAPUU[1], Ü. PECHTER[1], T. SUUROJA[2], E. GERSKEVITS[1], M.-M. MARJAMÄGI[1], E. SEPP[1], S. SUUROJA[1], J. OGANJAN[1], A. GARAJEV[1] and M. OTS[1]. [1]*Faculty of Medicine, University of Tartu; and* [2]*Faculty of Veterinary Medicine, Estonian Agricultural University, Tartu, Estonia*

We hypothesised that the effect of low-dose irradiation of rats with 5/6 nephrectomy stops the progress of focal segmental glomerulosclerosis and interstitial fibrosis. Wistar rats were studied during 8 wk after 5/6 nephrectomy. The left kidney was irradiated 24 h after operation in anaesthetised animals with 3 Gy or 1 Gy in a single dose. There was a group of operated control rats which were not irradiated, and healthy rats without surgery were the control group. We studied the following parameters: urine protein excretion rate (UprotV, mg/day), waking systolic blood pressure (SBP, mmHg), serum creatinine (SCr, μmol/l) and cystatin C (cyC, mg/l) concentrations, and kidney morphology for focal segmental glomerulosclerosis (FSGS, %), interstitial fibrosis (IF, %) and cytology. White blood cell count (WBC, $\times 10^9$/l) was performed at wk IV and VIII. Results are shown in the table as mean ±S.E.M.

Table.

Test Week	UprotV 4	UprotV 8	SBP 8	SCr 8	cyC 8	FSGS 8	IF 8	WBC 4
3 Gy (n = 7)	37.2±5.4†	63.0±22.5	169±3.2*	93.7±3.4*	1.2±0.1*	34.3±6.9*†	1.0±0.4*†	12.1±2.4
1 Gy (n = 8)	43.2±12.4*	64.3±31.0	166±6.5	98.4±4.0	1.2±0.1	50.0±4.2*	1.9±0.4*	9.8±1.5
Contr (n = 8)	107.2±33.0*	92.0±23.0*	180±7.4*	96.1±4.1*	1.6±0.1*	50.9±5.4*	2.2±0.3*	11.9±0.8
HContr (n = 9)	14.1±3.0	18.0±3.5	127±6.9	69.4±6.4	1.0±0.03	8.0±6.3	0±0	14.5±1.5

* $P < 0.05$ vs HContr, † $P < 0.05$ vs Contr

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P37 The sequential segmental analysis of a collection of malformed human hearts: personal experiences. By S. CRAATZ and E. KÜNZEL. *Institute of Anatomy, University of Leipzig, Germany*

To obtain interdisciplinary acceptance, a modern nomenclature of malformed hearts must be universally applicable. This requires systematisation, simplification and a defined terminology. Introduced a few decades ago the sequential segmental analysis provides an appropriate classification system to reach these objectives (Anderson & Ho, *Cardiol. Young* **7**, 1997). To gain practical experience we investigated a donated collection of 292 malformed human hearts according to the criteria of sequential segmental analysis. We registered the frequency of individual heart defects and compared the results with those obtained by previous classification methods. Our aim was to determine advantages and disadvantages comparing sequential segmental analysis with traditional classifications and to recommend a better practical usability.

Sequential segmental analysis is really appropriate for the description of complex cardiac malformations because it is a step-by-step approach based on the morphology of the heart. The method provides basic conditions to compare and systematise many different malformed hearts. However there are several distinct disadvantages concerning ambiguity of terms in some cases (e.g. atrioventricular connection with atrial isomerism), a limited diagnostic possibility of 'simple' malformations like ventricular septal defects or vascular anomalies and the exclusion of aetiological factors. Furthermore no exact statements can be made about spatial relations of segments to each other (e.g. ascending aorta and pulmonary trunk). Finally an exact topographical description of the position of specific anatomical structures (e.g. arterial valves) is restricted.

In conclusion, the sequential segmental analysis needs simplification for better practicability and easy documentation. A graphic system of defined and standardised symbols representing specific morphologic features of the different segments could be useful, and might be applied both in printed form and as a computer program. It could be helpful for cardiologists and cardiac surgeons as well as for pathologists and embryologists. Thus sequential segmental analysis may contribute to an interdisciplinary agreement of classification of malformed hearts.

P38 Improvement of sequential segmental analysis for human malformed hearts. By E. KÜNZEL, S. CRAATZ and K. SPANEL-BOROWSKI. *Institute of Anatomy, University of Leipzig, Leipzig, Germany*

Sequential segmental analysis (SSA) is a method accepted worldwide to classify human malformed hearts (reviewed by Anderson & Ho, *Cardiol. Young* **7**, 1997). Each heart is investigated stepwise in terms of its major developmental units in logical veno-arterial sequence. The segments are the visceroatrial situs, the atria, ventricles and great arteries. The distinction of chambers, valves and arterial trunks ensues from the morphologic method i.e. the structures are exclusively defined on the basis of their inherent morphological characteristics. Apart from the position and morphology of each segment special importance is attached to the atrioventricular and ventriculoarterial junctions.

Despite further evolution and wide acceptance the SSA requires systematisation and simplification. Because of its complexity the SSA realisation is time consuming in understanding before it can be put into practice. Since 292 malformed human hearts collected between 1955 and 1962 by Dr F. Spreer at the University's Institute of Pathology and donated to our Institute had to be recorded, we conceived a ready to use approach of the SSA technique. Instead of graphic charts pictographic symbols were used representing the 6 segments, i.e. venous, atrial, atrioventricular, ventricular, ventriculoarterial and arterial. The pictograms were logical, self-defining and memorable and illustrated terms like isomerism, left or right hand topology, concordant or discordant connections, and mirror-image arrangements. Legends to the pictograms substituted a wordy text.

In conclusion, the pictogram chart for malformed human hearts makes it easier to work with the SSA method and facilitates the diagnostic efforts of the pathologist. It offers a minimum database set that will allow step-by-step data acquisition and basic interpretation. Because of its simplicity and graphic nature our system may represent both a working basis for guidance in practice and a general visualisation tool for congenital heart disease.

P39 Anatomical and surgical aspects of the intramedullary osteosynthesis of proximal humerus fracture. A cadaver study of a new pinning technique. By E. KÜNZEL[1], W. SCHELLER[1], H. LILL[2] and W. SCHMIDT[1]. [1]*Institute of Anatomy, University of Leipzig; and* [2]*Department of Surgery III, University of Leipzig, Germany*

The prognosis of proximal humerus fracture depends primarily on the patient's age and the kind of fracture. Due to the muscular inactivity and osteoporotic bone structure of senescent patients the possibilities for stable osteosynthesis are often limited. The trend towards minimal osteosynthesis increasingly influences surgical treatment. From this point of view we present a new pinning technique which was tested with regard to injury of neurovascular structures. The risk of damaging important anatomical structures was investigated in the undissected shoulders of one cadaver. After longitudinal skin incision 8–10 cm distal to the acromion above the deltoid tuberosity we inserted a guiding wire in the direction of the humerus head. When it had reached its desired position immediately beneath the humerus calotte, the pin was inserted, pushed forward along the wire and opened.

After pin implantation the cadaver shoulders were carefully dissected in order to present the topographic interrelationships of anatomical structures in space. Concerning the possibility of injury to related structures due to incorrect surgical technique, we observed that the musculocutaneous nerve would be endangered if it shows high branching and an acute course within the coracobrachialis muscle near the compacta of the humerus' medial circumference. Parts of the radial nerve could be destroyed if there were a high ramification and distribution of branches to the long head of the triceps brachii muscle proximal to the radial groove. Furthermore it seems possible that branches of the axillary nerve innervating the deltoid muscle are endangered near the insertion point of the pin. Finally

there remains the risk of affecting branches of the axillary artery accompanying the aforementioned nerves.

In conclusion, complications could arise either because of an inadequate surgical technique or the presence of anatomical variations. The pinning technique described could reduce the former risk. In the cadaver studied our pinning of the proximal humerus shaft from laterally did not injure neurovascular structures. Nevertheless, our technique has to be applied in many more cases in order to evaluate the risks due to specific anatomical variations.

P40 Anatomical composition of the superior shoulder joint capsule. By I. KOLTS[1], L.-C. BUSCH[2], H. TOMUSK[1], E. RAJAVEE[1], A. ELLER[3], M. MERILA[3], M. RUSSLIES[4] and W. KÜHNEL[2]. [1]*Institute of Anatomy, University of Tartu, Estonia;* [2]*Institute of Anatomy, Medical University of Lübeck, Germany;* [3]*Clinic of Traumatology and Orthopaedics, University of Tartu, Estonia; and* [4]*Clinic of Traumatology and Orthopaedics, Medical University of Lübeck, Germany*

Recent anatomical and clinical works have revised some classical understandings about the ligamentous structures of the shoulder joint capsule. A bundle of capsular fibres between the Tuberculum minus et majus was proposed to be named as 'Lig. semicirculare humeri' (Clark & Harryman, *J. Bone Joint Surg*. **74-A**, 1992; Kolts et al. *Anat. Anz*. in press, 2000). Anatomy of the origin, composition and attachment of the coracohumeral ligament and relations with the coracoglenoid ligament were also revised. Most of the known ligaments of the shoulder joint lie anteriorly, while the superior and posterior shoulder joint capsule is poorly investigated. The aim of the present work was to study the fibrous framework of the superior shoulder joint capsule under the supraspinatus tendon.

36 alcohol-formalin-glycerol fixed cadaver shoulder joints (46–74 y old) were investigated. The soft tissues, acromion, acromioclavicular ligament and subacromial bursa were removed. Supraspinatus, infraspinatus, teres minor and subscapularis tendons were separated from the joint capsule. The joint capsule, coracohumeral ligament, coracoglenoid ligament and 'Lig. semicirculare humeri' were finely dissected.

The 'Lig. semicirculare humeri' was present in all the 36 preparations. The superior and inferior parts of the coracohumeral ligament coursed from the coracoid process and the coracoglenoid ligament laterally and inserted into the 'Lig. semicirculare humeri'. The superoposterior joint capsule was strengthened by a macroscopically recognisable ligament that arose from the scapula posterior to the supraglenoid tubercle. Medially it lay above the joint capsule and melted laterally with the capsular 'Lig. semicirculare humeri'. Its posterior margin coincided with the posterior edge of supraspinatus.

The new anatomical structure is similar to the coracohumeral ligament, but located on the opposite superoposterior side of the shoulder joint capsule. It ensures the structural and functional balance of the superior capsuloligamentous network. We propose to name the new ligament as 'Ligamentum glenocapsulare'.

P41 Axial pull-out and tangential strength of screws and hooks in the pedicle and lamina of thoracic vertebrae considering the bone mineral density. By L. HACKENBERG[1], T. J. FILLER[2], E. T. PEUKER[2] and U. LILJENQVIST[1]. [1]*Orthopaedic Department and* [2]*Institute of Anatomy, Westfalian Wilhelms-University, Münster, Germany*

Failure of pedicle screws, pedicle hooks and lamina hooks due to low bone mineral density in osteoporotic bone represents a serious complication in spinal surgery. The aim of this experimental study was the determination of the axial pull-out strength and tangential strength of pedicle screws compared with pedicle hooks and lamina hooks in the thoracic spine considering the bone mineral density.

15 human cadaveric thoracic spines were used. Fractures and osteolytic alterations of the vertebrae were excluded by radiographs at 2 levels. Bone mineral density of 5 vertebrae was measured by means of Q-CT. In 2 cases bone mineral density was determined as osteoporotic according to the WHO classification. All screws and hooks were implanted under visual control and in typical configuration of posterior idiopathic scoliosis instrumentation. Thus pedicle screws were compared with pedicle hooks between T4 and T8 and with supralamina hooks between T9 and T12. The implants' maximum pull-out strength in the axial direction, and the elastic and plastic dislocation in the tangential direction, were calculated.

The correlation of bone mineral density and axial pull-out strength was significant for both pedicle screws ($r = 0.907$) and pedicle hooks ($r = 0.873$) in the upper thoracic spine, and for pedicle screws in the lower thoracic spine ($r = 0.92$). The correlation for lamina hooks in the lower thoracic spine was not significant. The correlation of tangential dislocation of pedicle screws, pedicle hooks, and lamina hooks with bone mineral density was not significant. Overall the maximum pull-out strength ($P < 0.02$) and tangential elastic ($P < 0.0001$) and plastic ($P < 0.0001$) dislocation of pedicle screws was significantly higher than that of pedicle and lamina hooks.

Osteoporosis reduces the axial pull-out strength of pedicle screws and pedicle hooks in a signifcant manner whereas the influence on lamina hooks is not significant. This corresponds to findings of other authors. No significant influence of osteoporosis can be determined for the tangential strength of screws and hooks. Regardless of bone mineral density in the thoracic spine the axial and tangential stability of pedicle screws is significantly higher than that of hooks. The authors recommend the use of pedicle screws in the osteoporotic and nonosteoporotic thoracic spine as long as the pedicle size allows.

P42 Supraarticular, supramastoid and suprameatal crests on the outer surface of the temporal bone and the relation between them. By H. B. TURGUT[1], A. AML[1], T. PEKER[1] and C. PELIN[2]. [1]*Department of Anatomy, Gazi University Faculty of Medicine, Beşevler, Ankara; and* [2]*Department of Anatomy, Başkent University Faculty of Medicine, Bağlica, Ankara, Turkey*

The supraarticular, supramastoid and suprameatal crests on the outer surface of the squamous part of temporal bone are of clinical importance as they are accepted as landmarks for

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some surgical approaches to the temporomandibular joint, middle cranial fossa and mastoid air cells respectively. Because of their surgical importance the morphological structure, incidence and the relations between those 3 crests were studied on 442 skulls, 250 male and 192 female.

Suprameatal crests were observed commonly as a trace character on the male skulls (51.6% on the right side, 50.8% on the left side), though no such a crest was seen on most of the females (53.1% on the right side, 55.7% on the left side). Supramastoid crests were commonly observed as a small crest on male (45.6% right side, 47.6 left side), but as a trace character on female (53.6% right side, 50.5% left side) skulls. On the other hand supraarticular crests were commonly seen as a trace character both on male (54.8% right side, 62.4% left side) and female (69.8% right side, 71.4% left side) skulls. In the light of above data it was concluded that the crests on the male skulls were stronger than the ones on female skulls. The angulation between the crests were also examined. It was observed that the angulation between the supraarticular and supramastoid crests were larger in male skulls when compared with females. On the other hand no gender difference was observed when the angulation between suprameatal and supraarticular crests were examined.

P43 Anatomical aspects of computer assisted construction and manufacturing implants for bone defects of the human cranium. By J. BEINEMANN[1], C. LEMKE[1], W. FRIED[2], E. BELEITES[3], D. SCHUMANN[4], and W. LINSS[1]. [1]*Institute of Anatomy I*, [2]*Technical Institute*, [3]*Department of Otorhinolaryngology*, *and* [4]*Department of Maxillo Facial and Plastic Surgery*, *Friedrich Schiller University of Jena*, *Germany*

The reconstruction and the following substitution of damaged bone structures at the human head by implants confront physicians and technologists with many problems. The strict requirements on function and aesthetics among other things depend on the properties of the implant material, especially in compatibility and durability, and also in technical feasibility. All these aspects have to be considered in the context of time and cost efficiency. The currently available procedures for design, construction and manufacturing of complex curved surfaces with exact alignment to the area of defect can be extended with the method presented here.

We have developed a completely computer aided procedure for processing of patients data up to the integrated individual 3-D based manufacturing of implants without substantial interruptions. A virtual 3-D model of the skull with the damaged region is reconstructed from 2-D picture information of individual patient data and is compared with stored medical reference data. The most similar reference object for the patient is selected and a patient-specific implant is generated by application of mathematical methods in combination with modern CAE techniques. Thereby, enormous improvements are established in the treatment of skull defects especially in the restoration of complex structures. In this poster we present the method of the computer-based 3-D reconstruction of skull defects as the currently most convincing method to produce a definitive implant.

P44 The internal vertebral venous plexus prevents impression of the dural sac by bony parts of atlas and axis during atlanto-axial rotation. By H. VAN MAMEREN[1], E. M. REESINK[1], H. KINGMA[2], L. M. A. LATASTER[1] and J. T. WILMINK[3]. [1]*Department of Anatomy/Embryology*, [2]*Department of ENT*, *and* [3]*Department of Radiology*, *University of Maastricht*, *The Netherlands*

In vitro the cross section of the bony spinal canal in extreme atlanto-axial rotation is reduced to 61% at the level of the lateral atlanto-axial joints compared with the neutral position (Tucker & Taylor, *J. Bone Joint Surg*. **6**, 1998). This would lead to an unwanted impression of the dural sac, unless a compensatory mechanism exists. This study explores the volume of the bony spinal canal, of the extradural and intradural spaces and of the spinal cord in the upper cervical region during axial rotation of the head in vivo (5 persons without neck complaints). 60 consecutive 2 mm thick overcontiguous axial T1-weighted MR images (distance between slice centres 1 mm) were acquired. Three volunteers were examined. Two patients who had received intravenous gadolinium contrast injections for suspected intracranial pathology, and in whom MRI findings were normal, agreed to undergo 2 additional imaging sequences of the atlanto-axial region, one in the neutral position and one with rotated head.

During rotation the bony spinal canal proved to narrow by 40% maximally at the level of the lateral atlanto-axial joints, due to a scissor movement between atlas and axis. These rotate in opposite directions around the fluid-filled dural sac which is only minimally deformed. Extradural space changes were quantified in the 3 volunteers. In maximal rotation the extradural space in the atlanto-axial region became markedly asymmetric. Directly above the joints the 50%/50% left-right extradural cross section proportion in the neutral position was changed to 20% ipsilateral and 80% contralateral in maximal rotation. Directly below the joints the opposite occurred. The post-contrast studies in the 2 patients showed that the structures involved in the volume changes were of vascular (venous) origin. We conclude that the presence of a compressible and refillable internal vertebral venous plexus prevents deformation of the dural sac during head turning.

P45 Ossification of Civinini's ligament with lingual nerve entrapment. By E. T. PEUKER, G. FISCHER, T. SZUWART, F. PERA and T. J. FILLER. *Institute of Anatomy*, *Clinical Anatomy Division*, *Westfalian Wilhelms-University Münster*, *Germany*

A number of ligaments in the exocranial region of the foramen ovale are described in the scientific literature. Civinini described the pterygospinous ligament, normally connecting the posterior margin of the lateral pterygoid plate with the base of the spine of the sphenoid bone. This ligament could be more or less ossified. Complete ossification of this ligament is reported in 3 to 4.3%. The size of the resulting pterygospinous foramen varies from 2 to 12 mm. Occasionally the pterygospinous foramen is confused with the ossified pterygoalar ligament of Hyrtl. This ligament extends from the undersurface of the great wing of

the sphenoid bone, near the latero-anterior margin of the foramen spinosum, to the root of the lateral plate of the pterygoid process. The pterygoalar ligament is in a lateral position in relation to the external border of the foramen ovale and in close contact with the sphenotemporal osseous plane whereas the pterygospinous ligament runs more medially and at a greater distance from the base of the skull.

The body donated to the Institute of Anatomy was that of an 83 y old white male. It had been embalmed with a mixture of formaldehyde, chloral hydrate and sorbitum solution. During routine dissection, the masseter muscle, the vertical ramus of the mandible, the zygomatic arch, and the lateral pterygoid muscle were removed, the right mandibular nerve was exposed, and the branches were identified. An unusual course of the lingual nerve in combination with an entrapment of the nerve medial to the ossified pterygospinous ligament was observed. Furthermore, the nerve mobility was restricted by a more distal anastomosis with the inferior alveolar nerve. In addition, an anatomical variation of the auriculotemporal nerve was observed. It formed a loop around the maxillary and the medial meningeal arteries.

Anomalies of nerve courses are of significant practical importance for surgeons and neurologists. Entrapment of the lingual nerve may lead to lingual numbness or pain, associated with speech articulation impairment.

To our best knowledge this is the first description of an entrapment of the lingual nerve between an ossified pterygospinous ligament and the medial pterygoid muscle.

P46 Functional anatomy of the human Achilles tendon from a clinical viewpoint. By T. ZANTOP, W. PETERSEN and B. TILLMANN. *Department of Anatomy, Christian-Albrecht University of Kiel, Germany*

Ruptures of the human Achilles tendon (AT) occur most commonly in the middle third of the tendon. The aetiology still remains unclear. The purpose of this study was to investigate the structure and course of the AT with a focus on its typical region of rupture. The course of the human AT in different positions of the ankle joint was examined using MRI. The progress of the tendon fibres was analysed for 50 fixed ATs. Tonometry of the intratendinous pressure should be informative about regional differences along the tendon. The blood supply was investigated by comparing results of analysis of an injection preparation with immunolocalisation of laminin.

The insertions of the 3 parts of the triceps surae muscle could be distinguished by eye. Macroscopically fibres of the medial head of the gastrocnemius muscle inserted in the posterolateral margin, fibres of the lateral head in the anterolateral part, and the fibres of the soleus muscle are situated at the medial part of the calcaneus. The 3 bundles twisted around a longitudinal axis and cross approximately 3–4 cm above the calcaneal insertion in the region of the so called tendon waist. Here was found the highest hydrostatic intratendinous pressure under traction stress measured by an in vitro biomechanical tonometry. Immunohistochemistry shows a reduced blood supply in the typical area of rupture.

Our investigation shows a correlation between the length of the AT and degree of rotation. The more distal the musculo-tendinous junction of the soleus muscle is situated, the less the degree of rotation. The torsion of the 3 bundles of the human AT leads to a reduction in transverse diameter in the region of the tendon waist. The reduction in transverse diameter is the reason for the increased intratendinous pressure in this area. Shear stress is induced by the torsion, which depends on the structure and the position of the ankle joint. Of clinical relevance, the area of the tendon waist approximately 3–4 cm above its calcaneal insertion corresponds to the area where the human AT ruptures most commonly.

P47 The entheses of the anterior talofibular ligament of the human ankle joint. By T. KUMAI and M. BENJAMIN. *School of Biosciences, Cardiff University, UK*

The lateral ligament of the ankle joint consists of 3 distinct bands—the anterior and posterior talofibular ligaments and the calcaneofibular ligament. Of these the anterior talofibular ligament is the most commonly damaged, typically by excessive inversion involving an avulsion fracture of the fibular enthesis or an intraligament tear near its talar end. The purpose of the present study was to determine whether there are histological differences between the entheses at either end of the anterior talofibular ligament that can be related to the injury pattern. The entire ligament, including both entheses, was removed from 8 formalin fixed, elderly dissecting room cadavers (all males, ages 64–88 y) and prepared for routine histology. After further fixation in 10% neutral buffered formol saline and routine histology processing, the ligaments were serially sectioned longitudinally at 8 μm. Sections were collected systematically at 1 mm intervals and adjacent slides stained with haematoxylin and Alcian blue, Masson's trichrome or toluidine blue. Both entheses were fibrocartilaginous, but the quantity of uncalcified fibrocartilage was greater at the fibular end. In contrast, both the combined thickness of the calcified fibrocartilage and associated subchondral bone and the ratio of bone : marrow were considerably greater at the talar enthesis. Finally a sesamoid fibrocartilage was only present at its talar end, at the site where the ligament commonly ruptures. The presence of this fibrocartilage probably relates to the role of the talus in acting as a bony pulley for the ligament close to its insertion, when the foot is inverted and plantar flexed. There was no equivalent pulley or sesamoid fibrocartilage near the fibular end. By analogy with entheses elsewhere this cartilage covered pulley near the talar enthesis probably reduces stress concentration on the enthesis fibrocartilage itself. There are commonly signs of pathology in the region that suggest it may be subject to much wear and tear in daily life and thus be particularly vulnerable to inversion induced injuries. We conclude that the common patterns of injury in the anterior talofibular ligament relate directly to regional variations in its histological structure.

P48 Underfoot pressure distribution of patients with leg tumours. By J. LORKOWSKI. *Department of Anatomy, Collegium Medicum Jagiellonian University and Rehabilitation Centre "Zdrowie", Cracow, Poland,*

The cure of malignant bone tumours depends on early diagnosis (Funovics et al., *Radiologie* **39**, 1999). We performed an anatomical study of feet, based on underfoot

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pressure measurements, in 2 patients (both men) complaining of foot pain. It became evident that the cause of this pain was a tumour of the leg. The first patient was 32 y old and had a tumour on the distal 1/3 of the tibia and the second was 19 y old with a tumour on the distal 1/3 of the fibula. The control group consisted of 20 normal subjects. We used clinical histories, clinical examination, and postural pedobarography. The plantar pressure was determined by postural pedobarography at foot regions basing on the classification of Cavanagh et al. (*Foot Ankle* **7**, 1987). Comparing the painful leg with the normal one, the postural pedobarography of the first patient revealed: a decreased maximal underfoot pressure by 162 g/cm² (592 g/cm² normal, 430 g/cm² affected), a decreased mean underfoot pressure by 40 g/cm², a decreased foot contact area with the base by 55 cm², and a total lack of plantar pressure under foot regions III and IV. In the case of the second patient it was found: a decreased mean underfoot pressure by 4 g/cm², an increased maximal underfoot pressure by 37 g/cm², a decreased foot contact area with the base by 16 cm², an increased plantar pressure under foot regions I and II, and a decreased plantar pressure under foot regions III to VII and X. In this second patient compared with the control group, we ascertained on the normal leg increased plantar pressure under foot regions III and IV. Based on the pedobarographic examination results, we performed diagnostics that made it possible to recognise the neoplastic process and to initiate treatment.

Since examination by postural pedobarographic detects minimal changes in foot anatomy it could be one indicator for the necessity of orthopaedic diagnosis in the lower extremity.

P49 Estimation of the usefulness of photogrammetric and podometric examinations in diagnosis and treatment of selected diseases of the musculoskeletal system. By T. MAZUR[1], R. TOKARCZYK[2], J. LORKOWSKI[1,3] and E. SZCZYGIEL[1]. [1]*Rehabilitation Centre "Zdrowie"; [2]Academy of Mining and Metallurgy; and [3]Department of Anatomy, Collegium Medicum Jagiellonian University, Cracow, Poland*

The most frequent cause of pain involving the musculoskeletal system is an overload. The physical determinant of this phenomenon is the pressure force, its side and the time of its action. Each of these factors can increase or decrease the overload. The pressure force can change twice or exceptionally 3-fold but the side of its action can change 10-fold and more. However the duration time of the pressure can increase 100-fold and more. In spite of general opinion, it is not the pressure force but the side and the duration of pressure force action which determines the pathological changes of the movement system.

Measures of pressure force side have been made for a long time but only the photogrametric numerical system gives the possibility of solving this problem. This system includes the simultaneous registration at least of 2 pictures of 1 patient from 2 different points in space. The next stage is to mark the localisation of these points on the examined person's body. The final aim of the examination is to determine these point's interactions. Direct pressure measurement on podometric examination could evaluate the compensatory movements of vertical body posture, so it is possible to estimate pressure time duration.

This study presents the results of examinations of 20 patients with cervical spondylosis, lumbar spondylosis and lumbar discopathy. These results show clear differences from 20 controls.

P50 Estimation of the underfoot pressure distribution of patients with limb length inequality. By J. LORKOWSKI[1,2] and A. SKAWINA[1]. [1]*Department of Anatomy, Collegium Medicum Jagiellonian University; and [2]Rehabilitation Centre 'Zdrowie', Cracow, Poland*

The anatomical defect of limb length discrepancy is one of the reasons for back pain and lower extremity joint complaints because of biomechanical disturbances to the skeletal system. Generally, these problems intensify according to increasing limb length inequality (Bhave et al. *J. Bone Joint Surg*. **81A**, 1999; Kaufman et al., *J. Pediat. Orthop*. **16**, 1996). The purpose of this study was to estimate the underfoot pressure of patients with limb length discrepancy. We have studied 11 patients (5 women, 6 men), mean age 31 y, with documented limb length discrepancy of the lower extremities that ranged from 1.0 to 2.5 cm and as control group 20 healthy volunteers. The clinical examination and postural pedobarographic examination during bipedal and unipedal standing were used. The underfoot pressure was determined by pedobarography at defined foot regions according to the classification of Cavanagh et al. (*Foot Ankle* **7**, 1987). On postural pedobarography during unipedal standing it was found that there was a similar underfoot pressure distribution for the longer lower extremity as the shorter one. In the bipedal position, comparing a longer lower limb with a shorter one, there was: an increased mean underfoot pressure, an increased maximal plantar pressure under foot regions I and II (the heel), a decreased maximal plantar pressure under foot regions IV (lateral part of the midfoot), V, VI and VII, and a decreased foot contact area with the base. The compensatory strategy of this anatomical deformity was to apply the orthoses that correct the discrepancy between underfoot pressure of both feet. Since the changed underfoot pressure distribution of patients with limb length inequality exists, the total correction of limb length discrepancy by using orthoses seems to be indispensable for symmetric underfoot pressure distribution.

P51 Investigation and form analysis of the working surfaces of the hip joint. By A. RYNIEWICZ[1], A. RYNIEWICZ[2] and J. LORKOWSKI[3]. [1]*Academy of Mining and Metallurgy, Faculty of Mechanical Engineering and Robotics; [2]Cracow University of Technology Department of Production Engineering; and [3]Department of Anatomy, Collegium Medicum Jagiellonian University, Cracow, Poland*

When considering hip joint mechanics in relation to friction, wear, dynamic effects, activities, histological structure and continuity of life processes one can notice a marvellous adaptation to performed functions. The structure of a joint, proceeding physical chemistry reactions and a system of

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mutual interactions ensure reliable mechanism of lubrication.

An explanation of the lubrication mechanism would be of great significance for general knowledge. Understanding the mechanism of lubricating could facilitate prevention of hip joint arthritic changes, and optimise material selection and artificial hip joint construction. Taking up these issues is extremely important due to the relative prolongation of human life that requires longer maintenance of hip joint functionality in elderly people. The mechanism of lubricating biological bearings may be considered as perfect because it underwent verification through evolution. It should be emphasised that only biological constructions have a possibility of taking advantage of such an enormous amount of experience. New forms evolve and at the same time natural selection of faulty solutions and modifications according to life conditions take place. The formograph traces the 3D outline of the femoral head and the acetabulum to allow the changes in osteoarthritis to be quantified. This allows analysis of the shape of the articular surface.

P52 Estimation of the underfoot pressure of patients with rupture of the Achilles tendon. By J. LORKOWSKI[1,2] and T. MAZUR[2]. [1]*Department of Anatomy*, *Collegium Medicum Jagiellonian University; and* [2]*Rehabilitation Centre "Zdrowie"*, *Cracow*, *Poland*

The posttraumatic rupture of Achilles tendon changes the anatomy and the function of the foot and limits the possibility of the active plantar flexion in talocrural articulation (Yoho et al. *Clin. Podiatr. Med. Surg.* **16**, 1999). The aim of our study was to assess underfoot pressure of patients with partial rupture of the Achilles tendon. We examined 7 patients (2 women, 5 men) with rupture of at least 50% of Achilles tendon fibres, confirmed ultrasonographically and as a control group 25 volunteers with no known systemic diseases or foot pathology. The plantar pressure was determined by postural pedobarography (during bipedal standing on total plantar surface and on tiptoe) at foot regions, distinguished on the classification of Blomgren et al. (*J. Foot Surg.* **30**, 1991). On clinical examination we evaluated the possibility of rising onto tiptoe from a standing position; all patients with an Achilles tendon injury could not stand on tiptoe on the damaged foot. During bipedal standing on the total plantar surface, postural pedobarography in the case of Achilles tendon rupture revealed: a decreased mean underfoot pressure (by on average 8 g/cm^2), an increased maximal underfoot pressure (by 175 g/cm^2), a decreased maximal pressure under foot regions MT1, MT2, MT3, MT4 and MT5, and an increased maximal pressure under foot regions T and H. On standing bipedally on tiptoes there were found: a decreased mean underfoot pressure (by 138 g/cm^2), a decreased pressure under foot regions MT1, MT2, MT3, MT4 and MT5, and a subtotal lack of plantar pressure under the hallux of the foot with Achilles tendon injury compared with the normal foot. In our study using postural pedobarography decreased plantar pressure under the heads of metatarsal bones and increased plantar pressure under the heel of the foot with Achilles tendon rupture were found.

P53 The connective tissues and plastic surgery. By S. M. SAPIN. *Department of Surgery*, *Moscow Medical "Setchenov" Academy*, *Russia*

Investigations of the anatomy of the skin which formerly had theoretical significance now have important practical meaning. Scientific and educational papers have much information about the subdivisions of the skin, its layers, cell and tissue components, as well as the blood vessel and nerve supply in different regions of the human body. Investigation of the organisation of skin fibres and their interrelations is very important for surgical operations, including plastic surgery. It especially concerns the skin of the external (exposed) part of the body. This information is necessary for manipulations with skin of human body, especially the neck and dorsal part of the hands, where its thickness is very small. This information can also be interesting for the skin of mammary glands, because surgical operations there are common in medical practice. Ideas about the direction of collagen and elastic fibres of the skin are important not only for scientific curiosity. All facts answering the questions about interrelations of collagen and elastic fibres of different parts of human body would provide a significant advantage during plastic surgical operations. The tactics of such operations and their success very often depend on the knowledge of anatomy of our skin.

P54 Histological investigations after application of Tacho Comb® on serosal defects in a rabbit model. By W. SCHMIDT[1], J. WEISS[1], U. ROLLE[2], A. SCHNEIDER[2] and J. BENNEK[2]. [1]*Institute of Anatomy and* [2]*Department of Paediatric Surgery*, *University of Leipzig*, *Germany*

Disturbed healing of intraabdominal defects and the development of interenteric and enteroperitoneal adhesions are still unsolved problems, especially after relaparotomy. The aim of our study was to investigate whether or not the application of Tacho Comb on serosal defects of the caecal wall in primary and repeated laparotomy affects the course of healing.

Tacho Comb is a ready-to-use collagen patch coated with fibrin glue components. We removed part of the caecal serosa and of the parietal peritoneum of White Danish Landrace rabbits. Depending on the group, the resulting damage was covered by Tacho Comb sheets. The experimental group of rabbits with Tacho Comb at the defects was terminated after 4 wk. Macroscopically we found the locations with Tacho Comb completely covered by a new serosal layer. This part of the caecum was completely inconspicuous without findings of acute or chronic inflammation.

The staining methods used were Van Gieson and Crossmon staining. The Tacho Comb sheet was found histologically to be covered by a serosal layer with a small stroma containing many blood vessels. Especially at the luminal side of this sheet a lymphocyte wall had developed. An accumulation of macrophages containing multiple nucleoli was observed at the horse-derived collagen of the Tacho Comb. At special locations this collagen was spliced and appeared skyrocket shaped in close relation to the macrophages. The collagen was degraded by the macro-

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phages and the resulting amorphic mass formed a cystic process.

The histological features of the Tacho Comb sheet at the enteric wall after 4 wk were reminiscent of a foreign body granuloma but did not show any severe inflammatory signs. Further evaluation of later stages in the degradation of this foreign body is necessary.

P55 Atomic-force microscopic examinations of human enamel. By H. KOPP[1], B. KOPP[2], J. WEINGÄRTNER[3], V. BIENENGRÄBER[1], K.-P. SCHMITZ[2] and J. FANGHÄNEL[3]. [1]*Department of 0MR Surgery and* [2]*Institute for Biomedical Technology*, *University of Rostock; and* [3]*Institute of Anatomy*, *University of Greifswald*, *Germany*

On the basis of previous findings with the scanning electron microscope (SEM), this study investigated possible applications of the AFM (atomic force microscope) in assessing human enamel. 18 samples were taken from clinically intact permanent molars and premolars from subjects 12 to 34 y old. The samples were mechanically cleaned and partially etched with Vococid (Espe, Seefeld, Germany). The examination was conducted with an AFM, type TMX 900 (Zeiss, Oberkochem, Germany) under the following conditions: voltage 20 kV, cantilever spring constant 60 N/m, contact procedure with a horizontal scanning speed of 0.6 µm/s. Control examinations were conducted with a SEM, type DSM 960 A (Zeiss; voltage 20 kV, cathode distance 8 mm). Due to the high enamel hardness, the AFM technique allowed resolution in the nanometre range with good image sharpness. This high resolution capacity, together with user friendly software, made it possible to measure surface structures precisely. An average prism diameter of 5.9 µm was determined; the average difference in height between prismatic and interprismatic areas was 8.3 µm. Thus AFM is a valuable addition to the micromorphological examination techniques for enamel. This applies particulary to the measurement of prismatic structures, in addition to assessments of surface hardness (which were not addressed in this study).

P56 The role of haemodynamics in the development of the outflow tract of the heart (a preliminary assessment). By B. HILLEN[1], E. LOOTS[2], H. HOOGSTRATEN[2], W. LAMERS[3] and A. VELDMAN[2]. [1]*Department of Functional Anatomy*, *Utrecht University;* [2]*Department of Computational Mechanics*, *University of Groningen; and* [3]*Department of Anatomy*, *AMC Amsterdam*, *The Netherlands*

The question whether haemodynamic forces and mechanical stimuli modulate the morphogenesis of the vascular system, and if so to what extent, is a century old, especially for the outflow tract where a spiralling septum develops in and after a strong bend in the tube. Spiralling patterns of the flow in bends are well known. Of the mechanical stimuli that can potentially exert an effect on morphogenesis the most likely candidate is wall shear stress, and a number of genes that are expressed in the cardiovascular system have shear stress responsive elements in their regulatory units. Recent investigations have clearly shown that the disturbance of normal haemodynamic conditions results in maldevelopment of the heart. However an experimental model alone is necessary but not sufficient to identify the intermediate steps of the interaction between blood flow and tissue remodelling in the developing cardiovascular system, certainly at Reynolds numbers that are very small.

Therefore we created a model with a very simple geometry, using the ComFlo software that was recently developed by the Computational Mechanics Group in Groningen. ComFlo is a fully 3D computational fluid dynamics code that solves the Navier-Stokes equations in a cartesian grid. A first assessment of the possible influence of a strong curvature, with biological realistic dimensions, was made using steady flow conditions.

Since both the Reynolds number and the Womersley number (indicating the influence of the pulsatility on the velocity profile of the flow) are extremely low it is likely that these flow patterns do not differ significantly from those under pulsatile conditions. A first assessment of the effects of the strong curvature on the flow under the circumstances given showed only very small secondary velocities and negligible heterogeneity of the wall shear stress, indicating that the (time dependent) geometry may be an important factor.

P57 Location and extension of intima cushions in the infant parasellar carotid artery. By S. MENG[1], W. J. WENINGER[2], S. U. WENINGER[1], G. B. MÜLLER[1] and C. REITER[3]. [1]*Department of Anatomy*, *University of Vienna;* [2]*National Institute for Medical Research*, *Mill Hill*, *London*, *UK; and* [3]*Department of Forensic Medicine*, *University of Vienna*, *Austria*

In this study we describe and quantify intima cushions which occur in the parasellar internal carotid artery (pICA) of human infants. A total of 30 specimens were analysed using the 'EPI-3D' reconstruction method (Weninger, *Anat. Embryol.* **197**, 1998). One characteristic intima cushion was related to each segment of the pICA, wherefore they were termed the C5-cushion, C4-cushion and C3-cushion. The C5-cushion was located at the convex side of the posterior knee of the pICA. It occurred in 24 specimens, covered on average 30.7% of the wall circumference, and occluded 6.25% of the blood vessel's lumen. The C4-cushion was located at the bottom of the horizontal segment of the pICA. It occurred in 26 specimens covered on average 33% of the wall circumference, and occluded 8.5% of the lumen of the blood vessel. The C3-cushion was located at the concave side of the anterior knee of the pICA. It occurred in 19 specimens, covered on average 26.1% of the wall circumference, and occluded 6.5% of the lumen of the blood vessel. Our study provides for the first time detailed analyses of intima cushions within a naturally curved blood vessel like the pICA. It will contribute to the understanding of the mechanisms and forces that lead to the forming of intima cushions, but it also provides data about parasellar regions prone to atherosclerosis.

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P58 Zinc sulphate induced anosmia decreases the overall nerve density in the anterior cerebral artery in the rat. By J. C. M. VAN DENDEREN, R. L. A. W. BLEYS and B. HILLEN. *Department of Functional Anatomy, Rudolf Magnus Institute for Neurosciences, University Medical Center Utrecht, The Netherlands*

A dense perivascular plexus of sympathetic, parasympathetic and sensory nerve fibres innervates the circle of Willis. Differences in nerve density have been demonstrated in humans and in rats as well as local changes during ageing and disease. In humans the highest nerve densities were found in the posterior communicating and posterior cerebral arteries, whereas in rats the highest nerve density was found in the anterior cerebral artery. This pattern of topographical heterogeneity in nerve density could imply a functional role for these arteries since large fluctuations in flow occur in the corresponding vascular territories, i.e. the visual cortex in humans and the rhinencephalon in rats. Based on the above findings we hypothesise that local patterns of nerve density are influenced by changes or fluctuations in flow. To investigate this relationship an experimental olfactory deficit or anosmia was induced which would result in a decreased metabolic activity in the rhinencephalon, due to degeneration of the olfactory epithelium, and as a consequence a reduction of flow fluctuations in the vessels supplying this area.

Rats received an intranasal application of zinc sulphate or saline while anaesthetised with Hypnorm (0.01 ml/100 g bodyweight s.c.), twice a week for 2, 4, 6, or 8 wk. Subsequently the animals were anaesthetised with Nembutal (0.1 ml/100 g bodyweight i.p.), perfused, and whole mount preparations of the basal cerebral arteries were immunostained for the general neural marker protein gene product (PGP) 9.5. The nerve densities of the basal cerebral arteries were determined by image analysis and expressed as a percentage of vessel wall area. This analysis showed a significant reduction for all the time points in the overall nerve density in the anosmic group compared to the control group in the first part of the anterior cerebral artery (e.g. treatment for 4 wk 10.93 ± 0.29 vs 12.51 ± 0.44; $P < 0.01$), and the second part of the anterior cerebral artery which is just before the fusion of the right and left anterior cerebral arteries (e.g. treatment for 4 wk 11.34 ± 0.39 vs 13.44 ± 0.29; $P < 0.01$). Our results support the assumed relationship between haemodynamic factors and nerve densities.

P59 The arteries of the intracranial segment of the optic nerve. By B. SZABO and I. SZABO. *Department of Anatomy and Embryology, Cluj Napoca, Romania*

The arteries of the intracranial segment of the optic nerve were studied in 40 specimens obtained from 20 cadaver heads fixed in formaldehyde.

This part of the optic nerve was supplied in the most cases (52.5%) by a pial network made up of branches of the ophthalmic artery, anterior cerebral arteries and hypophyseal arteries. Branches of the internal carotid artery (15% of cases), anterior communicating artery (17.5%), posterior communicating artery (5%) and accessory anterior communicating artery (2.5%) were also found.

We have classified 5 main types of vascularisation, according to the arterial supply. (1) The optic nerve is supplied by 2 main arterial sources, branches of the ophthalmic and anterior cerebral arteries (20% of cases). (2) The optic nerve is supplied by 3 main arterial sources, branches of the ophthalmic, anterior cerebral and hypophyseal arteries (52.5% of cases). (3) The optic nerve is supplied by 4 main arterial sources, branches of the ophthalmic, anterior cerebral, hypophyseal and anterior communicating arteries (17.5% of cases). (4) The optic nerve is supplied by 5 main arterial sources, branches of the ophthalmic, anterior cerebral, hypophyseal, anterior communicating and internal carotid arteries (7.5% of cases). (5) The optic nerve is supplied by 6 main arterial sources, branches of the ophthalmic, anterior cerebral, hypophyseal, anterior communicating, internal carotid and posterior communicating arteries (2.5% of cases).

The branches of the anterior cerebral and anterior communicating arteries supplied only the superior parts of the optic nerve. Others supplied only the inferior part of the nerve, for example the branches of the hypophyseal arteries. The branches of the ophthalmic and internal carotid arteries supplied both superior and inferior parts of the optic nerve.

P60 Vertebral venous plexuses and anterior epidural space: comparisons between different species assessed from anatomical and histological studies. By O. PLAISANT[1], C. FALLET-BIANCO[2], P. COSTIOU[3], A.-L. DELEZOÏDE[4], J-F. UHL[1], C. GILLOT[1] and S. GLICKMAN[5]. [1]*Institut d'Anatomie, Paris V–Necker;* [2]*Laboratoire d'Anatomie Pathologie, Hôpital Sainte Anne, Paris;* [3]*Laboratoire d'Anatomie, Ecole Vétérinaire, Nantes;* [4]*Laboratoire de Foeto-pathologie, Hôpital Robert Debré, Paris, France; and* [5]*Department of Psychology, University of California, Berkeley, USA*

In a previous report (Plaisant et al. *It. J. Anat. Embryol.* **104**, 1999) the anterior epidural space was compared in the human and hyena. The aim of the present study was to compare more species and to use an injection technique that we had previously employed in humans for producing latex casts of the vertebral venous plexuses in animal cadavers.

For the latex casting, 1 adult cat and 3 adult rabbits (sacrificed by 5 ml intravenous injection of T61, Hoechst; active ingredients embutramide 20%, mebezonium 5%, tetracaine chlorohydrate 0.5% w/v and dimethylformamide 60% v/v) were injected with blue stained latex. The cat was injected through the saphenous vein after ligating the caudal vena cava beyond the renal veins. The entire venous system in the animal was easily injected (except for the hepatic portal system and the most superficial veins in the skin). The 3 rabbits were injected after ligating the caudal vena cava at the level of the heart, at the level of the diaphragm, between the liver and the renal veins, and at the level of the 2 common iliac veins. The injection was easily accomplished without pressure. The entire venous system was filled from the femoral vein through the vertebral venous plexuses to the azygos system and the heart. After the cat and the rabbits were dissected, the vertebral venous plexuses appeared to be completely filled with latex and the pattern of veins in the thoracolumbar region were not found to be dissimilar from the pattern previously reported for the human. This study confirms the existence in animals of an anastomosis between the caudal vena cava and the rostral vena cava or the heart through the vertebral venous

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plexuses. The blood can therefore circulate from the caudal to the rostral part of the body (or vice versa) because no valves exist in the vertebral venous plexuses.

For the second part of this study, trichrome histological sections of neonatal human, hyena, dog, and pig thoracolumbar vertebrae were analysed to describe the structures of the anterior epidural space. Sections of thoracolumbar spine were compared with respect to the structure of the anterior epidural space, the posterior longitudinal ligament, the internal vertebral venous plexuses, and the anterior longitudinal veins. No marked differences were again observed in any of the species studied, whether the tissues were neonatal or adult.

The presence of similar spinal morphologies in species that differ so widely in phylogeny and posture (i.e. bipedal vs quadrupedal) suggests a fairly remote common ancestry and raises questions about the functional significance of spinal morphology and vertebral venous plexuses.

P61 The arteries of the intraorbital segment of the optic nerve. By B. SZABO and I. SZABO. *Department of Anatomy and Embryology, Cluj Napoca, Romania*

We present the visual findings of the arteries of the human optic nerve of different ages. The study starts in embryonic period of the hyaloid vascular system and continues to fetal and adult ages, when the trunk of the ophthalmic artery, the central retinal artery and short posterior cilliary arteries can be found.

The arteries of intraorbital segments of the optic nerve were studied in 60 specimens (8 embryos, 12 fetuses and 40 adults) obtained from 30 cadaver heads, fixed in formaldehyde (the adult preparations were injected intraarterially). The early stages of the arterial blood supply of the optic nerve and eyeball are presented. We showed the hyaloid vascular system in 6–7 wk embryonic preparations. In fetal and adult preparations the difference was seen between the 2 parts of the optic nerve, situated anterior and posterior to the entrance of the central retinal artery in the trunk of the optic nerve. We found the origin of the central retinal artery was directly from the trunk of the ophthalmic artery in 88.8% of cases. The course of this artery was straight in 89% of cases and sinuous in 11% of cases. The sinuosity of the artery was related to the age of subjects. The mean length of the central retinal artery was 17 mm. The artery entered the inferomedial side of the optic nerve in 75% of cases, at a point situated on average 13 mm posterior to the eyeball.

The posterior cilliary arteries that also supply the trunk of the optic nerve were shown. In most cases we found 2 main arterial trunks (lateral and medial) in the same orbit (46.66%). The lateral trunk of the posterior ciliary arteries was found in all cases.

Beside its importance for descriptive anatomy, this study of the main arteries of the optic nerve is useful for the surgical approach to the retrobulbar region of the orbit.

P62 Incomplete double aortic arch. By P. C. BRUGGER. *Institute of Anatomy, Vienna, Austria*

Incomplete double aortic arch with interruption of the left arch proximal to the origin of the left common carotid artery is one of the rarest variations of the aortic arch system. With only 11 cases reported in the radiological literature, this is the first anatomical specimen to be described. The present case was observed in a female 89 y old dissecting room specimen. The heart was left sided and without anomalies. The great vessels of each arch arose independently with a separate right vertebral artery. The left sided ductus arteriosus was well developed, 4 cm long but not patent. Hidden within strands of connective tissue there was a 6 cm long atretic segment connecting both arches. The presence of this vestigial segment makes it the first case of a type D double aortic arch anomaly on record. Previously the existence of such an anomaly has only been postulated (Shuford et al. *Am. J. Roentgenol. Radium Ther, Nucl. Med.* **116**, 1972).

The sequence and topography of branches arising from the arches allow specification of the parts involved in forming the arches and an explanation of them in terms of embryological development. The regressive changes occurred in the initial part of the left aortic arch, prior to the origin of the left common carotid artery. As a consequence of homology, the left arch remnant should not be called an aberrant left brachiocephalic trunk. In contrast to the other cases on record there were no associated malformations, nor any history of dysphagia or dyspnea, though a vascular ring was formed by both arches and the atretic segment.

P63 The state of knowledge of clinically oriented anatomy in students just before they enter the clerkship phase. By C. J. A. H. PRINCE[1], H. VAN MAMEREN[2], N. HYLKEMA[1], A. J. J. A. SCHERPBIER[3], C. P. M. VAN DER VLEUTEN[4] and J. DRUKKER[2]. [1]*Skillslab*, [2]*Department of Anatomy/Embryology*, [3]*Institute of Medical Education, and* [4]*Department of Educational Development and Educational Research, University of Maastricht, The Netherlands*

Do medical students feel well prepared for clinical practice? At Maastricht Medical School students have indicated that they feel insecure when entering the clerkship phase. They claim to have difficulties in applying their knowledge and perceive shortcomings, particularly with respect to anatomy. We investigated whether students' knowledge of clinically oriented anatomy is actually deficient and if so, whether there are differences in the state of knowledge between Maastricht students, following a problem based curriculum, and students at the other Dutch medical schools. We also determined whether the score on questions formulated within the context of a patient problem differs from the score on questions presented without a clinical context. To this end we have examined beginning clerks (4th year students) at 7 of the 8 medical schools in the Netherlands.

Consultants of various clinical disciplines of the Maastricht Academic Hospital and general practitioners associated with the medical school were asked to provide subjects that students are likely to encounter during clerkships and that require application of knowledge of anatomy. We constructed patient problems with related anatomy questions on the basis of a selection from these subjects. Medical practitioners judged whether these problems and the questions were indeed relevant, reasonable and realistic. This resulted in 16 patient cases with a total of 142 questions; open questions as well as multiple choice and true/false questions. The examination also included 50

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true/false questions presented without a clinical context. All 4th year students of the 7 medical schools (n = 1661) in the Netherlands were invited to participate; a fee of Dfl 50.00 was offered. To date 361 students (77, 54, 57, 47, 56, 40 and 40, respectively), have taken the test. Their score was well below 70% suggesting a meaningful discrepancy between faculty's expectation and students' achievement. There were no significant differences between scores of Maastricht students and students in other medical schools on the patient case guided anatomy test and the set of questions without medical context. The younger students, and student demonstrators at anatomy practicals, had higher scores.

We conclude that Maastricht students (PBL education) are not inferior to other students (more theory oriented education) with respect to anatomical knowledge. Nevertheless many medical students enter the clerkship phase perceiving a lack of knowledge of anatomy.

P64 Giving colour to a new curriculum: bodypainting as a new tool in medical education. By W. J. OUDEGEEST, J. W. OP DEN AKKER, I. THUNNISSEN and B. HILLEN. *Department of Functional Anatomy*, *UMC Utrecht*, *The Netherlands*

The main goal of studying surface anatomy is to gain more insight in size, shape, projection and topographical relation of the abdominal and thoracic organs. It also makes students aware of the professional attitude towards physical examination and partial nudity in the early phase of their medical training. In the past our students were trained in surface anatomy using skinpencils. To improve the impact of the course we introduced bodypaint techniques. An elementary manual with points of reference was used to enable students to find the surface markings and then to paint the individual organs using the bodypaint techniques. The session dealing with the thoracic organs was combined with basic diagnostic techniques as percussion and auscultation.

To evaluate the bodypaint sessions the participants were asked to complete a questionnaire after each session. The questionnaire revealed that the basic bodypaint techniques were quite simple and highly motivating. Moreover, the educational impact was greatly enhanced, giving better insight in the topographical relation of the organs than in a conventional way.

In our opinion we succeeded in upgrading our surface anatomy course by leaving the line of conventional thinking in educational methods. By offering an attractive course, students were challenged to self-employment and creativity. We observed that educational goals could be accomplished much more easily in this way.

P65 The binding of monoclonal antibody to human platelet glycoprotein IIb-IIIa. By T. SZUWART, E. T. PEUKER, F. PERA and T. J. FILLER. *Institute of Anatomy*, *Westfälische Wilhelms-University Münster*, *Germany*

It is well known that the platelet receptor glycoprotein IIb-IIIa complex can bind to several adhesive proteins, such as fibrinogen, von Willebrand factor and fibronectin. The aim of the present study was to describe the binding of antiglycoprotein IIb-IIIa to unfixed platelets after adhesion in vitro. The experiments were the first attempt to demonstrate the uptake of monoclonal antibody to platelet glycoprotein (GP) IIb-IIIa using reflection contrast microscopy.

Blood samples were drawn from healthy volunteers by venipuncture and platelet rich plasma was obtained by centrifugation. After washing the platelets were transferred into incubation chambers. The platelets were treated with monoclonal antibody against GP IIb-IIIa (CD41, clone 5B12), and the ligand-receptor complexes were revealed by immunocytochemical techniques (FITC conjugate). Platelet adhesion was observed by means of a reflection contrast microscope (Leitz, Wetzlar, Germany) over a period of 90 min.

The platelets show disc-like or star-like adhesion patterns. Up to 60 min after the addition of antibodies no immunolabeling was localised on the platelets. After 65 min a markedly increased binding of glycoprotein IIb-IIIa was observed and after 70 min nearly all platelets were labelled. Therefore our results demonstrate that the incorporation of the antiglycoprotein IIb-IIIa is time-dependent.

P66 The pattern of distribution of cells producing plasminogen activator inhibitor-1 in vessel walls. By V. KŘÍŽKOVÁ[1], J. KOČOVÁ[1] and M. KORABEČNÁ[2]. [1]*Department of Histology and Embryology and* [2]*Department of Biology*, *Charles University*, *Faculty of Medicine in Pilsen*, *Czech Republic*

The aim of our work was to study and to compare the pattern of distribution of plasminogen activator inhibitor type 1 (PAI-1) expressing cells in vessel walls under normal and pathological conditions. We have chosen gene PAI-1, because the product of this gene takes a role in the regulation of fibrinolysis and its increasing activity has been observed during these pathological states: thrombosis and atherosclerosis. Control samples of veins, aorta and aneurysm of the abdominal aorta were obtained from organ donors (after multiple trauma). Varicose veins were taken from patients undergoing routine varicose veins stripping. We employed the method of in situ hybridisation (ISH). Our modification of this method allows the identification of PAI-1 mRNA in the cytoplasm of cells expressing it using a set of oligoprobes labelled with fluorescein. We compared the results of ISH with results obtained by classical histological methods to determine the types of cells producing PAI-1. In control samples of aorta we found endothelial cells (especially vasa vasorum), leiomyocytes and foam cells positive for PAI-1. By contrast, in an aortic aneurysm wall with a huge atherosclerotic plaque there were only rare PAI-1 positive cells because of damage to the vessel wall. Our control vein had endothelial cells and leiomyocytes PAI-1 positive. We confirmed PAI-1 positivity of endothelial cells and leiomyocytes in varicose vein, too. Here in addition to these cells we found degenerative forms of myocytes positive for PAI-1. We have found that the pattern of distribution of PAI-1 producing cells in all our samples is similar. Increased levels of PAI-1 mRNA in vessel walls under pathological conditions may be explained by increased stimulation of PAI-1 expressing cells. Localisation

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of PAI-1 producing cells does not only help in angiology in understanding the process of pathogenesis in vessel walls but it is also of great importance in evaluation of tumour expansion because PAI-1 expression is associated with factors that control extravascular fibrinolysis.

(This study was supported from grant: CEZ:J13/98:111400001).

P67 The effect of tocopherol in human platelet adhesion: in vitro studies using reflection contrast microscopy. By T. SZUWART, T. J. FILLER, F. PERA and E. T. PEUKER. *Institute of Anatomy*, *Westfälische Wilhelms-University of Münster*, *Germany*

Reflection contrast microscopy is a useful method for studying the adhesion process of unfixed cells. To achieve a better understanding of the role of tocopherol (vitamin E) in the adhesion of human platelets, the present study combines reflection contrast microscopy and an image analysis system.

Platelet rich plasma was treated with 1 mM α-tocopherol. After washing, the platelets were transferred into incubation chambers. Platelet adhesion was observed by inverted reflection contrast microscope (Leitz, Wetzlar, Germany) over a period of 15 min. The adhesion areas were evaluated and counted using the image analysis system KS 400 (Kontron, Eching, Germany).

The contact areas of the unfixed platelets were disc-like or star-like with pseudopodia. Compared with the control group, the treatment of platelets with tocopherol inhibited adhesion significantly ($P < 0.001$). Moreover the results showed that tocopherol does not inhibit the formation of pseudopodia ($P < 0.99$). This example shows that the correlation of dynamic morphological features (RCM) and quantitative aspects (computer assisted image analysis) can serve as an important model for platelet research.

P68 Different endostatin binding on angiogenic active and inactive endothelial cells. By A. SCHMIDT, W. BLOCH and K. ADDICKS. *Department of Anatomy*, *University of Cologne*, *Germany*

Endostatin is a C-terminal proteolytic fragment of collagen XVIII that causes an inhibition of tumour growth by an anti-angiogenic effect. The mechanisms suggested for the anti-angiogenic effect are inhibition of endothelial cell proliferation and migration of endothelial cells. Endostatin also induces apoptosis in tumour and endothelial cells. The regulatory pathways remain unknown, especially since a cellular binding site for endostatin has not been described. To gain an insight in the mechanism of action it is important to know where the binding sites of endostatin are and which are the target cells of endostatin binding. To analyse this we used embryonic stem cells and porcine aortic endothelial cells, and we also used normal and tumour tissue of kidney and bladder. For this experiment endostatin was biotinylated and the biotinylation was detected by Western Blot analysis. Cells (before and after fixation) and tissue were incubated with biotinylated endostatin for 4 h at 37 °C. To detect bound endostatin immunohistochemistry was performed. In embryonic stem cells endostatin bound predominantly to endothelial cells, endostatin binding endothelial cells showing a high proliferation ratio as detected by double staining with Ki67. Furthermore there were distinct binding characteristics of endostatin in bladder and kidney tumours. Apparently the angiogenically active embryonic endothelial cells need endostatin for their growth, differentiation or other processes and due to this, they express a receptor or a binding site for endostatin. This raises the question whether endostatin also binds to angiogenic inactive endothelial cells. The angiogenically inactive porcine aortic endothelial cells did not show a binding of endostatin. This could explain why there are no endostatin effects on the existing vascular system during long term endostatin treatment in rats. The differences in endostatin binding hint at a specific effect of endostatin on angiogenically active endothelial cells from embryos and tumours.

P69 Differences of laminin immunopositivity can indicate functional differences between rat brain vessels. By M. KÁLMÁN, A. SZABÓ and B. KISS. *Department of Anatomy*, *Histology and Embryology*, *Semmelweis University*, *Budapest*, *Hungary*

According to our results, there is an immunohistochemical staining against laminin which labels some types of rat brain vessel in preference to the others. The preferred types are the following ones: (1) the vessels of the newborn brain; (2) the vessels of lesioned brain area; (3) the vessels of some circumventricular organs. The animals were overdosed with ether and perfused transcardially with 4% phosphate-buffered paraformaldehyde solution. Serial coronal sections were processed for immunochemical staining against laminin or GFAP, using antibodies obtained from Sigma and Boehringer respectively. In newborns the vessels were intensely immunopositive to laminin throughout the brain. This immunopositivity disappeared almost completely during development; the main phase of the regression seemed to take place between the fourth and seventh postnatal days. The lesions were stab wounds to the brain after drilling the skull, performed under deep ketamine-xylazine anaesthesia, with a 1 wk survival period. The vessels were intensely laminin immunopositive in the territory of the glial reaction around the lesion, which was detected by immunochemical staining against GFAP. Electron microscopic investigation localised the immunostaining to the basal lamina of vessels. Of the circumventricular organs the vessels of the subfornical organ and area postrema were laminin immunopositive whereas vessels of the subcommissural organ, which has a complete blood-brain barrier in contrast to the previous ones, were not immunopositive. The reason why laminin immunostaining labels these vessels in preference to other ones is not clear, but the phenomenon was independent of the working dilution of antibody between 1:100 and 1:1000 and of the quality of perfusion. The possible explanations may be: (1) the immunostaining is effective where the perivascular space is relatively wide and the basal lamina is easy of access, i.e. at newly formed vessels and in the circumventricular organs; (2) the immunostaining labels preferentially those vessels in which the blood-brain barrier is incomplete, due either to their immature stage or to their special functions. We do not believe that our results suggest the absence of laminin in the immunonegative vessels. Most probably it is simply a laminin subtype which is missing or masked in these cases.

P70 Ultrastructural localisation of caveolin-1 in myocardial capillary endothelium by post-embedding immuno-electronmicroscopy. By M. REINER, E. JANSSEN, C. HOFFMANN, W. BLOCH and K. ADDICKS. *Department of Anatomy I, University of Cologne, Germany*

Recent research on caveolae has been mainly performed using biochemical isolation and purification methods. Ultrastructural evidence for many proteins and enzymes claimed to be associated with and enriched in caveolae still remains sparse, especially in whole tissue preparations. Most of the ultrastructural immunohistochemistry reported has been restricted to ultracryotomy or pre-embedding methods. Post-embedding immunoelectronmicroscopy on these 60–70 nm diameter membrane vesicles remains difficult due to the dilemma of preserving fine structure while retaining sufficient antigenicity but is, if successful, superior to other techniques.

We approached this problem applying a combined tannic acid/uranyl acetate 'en bloc' staining after mild aldehyde fixation of isolated perfused rat hearts followed by both LR-White and a modified epoxy resin embedding. After this treatment immunolabelling of thin sections on grids was carried out using polyclonal antibodies against caveolin-1. Detection was performed using 10 nm gold conjugated secondary antibodies. The best method for preserving fine structure, high contrast in thin sections, and acceptable immunoreactivity was catalyst induced polymerisation of LR-White resin at −20 °C, with the epoxy embedded sections providing higher detail.

This procedure enabled us to localise caveolin-1 in endothelial cells at high resolution in a reproducible manner and is a suitable tool for evaluating recent biochemical findings. It offers the possibility of performing functional studies on endothelial caveolae including observing alterations of their molecular characteristics and dynamics in situ, for example the redistribution of caveolin and associated proteins (e.g. eNOS) under inflammatory conditions induced by kinins. Furthermore a possible association of caveolin with microtubules was supported by our observations.

P71 Are there differences between young and old rats in the ultrastructural and biochemical response of myocardium to hypoxia and antioxidative protection? By K. WELT[1], G. FITZL[1], R. MARTIN[2], H. MARTIN[2], and C. MOZET[1]. [1]*Institute of Anatomy, and* [2]*Institute of Clinical Chemistry and Pathobiochemistry, University of Leipzig, Germany*

There are conflicting ultrastructural and biochemical data in the literature concerning hypoxia of the myocardium in young and old individuals, and the protective effects of Ginkgo extract require further investigation.

In experiments licensed by the government board for animal protection we used Wistar rats aged 3, 6, 18, 24 mo after 20 min respiratoric hypoxia at 5 vol% oxygen, after 5 h reoxygenation with and without antioxidative protection by Ginkgo extract (EGb 761). Analysis was by means of ultrastructural morphometry and biochemical parameters (SOD activity, MDA content), RT-PCR and immunohistochemical demonstration of NO synthases and SOD. The animals were killed after exposure to CO_2 by cervical vertebral dislocation.

Myocardial ultrastructure differed more strongly between 3 and 6 than between 18 and 24 mo of age. Lipid drops and mitochondrial degenerations were more prominent in the older rats. Hypoxia caused an increase of volume densities of SR, T-tubuli and mitochondria more strongly in young rats, and of lipid, vacuoles, mitochondrial destruction and average volume more at older stages. EGb protected some parameters, especially in the old rats.

RT-PCR products for i-NOS were lacking in all groups of young rats but were present in old rats, less in the protected hypoxic group. In young rats immunohistochemical i-NOS demonstration was negative in controls, weak after hypoxia, and strong after reoxygenation, and similar but weaker in the old groups. E-NOS expression behaved the reverse in the young groups and was strong in the old control and reoxygenated group. EGb showed no influence. SOD activity was stronger in old than in young controls, and elevated in young rats after hypoxia/reoxygenation, especially after EGb-pretreatment. MDA was higher in young than in old rats, decreased more after hypoxia in young rats, and increased after EGb in young and decreased in old animals. Immunohistochemical demonstration of CuZn SOD showed lowest staining in the hypoxic and stronger in the control and reoxygenated group, and in the old group generally weaker. Mn SOD immunostaining was less intense in the old than the young rat myocardium and increased in the hypoxic groups. EGb seemed to strengthen both SODs in the reoxygenated groups.

The myocardial response to acute hypoxia differs in young and old rats, since different morphological and biochemical parameters are affected. Ginkgo extract protects different ultrastructural and biochemical parameters in young than in old animals.

P72 Invasive pericytes in a MDA-MB231 tumour angiogenesis assay. By D. LAUER, M. PAPOUTSI, J. WILTING, B. CHRIST and H. KURZ. *Institute of Anatomy II, University of Freiburg, Germany*

The involvement of pericytes in physiological or tumour angiogenesis is a matter of debate. We studied the expression of pericyte, smooth muscle cell and matrix markers in experimental tumours of the mammary ductal adenoma MDA-MB231 cell line that were grown on chick or quail chorioallantoic membrane (CAM) for 7 d (Papoutsi et al., *Histochem. Cell Biol.* **113**, 2000). The expression pattern and colocalisation of fibronectin and laminin, of β1 integrin and endothelial markers HT7 and QH1, and of smooth muscle actin (αSMA) and desmin were analysed with conventional and confocal laser scanning microscopy.

The CAM arterial wall showed strong αSMA signal in all smooth muscle cell layers, but only the innermost layer was desmin-positive. Ramified cells with delicate desmin staining accompanied most minor vessels and were also seen basal to the capillary plexus indicating the presence of pericytes. Distribution of matrix components and β1 integrin was well differentiated outside, but not inside tumour spheroids. In the tumours, a diffuse αSMA signal without definite relationship to vascular structures was detected. Strongly

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desmin positive cells were frequent in the basal region of small tumour nodules and were scattered everywhere in larger tumour masses. They were occasionally associated with vascular structures, but frequently appeared as single migrating cells. We conclude that (1) pericytes stabilise the normal capillary network and microvessels of the differentiated CAM; (2) pericyte like cells may be attracted by MDA-MB231 cells during tumour angiogenesis, but fail to interact properly with endothelial cells in the tumour environment.

P73 Gap junctions in haematopoietic stroma cells. By C. LEMKE[1], A. MUELLER[3], W. RICHTER[2], E. BAUMANN[1] and W. LINSS[1]. *Institutes of* [1]*Anatomy I and* [2]*Ultrastructure Research and* [3]*Childrens Hospital, Friedrich Schiller University of Jena, Germany*

Rosendaal (*Microsc. Res Tech.* **31**, 1995) first described gap junctions in haematopoietic cells. We are interested in knowing if gap junctions between haematopoietic progenitor cells and the bone marrow stroma environment are involved in the regulation of haematopoiesis. Therefore we visualised gap junction proteins in cultured human bone marrow stroma cells by means of confocal immunofluorescence microscopy, transmission electron microscopy and the freeze fracture replica labelling technique.

We initially searched for the existence of the gap junction protein connexin 43 in our model system by immunoblotting. Immunofluorescence localisation of connexin 43 with an anti-connexin 43 antibody revealed fine punctate labelling preferentially in the region of contact between neighbouring cells.

This fact could be further ascertained by the freeze fracture technique. Morphologically identifiable junctional structures bound the connexin 43 antibody. Transmission electron microscopy revealed that gap junctions were often combined with other intercellular junctions including tight junctions and desmosomes.

P74 Pathomorphological changes and disseminated bacterial microfoci in an experimental rat model of sepsis. By H. TAPFER[1], A. LIIGANT[1], K. TAMME[2], P. NAABER[3], I. SMIDT[3], R. TALVIK[2] and M. MIKELSAAR[3]. [1]*Institute of Anatomy*, [2]*Clinic of Anaesthesiology and Intensive Care, and* [3]*Institute of Microbiology, University of Tartu, Estonia*

Our previous studies of septic shock in Man (Talvik et al. *Intensive Care Med.* **24**, 1998) revealed massive penetration of bacteria into the tissues of several organs. The aim of this study was to detect the dissemination of *E. coli* during early and late sepsis in an experimental rat model of septic shock.

The histopathological changes in multiple organs, counts of disseminated bacteria and haematological values were determined. Wistar rats (n = 27) were inoculated with *E. coli* (1.5×10^8) intraperitoneally. Uninfected animals (n = 6) served as control. Different groups of rats were killed 24 h (n = 6), 2 d (n = 6) and 5 d (n = 8) after inoculation. Bacteria were stained with a modified Gram method, their number estimated semiquantitatively and a Contamination Index (CI) calculated.

To evaluate the systemic lesion, tissue samples were taken from several organs. The different levels of severity ranged from mild vascular changes to extensive destruction-necrosis and were observed in lungs, liver and kidneys after 24 h. All the tissues were contaminated with foci of cocci, located mostly intravascularly and in the interstitial spaces, but also within macrophages and parenchymal cells. Polymorphonuclear infiltration around the cocci was not found. This was probably due to the decreased activity of macrophages.

The different rates of bacterial clearance in the blood and tissues were probably caused by microcirculatory disturbances due to vascular stasis that resulted in red blood cell aggregation. In the sites of obstruction, uncontrolled bacterial growth may be present. A significant negative correlation was found between the duration of sepsis and CI of all organs. We suggest that those animals which survive the early sepsis gradually clear the bacteria from their tissues.

P75 Virus particles detected during osteogenesis of mouse calvaria. By B. ZIMMERMANN. *Institute of Anatomy and Institute of Clinical Pharmacology, Department of Toxicology, Free University of Berlin, Berlin*

During systematic examination of development and growth of mouse calvariae, virus particles were detected electron microscopically. Mice, strain NMRI, were obtained from the breeder Harlan-Winkelmann, Borchem (Germany), and mated in the animal house of our institute. Calvaria from d 18 fetuses and from different stages until d 26 post partum and from adult mice were studied. Pieces containing the area of the sagittal suture were fixed in glutaraldehyde plus 1% tannic acid, postfixed in OsO_4 and embedded in Epon. Frontal thin sections were examined with a Siemens Elmiskop 101. Virus particles were found in the pericellular space of many, but not all osteogenic cells, mainly around osteocytes embedded in the mineralised bony matrix. All viruses found were regular in size, spherical and showing a diameter of 120 nm. They were surrounded by a darkly stained membrane and showed an inner core. However although examinations of 22 different individual calvariae has been done, only 1 specimen obtained from a d 21 pp mouse was virus-positive. It is questionable whether this observation is due to an external viral infection or an endogenous virus production. In any case, the isolated occurrence of one case may indicate a very short event. Reports in the literature are scanty. Nevertheless, virus infected material may influence experimental approaches and may be dangerous for the people who do the work.

P76 β1 Integrins in cartilage matrix. By M. SHAKIBAEI and H.-J. MERKER. *Institut für Anatomie, Freie Universität Berlin, Germany*

Integrins are cell surface receptors which mediate cell attachment to extracellular matrix components. The pericellular matrix in cartilage is not only a mechanical framework, but is important for chondrocyte differentiation and stabilisation of the phenotype. The interaction between chondrocytes and pericellular matrix is mediated, in part, by integrin receptors.

We have previously demonstrated the presence of β1 integrins in the cartilage matrix of organoid culture of limb buds from 12 d old mouse embryos by immunohistological

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methods. In order to corroborate these findings, we further investigated the distribution of integrins in cartilage matrix by immunoelectron microscopy and immunoprecipitation methods. Cartilage from the limb buds of 17 d old mouse embryos was treated with collagenase, and the cell- and cellular protein-free supernatant removed and used for immunoprecipitation experiments. Immunoprecipitation with anti-β1, -α1, -α3, and -$\alpha 5\beta$1 integrins and anti-collagen type II antibodies followed by immunoblotting with the same antibodies demonstrated the presence of these integrins and collagen type II in the supernatant. The integrins found in the cartilage matrix might either be secreted or shed by the cells.

Whether integrins have a function in cartilage matrix such as interlinking, matrix organisation or stabilisation of matrix components, remains to be elucidated.

P77 A new staining method for connective tissue: orcein-picroindigocarmine. By P. STEVEN, F. PAULSEN and B. TILLMANN. *Department of Anatomy, Christian Albrecht University of Kiel, Germany*

A new colour combination of orcein, indigo carmine and picric acid was developed for histological applications. The new technique was tested on different human tissues.

Colours ranging from red to brown, yellow, green and blue are observed in paraffin-embedded stained material. Nuclear structures in all tissues are stained dark brown to dark blue. Squamous epithelium is stained light brown with varying shades of blue in upper cornified layers. Ciliated epithelium, on the other hand, is tinted blue grey. When connective tissue is stained, collagen fibrils appear strongly blue next to elastic fibres, which take on a rust brown tinge. Connective tissue cell components all colour brown. The matrix of hyaline cartilage is stained in different shades of blue with the chondrocytes rust brown. Sections of bone components appear dark blue to dark green. Skeletal muscle cells colour yellow and green with blue collagenous septa.

Orcein-picroindigocarmine staining is suitable for distinguishing connective tissue components such as elastic fibres and collagen fibrils in a slice preparation. It also makes it possible to render chondrocytes in cartilage visible in contrast to the cartilage matrix.

Orcein-picroindigocarmine could represent an alternative for histological investigations of these tissues, since the colours complement one another very well. The result was aesthetic staining colouring that could supplement histological investigations and provide an alternative to other staining materials.

P78 Localisation of the NO-cGMP signalling pathway in the odontoblasts cells of the rat molar dentine-pulp complex. By Y. KORKMAZ[1,2], W. BLOCH[1], M. A. BAUMANN[2], H. SCHRÖDER[1] and K. ADDICKS[1]. [1]*Institute for Anatomy and* [2]*Dental School, University of Cologne, Germany*

The inter- and intracellular messenger molecule nitric oxide (NO) is synthesised by NO synthase (NOS-I, -II and -III) and mediates cell-cell communication. NO acts via the soluble isoform of guanylate cyclase (sGC) which catalyses the formation of intracellular cyclic guanosine monophosphate (cGMP) within target cells. Based on its localisation in bone cells and its involvement in regulating osteoblast proliferation and differentiation, it was hypothesised that NO-cGMP signalling pathway molecules exist in odontoblasts and play a role in controlling dentinogenesis.

To investigate the localisation of the NO-cGMP signaling pathway in odontoblasts of the dentine-pulp complex, rat molars were perfusion- and post-fixed, decalcified in 4N formic acid and cryosectioned at 40 µm. Free floating sections were immunolabelled with antisera against NOS-I, -II, -III, sGC and cGMP and stained by nicotinamide adenine dinucleotide phosphate-diaphorase (NADPH-d) histochemistry.

Labelling for NOS-I, NOS-II, sGC and cGMP was detected in both the odontoblast cell bodies and in their processes within dentinal tubules. In addition, NADPH-d staining could be found in dentinal tubules. This immunohistochemical and histochemical localisation of the NO-cGMP signal pathway molecules gives strong evidence for a physiological role of a NO-cGMP signalling pathway in odontoblasts. It can be speculated that NO-cGMP signalling pathway molecules may be involved in regulating odontoblast proliferation, differentiation and matrix biomineralisation.

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P79 Suitable in vitro model for testing amelogenins. By N. PISCHON[1], H. YONEKAWA[2] and B. ZIMMERMANN[3]. [1]*Department of Periodontology, Charité Hospital, Humboldt-University, Berlin;* [2]*Saitama Medical School, Moroyamamachi, Japan; and* [3]*Institute of Anatomy, Free University of Berlin, Berlin*

Amelogenins are enamel matrix proteins which may play a crucial role in tooth development. It has been shown that isolated enamel matrix proteins (EMPs), commercially available as Emdogain (Biora, Bad Homburg, Germany), promote periodontal wound healing in the course of surgical therapy of severe periodontitis. Data showing positive effects in experimental approaches with regard to growth promotion are scanty. The study objective was to determine the effects of Emdogain on growth, matrix synthesis and mineralisation of osteoblasts and periodontal ligament cells from different sources. Cells were isolated by enzymatic means and propagated as monolayers. Proliferation and metabolic activity were measured under the influence of Emdogain using monolayer cultures; matrix synthesis and mineralisation were studied in organoid cultures. Emdogain did not show any positive effects either on cell proliferation and metabolic activity or on mineralisation and activity of alkaline phosphatase (AP). However, incorporation of ^{3}H-proline was significantly increased but only in human bone cells indicating a positive effect on collagen synthesis. In a second approach we used organ cultures of mandibles of d 18 mouse fetuses. Small pieces of mandibles free of skin were grown at the medium/air interface for 6 d and treated with different concentrations of Emdogain. Calcium content and activity of AP increased significantly and dose-dependently after treatment. Maximum values were obtained after 2 mg/ml, lower values at higher as well as lower concentrations indicating a very specific effect. Electron microscopically, an increase in cell activity, matrix deposition and

mineralisation was obvious. Hence mandible organ cultures are an appropriate model for investigating EMPs.

P80 Immunolocalisation of leptin in the human ovary. By S. LÖFFLER, G. AUST and K. SPANEL-BOROWSKI. *Institute of Anatomy*, *University of Leipzig*, *Germany*

Leptin, the 'obese protein' because of its key role in body weight control, was originally discovered in adipose tissue. Other tissues with leptin production have been found, e.g. the placenta and cultured granulosa cells from preovulatory follicles obtained from women under in vitro fertilisation therapy. Whether or whether not leptin occurs in the intact ovary has not been studied. Since leptin receptors of both the long form OB-R and the 2 short isoforms are found in human follicle cells, an autocrine and paracrine loop is suggested for steroid production.

21 human ovaries were collected in collaboration with the Institute of Pathology (Leipzig) or of Forensic Medicine (Heidelberg). Histological sections were HE stained for the classification of the ovarian cycle and used for simple (leptin) and double (leptin and leucocyte common antigen, LCA) immunolabelling with the avidin-biotin complex technique. Intact and regressing antral follicles displayed a stronger leptin response in the thecal than in the granulosal layer. The epithelioid appearance of leptin-positive thecal cells became fibroblast-like in mature follicles and in corpora lutea. The leptin-positive cells were noted in high number in the septa of developing corpora lutea (n = 4) and in low number between luteal cells of the subsequent secretory stage (n = 5). The leptin-positive cells appeared to be larger than LCA-positive leukocytes as detected by double immunolabelling. As regards polycystic ovaries (n = 4), the hypertrophic theca of large antral follicles was richer in epithelioid leptin-positive cells than the theca of normal antral follicles. In conclusion, our finding of leptin positive cells in various ovarian regions has to be confirmed for the mRNA level both for leptin and leptin receptors in order to verify para- and autocrine leptin function.

P81 Morphological and histological examination of cattle ovaries after repeated punctures for oocyte aspiration. By J. KURYKIN[1], L. MAJAS[1], M. AUNAPUU[2] and H. KÜBAR[1]. [1]*Faculty of Veterinary Medicine*, *Estonian Agricultural University*, *Tartu*; *and* [2]*Faculty of Medicine*, *University of Tartu*, *Estonia*

The aim of the study was to investigate morphologically and histologically the aberrations in cattle ovaries after repeated punctures for aspiration of oocytes. Transvaginal punctures of ovaries were performed under rectal control once a week, using the aspiration device and technique described by Hill (*Theriogenology* **43**, 1995) in 9 oocyte donors (7 cows and 2 heifers) for 11 wk. After the last puncture session 6 cows and both heifers were slaughtered and their ovaries examined. In the ovaries of 3 cows slaughtered on d 4 after the last puncture session visible points of previous intrusions of the puncture needle and 3 haematomas 10 to 20 mm were revealed. In 1 ovary a corpus luteum (CL) was formed. On d 6 ovaries of 1 cow and 2 heifers had normal form, consistency and colour with few indications of intrusions. Follicles of different size were present. In the ovaries of both heifers, CL were formed. On d 12, in 1 cow the remnants of haematoma in one ovary and follicular growth in both ovaries were observed. On d 19 in 1 cow some follicles were seen in both ovaries, and CL and luteal cyst (3 mm thin wall, cavity 24 mm in diameter) were present in 1 ovary. No adhesions to the tissue surrounding ovaries and other abnormalities were found. Histological examination of the ovaries of the heifers slaughtered on d 6 revealed connective tissue cells in the cortex, minor hardening of the stroma and infiltration of leukocytes. In the cow slaughtered on d 19 a small increase in the amount of connective tissue in one ovary was found. The gross and histological examinations of ovaries indicated the presence of traumas associated with multiple punctures. The follicular growth and corpora lutea formation prove that these traumas are minor and do not exert significant detrimental effect on subsequent ovarian function of oocyte donors.

P82 Immunolocalisation of substance P and tyrosine hydroxylase in the bovine corpus luteum. By I. REIBIGER, R. BEYER, G. TSCHEUDSCHILSUREN, C. GEBHARDT and K. SPANEL-BOROWSKI. *Institute of Anatomy*, *University of Leipzig*, *Germany*

The steroidogenic function of the bovine corpus luteum may be influenced by peptidergic and adrenergic innervation according to the literature. However neither substance P (SP) nor tyrosine hydroxylase (TH) are located in the corpus luteum itself, although abundant nerve fibres have been found in the ovarian cortex and the medulla.

Bovine corpora lutea in different stages of the oestrus cycle were studied for the occurrence of both transmitters using localisation by immunofluorescence. Substance P-immunoreactive fibre-like structures were clearly seen in the corpus luteum, more in the stage of development (n = 7) than the stages of secretion (n = 6) and regression (n = 6). In the stage of development, the SP-positive structures formed a network in the luteinising granulosal layer. Some positive fibres were usually noted in the former theca. Fibres with TH-immunoreactivity were also apparent in corpora lutea throughout the oestrus cycle, yet most distinct in the stage of secretion. Fibres were mainly associated with blood vessels and thus appeared not to be coexistent with SP-positive structures. The presence of SP and TH in the corpus luteum and their changes in amount during the oestrus cycle were confirmed with the dot blot method. In conclusion, the structure and mechanism where and how SP and TH are cyclically expressed in the bovine corpus luteum remain to be clarified.

P83 Glycoconjugates in the apical membrane of brush cells possess a specialised, but species-specific composition—a lectin histochemical study in rats, guinea pigs, and mice. By A. GEBERT, A. GEBHARD, S. FASSBENDER, K. WERNER and K. AL-SAMIR. *Hannover Medical School*, *Centre of Anatomy*, *Germany*

Intestinal brush cells are epithelial cells that possess unique ultrastructural characteristics and are assumed to represent chemoreceptors of the gut mucosa. They comprise a small population of epithelial cells lining the intestine and in many aspects resemble the receptor cells of taste buds. To characterise glycoconjugates possibly involved in sensory functions we investigated brush cells in the ileum of rats,

guinea pigs, and mice using thin section electron microscopy and lectin histochemistry by confocal light microscopy.

Brush cells of rats were selectively labelled by the sialic acid-specific lectin derived from *Maackia amurensis* (MAA), those of guinea pigs by the D-galactose-specific lectin derived from *Bandeiraea simplicifolia* (isolectin B4, BS-I-B4), and those of mice by the L-fucose-specific lectin derived from *Ulex europaeus* (UEA-I). Lectin binding sites were consistently located in the glycocalyx of the apical membrane and in that of cytoplasmic vesicles. In vivo lectin labelling revealed that the glycoconjugates of the apical membrane are accessible under physiological conditions, that brush cells do not endocytose and that they possibly possess a high membrane turnover.

The results show that specialisations exist in the composition of glycoconjugates forming the glycocalyx of brush cells in all species investigated. The presence of brush cell specific glycoconjugates would be in accordance with the current hypothesis of a receptive function of brush cells. Differences in the specific glycosylation patterns between rats, guinea pigs and mice indicate species specific adaptations.

P84 Calbindin D-28K and parvalbumin immunocytochemisry in Herbst mechanoreceptors of the duck. By C. CHOUCHKOV and A. DANDOV. *Department of Anatomy*, *Faculty of Medicine*, *Thracian University*, *Stara Zagora*, *Bulgaria*

Calbindin D-28K (CB) and parvalbumin (PV) are members of the calcium binding proteins (CaBP) which are involved in transduction, neurotransmitter release, rapid adaptation and regulation of intracellular energy metabolism in both the central and peripheral nervous system, including mechanoreceptors. Recent studies have demonstrated CaBP in Herbst mechanoreceptors, but the results only showed light microscopy visualised immunoreactivity (IR) in these rapidly adapting sensory corpuscles.

In this study we used electron microscopy immunocytochemical techniques to demonstrate the precise localisation of CB and PV in different cellular and nerve elements of mechanoreceptors. One or 2 wk old ducks were anaesthetised with chloroform and small pieces of the skin on the upper beak were rapidly removed and, after rinsing in 0.1 M phosphate buffer (pH 7.2), fixed in 4% paraformaldehyde and 2% glutaraldehyde, and processed for monoclonal immunocytochemistry. For control purposes some of the sections were treated in the same way omitting the primary antibodies.

The investigated CaBP showed a remarkably homologous pattern of distribution. The most prominent IR was observed in modified Schwann cells, also known as inner core cells. The IR increased progressively from the periphery of the inner core towards the central part of the receptor, especially in the close proximity of the nerve ending. The CB-IR produced diffuse and more or less intense staining of the receptor nerve fibre. The nonmyelinated part of the fibre and its nerve ending showed more pronounced IR when compared with its myelinated part. The perineural capsule cells showed weaker IR whereas the cells of the subcapsular space did not display any IR.

The presented results suggest that despite an ill defined physiological significance of CaBP in rapidly adapting mechanoreceptors, the discrepancies in IR reflect the differences in receptor structures. Thus the most pronounced IR in the nonmyelinated part and its ending as well as the IR of correlated lamellae of modified Schwann cells support the assumption that calcium ions play an important role in mechano-elecrtric transduction.

P85 Localisation of $VPAC_2$ mRNA expression in human trachea and bronchi. By D. A. GRONEBERG[1], P. HARTMANN[1], and A. FISCHER[1,2]. [1]*Institute for Anatomy and Cell Biology*, *University of Giessen; and* [2]*Department of Pediatrics*, *Research Division of Allergy*, *Charité*, *Humboldt-University*, *Berlin*, *Germany*

Vasoactive intestinal polypeptide (VIP) is a neurotransmitter of the i-NANC system and influences all aspects of mammalian airway function. It binds to 2 distinct receptors. The $VPAC_2$ receptor, formerly known as VIP_2 receptor is highly selective and activated by the cyclic peptides Ro 25-1553 and Ro 25-1392. Since VIP binding sites have only been demonstrated by ligand binding techniques, we studied the distribution of vasoactive intestinal polypeptide receptor $VPAC_2$ mRNA in human trachea and bronchi.

A Northern blot demonstrated high levels of $VPAC_2$ mRNA in human airway tissue. Using a human specific $VPAC_2$ cRNA probe we investigated the $VPAC_2$ mRNA expression in human lung by nonradioactive in situ hybridisation. High resolution interference contrast microscopy revealed positive $VPAC_2$ mRNA signals in tracheal and bronchial epithelial cells. There was also marked staining of acinar and secretory cells of submucosal glands. No signals were obtained in airway and vascular smooth muscle myocytes and endothelial cells. Our findings demonstrate the cellular localisation of a specfic VIP receptor in human larger airways and extend the knowledge of pulmonary pathways of VIP signalling.

P86 The human and murine homologues of yeast vacuolar protein sorting 29. By A. J. EDGAR and J. M. POLAK. *Department of Histochemistry*, *Imperial College School of Medicine*, *London*, *UK*

The subcellular organelles that comprise the secretory and endocytic pathways perform the essential function of compartmentalising biochemical reactions. Vesicle formation is mediated by cytoplasmic coat proteins that are recruited on to specific target membranes. We have cloned the human and murine homologues of the yeast, *Saccharomyces cerevisiae*, vacuolar protein sorting 29 (VPS29), a component of the sorting machinery required for prevacuolar/late endosome to Golgi retrieval of the VPS receptor VPS10. VPS29 together with VPS26 and VPS35 form a cargo recognition and concentration subcomplex, termed the inner shell of the retromer coat, prior to the addition of the membrane deforming outer shell. The human and murine cDNAs are 990 bp (GenBank accession Nos. AF193795 & AF193794 respectively). They encode 182 residue proteins (AAF04596 & AAF04595) of 20.5 kDa and pI 6.29 that are identical except for the C-terminal residue which is proline in human and serine in mouse. The 10.5 kb human VPS29 gene is located on chromosome 12q24 and consists of 4 exons. Rather unusually, only the initiation

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methionine codon is found in exon 1. The human protein has 43% identity and 57% similarity to the yeast protein. In humans most of the components of this membrane coat complex have now been identified. The identity of the protein sorting receptors involved has yet to be determined, however, they may include the mannose-6-phosphate and epidermal growth factor receptors.

(This work was supported by GalaxoWellcome and the Julia Polak Lung Transplant Fund).

P87 The effects of micro-injecting P40^{phox} on the actin cytoskeleton of a mouse monocyte macrophage cell line (J774). By M. M. BIRD, D.-M. SHAO and A. W. SEGAL. *Department of Biomedical Sciences, Queen Mary and Westfield College; and Department of Medicine, Rayne Institute, University College London, UK*

Macrophages play a crucial role in host defence mechanisms against viral and bacterial pathogens by initiating the detection, phagocytosis and destruction of pathogens and the initiation of an immune response. The actin cytoskeleton is made up of filaments and binding proteins known to play an important role in maintaining cell shape and in cell movement. It is also known that the small GTP binding protein p21Rac is involved in both the NADPH oxidase, which generates microbicidal superoxide in phagocytes, and in the organisation of the F-actin cytoskeleton. The target proteins mediating this effect are unknown but the p40^{phox} protein is located within the cytosol and is translocated to the cell membrane when it is activated, making it a candidate for this role.

J774 macrophages (1×10^5/ml) suspended in Ham's F-12 culture medium were plated out onto uncoated 13 mm glass coverslips and maintained in a humidified chamber at 37 °C for 24 h. FITC-labelled IgG was added to the injection buffer as a marker to identify injected cells in control experiments and with P40^{phox} protein at a concentration of 0.5 mg/ml in the experimental micro-injections. The volume of fluid injected into the cells was restricted to a maximum of 10% of the total cell volume. Following micro-injection the cells were fixed in 3.7% formaldehyde, permeabilised and then immersed in TRITC-labelled phalloidin to identify the actin cytoskeleton.

In control material the actin cytoskeleton was dispersed throughout the cell cytoplasm with some accumulations associated with the cell membrane. Following micro-injection with p40^{phox} the J774 macrophage cytoskeleton became polarised and substantial increases in lamellipodia and membrane ruffling were observed in fluorescence studies. These findings suggest that p40^{phox} may also provide a link between the NADPH oxidase and the actin cytoskeleton.

P88 The human homologue of yeast vacuolar protein sorting 35. By A. J. EDGAR and J. M. POLAK. *Department of Histochemistry, Imperial College School of Medicine, London, UK*

The secretory pathway consists of distinct membrane-enclosed compartments. The maintenance of the identity of each organelle depends on the correct localisation and retention of its resident proteins. In yeast, resident membrane proteins of the trans-Golgi network (TGN) are selectively retrieved from a prevacuolar/late endosome compartment. Proper cycling of a number of these resident membrane proteins depends on vacuolar protein sorting 35 (VPS35). Mutations located in various regions of yeast VPS35 have been shown to disrupt the retrieval of different resident proteins, suggesting that VPS35 is involved in cargo selection in the prevacuolar compartment prior to transportation to the TGN. We have cloned the human homologue of VPS35. The cDNA is 2684 bp (GenBank Accession No. AF191298) encoding a 796 residue protein (AAF02778) of 92 kDa and pI 5.32). The protein is predicted to be predominantly alpha-helical. Overall it has 32% identity to the yeast protein and this conservation reaches 70% similarity in the N-terminal domain. Since in yeast, VPS35 interacts with VPS29 as part of the retromeric coat complex it will be of interest to determine whether the 2 human homologues also interact.

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

P89 Human skin cells exhibit variable alignment responses to substratum topography. By J. SUTHERLAND[1], M. ROBERTSON[2], W. MONAGHAN[2], M. RIEHLE[2] and S. BRITLAND[1]. [1]*School of Pharmacy, University of Bradford; and* [2]*Department.of Electronics and Electrical Engineering, University of Glasgow, UK*

Oriented cell movement is a fundamental process in morphogenesis and wound repair. The adhesive and microtopographic properties of the cellular substratum represent 2 interdependent morphogenetic guidance cues that have previously been shown to induce alignment in a variety of cell-types in culture, possibly as a prelude to oriented polarity or cell motility. In the present study we have examined the influence of model microtopographic cues on the alignment responses of human skin cells which are known to participate in dermal wound healing.

Model topographic guidance cues (gratings) were micro-engineered on the surface of cell culture substrates using techniques adapted from the microelectronics industry. These substrates were derivatised with relevant molecular components of the extracellular matrix prior to cell culture. Cells were prepared from tissue samples kindly offered by patients undergoing elective surgery as approved by the Local Research Ethics Committee. Dermal fibroblasts, melanocytes, putative myofibroblasts (from Dupuytren's nodules), endothelial cells (HSVECs) and keratinocytes were plated at 1×10^4 cells/cm^2 in hepes-buffered media and maintained at 37 °C for 24–48 h prior to analysis of alignment using an eyepiece protractor graticule.

Within the parameters of this experimental design all cells were capable of aligning to microtopography. The degree of alignment was dependent upon cell type, groove width and groove depth. Generally the proportion of aligned cells increased with groove depth for all groove widths. Although less clear cut, narrower grooves were associated with increased cell alignment. Fibroblasts were consistently the most sensitive to surface topography even detecting features 95 nm high at 1, 2 and 4 μm groove widths. Interestingly, fluorescent staining of the keratinocyte cytoskeleton revealed apparently 'non-aligned' cells with filamentous actin and other intracellular components that were in fact aligned

to the substrate. This observation suggests that to some extent cell alignment to substratum topography may result from 'informed' choice.

The present study has shown that substratum microtopography can influence the behaviour of primary human skin cells.

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P90 The human and mouse homologues of rat prolactin regulatory element binding protein. By A. J. EDGAR and J. M. POLAK. *Department of Histochemistry, Imperial College School of Medicine, London, UK*

The prolactin regulatory element binding protein (PREB) is a transcription factor that was first identified by expression cloning from the rat pituitary. PREB binds to the prolactin promoter in a sequence specific manner and stimulates both basal and protein kinase A transcriptional activity in pituitary cells. Although prolactin is specifically expressed in pituitary lactotropic cells, PREB mRNA is strongly expressed in heart, skeletal muscle and pancreas, suggesting that it also regulates other genes. We have cloned the human and mouse homologues of rat PREB from lung (GenBank accession no. AF203687 and AF150808). The human 2059 bp cDNA encodes a 417 residue protein (AAF19192) of 45.5 kDa and pI 8.02. It has 90% identity and 98% similarity to the rat protein. The structural motifs of the human PREB protein include: 3 putative WD (or beta-transducin) motifs (residues 145–182, 186–223, 291–328), a glutamine rich region, residues 86–157 and proline/glutamine (P/Q) rich region (residues 223–279). There is a t814a polymorphism which results in the conserved valine228 being replaced by a glutamic acid residue. The gene is located on chromosome 2. Rather intriguingly, the polyadenylation signal of this cDNA overlaps with the polyadenylation signal of the lung alpha/beta hydrolase fold protein-1 (LABH-1) encoded by the opposite strand. PREB may play a significant homeostatic role in transcriptional regulation in other tissues in which it is expressed.

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P91 Cellular components of human skin are differentially motile in primary culture. By J. SUTHERLAND[1], M. ROBERTSON[2], W. MONAGHAN[2], M. RIEHLE[2] and S. BRITLAND[1]. [1]*School of Pharmacy, University of Bradford, Bradford and* [2]*Department of Electronics and Electrical Engineering, University of Glasgow, Glasgow, UK*

The pathogenesis of unsightly scarring, which may occur for example after traumatic excisional dermal injuries, is of enormous clinical relevance but is not yet fully understood. A pivotal process involved in both this and other types of wound repair is oriented cell migration both through and over a wound bed comprised of adhesive extracellular matrix components. In the present study we have characterised the motility capability of some of the key cellular components of human skin involved in dermal wound repair.

Primary cultured cells were prepared from tissue samples kindly donated by patients undergoing elective surgery as approved by the Local Research Ethics Committee. Dermal fibroblasts, myofibroblasts, keratinocytes and melanocytes were isolated, amplified and stored according to methods established in our laboratory. Cells were routinely cultured for 24–48 h on fetal calf serum-treated tissue culture plastic before quantitative analysis was attempted. Semi-automated video microscopal image analysis of cell motility allowed calculation of a range of motility parameters including mean velocity and persistence.

Fibroblasts, myofibroblasts and keratinocytes were all found to display conspicuous motile behaviour. Cell motility was greatest in keratinocyte populations which had a mean velocity of 5.31 ± 0.54 μm/h (mean ± S.E.). Qualitative observations suggested that motile behaviour in keratinocytes was modulated by whether clustering in colonies or isolated and whether having a spread or rounded morphology. Fibroblasts and myofibroblasts had a similar velocity of 3.55 ± 0.46 μm/h and 3.67 ± 1.02 μm/h respectively and shared a similar spread stellate morphology. In stark contrast melanocytes were virtually stationary with a mean velocity of only 0.47 ± 0.04 μm/h. This small degree of movement was mainly derived from oscillations around the cell body and typically associated with probing behaviour.

These experiments have revealed that cells of fundamental importance during human dermal wound repair are capable of displaying highly motile behaviour in culture. However, within the constraints of this experimental design, the degree of motility was unequal between the cells possibly reflecting their differentiated function in vivo.

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P92 Cultivation of normal human epidermal keratinocytes and in vitro transfection with lipid/DNA complexes. By S. ZELLMER[1], J. SALVETTER[2], A. SUROVOJ[3], F. GAUNITZ[1], A. BEKELE[4], R. GEBHARDT[1] and D. REISSIG[2]. [1]*Institute of Biochemistry and* [2]*Institute of Anatomy, Medical Faculty, University of Leipzig, Germany;* [3]*Russian Academy of Sciences, Institute of Bioorganic Chemistry, Moscow, Russia; and* [4]*Department of Anatomy, Gondar College of Medical Sciences, Ethiopia*

The keratinocytes of the stem cell system can be isolated and cultivated ex vivo. Therefore, this cell system is an ideal target for a therapy of epidermal defects by gene transfer. Two different approaches for transfection of keratinocytes in vitro exist: the use of viral transfection systems and the incubation with lipid/DNA complexes.

We have isolated human keratinocytes from the basal epidermal layer. Cells were grown in serum free medium. The development of individual clones was followed by light microscopy. Cell cultures with highly proliferating keratinocytes expressed cytokeratin 19, a marker of epidermal stem cells as well as β_1 and β_4 integrin. The development of hemidesmosomes was followed by electron microscopy.

These keratinocytes were transfected with 2 different cationic lipid systems, Lipofectamine and Effectene. The transfection efficiency of Effectene was 20 fold superior over Lipofectamine, determined by the expression of a luciferase marker gene. Transfection of individual cells was followed with the GFP marker gene.

The presented study demonstrates that lipid/DNA complexes can be used for efficient transfection of human epidermal keratinocytes.

P93 P19 cells: a cell line with a potential use in the development of a bioassay system consisting of electrogenic cells cultured over microfabricated extracellular recording devices. By M. C. DENYER[1], E. C. SCHMIDT[2], S. T. BRITLAND[1], M. SCHOLL[3], C. SPROESSLER[4], A. OFFENHAEUSSER[4], M. HARA[5] and W. KNOLL[4]. [1]*School of Pharmacy, University of Bradford, UK;* [2]*MPI for Biochemistry and Membrane Chemistry, Martinsried;* [3]*Institute for Physiological Chemistry, School of Medicine, Johannes Gutenberg University, Mainz;* [4]*MPI Polymerchemie, Mainz, Germany; and* [5]*Laboratory for Exotic Nano-Materials, RIKEN, Saitama, Japan*

In recent years it has become possible to examine signal processing and construct bioassays consisting of cardiac myocytes or patterned neuronal networks cultured over microfabricated electrophysiological recording devices. However the cells used in these systems are currently primary cells derived from neonatal rats, thus these systems can prove expensive to run and may raise ethical opposition because of the heavy use of animals. These are both problems that can be circumvented by developing a system using electrogenic cells derived from cell lines. We are currently examining the use of P19 cells. P19 cells were originally derived from an embryonic mouse carcinoma (McBurney & Rogers, *Dev. Biol.* **89**, 1982). They have a phenotype similar to the primitive ectoderm and have the remarkable ability to be differentiated irreversibly in the presence of retinoic acid into neuronal type cells or cardiac myocyte cells in the presence of DMSO or 3,5,3′ triiodo-L-thyronine. To demonstrate that p19 neuronal cells can be used in bioassays we first need to demonstrate that P19 neurons can be patterned so that cell bodies can be positioned over recording sites. In this study we examined the patterning of P19 neurons on glass substrates using a combination of silane chemistry and microcontact printing. The patterns presented to the cells consisted of 5 μm wide tracks of a synthetic analogue of the laminin a-chain PA22a crossing at 100 μm intervals. We found that P19 cells responded to the guidance cues by preferentially adhering to the adhesive pattern and migrating to the crossing points of the pattern. The patterned neuronal networks formed proved remarkably stable, with patterned cells surviving in low density culture for periods in excess of 16 d. We are now in the process of patterning P19 neurons onto recording devices. An additional benefit of using P19 cells in a bioassay is that they can be transfected with the cDNA encoding for specific receptors. This may allow transfected P19 cells to be used in assays tailored to specific compounds.

P94 The chick homologue of the mammalian gene *kif 5C* was cloned from a subtractive library of embryonic chick cDNA. By V. DATHE, F. PRÖLS, M. AST and B. BRAND-SABERI. *Department of Anatomy II, Albert-Ludwigs-Universität Freiburg, Germany*

In our search for new genes important in vertebrate development we prepared a subtractive cDNA library based on the complete RNA of chick embryos (*Gallus gallus domesticus*) at stage HH 11–13 and younger than stage HH 10.

For screening the library we chose the method of in situ hybridisation of chick embryos using a digoxigenin labelled RNA probe from cDNA templates. The embryos were analysed carefully for a distinct expression pattern indicating developmental relevance. Selected cDNA clones were used for screening a chick cDNA phage library in order to find full length clones of the genes of interest.

We used this experimental procedure because subtractive cDNA libraries are a potent instrument to enrich and isolate rare cDNA species, belonging to mRNAs of a low copy number. The in situ hybridisation allows a quick and direct view of the expression patterns which is helpful for choosing attractive clones.

A good example of the success of this experimental concept was the cloning of the chick homologue of *kif 5C* which has previously only been described for mammalian species, including humans. This gene encodes for a protein (kinesin heavy chain 5C) belonging to the kinesin family. Kinesin proteins are essentially involved in microtubule-dependent intracellular transport processes. The *kif 5C* of mouse is reported to be expressed only in neuronal tissues (Xia et al., *Genomics* **52**, 1998). Corresponding with those results we found a distinct neuronal expression pattern of *kif 5C* in chick embryos. This gene is expressed in the dorsal root and cranial ganglia. Expression of *kif 5C* may be correlated with the onset of axonal transport in the developing nervous system. *Kif 5C* is also expressed in Hensen's node, prochordal mesoderm, dermomyotome and Wolffian duct.

We believe that *kif 5C* represents a good marker helpful in further observation of neuronal development in the chick as a model organism which may yield important information about kinesin function in vertebrate development in general.

P95 Distribution of somitic myoblasts within developing avian limb muscles. By D. J. R. EVANS and E. REES. *School of Biosciences, Cardiff University, UK*

Previous studies have demonstrated that avian limb muscles are formed from myogenic precursor cells (myoblasts) that have migrated from adjacent somites into the developing limb bud. The aim of the present study is to reveal the contribution made by somitic myoblasts to all muscles within the avian limb and define the spatial and temporal relations among individual populations of muscle precursors within each somite. We utilised the *LacZ* reporter gene, encoding for bacterial β-galactosidase to label and follow individual muscle populations. The gene was introduced into cells via a replication deficient retroviral vector. Injections of retrovirus were made into precisely recorded individual somites of stage 14–18 White Leghorn chick embryos. Following cervical dislocation at stage 36, embryos were fixed and stained for β-galactosidase to identify infected cells and their descendants. The results of injections into somites adjacent to the developing limb bud confirmed that somites 26 to 33 are the source of myoblasts for all limb muscles with injections made into more cranially or caudally placed somites never resulting in labelled limb muscles. Injections into single somites consistently resulted in more than one labelled limb muscle and in many cases, progenitors from individual somites made contributions to muscles within both the thigh and shank regions. Inte-

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restingly however, injections made into the same somite, in different embryos, at increasingly later stages resulted in labelling being progressively restricted to more distally situated muscles. In most instances both primary and presumptive secondary myotubes were labelled within each muscle, indicating that at least some of the progenitors for both these myotube populations arise from the same somite. Interestingly though, the precursors of the secondary myotubes may already be spatially segregated within the somite as several injections resulted in labelling of only presumptive secondary myotubes. Overall the pattern of labelled muscles was highly selective, indicating that the distribution of myoblasts throughout the limb may not be a random phenomenon.

(Supported by The Wellcome Trust).

P96 Lymphangioblasts of the avian somite give rise to the lymphatic endothelium of the wing. By M. PAPOUTSI, M. SCHNEIDER, K. OTHMAN-HASSAN, B. CHRIST and J. WILTING. *Anatomisches Institut II der Albert-Ludwigs-Universität Freiburg, Germany*

By grafting chick limb buds homotopically into quail embryos we have recently shown that there are lymphangioblasts in the early avian wing bud, but fate map studies on the origin of these cells have not yet been performed. The lymphatics in the wing of 10 d old chick and quail embryos are characterised both by the position along with all major blood vascular routes and by Vascular Endothelial Growth Factor Receptor-3 (VEGFR-3) expression. In the quail the endothelium of both the blood vessels and the lymphatics can be marked with the QH1 antibody. We have grafted dorsal halves of epithelial somites of 2 d old quail embryos homotopically into chick embryos. The grafting was performed at the wing level and the host embryos were reincubated until d 10. The chimeric wings were studied with the QH1 antibody alone and with double staining consisting of VEGFR-3 in situ hybridisation and QH1 immunofluorescence. Our results show that in the wing the endothelium of both the blood vessels and the lymphatics is derived from the somites. QH1-positive endothelial cells form the vasculature of the chimeric wings. Chimeric lymphatics of the wing can be identified because of their typical position and their VEGFR-3 and QH1 double positivity. This shows that not only the blood vascular but also the lymphatic endothelial cells of the avian wing arise from the paraxial/somitic mesoderm.

P97 The role of apoptosis and necrosis during midgut development in A2G mice. By L. CHIRCOR, C. KAPETANOS, S. B. RIZWAN and E. GRISBOLAKI. *Embryology Department, Faculty of Medicine, "Ovidius" University, Constanta, Romania*

The purpose of this study was to analyse the development of the small intestine by the recently described phenomenon of apoptosis in order to understand the modulating process of development.

The authors used A2G mice and the study was done according to the international rules of animal care. The number of animals was justified by statistical criteria. Pregnant A2G mice were administered phenytoin (25 mg/100 g body weight) orally on 5 consecutive gestational days, 8–12 (group F1) and 10–14 (group F2); or alternatively by intravenous administration (25 mg/100 g body weight) for 5 consecutive gestational days, 8–12 (group F3) and 10–14 (group F4). The control group was maintained until day 14. After general anaesthesia all mice were killed by cervical dislocation at d 14 and the fetuses were removed from the uterus. A complete morphological examination was performed in each case. In order to detect apoptotic cells we performed a histological study of the midgut using H&E staining and epoxy semithin sections stained with toluidine blue. The incidence of apoptotic bodies in intestinal cells was assessed in longitudinal sections of the crypts of the midgut.

In the midgut tissues of the control group, newly formed rounded apoptotic bodies and partly degraded phagocytosed bodies existed as markers of apoptosis. The results strongly suggest that the endoderm of the midgut represents an important process of apoptosis during embryonic period, apoptosis which has an important role in regulating the midgut lumen size. Midgut malformations exist at all phenytoin groups in statistically significant numbers ($t < 0.005$). The histology of the malformed tissues showed hyperaemia and vascular ectopy or necrosis. Phenytoin is capable of inducing both necrosis and apoptosis. The type of cell death produced depends on the severity of the insult.

P98 Cardiac morphometric and 3D reconstruction in human embryos of Carnegie stages 16 to 18. By L. A. ARRÁEZ-AYBAR, D. G. MARANTOS, F. GONZÁLEZ-LORRIO and J. JIMÉNEZ-COLLADO. *Instituto de Embriología, Departamento de Ciencias Morfológicas II, Facultad de Medicina. Universidad Complutense, Madrid, Spain*

The morphogenic processes underlying cardiac development and differentiation have been widely described and experimentally studied (Ost'ádal, *The Developing Heart*, Lippincott 1997). However in the quantitative studies there is a remarkably high prevalence of studies in the fetal period compared with a scarcity in the embryonic period (Mandarin-de-Lacerda, *Ann. Anat.* **177**, 1995). We have collected data and achieved a quantitative analysis of the embryonic phase of the period of cardiac organogenesis in an attempt to establish specific patterns in the different Carnegie stages (O'Rahilly et al. *Bull. Ass. Anat.* **65**, 1981). We present a morphometric study and 3D reconstruction of the developing heart in 12 human embryos from Carnegie stages 16 to 18. The cardiac volume was determined by morphometric methods.

Computerised image analysis was applied using a methodology elaborated by us (Arráez-Aybar et al. *Surg. Radiol. Anat.* **16**, 1994) for the impartial detection and extraction of functionally homologous regions in each consecutive section of a serially cut specimen. The image of the structure was automatically registered by the computer by discriminative significance calculation of the consecutive points in a drawing. This was followed by quantitative analysis of size and shape and 3D reconstruction of these areas and also of the whole structure of the heart.

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P99 Developmental notes on the urinary bladder wall in man and some cetaceans (*Stenella longirostris*, *Stenella attenuata*). By J. KOČOVÁ[1], Z. TONAR[1], V. KRÍŽKOVÁ[1] and M. KLIMA[2]. [1]*Department of Histology and Embryology, Charles University Faculty of Medicine, Pilsen, Czech Republic; and* [2]*Zentrum der Morphologie, J. W. Goethe University, Frankfurt-am-Main, Germany*

Although the principles of formation of the urinary bladder and primitive urethra are relatively well known in humans we lack a more detailed description of these organs' histogenesis. We investigated this using the serial sections of human embryos and fetuses of crown-rump lengths (CRL) 4, 5, 9, 14, 18, 58, 60, 69, 70, 90 and 95 mm. The early development of the urogenital sinus was also studied in 5 stages of 2 cetacean species (*S. longirostris*, *S. attenuata*) in which the process is unknown. General staining methods for light microscopic study were used (haematoxylin and eosin, Mallory's and modified green trichrome).

In human embryos the urogenital membrane persisted until CRL of 14 mm when it shows degenerative changes. The vesico-urethral canal in 18 mm embryos was already lined by transitional epithelium and the mesodermal splanchnopleuric mesenchyme was giving rise to the first primordia of the longitudinal smooth muscle layer in the cranial part of the originating bladder. All 3 layers were present in the 58 mm fetus, especially in the cranial and lateral parts of the bladder wall. The inner longitudinal layer was the finest layer and rather discontinuous. The inner longitudinal and middle circular muscle layers of the bladder became thickened at the neck of the bladder, especially ventrally, and were continuous with the muscle layer of the primitive female urethra (CRLs 58, 60, 69, 70 mm). The iliac part of the fragmented posterior lymph sac (the rudiment of the iliac group of lymph nodes) and the vesical venous plexus became important parts of the posterior wall of the bladder in fetuses of 70 and 90 mm, with lymph node primordia at 95 mm.

The terminal part of the hindgut formed the cloaca in 7.5 mm embyros of *S. attenuata*. The cloaca became divided by the urorectal septum at CRL of 9.2 mm, when the cloacal membrane persisted and both the ureter and the mesonephric duct entered the ventral part of the cloaca separately. In *S. longirostris* embryos of 12.3 and 12.5 mm the urogenital membrane started to disintegrate. The bladder of *S. attenuata* was still rather collapsed in 38.5 mm fetuses and only a fine rudiment of the muscle layer could be seen.

The findings of this study are planned to be used in our collaboration with biomechanicists modelling the structure of the human urinary system.

(The study was supported from grant GACR 106/00/1733).

P100 Morphological changes in the induction of supernumerary neural tubes in the chick (*Gallus domesticus*). By L. J. ROBINSON. *Department of Pre-Clinical Sciences, University of Leicester, UK*

Previous investigations have revealed that wounds inflicted on early embryos heal quickly, and tissue integrity is then restored. Both normal and wounded embryonic development of the nervous system depends on a fine balance between cell proliferation, cell migration, cell orientation and differentiation of cell types.

As neurulation progresses 'signalling inputs' from the anterior/posterior and dorsal/ventral axes define the patterning of the neuroectoderm. The notochord is a source for a 'signalling input'.

Chick embryos were prepared by New culture (New 1955). Following notochord wound healing, stage 10–11 chick embryos were re-incubated for 1–24 h. The notochord was wounded in the area of somites 1–6. A series of control embryos without notochordal wounds were also explanted. The embryos were prepared for light and scanning electron microscopy (SEM).

In whole mount experimental embryos there was little evidence of external abnormality. In cross sections, a range of gross neural tube abnormalities was observed.

At neural tube levels posterior to the notochordal wound the integrity of the limiting membrane was intact although a few blebs were occasionally present on the basal (outer) surface of the dorsal lateral neuroepithelia. The cells of the floorplate appeared to thin and flatten and luminal surface blebs were present. The ventral lateral region increased in thickness and bulged bilaterally into the lumen of the neural tube. The luminal surface also displayed excess blebbing. At dorsal lateral levels of the tube, the cells appeared to be delaminating and moving into the lumen of the tube in a ventral direction. The roofplate remained a coherent structure.

At neural tube levels anterior to the notochordal wound, the morphological changes appeared to be at a more advanced stage. The limiting basement membrane at some dorsal lateral levels had broken down and cells were concurrently migrating away from and into the neural tube The whole lumen of the neural tube was filled by migrating cells and blebs. They appeared to be rearranging themselves into numerous concentric patterns. The lateral edges of the roofplate section rounded up and formed a smaller separate neural tube.

P101 Influence of B-193 on development of bones of the skull. By A. ANASIEWICZ, J. WYSOKIŃSKA-MISZCZUK, T. TOMASZEWSKI, J. KLATKA, R. CZABAK-GARBACZ, A. NADULSKA and B. BUCZARSKI. *University Medical School, Lublin, Poland*

The aim of this study was to investigate the influence of B-193 on the development of bones of the skull of rat fetuses of the Wistar strain.

The new chemical compound B-193 (9-methyl-2[-3-(4-phenyl-1-piperazinylpropyl)]-1,2,3,4-tetrahydro-β-carbolin-1-one) is characterised by a potent antiserotonin action. It resembles the atypical antidepressant drug trazodone in its chemical structure, in particular in the phenylpiperazine group connected by means of a propyl chain with the cycloamide nitrogen atom of the heterocyclic system. B-193 was synthesised at the Institute of Pharmacology, Polish Academy of Sciences in Cracow.

Studies were performed as recommended by WHO Technical Report Series No 364/1967 *Principles for Testing of Drugs for Teratogenicity*. Macroscopic external evaluation of fetuses, both sectional and skeletal according to

Dawson's and Peter's methods were employed. The evaluation of birth defects of internal organs was carried out according to Wilson's technique in Barrow's and Taylor's modification.

Pregnant females (10–12 rats in each group) were treated with 1/50, 1/100, 1/250, 1/500, 1/1000 of LD_{50} (650 mg/kg body mass) of B-193 by gavage on each of days 7–14 of gestation. The following controls were used: UC, untreated control, pregnant females not receiving any drugs; TC, pregnant females treated with an equal volume of H_2O by gavage; TCc, pregnant females treated with an equal volume of carboxymethylocellulose by gavage; and ST, pregnant females receiving chlormethine hydrochloride (0.26 mg/kg body mass intraperitoneally) as a standard teratogen.

Females were euthanised and caesarean section was performed on last day of gestation and malformations of fetuses were determinated by gross examination and by Alcian blue with Alizarin red double skeletal staining.

On the basis of this study it was found that B-193 in all doses had teratogenic effects. The highest incidence of malformed fetuses with abnormal bones of the skull or without bones of the palate occurred after treatment with 1/250 of LD_{50} of B-193, and those with abnormal bones of palate after treatment with 1/100 of LD_{50} of B-193.

P102 Blood plasma results of gravid rats at the day of physiological conclusion of embryonic processes palatini after 2 week application of folate and thiocyanate. By J. WEINGÄRTNER[1], V. BIENENGRÄBER[2], S. BAYER[1], H.-J. ROSE[1], T. KOPPE[1] and J. FANGHÄNEL[1]. [1]*Institute of Anatomy*, *University of Greifswald; and* [2]*Department of OMF Surgery*, *University of Rostock*, *Germany*

Folic acid is considered to prevent cleft palate successfully. There is a question whether either an insufficiency of folate or a hyperhomocyteinaemia is primarily responsible for deficient palatinal closure during embryogenesis. In a first study with female primagravida rats the effects of folic acid and thiocyanate on the values of folic acid and homocysteine in maternal blood plasma at d 14 of pregnancy were examined. The animals were injected subcutaneously with folic acid (group 1; $n = 12$) on d 1, 4, 7, 10, 13 of pregnancy and with thiocyanate (group 2; $n = 10$) on d 10, 13 of pregnancy: the dose was 3.2 mg per 100 g of body mass. At the same dates nonpregnant animals were treated with folate acid (group 11; $n = 14$) and thiocyanate (group 22; $n = 10$). In addition a nonpregnant, untreated group (group 10; $n = 12$) was examined. First results show that homocysteine in blood plasma of group 10 is a weight related quantity and it correlates with $r = 0.783$ at $P < 0.003$. Otherwise no significant differences of the levels of homocysteine in groups 10, 11, 22 were found. The values of homocysteine of groups 1, 2 were significantly increased compared to group 10. Though folic acid had been applied at 0.016 mg per 100 g of body mass a significant decrease of blood values was found for groups 1, 2 in comparison with group 10. The values for group 2 were nonsignificantly higher than type 1. Those observations at the time of embryonic palate closure stress the increased need for folic acid during pregnancy. In a second study dams and fetuses up to d 21 of pregnancy will be looked into. Thus an effect of treatment of nonpregnant groups can be determined. However conclusions concerning values in blood plasma of pregnant groups cannot be drawn. The examination of a pregnant, untreated group be undertaken later.

P103 Topography of the developing pelvic region in the mouse (*Mus musculus*). By B. STRAUSS, S. MENG, T. P. T. TRUONG, M. A. DONAT, J. STREICHER and G. B. MÜLLER. *Integrative Morphology Group*, *Department of Anatomy*, *University of Vienna*, *Austria*

Over the past 20 y a great amount of data concerning the molecular differentiation and control of the vertebrate body axis and the limb have been published. In contrast information about the development of those structures that connect the limbs with the body axis, namely the pectoral and pelvic girdles, is still very limited. Which factors determine position, shape, and orientation of the elements is as yet unknown. No molecular or experimental studies explicitly addressing these questions are available. The reason for this might be that girdle regions are difficult to access in the embryo, and have a 3-dimensionally complex topology that has dynamic relationships with neighbouring structures. Moreover in most species, the condensation of defined precartilage elements is a fast process that takes place more in a single morphogenetic event, as compared with the sequential mode of skeletal differentiation in the external limb bud. In order to address some of the open questions related to girdle formation we investigated the development of the pelvic region in mouse embryos of stages 11.5 (d.p.c) to 12.5 (d.p.c.) prior to and throughout the condensation period of the prospective pelvic elements. Since there are some indications that the anterior proximal limb bud mesenchyme contributes to pelvic elements we created 3D reconstructions of this region from serial sections, following the method by Streicher et al. (*Anat. Rec.* **248**, 1997). Cellular distribution, local differences in density, and the spatial relationships between cell masses, blood sinuses, nerves, and the body cavity are shown in 3D computer models. In addition, reconstructions of the distribution of BrdU-pulse labelled cells demonstrate the proliferation dynamics of this region. The resulting 3D models provide information about the cellular origin, patterning, and morphogenetic mechanisms of early pelvis development, and help to define strategies for further molecular experimentation.

P104 The development of menisci of the knee joint in human embryos and early fetuses. By W. RATAJCZAK, W. WOŹNIAK and M. JAKUBOWICZ. *Department of Anatomy*, *University School of Medical Sciences*, *Poznań*, *Poland*

Interpretations of the developmental findings on knee menisci and the formation of their final shape are still controversial (Clark & Ogden, *J. Bone Jt Surg*, **65-A**, 1983; O'Rahilly & Gardner, *Anat. Embryol.*, **148**, 1975; Merida-Velasco et al. *Anat.Rec.* **248**, 1997).The aim of the study was to trace the development of the menisci of the knee joint in staged human embryos and early fetuses. Investigations were carried out on staged human embryos and fetuses from

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the Collection of the Department of Anatomy in Poznań. Embryos were staged according to Carnegie stages. 25 serially sectioned embryos of developmental stages 18–23 and 6 fetuses aged 9 and 10 wk have been studied.

It was found that in embryos at stage 19 the primordia of menisci and cruciate ligaments were recognisable. During stage 21 the menisci were well defined and could be measured. The incipient cavitation between the patella and femur was observed at stage 21. In embryos of stage 23 all intraarticular elements (articular surfaces, menisci and their ligaments, cruciate ligaments) of the knee joint were present.

Early developing menisci occupied a broad area of the articular surfaces of the tibia (from 50% to 96.01% for the medial meniscus and from 77.5% to 99.6% for the lateral meniscus). Based on the study performed it is suggested that the discoid meniscus is not a developmental anomaly but it is a stage in the development (arrested phase).

P105 Trophoblast changes in the small for date infants. By L. CHIRCOR, M. ALEXIOU, C. KAPETANOS and E. GRISBOLAKI. *Embryology Department*, *Medicine Faculty*, '*Ovidius*' *University*, *Constanta*, *Romania*

The authors analysed trophoblastic changes in order to establish clinical correlations with predictive values for the small for date infants.

This study relates to 34 small for date infants at gestational age 38–42 wk with weight $<$ 10th centile, born at Constanta County Clinic Hospital during 1995–1999. The method consisted of clinical evaluation of the newborn, macroscopic study of the placenta, light microscopic study of the placenta, and electron microscopic study of the placental bed containing spiral arteries.

The small for date infants' placentae showed a reduced chorionic surface area in 34 cases (89.9%) and numerous villous cytotrophoblastic cells with reduced fetal villous capillaries in 30 cases (80%). Physiological trophoblast invasion was restricted to the decidual segment of the spiral arteries in 16 cases (47%). Focal syncytial necrosis appeared on electron microscopic examination in 25 cases (65.5%) and thick trophoblastic basement membranes in 35 cases (92%).

All 34 cases presented abnormalities of the placentation including histological changes in the trophoblast and spiral arteries and were associated with small for date infants. The retardation of placental growth and placental ischaemic lesions may lead to the occurrence of small for date infants.

P106 The effect of spatial autocorrelation between structures on sequential dipole morphometric analysis. By N. T. JAMES. *SigmaMetrics Statistical Consulting Services*, *Sheffield*, *UK*

The use of pairs of finite sized quadrats for spatial sampling has long been used in ecology (Pielou, *An Introduction to Mathematical Ecology*, Wiley, 1969) and has been adapted as a point counting process for stereology (James & Čabrić, *Exp. Neurol.* **76**, 1982; James, *Morphometry*, *Applications to Medical Sciences*, Macmillan, 1996) where dipole ends act as infinitely small quadrats in a 'hit' or 'miss' counting process.

As in all forms of statistical estimation spatial statistics depend on underlying distributional assumptions which require testing to determine test validity or bias. Particularly important are the assumptions of homogeneity of variance (for example, 2 test populations having similar variances) or the presence of autocorrelation between data items or test objects.

By analogy with the effects on the analysis of autocorrelated data in unidimensional data analysis where autocorrelation influences statistical significance levels (Cliff & Ord, *Spatial Autocorrelation*, Pion Books, 1973), in the present study autocorrelation appeared even more significant. In synthetic populations mimicking anatomical and histological structures, positive autocorrelation between component test items were found to increase sampling variability with a consequent spurious decrease in significance levels. Conversely, negative autocorrelation in test populations was found to increase statistical significance levels spuriously. Heterogeneity of spatial variance within a population of test objects is also an important factor which induces alterations in statistical significance levels. An important conclusion of the present study is that experimental design and the construction of adequate sampling regimes are at least as important as in classical statistical methodologies and that detailed quantitative knowledge of anatomical structure remains as important as ever in designing experiments.

P107 Combined 3D reconstruction of multimodal data from histological sections. By J. STREICHER, B. STRAUSS, M. A. DONAT, T. P. T. TRUONG, S. MENG and G. B. MÜLLER. *Integrative Morphology Group*, *Department of Anatomy*, *University of Vienna*, *Austria*

The 3-dimensional visual synthesis of molecular, cellular, and morphological data, such as detected by immunostaining, in situ hybridisation, and conventional staining is essential for integrated biomedical research. Currently sectional histological preparations still offer the broadest spectrum of detection and visualisation modes (brightfield, fluorescence, phase contrast, and dark field imaging). However series of microscopical sections are burdened by shortcomings such as misalignment, distortion and staining variation, which impede their straightforward reintegration into combined 3D visualisations. Starting from an earlier concept for 3D reconstruction of conventionally stained histological serial sections (Streicher, *Anat. Rec.* **248**, 1997) we now present an advanced version, which provides the means for a combined visualisation of multiple signal types (Streicher, *Nature Genetics* **25**, 2000). Drill holes introduced into a permanent embedding medium prior to sectioning serve as external markers for automatic macro-driven congruencing (realignment plus rectification) of digital images captured from the microscopic sections. These markers have to be visible only in one of the viewing modes (e.g. in the phase contrast view), whereas all additional views (fluorescence or brightfield) which show different

aspects of the same section are automatically congruenced in accordance. This results in corresponding stacks of aligned series of images, each containing one type of view. Compensation for staining variation is automatically performed for each stack during the subsequent segmentation of the elements of interest by dynamic thresholding algorithms. With this technique any number of parameters (gene and molecule expression, cell activity, morphological aspects etc.) revealed by different detection modes can be collected from the same specimen as coordinated data sets. The reintegration of all sectional representations throughout the entire series is then performed using a commercially available software package (Velocity, Image3). The resulting 3D models of each aspect can be viewed simultaneously in any combination and from any point of view, providing the means for detailed quantitative and qualitative analyses of spatial relations. Documentation is possible in all formats ranging from conventional prints of single images through stereoscopic views and movies up to virtual reality scenes. In summary, the present method allows us to acquire multimodal data from serial histological sections and recombines them into coordinated, registered and staining compensated 3D datasets that can be visualised, analysed and published in digital formats.

P108 A modification of the 2 sample Student *t* test for studying nearest neighbour 3D distances. By N. T. JAMES. *SigmaMetrics Statistical Consulting Services, Sheffield, UK*

Random distributions of objects in both 2 and 3 dimensions possess exact properties which can be used as standards against which the distributions of anatomical structures can be compared. Whilst spatial statistics have long been used to study the distribution of structures in 2 dimensions the study of spatial distributions in 3 dimensions is less common. A disadvantage of studying spatial distributions in 3 dimensions has been the lack of 3D data rather than 2D plane images. With modern computer technology and microscopical techniques (e.g. confocal scanning microscopy) 3D data are now readily available. Adequate statistical technology is therefore required for 3D experimental design and data analysis. The 2 sample Student *t* test can be modified to allow for the average distance between dilute populations of test objects (their centres being regarded as points) first discovered by Hertz (in 1909) and reconfirmed by Underwood (*Quantitative Stereology*, *Addison-Wesley*, 1970), to be

$$\frac{0.554}{\sqrt[3]{N_v}}$$

where N_v is the mean empirical density per unit volume. The modified Student *t* test function T is given by the expression

$$T = \frac{\bar{x} - 0.554}{\sqrt{(s^2/n)}}$$

where $\bar{x}$, n and s^2 are the empirical mean, sample size and sample variance respectively. Prior to testing, all supporting assumptions must have been demonstrated to be valid by specific tests. Significant differences between empirical and infinitely large theoretical control populations indicate whether 3D populations of test objects have a contagious or hyperdispersed distribution. Large sample distributions can be studied using the analogue of the *z*-test.

P109 Relative specific labelling: a more efficient way of quantifying immunogold particles and testing for non-random distributions between intracellular compartments. By T. M. MAYHEW[1], J. M. LUCOCQ[2] and G. GRIFFITHS[3]. [1]*School of Biomedical Sciences, University of Nottingham;* [2]*Department of Anatomy and Physiology, University of Dundee, UK; and* [3]*European Molecular Biology Organisation, Heidelberg, Germany*

In immunoelectron microscopy gold particles are used to localise antigens in different intracellular compartments, and by counting those particles it is possible to quantify labelling distributions between compartments. Currently this usually involves estimating labelling densities (LD) which are calculated by referring gold counts to compartment volumes (golds/μm^3), profile areas (golds/μm^2) or trace lengths (golds/μm). Here we present a simpler and more efficient alternative for counting immunogold particles (in organelles or on membranes or filaments) and statistically evaluating labelling distributions. The statistical tests address 2 questions: (1) does the labelling pattern within a group (control or treated) conform to that expected for a random distribution? and (2) do patterns vary between groups (control versus treated)? The approach requires random sampling of cells, sections and compartments. In the case of organelle compartments, numbers of gold particles lying on identified compartments can be used to generate an *observed* frequency distribution. By randomly superimposing a lattice of test points on the same cell profiles the frequencies with which test points overlie the same compartments can be determined. Conveniently, this provides a corresponding *expected* distribution (i.e. that which would be found if gold labelling was random) because random points hit compartments on sections with probabilities determined by profile areas. A similar approach has been used to analyse electron microscopic autoradiographs (Williams, *Quantitative Methods in Biology*, North-Holland, 1977). By replacing test points with test lines and counting sites at which lines intersect different membrane or filament traces, analogous procedures provide observed and expected distributions of gold labelling for different categories of membrane or filament. If lattice constants are known, LD values can be calculated by dividing gold counts by point or intersection counts. However dividing observed by expected distributions provides indices of the relative specific labelling (observed golds/expected golds) of individual compartments without needing to know the lattice constants. Observed and expected distributions within a group can be compared using χ^2 analysis to test whether or not the observed distribution differs from random and, if it is nonrandom, to identify compartments which are preferentially labelled. Contingency table analysis can be used to compare directly the observed labelling distributions in different groups of cells. The main variations of the method, for organelle and membrane (or filament) compartments, will be presented and illustrated using specimen examples.

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P110 Pressure overload induced cardiac myocyte hypertrophy in Sprague Dawley rats. By K. A. LINEHAN, A.-M. L. SEYMOUR and P. E. WILLIAMS. *Department of Biological Sciences*, *University of Hull*, *UK*

Left ventricular hypertrophy is one of the most important risk factors associated with heart failure and sudden cardiac death. The aim of this study was to quantify both the magnitude and variation of myocyte hypertrophy in the left ventricle as a result of pressure overload. Adult male rats were maintained in accordance with Home Office guidelines. Cardiac hypertrophy was induced by constriction of the abdominal aorta interrenally, following anaesthesia with an intraperitoneal injection of a mixture of ketamine, medetomine and sterile water. Controls were subjected to the same procedure without the constriction of the abdominal aorta. Nine or 15 wk postsurgery, following an overdose of sodium pentobarbitone, hearts were removed and arrested in cardioplegic solution. The precise location of the equator of each heart was calculated and 12 μm cryostat sections were cut and stained. Myocyte diameter was assessed using the trace facility of the image analyser. Ten random cells in 9 specific regions of the cross section were measured. This method allowed the evaluation of the extent and variation of hypertrophy throughout the subendocardium of the left ventricle. Following 9 wk of banding, mean myocyte size significantly increased by 38% (experimental $244 \pm 12\ \mu m^2$ vs control $363 \pm 25\ \mu m^2$; mean $\pm$ S.E., $P < 0.05$). Within the cross section of subendocardium all myocytes did not hypertrophy to the same extent, with myocytes in the free wall of the left ventricle showing greater hypertrophy than those adjacent to the interventricular septum. It is possible that myocytes in the free wall are exposed to higher pressures during the cardiac cycle and thus hypertrophy to a greater extent as a result of pressure overload. Fifteen weeks postsurgery there was no further increase in mean myocyte size, despite the extended duration of pressure overload. This raises the possibility that the degree to which a myocyte can hypertrophy in response to a given pressure overload is limited.

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