Proceedings of the Anatomical Society of Great Britain and Ireland, the Nederlandse Anatomen Vereniging and the Anatomical Society of Southern Africa

A joint meeting of the Anatomical Society of Great Britain and Ireland, the Nederlandse Anatomen Vereniging and the Anatomical Society of Southern Africa was held from 15 to 17 April 1998 at Rolduc, Limburg, The Netherlands. It included symposia on 'Recent advances in human evolution research' on Wednesday 15 April and on 'Adaptation of cells and tissues to mechanical stimuli' on Thursday 16 April. The first EFEM Lecture was given by Professor J. Voogd of Erasmus University Rotterdam on 'Transformations and interactions of cerebellar maps' on Friday 17 April. The following are abstracts of communications and demonstrations presented at the meeting.

TALKS

1 Human evolution: species diversity and relationships. By B. A. Wood. *Department of Anthropology, George Washington University, USA.*

The combination of new fossil discoveries, improvements in the way that fossil evidence is analysed and relevant information from molecular biology have brought about what amounts to a revolution in our understanding of human evolutionary history. There is now widespread acknowledgement that the fossil record is a good deal more complex than was understood to be the case only a decade or so ago. There has been a steady and unrelenting trend to recognise more rather than fewer species. Coupled with the fact that the primate palaeontological record seems to consistently underestimate species diversity it is apparent that the 3 living African 'apes', *Gorilla*, *Pan* and *Homo*, represent a considerably impoverished sample of the diversity that existed in the Plio-Pleistocene.

The term 'hominin' refers to members of the tribe *Hominini*, the living members of which are *Homo sapiens* and the 2 or 3 species of *Pan*. Fossil hominin species are morphologically and adaptively diverse. They include creatures that are 'apelike' in many aspects of their postcranial skeleton and in their growth and development, others that have a specialised masticatory system with diminutive incisors and canines combined with much enlarged premolars and molars, and anatomically modern humans which share our own specialised locomotor system and dependence on culture.

Once it became apparent that hominin species were not simply 'time-successive' rungs on an evolutionary ladder, it was clear that methods needed to be developed to both recognise species in the fossil record and then establish the nature of the relationships between them. The simplest statement that can be made about the relationships between fossil hominin species is a cladistic one. This groups species together on the basis of whether they possess shared-derived characters. The method is predicated by two principles. The first is that morphology is an effective proxy for genetic propinquity, so that closely related species will share morphologies (called synapomorphies) that discriminate them from other groups of species. The second is that of parsimony, which states that evolutionary change (i.e. morphological modifications) should be minimised. Thus if 2 taxa share a morphological feature, or 'character', then it is assumed that they will have inherited it from a common ancestor. The alternative hypothesis, that characters evolved independently (i.e. that they are convergent), is evidently less parsimonious. Parsimony will support the branching pattern, or cladogram, that minimises the amount of convergent evolution. When these methods are applied to the hominin fossil record, there is substantial character conflict and this presentation will discuss the implications of this for our attempts to investigate human evolutionary history.

2 Recent developments in human locomotion and its evolution. By M. M. Gunther. *Department of Human Anatomy and Cell Biology, University of Liverpool, UK*

The origin of hominid bipedality still remains one of the most controversial issues in paleoanthropology. There are many different approaches in uncovering this important event in human evolution. Most investigations are based on comparative and functional analyses of the postcranial primate anatomy, theoretical biomechanics and energetics in bipedal walking, possible influences of paleo-environmental factors, and last but not least, detailed interpretations of fossil remains. However for the complete reconstruction of locomotor behaviour it is mandatory to know all physical parameters (i.e. length, mass, centre of gravity, moment of inertia) of each body segment. The fossil record of incomplete skeletal remains does not yield such data.

Since 1991 the 'Primate Evolution and Morphology Group' (PREMOG) in Liverpool has employed 2 basic methods to circumvent this problem. (1) The inductive approach is based on 3-D measurements of segment motion and external forces to analyse the kinematics (based on video film) and kinetics (based on force and pressure measuring systems) of voluntary bipedal gaits in humans and chimpanzees. *Pan troglodytes* was the first selected nonhuman primate species because of its taxonomically close relationship with *Homo sapiens* and its facultative bipedal walking mode. The results of the kinetic analyses alone show a species-typical force distribution. If however humans imitate the bipedal gait of a chimpanzee, then the crucial vertical force component does indeed resemble that of chimpanzees themselves. This suggests that the pattern of force distribution depends on the assumed walking posture. (2) The deductive approach on the other hand is based on computer simulations (ADAMS/ANDROID 'predictive dynamic modelling'). Applying the efficient human bipedal walking pattern (i.e. straight legs rather than bent-hip bent-knee bipedal posture) to the physical parameters of a chimpanzee results in a large inefficient increase in body

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rotation around the longitudinal axis. This leads to a further reduction of the effective stride length and an increase in energy expenditure.

Finally, by adapting these models progressively to represent the known or estimated measurements of the anatomy of the fossil species it was now possible to predict the mechanical consequences of various hypotheses of the fossil's behaviour. Thus for example, one alternative fossil model was equipped with chimpanzee-like mass distribution and then run through its joint motion typical of humans imitating chimpanzee bipedalism. A combination of all possible models showed that the reconstructed proportions of the fossil in question (the most complete postcranial 3.2 Myr early hominid skeleton, AL-288–1 'Lucy', *Australopithecus afarensis*) are incompatible with the kinematics of chimpanzee bipedalism. Although less efficient than modern humans, this fossil model is indeed capable of either erect or 'bent-hip, bent-knee' human gait. This unique methodology is now applied to a much more recent hominid (the remarkably complete 1.53 Myr juvenile skeleton KNM-WT 15 000, *Homo ergaster*) to shed light on the disputed reconstruction of its body size and trunk shape.

3 Evolution of the human hand. By M. W. Marzke (introduced by B. A. Wood), *Department of Anthropology*, *Arizona State University*, *Tempe*, *USA*.

Napier's classic study of fossil hand bones from Olduvai Gorge (*Nature* **196**, 1962) established interest among physical anthropologists in the relation of hand morphology to precision and power gripping capabilities and to stone tool making. His study combined 3 approaches: (1) comparison of living and fossil human and nonhuman primate hand morphology, (2) experimental manufacture of prehistoric tools and (3) observation of hand use in nonhuman primates. Since then studies of the evolution of the human hand have emphasised his first approach, and have been focused to a large extent on anatomical regions preserved in fossil hands. Metacarpals and phalanges found at Swartkrans have been examined for evidence of precision gripping capabilities, particularly in the thumb. *Australopithecus afarensis* hand fossils include well preserved carpometacarpal joint surfaces, which have been investigated for evidence of movement ranges consistent with human power and precision grip capabilities. Studies of hand remains from Sterkfontein and from several later neanderthal sites have concentrated on the thumb and on the finger metacarpals. Almost all studies have sought evidence for skeletal features associated with the modern human flexor pollicis longus muscle. In our investigation we are applying Napier's second and third approaches, as well as the first, to the identification and interpretation of differences among fossil and living hominids and nonhuman primates in hand morphology. Electromyographic analysis of muscle recruitment during stone tool manufacture by archaeologists has revealed the importance of forceful precision grips, which require a complex of features that have not yet been examined as thoroughly as those associated with fine precision manipulation and with strong power gripping of objects. This EMG analysis has also highlighted the importance of the fifth finger in both precision and power manipulation of prehistoric tools, as well as the major role of several intrinsic muscles in these behaviours. Comparisons of physiological cross sectional areas and moment arms of these muscles indicate that several of them have substantially larger torque potential in humans than in chimpanzees. Observations of hand grips and movements during manipulative behaviour in chimpanzees reveal behaviours in addition to tool making that may explain some human morphological features and are putting into relief the grips and associated morphology essential to tool making. The results of these studies, together with recent discoveries of extensive hand remains from Middle Pleistocene hominids, challenge anatomists to obtain and analyse quantitative 3-D data on bones and joints from all hand regions highlighted by the studies, and to develop models for use in more comprehensive and productive functional analyses of fossil hand morphology in the future.

4 Microstructure of enamel and dentine in fossil primates from East Africa. By M. C. Dean (introduced by B. A. Wood) *Department of Anatomy and Developmental Biology*, *University College London*, *UK*.

Experimental studies have concluded that daily incremental markings exist in both enamel and dentine. While some teeth begin to mineralise before birth other teeth are still mineralising around the time of skeletal maturity in primates. There is therefore potential for retrieving chronological information from histological sections of teeth about both enamel and dentine formation and about the sequence and period of dental development. Ground sections of fossil teeth attributed to the early Miocene hominoid *Proconsul*, from Rusinga Island, Kenya, reveal information about each of these. Surprisingly molar tooth enamel is thick in these hominoids and rates of cuspal enamel formation are fast (4 mm to 6 mm per day). Dentine formation on the other hand is slow (1 mm to 3 mm per day). Rates of enamel and dentine formation are not near equal, as they are in humans, and not therefore tightly linked to each other in this way among primates. A smaller dentine cap relative to body size may be part of the mechanism of thickening enamel over the cusps in some primates. The period of dental development in *Proconsul* was probably between 6 and 7 years, broadly equivalent to that of extant Old World monkeys, although the influence of body size on this is unclear. It may turn out that the earliest hominoids had periods of growth and development similar to Old World monkeys and that the earliest hominids (*Australopithecus* from the Plio-Pleistocene of East Africa) had periods of development similar to modern great apes. Evidence for prolongation of the growth period and of increased brain size, which may be interrelated, might not then be good ways of assessing the hominoid or hominid status of fossil remains.

5 New approaches for the analysis of morphological variation: applications to the study of primate cranial growth and evolution. By P. O'Higgins. *Department of Anatomy and Developmental Biology*, *University College London*, *UK*.

This review will outline some important new developments in the geometric analysis of variations in form. The methods are potentially applicable in any situation in which morphological differences and their correlates are of interest and

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so are likely to prove of value in disciplines as diverse as developmental biology, diagnostic imaging and evolutionary biology. In this presentation the methods are outlined in the context of studies of cranial evolution and growth in primates.

Differences between adult crania arise through the accumulation of differences in developmental patterning and growth during evolutionary divergence. In interpreting variations it is therefore important to address the mechanisms by which they arise. Such knowledge might be turned to the study of evolutionary adaptation and clarification of issues relating to phylogeny. Amongst primates a significant proportion of the differences in adult cranial form arise postnatally due to differences in patterns of growth (allometry). Statistical modelling of growth is therefore of potential benefit in understanding differences amongst adults.

Classically growth modelling is approached through the analysis of linear and angular measurements taken between defined anatomical landmarks. However for practical and statistical reasons such studies usually fail to enable ready visual interpretation of analytical results. These difficulties have lead in recent years to the development of a new class of approaches to morphological analysis based on the landmark coordinates themselves. These developments have taken their inspiration from the early work of D'Arcy Thompson (1917) in which differences between forms are expressed in terms of deformations of Cartesian grids. Although Thompson's ideas were first mooted in the early part of this century, it is only now that a general statistical and morphometric consensus has been reached concerning appropriate methods for the geometric analysis of variations in form using landmark data. Collectively these are known as the methods of geometric morphometrics since the geometry of objects is preserved throughout the analysis enabling ready visualisation of findings at every stage.

In this review the statistical properties of the shape spaces spanned by landmark coordinates will be considered in the context of cranial variation in primates. These studies will be used to illustrate appropriate approaches to the analysis and visualisation of changes in morphology due to growth and evolution and to indicate possible directions for future work.

6 Vertebrate evolution: Haeckel revisited. By M. K. Richardson[1], L. Selwood[2], G. V. Wright[3] and C. Pieau[4]. [1]*Department of Anatomy and Developmental Biology, St. George's Hospital Medical School, London, UK,* [2]*School of Zoology, La Trobe University, Australia,* [3]*Department of Anatomy and Physiology, Atlantic Veterinary College, University of Prince Edward Island, Canada and* [4]*Département Dynamique du Génome et Evolution, Institut Jacques Monod, Paris, France.*

Ernst Haeckel's embryo drawings are among the most famous images in biology. Originally intended to show the close affinities between man and animals, they have become the supreme statement on phenotypic divergence during development. Recent work supports claims made in Haeckel's lifetime that the drawings exaggerate similarities among early embryos of different species. Haeckel's defence was that he was trying to show the homologies in early embryos. But the problem goes deeper; as we report here, he made some serious mistakes in later stages too. Surprisingly, we also find that Gavin de Beer used artistic licence in his embryo drawings. Thus de Beer's 'dogfish' embryo has had structures added or removed to increase the resemblance to other species. Stylised embryo drawings are useful for showing the homologies among species. However they create uncertainty about the true nature of phenotypic divergence in development. We have therefore recreated Haeckel's drawings using real specimens from an extended range of species. They suggest that a crucial point about vertebrate evolution may have been overlooked: different developmental stages are targets for natural selection in different groups. Some vertebrate taxa (e.g. placental mammals) show evolution principally through the modification of late developmental stages, whereas others (e.g. amphibians and lepidosaurs) show important modifications to earlier, phylotypic, stages.

7 Postnatal development of the permanent tooth enamel organs in the horse. By M. Hornsveld. *Department of Veterinary Anatomy, University of Pretoria, South Africa.*

Although aspects of the enamel organs of the horse have been studied in detail, the gross anatomical features at different stages of development are inadequately described. Normal permanent tooth eruption follows a fixed sequence which is only completed some time after the animal reaches puberty. The enamel organs pass through various stages of development in the 7 mo (minimum) to 30 mo (maximum) preceding eruption of the permanent tooth. These stages include the development of the enamel organ from the regional gingiva, the 'bud', 'cap' and 'bell' phases as well as further developmental phases in which the organs' position changes. The developing enamel organs and the unerupted portions of the teeth lie in close proximity to important structures which run in the infraorbital and mandibular canals. The unerupted portions of the upper cheek teeth and/or their enamel organs may also lie adjacent to the rostral and caudal maxillary sinuses; in the young animal they largely occupy these sinuses. In both the upper and lower dental arches the spatial relationships between the proximal ends of the teeth and other osseous structures, e.g. the external layer of the overlying bone, are also clinically important. Recently developed techniques in equine dentistry favour percutaneous approaches for procedures involving the embedded portions of the reserve crowns and the true roots. In the case of the upper dental arch the approach is generally performed laterally, via the paranasal sinuses, while the teeth of the lower dental arch are approached either laterally or from the ventral margin of the mandible. In addition to a sound knowledge of the relevant surgical anatomy, a thorough understanding of enamel organ development is required before dental surgery and the interpretation of clinical and radiographic findings can be attempted; such an understanding also facilitates the comprehension of pathological conditions of the teeth and periodontal ligament, and other phenomena such as dental drift and continuous eruption. Heads for this study were obtained from 7 Thoroughbred cadavers, ranging in age from approximately 6 mo to approximately 4 y at death. After the soft tissues had been removed, the embedded portions of all the teeth, enamel organs and other relevant

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structures were exposed in situ by removing the overlying bone. Specimens were fixed by immersion in a 10% formalin solution and the gross anatomical features were recorded using digital photography.

8 Skin morphology and its role in thermoregulation in mole-rats, *Heterocephalus glaber* (Rüppell, 1842) and *Cryptomys hottentotus* (Lesson, 1826). By T. J. M. DALY[1] and R. BUFFENSTEIN[2]. *Departments of* [1]*Anatomical Sciences and* [2]*Physiology, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa.*

The skin structure of 2 Bathyergid rodents, the naked mole-rat (*Heterocephalus glaber*) and the common mole-rat (*Cryptomys hottentotus*) is compared, to investigate whether thermoregulatory differences may be attributed to different skin features. The skin was removed from the dorsal region of 5 adult male naked mole-rats (~ 30 g, 2-y-old) housed in the Central Animal Unit of the University of the Witwatersrand at room temperature (32 °C) and 2 field-caught male common mole-rats (~ 120 g, age unknown; Animal Ethics Clearance certificate no. 94-67-1). The tissue was fixed in 10% buffered formalin and routinely processed for light microscopy. In addition paraffin sections of skin were treated with the Masson-Fontana reducing method in order to demonstrate both formed melanin and melanin precursors. Control sections of naked mole-rat, common mole-rat and human skin were bleached with either potassium permanganate and oxalic acid or hydrogen peroxide and counterstained in neutral red. Pieces of skin tissue were also placed in 2.5% glutaraldehyde for 2 h, postfixed in 1% osmium tetroxide and routinely processed for transmission electron microscopy. Histological and ultrastructural studies of the dorsal skin of these closely related species showed morphological and structural similarities. However, differences in the degree of skin folding, thickness of the integument and dermal infrastructure were evident. The skin of the common mole-rat conforms with expected morphological/histological arrangements that are commonly found in mammalian skin. Many features of the skin of the naked mole-rat, such as the lack of an insulating layer and the loosely folded morphological arrangement, contribute to poikilothermic responses to changing temperatures of this mammal. Further evidence for poikilothermy in the naked mole-rat is indicated by the presence of pigment containing cells in the dermis, rather than the epidermis as commonly occurs in homeotherms. Lack of fur is compensated by a thicker epidermal layer and a marked reduction in sweat glands with none present in the dorsal region in naked mole-rats. Differences in skin morphology thus contribute substantially to the different thermoregulatory abilities of the 2 Bathyergids. The skin morphology contributes substantially to the poor thermoinsulatory ability of the animals whilst simultaneously facilitating heat transfer from the environment to the animal by thigmothermy and/or other behavioural means.

9 Incidence of Paneth cells in the small intestine of the mouse. By R. R. ETTARH. *Department of Human Anatomy and Physiology, University College, Dublin, Ireland*

Paneth cells are granule-containing cells found in the mucosal epithelium of the small intestine. These cells are usually located at the bases of the crypts of Lieberkuhn, and in histological sections are readily identified by their content of secretory granules positioned within the apical part of the cell. Although the function(s) of Paneth cells is not completely understood, profiles of these cells are present in a majority of crypt profiles in histological sections of the small intestine. Thus some crypt profiles do not show Paneth cells. Although it has been suggested that nearly all the crypts in the rat intestine may contain Paneth cells (Lopez-Lewellyn & Erlandsen, *Amer. J. Anatomy* **158**, 1980), there has been no study to show whether or not Paneth cells are present in every crypt. This investigation therefore examined Paneth cell occurrence in the small intestine of the mouse. Adult male CD-1 mice were killed by cervical dislocation and the small intestine removed, divided into 4 equal segments and samples taken at the midpoint of each segment. Samples were processed routinely for histology and up to 20 consecutive sections cut and stained. In each light microscopic section 20 crypt profiles were examined and scored for the presence of Paneth cells. Results show that the values for the percentage of crypt profiles which contain Paneth cells, the number of Paneth cell profiles per crypt profile and the minimum number of whole Paneth cells per crypt are all greater in distal than in proximal segments. Results also show that for each crypt Paneth cell profiles eventually appear within the crypt and it is therefore concluded that these cells are present in every crypt in the small intestine of the mouse.

10 Nuclear shaping during spermiogenesis in 2 nonpasserine birds, the ostrich (*Struthio camelus*) and sacred ibis (*Threskiornis aethiopicus*). By J. T. SOLEY. *Department of Anatomy, Faculty of Veterinary Science, University of Pretoria, South Africa.*

The process of chromatin condensation observed during spermiogenesis in birds is known to conform to the general pattern described in various vertebrate and invertebrate species. The change in nuclear shape which accompanies this process has been the subject of numerous studies and diverse opinions have been expressed regarding the mechanisms involved in effecting nuclear shaping. In this paper the process of nuclear morphogenesis in 2 non-passerine bird species, the ostrich and sacred ibis, is compared. Testis samples were obtained from 8 sexually mature ostriches slaughtered at the Oudtshoorn abattoir, South Africa. The material was immersion-fixed in 4% glutaraldehyde in Millonig's phosphate buffer for 24 h and routinely prepared for transmission electron microscopy (TEM) using standard techniques. The testes of 2 sexually mature sacred ibises which had been fixed whole in buffered formalin were cut into small blocks, fixed for an additional 24 h in 4% glutaraldehyde in Millonig's phosphate buffer and further processed for TEM as for the ostrich material. For comparative purposes spermatids were graded according to nuclear structure into 4 phases of development: (I) those with round nuclei, (II) those with irregular-shaped nuclei, (III) those with elongated nuclei containing coarse chromatin granules and (IV) those with elongated nuclei containing homogeneous, dense chromatin. Phase I spermatids of both species displayed similar nuclear characteristics. The relatively pale round nucleus revealed scattered marginal clumps of chromatin, intervening clear areas of

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flocculant material and a clearly defined region of fine granular material. Phase II ostrich spermatids displayed irregular shaped nuclei due to localised constriction of the nucleolemma by small groups of circularly arranged microtubules. The arrangement of the chromatin was generally similar to that of phase I spermatids. Phase II ibis spermatids contained round or pear shaped nuclei with a distinct band of moderately electron dense marginal chromatin. No microtubules were apparent in the perinuclear cytoplasm. Early phase III ostrich spermatids revealed an elongated nucleus filled with evenly dispersed fine granular material. The nuclear contents were observed to shrink away from the nucleolemma and to systematically aggregate by forming dense granules and moderately dense rods/filaments. Some rods appeared hollow. A well-developed circular manchette was present during all stages of this phase. Ibis spermatids, in contrast, displayed increased marginal accumulation of fine granular chromatin material, which resulted in the formation of an ever shrinking central nuclear cavity or series of cavities. The fine nuclear material was progressively transformed into coarse, round, dense granules which eventually became tightly packed. Manchette microtubules were absent. In both species phase IV spermatids possessed elongated nuclei filled with a homogeneous dense mass of condensed chromatin. In ostrich spermatids this phase was characterised by the presence of a longitudinal manchette. This structure was absent in ibis spermatids. Bearing in mind that different fixation techniques were employed, it would appear from these findings that the process of chromatin condensation and nuclear shaping differs significantly between the 2 bird species studied. The results also question the role of manchette microtubules in nuclear transformation in non-passerine birds.

11 Tendon excursion measurements with colour Doppler imaging: a calibration study on embalmed human specimens. By H. M. Buyruk, W. P. J. Holland, C. J. Snijders, J. S. Laméris, E. Hoorn, R. Stoeckart and H. J. Stam. *Institute of Rehabilitation, Central Instrumentation Department, Department of Biomedical Physics and Technology, Department of Radiology; Central Department of Automatization and Information and Department of Anatomy, Erasmus University Rotterdam, The Netherlands.*

Assessment of tendon excursion is important, however existing noninvasive methods such as excursion calculations from joint rotation angles and joint diameters are not precise. Recently the use of colour Doppler imaging (CDI) in the musculoskeletal system has been reported by our group. In our earlier studies CDI has been successfully used for imaging of tendon function and for measurement of tendon excursion velocity. As demonstrated by pilot studies, with CDI it is possible to obtain values for tendon excursion from the integration of continuous velocity measurements. The aim of the present study was to assess the applicability of tendon excursion measurement by CDI on human specimens, and to assess the correlation between values measured by Doppler and by a digital mechanical displacement meter. The digital flexor muscles of the forearm were separately connected to a mass of 1 kg with a steel wire running over a pulley. This weight moved the telescopic end of a digital displacement meter up and down during passive extension and flexion of the fingers. Excursion was measured with a pulsed multichannel CDI scanner on the same arm. Statistical analysis showed Doppler measurements to be 3% less than displacement meter measurements ($P < 0.001$) for both flexion and extension. In conclusion assessment of finger tendon excursion with CDI correlated well with the displacement meter, the latter being considered the most accurate method in cadaver studies.

12 Assessment of fluid status in knee injury using electrical impedance measurements. By C. L. Grieve and Y. Stone. *Department of Anatomy, University of Pretoria, South Africa.*

Clinical evaluation of the extent of inflammation and oedema of injured knees can be both subjective and difficult. This information may play an important role in the decisions taken with regard to injury management and treatment. A valid technique of assessing the extent of fluid retention within the injured tissue would provide this information. Bioelectrical impedance analysis (BIA) is said to be a simple, safe and noninvasive method of determining the volume of body fluid, on a total body or a local scale. BIA is based upon the principle that electrical conductivity of fat-free mass—including body fluid—is greater than the conductivity of body fat, which is generally anhydrous. The purpose of the present study was to determine whether local measurements of impedance have any value in indicating oedema in injured knees.

Electrical impedance measurements at 3 different AC frequencies (1 kHz, 50 kHz, 100 kHz) were performed on 12 volunteers with unilateral effusion-type knee injuries. All volunteers gave informed consent to participation in this study. Two impedance measurement planes were used, across the knee between lateral and medial femoral epicondyles, and longitudinally on the anterior surface. For the longitudinal measurement electrodes were placed 10 cm superior and 10 cm inferior to the midpatella point. To standardise this study, haemarthrosis injuries were not included. As a control identical impedance measurements were performed on the noninjured knee in each volunteer. The midpatella circumferences of both injured and control knees were also measured as a crude indication of the extent of oedema. To determine whether differences in impedance measurement were associated with knee oedema, and were not just a result of normal variation, 12 further uninjured volunteers were matched with the above group. In this group circumference and electrical impedance measurements were performed on both uninjured knees. Paired 2-tailed t tests were performed to assess any significance in difference between mean measurements ($P \leqslant 0.05$).

The circumference measurement of the injured knee was significantly greater than that of the uninjured knee, indicating a significant degree of oedema. On average the injured knee had a circumference 2.5 cm greater than the control knee: mean $\pm$ S.D. being 39.4 ± 5.0 cm and 37.0 ± 4.7 for the injured and uninjured knees respectively. For all electrical impedance measurements (1, 50, 100 kHz, longitudinal and cross sectional) the injured knee impedance was significantly lower (t test) and strongly correlated ($r = 0.58$

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to $r = 0.66$) to the control knee in the same person. In the uninjured volunteers, the circumferences of the 2 normal knees were not significantly different (mean $\pm$ S.D. being 37.8 ± 2.4 cm and 37.6 ± 2.4 cm for left and right knees respectively,). For the same electrical impedance measurements shown above, the knee impedance was not significantly different (t test) and was strongly correlated ($r = 0.57$ to $r = 0.81$) for left and right knees in the same person. The correlation coefficients were lower between cross sectional measurements in both the injured an uninjured groups, this possibly being due to a lack of standardisation for conductor length.

The results from this study show that electrical impedance measurements do indicate differences of oedema between injured and uninjured knees. A longitudinal rehabilitation study should be undertaken to see if these differences could be related to clinical and therapeutic findings.

13 Attitudes of medical and allied medical students to the teaching, learning and assessment of anatomy. By R. Asvat, K. Kuykendall and D. Manning. *Department of Anatomical Sciences, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa.*

The course in anatomy for medical and allied medical students (dental; physiotherapy, PT; occupational therapy, OT) taught in the Department of Anatomical Sciences, University of the Witwatersrand is currently placed in the second year of study. It consists of a comprehensive programme including 240 h of full body dissection, 100 h of practical histology and 120 h of lectures in gross anatomy, histology and embryology. The homeopathy and chiropractic course differs from that of the medical, dental and therapy course in that histology is taught in the first year and full body dissection is carried out in the second year of their curriculum. The present study forms part of an extensive investigation into the background and attitudes of students in medicine and other allied health disciplines in order to identify factors that may influence trends in academic perfomance. All students beginning the third year of study were invited to complete an anonymous questionnaire focusing on the previous year's anatomy course. Information was requested in 3 categories: (A) students background: age, gender, family, language and schooling; (B) study patterns: hours of study, recreation and class position; (C) anatomy course and career: 28 Likert scale value judgements covering course content (cognitive, value and behavioural domains), process (various teaching-learning strategies) and assessment. 237 questionnaires were returned in total: 128 medical, 20 dental, 25 occupational therapy, 25 physiotherapy (198 out of 450) and 39 from 80 homeopathy and chiropractic students. 37.2% of the respondents spent less than 10 h per week studying anatomy. 46.9% of medical, 50% of dental, 64% of OT, 80% of PT and 30.8% of homeopathy and chiropractic students felt that their results had not justified their effort. The volume of anatomy had hampered performance in other subjects for 54.7% of medical students, 60% of dental, 88% of OT, 72% of PT and 51.3% of homeopathy and chiropractic students. The extensive self-discipline required had been a problem for 33% of medical, 45% of dental, 28% of OT, 40% of PT and 18% of homeopathy and chiropractic students. While the majority of students understood the relevance of the material, 29% of medical, 40% of dental, 44% of OT, 44% of PT and only 8% of homeopathy and chiropractic students rated it as too difficult. Dissection was rated as a useful way of learning by 52% of medical, 40% of dental, 72% of OT, 68% of PT and 95% of homeopathy and chiropractic student as opposed to lectures (medical 11%, dental 20%, OT 16%, PT 16%, homeopathy and chiropractic students 21%). The practical 'spotter' tests were rated as most useful by homeopathy and chiropractic students (85%) followed by the PT (76%), medical (65%), OT (56%) and dental (55%). Written essay tests were rated most useful once again by the homeopathy and chiropractic students (87%) followed by dental (70%), OT (56%), medical (48%) and PT (48%) students. This study has helped to highlight areas for attention in curriculum development such as improved usage of lecture time. The breakdown of the anatomy programme over a period of 2 y, as for the homeopathy and chiropractic students, seems to have a positive effect on the attitudes of these students with regard to anatomy.

14 Cadaver profiles at University of Stellenbosch and University of Cape Town Medical Schools. By B. C. J. Labuschagne[1] and C. P. Slater[2]. [1]*Department of Anatomy and Histology, University of Stellenbosch and* [2]*Department of Anatomy and Cell Biology, University of Cape Town, South Africa.*

Limited information is available on the characteristics of cadavers at medical schools world wide. To contribute to this research data were compiled on the characteristics of cadaver donors at 2 medical schools located in Cape Town situated approximately 20 km apart at the University of Stellenbosch (US) and the University of Cape Town (UCT). The objective of the study was to identify characteristics of donors (population group, gender, age at death, cause of death), establish profile changes, compare the data at the 2 medical schools, relate certain characteristics with that of the regional and general population, and to analyse bequest data. The period covered in the survey 1956–1996 for US and 1911–1996 for UCT, and includes information on 1698 (US) and 4277 cadavers (UCT). Applications for cadaver donations are essentially limited to the Cape Town metropolitan area. Donors to both programmes were predominantly coloured (62.6% at US, 48.0% at UCT). Whites contributed 22.1% (US) and 40.0% (UCT), and blacks 15.3% (US) and 12.0% (UCT). A considerable increase in donors from the white population group occurred during the latter part of the programme at both US and UCT. Donors to both programs were predominantly male (68.4% at US, 68.2% at UCT) with the highest female to male ratio in the white population group (1:1.4 at US, 1:1.6 at UCT) and the lowest female to male ratio in the black population group (1:4.9 at US, 1:5.9 at UCT). An increase in the percentage of female donors also occurred during the latter parts of both donation programmes. The mean age at death for donors from the coloured population group was 51.2 y (US) and 50.6 y (UCT), for the black population group 51.0 y (US) and 49.4 y (UCT), and for white donors 69.9 years (US) and 71.6 y (UCT). The disease

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profile at the US donor programme revealed the following: vascular disease (heart disease plus cerebrovascular disease) the major cause of death among the white donor group (48.3% deaths); cancer the leading cause of death in the coloured (24.3%) and black donor groups (28.3%); cervical cancer the leading cause of cancer deaths in coloured (31.5%) and black female donors (30.8%); breast cancer the leading cause of cancer deaths in white female donors (22.9%); pulmonary tuberculosis contributed to the death of 13.0% coloured donors and 13.9% black donors compared to 0.5% for white donors. Death due to cerebrovascular disease and pneumonia had the highest average age at death in all 3 population groups while infectious disease (tuberculosis, septicaemia) or conditions associated with a high mortality (pancreatitis, diabetes, epilepsy) had low average ages at death. The cadaver profiles at both medical schools, despite the fact that certain criteria exclude acceptance into the donor programme, reflect the health status of the different population groups in South Africa. The profiles of the coloured and black groups reflect that of disadvantaged population groups (a high prevalence of infectious disease, relatively young populations), while the white profile is that of a privileged population group (a high prevalence of degenerative disease, ageing population).

15 An unusual case of aberrant ureter in a male patient. By G. J. Louw[1], J. H. Naudé[2] and D. MacGillivray[2]. [1]*Department of Anatomy and Cell Biology*, [2]*Department of Urology*, *University of Cape Town*, *South Africa*.

A 42-y-old fertile male presented with a painful mass in his right loin. He suffered from unilateral cryptorchidism (right). A laparotomy was performed and a 240 g mass (105×55 mm) was excised from the right pararenal region. The tumour was identified histopathologically as a seminoma with high mitotic activity. Tumour markers were elevated in the patient and it was suspected that non-seminomatous elements were present: he was referred to a State hospital for further investigation. Computerised tomographic scans revealed a further mass in the right pararenal region (80×64 mm) and enlarged external iliac lymph nodes. Chemotherapy was initiated and continued for 4 mo, after which the mass halved in size, the lymph nodes were less enlarged, and the tumour markers returned to normal levels. The tumour was excised again at laparotomy and at this time an aberrant right ureter was observed in the patient, passing *posterior* to the common iliac vessels. Histopathology of the tumour indicated that it was no longer viable.

Between 4 and 6 w of development in the embryo, the mesonephric duct branches to form the ureteric bud, which grows dorsally and then cranially, pushing into the metanephric mass of intermediate mesoderm, after which time further development of the renal pelvis and calyces follows. The common iliac artery on each side of the body develops from a stem connecting the umbilical artery, originally a ventral branch of the dorsal aorta, to the dorsal intersegmental branches. The posterior cardinal veins unite caudally and form the external and internal iliac veins.

The metanephros originates at the upper sacral segments, receiving its blood supply from this level. It then undergoes a relative change in position, the 'ascent' of the kidney, from its original pelvic to its final lumbar position (between 5 and 8 weeks of development). The 'ascent' is probably due to the rapid longitudinal growth of lumbar and sacral segments of the body wall, and a reduction in the lumbar flexion of the embryo.

A possible explanation for the unusual route followed by the ureter in this patient is that during the 'ascent' of the kidney, the developing common iliac vessels moved anteriorly over the elongating ureteric stem.

16 Roux revisited. By B. Hillen. *Department of Functional Anatomy*, *Rudolf Magnus Institute for Neurosciences*, *Utrecht*, *The Netherlands*.

In 1895, well over 100 y ago, Willem Roux published his collected work on the 'Developmental mechanics of organisms' in 2 volumes. Volume 1 is largely dedicated to 'Functional Adaptation', a condensation of his investigations into the causes of size and shape of organs and tissues, postulating the influence of the functional demand on the shaping of organs and tissues. In his classical work he discusses blood vessels, muscles and bone. This work has been a source of inspiration for many investigators over the years. Over the past 2 decades there has been a significant reappraisal of these hypotheses and concepts. New tools have been brought in from various disciplines to study these phenomena in more detail in the search for causal relationships. Contributions from the field of computer modelling have enhanced our insight into the mechanics of complicated shapes; molecular biology methods have demonstrated that mechanical stimuli can initiate cascades of messages within and between cells; and modern imaging techniques offer the possibility to study changes of shape of living organisms in great detail.

Roux's contributions to the vascular system focused on the spatial distribution: he 'dissected' the vascular tree with ruler and compass, looking for the regularities in the design. His explanation of the observations was largely founded on haemodynamic forces interacting with the vessel wall. Building on Roux's observations and hypothesis Thoma (1893) formulated his histomechanical laws where both flow and pressure play an active role in the moulding of the vascular tree. In 1926 Murray applied the principles of optimal design to the vascular system and concluded that in an optimal vascular tree the wall shear stress is uniform throughout the network. Since then many investigators have established that wall shear stress is fairly constant in various vascular beds and various species. In a theoretical analysis it emerges that if the wall shear stress per se were to be the regulating factor, all collateral pathways in the vascular tree would be eliminated. Thus the existence of well known collateral systems (palmar arches, circle of Willis, mesenteric arcades) seem to contradict the regulating role of wall shear stress, even though in these vessels the wall shear stress seems to be fairly constant. Recent experiments in our group have shown that arterial remodelling is a thresholded response. This threshold mechanism provides a plausible explanation for the existence of collaterals in a system that is regulated by a uniform wall shear stress.

If we consider the wall shear stress distribution on a smaller scale, for instance at branching sites, arterial

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confluences or bends, it becomes clear that the local geometry influences the local flow patterns. Flow velocity profiles become distorted by the change of direction and/or change of cross-sectional area of the vessels. These distortions imply that at this scale wall shear stress will never be uniformly distributed along the vessel wall. This may introduce local growth (positive or negative) of the vessel wall, resulting in local adaptation. The most intriguing finding is however the location of early atherosclerotic lesions in areas where shear stress is low, while at the same time the vessel wall opposite the lesion can exhibit compensatory growth due to the increased wall shear stress.

In conclusion, the mechanical effects of the flow on the vessel wall are confusing. The molecular biology of the vessel wall may well provide clues to the underlying mechanisms.

17 Release of vasoactive substances from vascular endothelial cells. By G. Burnstock. *Autonomic Neuroscience Institute, Royal Free Hospital Medical School and University College London, UK*

Local mechanisms of control of vascular tone will be described, with particular emphasis on the release of vasoactive substances such as ATP, substance P, acetylcholine and vasopressin from endothelial cells during shear stress produced by changes in blood flow. A role for ATP released from epithelial cells lining tubes such as ureter and sacs such as urinary bladder in mechanosensory transduction will be considered and also the possibility that mechanically induced release of ATP from odontoblasts is associated with tooth pain.

18 Regulatory mechanisms for adaptive remodelling. By R. Huiskes. *Orthopaedic Research Laboratory, University of Nijmegen, The Netherlands.*

In 1892 Wolff described his ideas about the capacity of bone to adapt its mass and structure to mechanical requirements imposed by functional loading. These became known as 'Wolff's Law' of bone remodelling. Although supposedly essential for understanding the biological behaviour of bone as a result of orthopaedic treatment, the 'Law' has never been more than an anecdotal series of observations, useless for quantitative predictions. Recent developments of new theories and computer methods however have changed that.

Strain-adaptive bone-remodelling theory is based on the hypotheses of Roux (1881), who described bone remodelling as a biological control process, governed by local bone deformations and enacted by bone cells. The theory proposes a mathematical description of this process, quantitatively relating local internal loading variables to resorption and formation of bone mass. In combination with advanced computer methods of stress analysis, suitable for evaluation of local internal loading variables in irregular structures as a result of external loads, shape and constitutive characteristics of its material, the theory can be used to build computer simulation models. These can be used to analyse bone remodelling behaviour in growth and as a result of orthopaedic treatments.

In the presentation, development and validation of theories and simulation models will be discussed. It will be shown that these tools are useful in the search for the 'design rationale' of bone structure and shape. Applications emphasised are studies of bone resorption and formation patterns around orthopaedic implants such as artificial joint replacements, to predict their long term effects as a result of implant design, surgical and patient factors.

19 Characteristics of a synovial joint. By R. V. Putz, F. Eckstein, S. Milz and M. Müller-Gerbl (introduced by B. Hillen). *Anatomische Anstalt München, Ludwig-Maximilians-Universität, Germany.*

The shape of bones and synovial joints is one of the most miraculous and intriguing phenomena in nature. One of the reasons for this is that we do not really understand yet the principles that determine their structure and the interaction between the genome and functional influences (the mechanical forces acting on the synovial joints) in the expression of phenotype.

One approach to the understanding of biological shapes is to try to correlate different parameters. Although this way is risky in terms of showing causality, it seems to be an appropriate method for estimating the relevance of data as a basis for various approaches. Starting from morphology it appears possible to create hypotheses which can be investigated experimentally or theoretically in a model.

Therefore during the last few years we have investigated 4 aspects of the larger joints: (1) the distribution of thickness and mineralisation of the subchondral bone plate; (2) the distribution of thickness of hyaline cartilage; (3) the geometry of larger joints in terms of their congruity/incongruity; and additionally (4) we investigated the contact areas in some joints under increasing load.

The thickness of the subchondral plate measured in contact radiographs in specimens is distributed generally corresponding to the mineralisation assessed by CT-OAM. Some distribution patterns are recognisable in different joints that change with age. The pressure distribution in some larger joints, e.g. the knee joint, seems to be reflected in the distribution of the mineralisation. Changes take place with alterations of the joint reaction force (e.g. genu varum correction osteotomy). The thickness of hyaline cartilage can be measured elegantly by MRI in the living subject using special gradient echo sequences that allow for a precise delineation between the cartilage and the mineralisation zone. In specimens A-scan ultrasound can be used as a nondestructive method. The distribution of cartilage does not correspond with the mineralisation pattern in each joint. Furthermore there are significant differences between the distribution patterns in the upper and in the lower limb concerning the area distribution of the cartilage. The geometry of the larger joints determines the particular kind of pressure transmission between the two parts of a joint. Convex as well as concave incongruity influence largely the contact areas where pressure is transmitted. It is clearly shown in the humero-ulnar joint that there is a correspondence of contact areas with an appropriate regulation of local bone density that also can be predicted in a finite element model.

Finally, the subarticular trabecular bone shows a characteristic orientation, supporting the load transmission role of some larger joints. Reinforcing plates can be demonstrated in these parts of some joints which are subjected to bending stress (e.g. posterior margin of glenoid

cavity). We conclude that the distribution patterns of some characteristic elements of the larger joints depend on local mechanical influences.

20 The expression of autocrine and systemic growth factors by muscle in response to stretch and overload. By G. GOLDSPINK. *Department of Anatomy and Developmental Biology. Royal Free Hospital School of Medicine, University of London, UK*

The study of the underlying mechanisms via which cells respond to mechanical stimuli, i.e. the link between the mechanical stimulus and gene expression, represent a new and important area in the morphological sciences. Several cell types ('mechanocytes'), e.g. osteoblasts and fibroblasts as well as smooth, cardiac and skeletal muscle cells, are activated by mechanical strain and there is now mounting evidence that this involves the cytoskeleton. Muscle offers one of the best examples of studying this type of mechanotransduction as the mechanical activity generated by and imposed upon muscle tissue can be accurately controlled and measured in both in vitro and in vivo systems. Muscle is highly responsive to changes in functional demands. Overload leads to hypertrophy whilst decreased load, force generation and immobilisation with the muscle in the shortened position leads to atrophy. For instance it has been shown that stretch is an important mechanical signal for the addition of new sarcomeres in series and in the production of more actin and myosin filaments. This is preceded by upregulation of transcription of the appropriate genes some of which, e.g. the myosin isoforms, change the muscle phenotype (Goldspink et al. *Amer. J. Physiol.* **262**, 1992; Yang et al. *J. Anat.* **190**, 1997).

The switch in the expression of myosin heavy chain genes which encode different molecular motors will be discussed in relation to the physical signals that induce fibre type transition. As far as increase in mass is concerned, our group have cloned the cDNA of a splice variant of IGF-1 that is produced by active muscle and appears to be the factor that controls *local* tissue repair, maintenance and remodelling. From its sequence it can be seen that it is derived from the IGF-1 gene by alternative splicing but it has different exons to the liver isoforms (Yang et al. *J. Muscle Res. Cell Motil.* **17**, 1996). It has a 52 base insert in the E domain which alters the reading frame of the 3′ end. Therefore this splice variant of IGF-1 is likely to bind to a different binding protein, e.g. BP5 which only exists in the interstitial tissue spaces of muscle, neuronal tissue and bone. This would be expected to localise its action as it would be unstable in the unbound form which is important as its production would not disturb unduly the glucose homeostasis. This new growth factor has been called mechano growth factor (MGF) to distinguish it from the liver IGFs which have a systemic mode of action. Although the liver is usually thought of as the source of circulating IGF-l, it has recently been shown that during exercise skeletal muscle not only produces much of the circulating IGF-l but active musculature also utilises most of the IGF-l produced (Brahm et al. *Calcified Tissue* **60**, 1997). Indeed we have cloned both autocrine and endocrine IGF-1s (Yang et al. *J. Muscle Res. Cell Motil.* 17, 1996) both of which are upregulated in cardiac as well as skeletal muscle when subjected to overload. It has been shown that in contrast to normal muscle MGF is not detectable in dystrophic mdx muscles even when subjected to stretch and stretch combined with electrical stimulation. This is true for muscular dystrophies that are due to the lack of dystrophin (sex-linked) and due to a laminin deficiency (autosomal), thus indicating that the dystrophin cytoskeletal complex may be involved in the mechanotransduction mechanism. When this complex is defective the necessary systemic as well as autocrine IGF-1 growth factors required for local repair are not produced and the ensuing cell death results in progressive loss of muscle mass. Supported by Action Research UK.

21 Fibrocartilage in tendons and ligaments. By M. BENJAMIN and J. R. RALPHS. *Anatomy Unit, School of Molecular and Medical Biosciences, University of Wales Cardiff, UK*

Typically, tendons and ligaments are dominated by collagen fibres and have a relatively small number of fibroblasts scattered throughout the extracellular matrix. Although they are often considered to be simple rope-like structures of uniform composition along their length, their structure is highly specialised at certain sites where they are subject to compression and/or shear. Thus the entheses of most tendons and ligaments (i.e. their junction with the bone) and the regions where they change direction by wrapping around pulleys or passing beneath retinacula are fibrocartilaginous rather than fibrous. Enthesis fibrocartilage is characteristic of tendons and ligaments that attach to epiphyses and apophyses and helps to create a gradual change in mechanical properties at the bony interface and thus reduce stress concentration. It is significant that ruptures rarely occur at such sites. The enthesis fibrocartilage initially develops from the cartilage of the bone anlagen, but is later replaced by fibrocartilage that develops by tendon/ligament metaplasia—probably in response to mechanical load. Fibrocartilage in 'wrap-around' tendons protects them from compressive and shearing forces where they change the direction of muscle pull, e.g. around the malleoli of the ankle. It can be restricted to the epitenon or endotenon or be present throughout the tendon fascicles. At either fibrocartilage site the tendons or ligaments have an extracellular matrix that contains molecules typical of hyaline cartilage—e.g. type II collagen and aggrecan. In the human Achilles tendon at least, the mRNA for aggrecan can be readily demonstrated in the tendon fibrocartilages but not in the mid tendon. Equally the message for versican (a large proteoglycan typical of fibrous tissues) is characteristic of purely fibrous, midtendon—but not tendon fibrocartilage. In addition to the pronounced differences in the extracellular matrix there are also marked differences in cell shape and cell-cell communication between fibrous and fibrocartilaginous regions. These also may relate to the response of tendons and ligaments to mechanical load.

22 Cellular mechanisms of Wolff's law of bone adaptations. By J. KLEIN-NULEND. *Department of Oral Cell Biology, ACTA-Vrije Universiteit, Amsterdam, The Netherlands.*

Bone cells, in particular osteocytes, are extremely sensitive to mechanical stress, a quality that is probably linked to the process of mechanical adaptation (Wolff's Law). It is currently believed that this regulatory process is governed

by osteocytes and bone lining cells. Mechanical stress produces flow of interstitial fluid in the bone lacunar-canalicular network along the surface of osteocytes and lining cells, which is probably the physiological signal for bone cell adaptive responses in vivo. Osteocytes have been shown to be particularly sensitive to fluid flow and less sensitive to hydrostatic compression (Klein-Nulend et al. *FASEB J.* **9**, 1995). This observation fits the theory developed by Cowin and associates that, rather than direct cell strain, the flow of fluid through the osteocyte canaliculi resulting from mechanical strain is the physical stimulus that activates the osteocytes (Cowin et al. *J. Biomech. Engineer*. **113**, 1991).

The response of bone cells in culture to fluid flow includes prostaglandin synthesis (Klein-Nulend et al. *J. Cell Physiol.* **168**, 1996) and expression of inducible prostaglandin G/H synthase (PGHS-2 or inducible cyclooxygenase, COX-2; Klein-Nulend et al. *J. Bone Miner. Res.* **12**, 1997), an enzyme that mediates the induction of bone formation by mechanical loading in vivo (Forwood, *J. Bone Miner. Res.* **11**, 1996). In addition the response of osteocytes to fluid flow includes a rapid production of nitric oxide (Klein-Nulend et al. *Biochem. Biophys. Res. Commun.* 217, 1995) and expression of endothelial nitric oxide synthase (Klein-Nulend et al. *J. Bone Miner. Res.* **12**, 1997). Nitric oxide has been shown to mediate the mechanical effects in bone, leading to enhanced prostaglandin E_2 release (Klein-Nulend et al. *Biochem. Biophys. Res. Commun.* **217**, 1995). Disruption of the actin cytoskeleton abolishes the prostaglandin response to stress in osteocytes, suggesting that the cytoskeleton is involved in cellular mechanotransduction. Experiments using various specific blockers acting at various cellular structures possibly involved in the the induction of prostaglandin production (phospholipase A_2, phospholipase C, protein kinase C, stretch-activated channels, intracellular Ca^{2+}) show that fluid flow raises Ca^{2+} by an enhanced entry through stretch-activated ion channels in combination with Ca^{2+} and inositol trisphosphate (the product of phospholipase C) induced Ca^{2+} release from intracellular stores. Ca^{2+} and protein kinase C then stimulate phospholipase A_2 activity, arachidonic acid production, and ultimately prostaglandin release.

The present data support the hypothesis that stress on bone causes fluid flow in the lacunar-canalicular system, which stimulates osteocytes to produce prostaglandins that induce an osteogenic response.

23 Micro-density and macro-training in Thoroughbred racehorse cannon bone. By A. Boyde[1], C. M. Riggs[2], and S. J. Jones[1]. [1]*Department of Anatomy, University College London and* [2]*Department of Veterinary Clinical Studies and Animal Husbandry, University of Liverpool, UK*

The typical course of the distal regions of common distal condylar fractures of equine third metacarpal bones can be explained by anisotropic structural features. In the present study we examined changes induced by 'training'. Diaphyseal and distal parts of cannon bones (McIIIs) were obtained from a controlled experiment at Bristol (by courtesy of A. E. Goodship and colleagues) in which 6 pairs of 2-y-old unbroken Thoroughbred fillies were subjected to strongly contrasting exercise routines over 19 wk: either high intensity exercise including treadmill work, or walking. Digital radiographs of 2–5 mm thick sawn slices were recorded before embedding them in PMMA. The blocks were trimmed, micromilled, studied by confocal fluorescence scanning laser microscopy (CFSLM) to map very low dose calcein growth labels, then carbon coated and studied by backscattered electron microscopy and image analysis (BSE-SEM). We analysed the microdensity of the bone tissue proper using quantitative backscattered electron imaging (QBSE). We mapped the bone volume fraction (BVF) in 2.7 mm square fields and the relative degree of mineralisation within cubic micrometre volumes in a 21 μm square grid pattern for entire 60 mm distal-end block surfaces of the McIIIs.

Many prior investigators have concluded that microdamage somehow constitutes a signal, recognised in the chain of events in intercellular communication between bone cells, that leads to osteoclastic resorption of the impaired bone and its eventual replacement by new bone. Albeit rarely, we were able to find microdamage, and even at great distances from the articular surface in cancellous bone in 'trained' horses, but the repair responses were initiated as rapidly formed lamellar bone (bandaging) without prior osteoclastic resorption. Nearby more abundant repair tissue was less mature in type (microcallus). The densification (increased BVF) of distal cancellous bone in the trained animals also involved the initial formation of lamellar bone over resting surfaces accompanied by immature bone in the larger compartments of prior 'marrow' space formed as strands and sheets with more highly mineralised centres. Addition of bone to cancellous surfaces was also found along the potential condylar fracture propagation track within the parallel-sagittal-plate zone as a biological response to exercise. The stiffness and brittleness of bone tissue depend upon the extent to which the water space in the collagenous matrix is replaced with bone mineral. The more loaded zones, e.g. the palmar subchondral regions in the condyles, have a higher BVF but a lower level of mineralisation—implying that the more solid bone fabric is, at least initially, made of a more compliant material.

Documenting differences in mineralised articular cartilage may help us to understand variability in both fracture incidence and joint problems. Critical events leading to a prospective stress fracture occur within the thin layer of calcified cartilage. The very large field QBSE tissue density surveys showed substantial regional variations both in the thickness and the degree of mineralisation of the mineralised layer of the articular cartilage. This thickens by extension of its mineralising front into the cartilage, but is locally thinned from the bone side by osteoclastic resorption. Focal excess resorption at the bone:cartilage interface would lead to weakening, such that an overload event occurring synchronously would easily lead to extension of a microcrack into the subchondral bone. Weakening by temporally and spatially prodigious resorption would be expected to be a major problem in acute disuse.

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24 Form and function—a three-dimensional finite element analysis of the scapula,. By T. C. LEE[1,2], D. LACROIX[2], L. A. MURPHY[2] and P. J. PRENDERGAST[2]. [1]*Department of Anatomy, Royal College of Surgeons in Ireland and* [2]*Department of Mechanical Engineering, Trinity College, Dublin, Ireland.*

Mechanical forces play an important role in shaping tissues as shown by evolutionary progression and individual adaptation. The finite element method has been extensively used to analyse musculoskeletal elements because of its ability to simulate with complex geometries and material properties. In this study we have applied the method to identify the deformations and stress distributions in the scapula when the arm is abducted to 90°. An embalmed cadaveric scapula was immersed in water and CT scanned in the sagittal and coronal planes. The images were used to create a 3D brick and wedge finite element mesh using the finite element program MARC. A Hounsfield number was retrieved from each 2D pixel to calculate bone density and hence Young's modulus of elasticity using standard equations. Muscle attachments, orientation and forces at 90° abduction were derived from van der Helm's work (*J. Biomech.* **27**,1994). Experimental strain measurements were taken using rosette gauges mounted on a scapula loaded in an Instron testing machine; these were sufficiently close to the finite element model predictions to confirm the model's validity. Finite element analysis predicted that most of the bone in the scapula was under low stress (< 4 MPa) but that both compressive and tensile stresses were maximal at the spine and along the lateral border, in the range 20–24 MPa which are comparable in magnitude to those in the proximal femur. Under the loading configuration in this study, the lateral border was in tension on its dorsal surface and in compression on its costal surface indicating a bending deformation. Hence the scapula may be analysed as an 'I beam' with the spine and lateral border functioning as divergent flanges to support the glenohumeral load. Between these 2 flanges lies the infraspinous fossa with stresses of less than 4 MPa corresponding to the web of the beam. The morphologies of the thick cortical bone of the spine and lateral border and the thin translucent infraspinous fossa reflect these functional stresses.

25 Flexor digitorum superficialis tendon compression mechanism induces compression differentiated areas in flexor digitorum profundus of the rat. By J-B JAQUET[1], E. T. WALBEEHM[2], R. A. BROWN[1], S. E. R. HOVIUS[2] and D. A. MCGROUTHER[1] (introduced by Chr. VERMEIJ-KEERS). [1]*Department of Plastic and Reconstructive Surgery, University College of London, UK,* [2]*Department of Plastic and Reconstructive Surgery, Erasmus University Rotterdam, The Netherlands.*

Anatomically the digital flexor tendons have a complex interrelationship in which the flexor digitorum superficialis (FDS) seems to encircle flexor digitorum profundus (FDP) like a Chinese finger trap. By proximal loading, the tunnel formed by the 2 slips of FDS narrows causing a compression force by FDS on the FDP. The ultrastructure of FDP in this compression zone has been examined by scanning and transmission electron microscopy and compared with tendon matrix proximally and distally. By examining whole tendon segments in rats, it was possible to visualise the entire fibre arrangement across the tendon. For this study ex-breeder Sprague Dawley rats, were euthanised 'lege artis'. Sixty pairs of rat flexor tendons were used to examine anatomical, ultrastructural and histological features. The flexor tendons of the second, third and fourth digits of the left and right forelimb paw were approached through separate midlateral incisions. The synovial sheaths and pulleys were incised longitudinally at the lateral aspect, to expose the flexor tendons. The flexor tendons were incised transversely proximal to Camper's chiasm and at their insertion.

By SEM well-defined FDP compression related indentations were found. A compression related ring was distinguishable starting proximally, dorsal to FDS bifurca, and ending distally, volar to Camper's chiasm. At the FDS bifurca FDP collagen fibres also start to spiral parallel to the two FDS slips. Frequency analyses of FDP collagen fibril diameters on TEM, pointed out a change from a normal biphasic (50–100 nm and 190–240 nm) fibril distribution to a single peak in fibril diameter (60–70 nm) in the tendon compression area. The TEM views also showed a combination of longitudinal and transverse cut collagen fibrils. Furthermore the compressed areas were stained specifically by Masson trichrome light green dye, suggesting a greater proteoglycan content associated with chondroid changes. Chondrocyte like cells were not detectable in the FDS-FDP interaction area. Between pressure and tension areas an intermediate transition zone was perceptible, showing a downward shift of the large collagen fibril diameter area (130–200 nm). Just distal to Camper's chiasm the morphology of the FDP showed a typical tendinous aspect again, in spite of the continuing spiralling of FDP collagen fibres.

The results suggest fibrocartilaginous specialisation of FDP, caused by the FDS tendon compression mechanism. Zone II tendon repair is not only influenced by longitudinal forces, but also by FDS tendon compression forces. The fibrocartilage-differentiated ring around FDP may be a particularly vulnerable zone after flexor tendon repairs in zone II. It is likely that the different ultrastructural areas shown here have different healing potentials.

26 Altered blood flow patterns in chick embryos result in cardiac congenital anomalies. By B. HOGERS, M. L. A. BROEKHUIZEN, M. C. DERUITER, A. C. GITTENBERGER-DE GROOT and R. E. POELMANN. *Department of Anatomy and Embryology, Leiden University Medical Center, The Netherlands.*

Heart malformations are the most common congenital defects among newborns and are often life threatening. There is a need for extended knowledge of the developmental mechanisms during normal and abnormal heart development to diagnose and treat these malformations. The effect of placental blood flow on human heart development was studied by developing an embryonic chicken model in which blood flow patterns were marked with Indian ink injections, and the effect of blood flow alterations on normal heart development was studied.

Abnormal intracardiac blood flow results in serious intracardiac and pharyngeal arch artery malformations com-

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parable to defects observed in embryonic chicken models by interfering with other developmental mechanisms, like neural crest ablation, cervical flexure experiments, and excessive retinoic acid treatment. We tried to find similar pathways of pathogenesis by extensive morphological investigation after prevention of venous return, using SEM, immunohistochemistry, and haemodynamic measurements. Chick embryo yolk sac veins were permanently clipped in ovo and evaluated during the looping stages (18–24) and at postseptation stages (34–45).

We postulate that shear stress induced decreases in expression of endothelin-1 and TGFβ2,3 and the receptor TGFβRII in the early stages, resulting in impairment of the epithelial/mesenchymal transformation necessary for proper AV and outflow cushion development. This in turn resulted in a lack of myocardialisation of the outflow tract septum. Impaired cardiac looping, due among other things to hypotrophy of the compact layer of the myocardium, prevented alignment of the cardiac septa in the inner curvature of the heart and resulted in ventricular septal defects and a double outlet to the right ventricle. Abnormal cardiac morphology altered haemodynamics and induced pharyngeal arch artery malformations at later stages.

A pulsed Doppler velocity meter and servo-null system was used to determine haemodynamic parameters (blood flow velocity and arterial pressure) at stages 24 and 34 in clipped embryos and normal controls. Only a decrease in peak acceleration was found at stage 24, which correlates well with the small AV cushions and thin myocardium found morphologically. At stage 34 peak systolic and mean velocities, peak systolic and mean blood flow (and thus stroke volume) were increased, while heart rate was reduced. This indicates that the heart is able to compensate its function, despite (or at the expense of) cardiac malformations. Another possibility is that the embryolethality usually observed after stage 24 is exclusively determined by those embryos with decreased cardiac function, leaving the minor malformations to survive until stage 34.

27 Relationship between Sonic Hedgehog (Shh) and Bone morphogenetic protein 2 (Bmp-2) in patterning the chick embryonic limb. By G. Drossopoulou[1], A. P. McMahon[2] and C. Tickle[1] (introduced by J. P. Bennett). [1]*Department of Anatomy and Developmental Biology, University College London, UK and* [2]*Department of Cellular and Molecular Biology, Harvard University, Cambridge, USA.*

How cells and tissues become arranged in the vertebrate body plan during embryonic development is a fundamental problem. A good model is the establishment of pattern along the anteroposterior axis of the chick embryonic limb. Transcripts of *sonic hedgehog* (Shh), the vertebrate homologue of the fruitfly *hedgehog* gene, map to the polarising region, a small group of mesenchymal cells at the posterior of the limb bud. When the polarising region is grafted at the anterior margin of a second bud, this can induce digit duplications. Other molecules involved in the signalling cascade include BMP-2 and also genes that belong to the Hox-d complex. Beads soaked in Shh protein can induce digit duplications in the chick embryonic wing in a dose dependent manner. Digit duplication occurs in a 2 step process: in the first 14 h tissue appears to be primed, and in the next 10 h additional duplicate digits are specified in an anterior to posterior sequence with additional anterior digits being specified first and then promoted. There is no induction of Bmp-2 and Hoxd-13 in the priming phase but by 16 hours, at the start of the promotion phase, Bmp-2 and Hoxd-13 are expressed anteriorly. Tissue next to Shh beads has no polarising activity suggesting that any downstream molecules require continuous exposure to Shh to exert their effects. Bmps make good candidates for the downstream signal. One possible way in which Shh and Bmps cooperate is by Shh maintaining Bmp-2 expression. When an Shh bead is removed 16 h after application, at the start of the promotion phase, mesenchymal expression of Bmp-2 is lost. However when the implanted beads are removed after 24 h, at the end of the promotion phase, then the ectopic Bmp-2 domain is maintained. These results show that the constant presence of Shh during the promotion phase is needed in order to establish a stable Bmp-2 domain. Induction of a full duplicated digit pattern correlates with irreversible activation of Bmp-2 expression. This same time requirement for irreversible induction of Bmp-2 expression by Shh is seen when mesenchymal tissue adjacent to an Shh bead is grafted to a new host. Mesenchyme cells only stably express Bmp-2 when transplanted after a 24 h exposure to Shh. These results raise the possibility that the function of Shh is to maintain Bmp-2 expression during the promotion phase. However applications of beads soaked in Bmp-2, or cells expressing Bmp-2, do not duplicate the limb. This suggests that Bmps may only be able to act after Shh priming. To test this we implanted beads soaked in Shh, removed them 16 h later, and replaced them with beads soaked in different concentrations of Bmp-2. Control experiments were also performed in which the Shh bead was removed at 16 h and no Bmp-2 was added. Addition of Bmp-2 seems to enhance polarisation indicating that sequential signalling by Shh and then Bmp-2 may pattern the digits.

28 Horizontal eye movement representation in the flocculus; retrograde transneuronal tracing with rabies virus. By N. M. Gerrits[1], W. M. Graf[2], N. Yatim[2] and G. Ugolini[3]. [1]*Department of Anatomy, Erasmus University Rotterdam, The Netherlands,* [2]*Laboratoire de Physiologie de la Perception et de l'Action, CNRS, College de France, Paris, France and* [3]*Laboratoire de Génétique des Virus CNRS, Gif-sur-Yvette, France*

Retrograde transneuronal tracing with rabies virus (CVS strain) was used to visualise the neuronal circuitry involved in horizontal eye movement control. After unilateral injection (1 µl) into the medial rectus eye muscle (MR) of guinea pigs, viral transfer was studied immunohistochemically at 6 h intervals from 1.5 to 5 d post inoculation.

After sequential labelling of MR motoneurons (1.5 d) and premotoneurons in the vestibular nuclei (2.5 d), labeled Purkinje cells (PC) appeared at 3.5 d in the ipsilateral flocculus (FL) in a single band that ran diagonally from caudomedial to rostrolateral in an intermediate position. This band corresponds to the so-called 'horizontal zone'. In some cases labelling continued laterally across the posterolateral fissure into the ventral paraflocculus. At longer survival times (between 3.5 and 5 d), the initial band became slightly broader and additional separate bands of labelled

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PCs appeared in the rostromedial and caudolateral FL. At these times an intermediately positioned band also appeared in the contralateral FL that mirrored the one initially labelled in the ipsilateral FL. The time difference in the appearance of these bands reflects a similar time difference in labelling in ipsi- versus contralateral magnocellular medial vestibular nucleus neurons. After 4 d the empty areas adjacent to the 'horizontal zone' became filled with labeled PCs that were at first scattered, but at 5 d covered basically all areas. This increase suggests involvement of PCs belonging to the 'vertical zones' in horizontal eye movement circuits via neurons in the vestibular or deep cerebellar nuclei.

29 The cavernous sinus nerve plexus in humans. By R. L. A. W. Bleys, L. M. Janssen and G. J. Groen. *Department of Functional Anatomy, Rudolf Magnus Institute for Neurosciences, Utrecht University, The Netherlands.*

Neural pathways innervating intracranial structures, such as cerebral arteries and dura mater, come together in the cavernous sinus (CS) and surroundings. Here the internal carotid artery, several cranial nerves, and the pterygopalatine ganglion are in close proximity, which makes it likely that that these structures are interconnected. In comparison with gross anatomical aspects the autonomic nerve plexus and neural connectivity in the human CS has been less extensively investigated. To determine the extent of neural connections in the human CS and surroundings 7 specimens were stained as whole-mount preparations by a sensitive acetylcholinesterase method. The CS was dissected by a combined medial, superior and lateral approach. In addition 1 specimen was cryosectioned in a frontal plane (section thickness 25 μm) and also stained for acetylcholinesterase. The cavernous sinus nerve plexus was located mainly around the abducens nerve and medial to the ophthalmic nerve. We named this part of the plexus the cavernous plexus proper (CPP). The CPP had a lateral extension just underneath the outermost (dural) layer of the lateral CS wall, lateral to the trochlear and ophthalmic nerves. Nerves running between the oculomotor, trochlear, and ophthalmic nerves connected the CPP to the lateral extension. Small ganglia were found in the CPP around the abducens nerve at the points of confluence of several nerve fibre bundles. Additional sectioning of the ganglia demonstrated neural cell bodies. The CPP was connected to the large branches of the internal carotid nerve, the pterygopalatine ganglion and the trigeminal ganglion. From both the CPP and the lateral extension nerve branches ran along the oculomotor, trochlear, ophthalmic and abducens nerves into the orbit. Furthermore the CPP had multiple connections to the nerves around the internal carotid artery that continued towards the cerebral arteries. The numerous connections of the cavernous plexus with functionally defined neural structures strongly suggest a mixed constitution of the plexus, i.e. consisting of sympathetic, parasympathetic and sensory nerves. It is also likely that the cavernous nerve plexus distributes nerve fibres to the orbit and to the cerebral arteries. The complexity of the neural organisation in this area aids in explaining symptoms of pain syndromes such as cluster headache.

30 Annexin V transcription and annexin V distribution during mammalian development and its association with apoptotic cell death, By S. M. Van Den Eijnde[1], H. Heus[2], L. Boshart[2], C. I. de Zeeuw[3], C. P. M. Reutelingsperger[4] and Chr. Vermeij-Keers[1,3]. [1]*Institute of Plastic and Reconstructive Surgery,* [2]*Department of Clinical Genetics and* [3]*Department of Anatomy, Erasmus University Rotterdam, and* [4]*Department of Biochemistry, Cardiovascular Research Institute, Limburg State University, The Netherlands.*

The members of the phylogenetically old annexin family are functionally characterised by their calcium and phospholipid binding capacity. Of this protein family annexin V is the member with the highest affinity for phosphatidylserine (PS). In adult specimens annexin V is detected in blood, amniotic fluid and seminal plasma as well as in a variety of tissues where it mainly localises in cells that are involved in exo- and endocytotic processes, or that have a barrier function between tissues and blood or extracellular fluid. In vitro annexin V has shown to be a potent blood clotting inhibitor, and in vivo it appears to inhibit interleukin-1β-induced pyresis. Hence the physiological role of annexin V is sought in processes like haemostasis and inflammation.

Recently we have demonstrated by perfusing viable specimens with annexin V that the distribution of PS across the 2 layers of the plasma membrane is tightly regulated in vivo (van den Eijnde et al. *Cell Death Different*. **4**, 1997). In developing mammals, birds and insects PS is exposed at the outer leaflet of the plasma membrane of apoptotic cells, whereas viable cells hide this aminophospholipid in the plasma membrane leaflet facing the cytosol (van den Eijnde et al. *Apoptosis* **3**, 1998). The ubiquitous nature of PS exposure by apoptotic cells suggests an important physiological role for this plasma membrane alteration in vivo. Triggering of rapid internalisation of apoptotic cells by phagocytes is such a role suggested in the literature.

The specificity by which the exogenous annexin V appears to bind to apoptotic cells in vivo lead us to the hypothesis that the externalised PS molecules, in addition to triggering phagocytosis, may also influence the immunological inertness of this phagocytic activity by binding endogenous annexin V. Testing this hypothesis however starts with establishing whether and to what extent endogenous annexin V correlates as strictly with apoptosis as we have found for the administered exogenous annexin V. First results from in situ hybridisation experiments for annexin V mRNA in whole mount mouse embryos and immunohistology on sectioned mouse and human embryos indeed points in this direction.

31 Holoprosencephaly: the neurectoderm predicts the face. By Chr. Vermeij-Keers, W. Zuidervaart, J. M. Vaandrager and J. C. van der Meulen. *Institute of Plastic Surgery, Erasmus University and Craniofacial Centre, University Hospital Rotterdam, The Netherlands.*

In the literature various classifications concerning holoprosencephaly, i.e. failure of proper cleavage of the prosencephalon with a deficit in the midline facial development, are available. The most cited classification (DeMeyer &

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Zeman, *Confin.Neurol.* **23**, 1963) discriminates alobar (arhinencephaly), semilobar and lobar holoprosencephalies. The lobar form has been subdivided into: (A) with midline continuity of frontal neocortex, and (B) with complete separation of the neocortex across the midline, and agenesis, a- or hypoplasia of the rhinencephalon and/or corpus callosum.

During normal embryogenesis of the prosencephalic neurectoderm including the eyes, the following developmental sequence occurs: telencephalic hemispheres (5–6 mm crown-rump length, CRL), rhinencephalon (11 mm CRL) and corpus callosum (54 mm CRL). As a consequence, holoprosencephaly: (I) agenesis of both eyes and orbits, (II) cyclopia (a single eye or closely approximated eyes, with all integrades, in a single orbit) and (III) nonsynostotic hypotelorism can be defined as early or primary dysplasias of the cerebrocraniofacial group ($\leqslant$ 17 mm CRL).

In all holoprosencepalic cases under study (dissections of human fetuses, n = 13; clinical reports of neonates; CT-scans; autopsies, n = 4; and dry human fetal cyclopic skulls, n = 2) the skull midline structures, such as both premaxillae, nasal septum, and ethmoid were agenetic (n = 1), a- or hypoplastic (n = 18). Moreover the brain features exhibited agenesis of the interhemispheric fissure, i.e. complete form of holoprosencephaly (n = 5), and agenesis of the anterior interhemispheric fissure, i.e. incomplete form of holoprosencephaly (n = 12).

Embryologically the area between the primordia of both eyes and the nasal placodes is agenetic, a- or hypoplastic, as a result of insufficient outgrowth of the prosencephalic neurectoderm and midline mesectoderm in presomite developmental stages. Hence from an embryological point of view, the term lobar holoprosencephaly is a contradiction in terms.

32 Transmission routes of prion disease. By C. J. G. Wensing. *Institute of Animal Science and Health, Lelystad, The Netherlands*

In the mid eighties a new spongiform encephalopathy in cattle was discovered in the United Kingdom. So far more than 170000 clinical cases of this new disease have been diagnosed, causing a detrimental impact on the European cattle industry and great concern about possible effects on human health. Transmissible spongiform encephalopathies (TSEs) have been known for a long time and the diseases best known were scrapie in sheep, Creutzfeld-Jacob disease and Kuru in man and wasting disease in mink.

TSEs or prion diseases are believed to be caused by unconventional transmissible agents which are extremely resistant to thermal and other physical treatment. About the nature of the agents 3 hypotheses have been postulated: the virus, virino and prion hypotheses, of which the last has gained wide acceptance in recent years.

A pivotal role has been assigned to the prion protein (PrP) in the pathogenesis of these diseases. This prion protein causes the conversion of the normal isoform of the prion protein into the pathological form. The accumulation of the abnormal protein causes vacuolation (spongiform changes) of the grey matter neuropil leading to neuronal degeneration in several nuclei of the caudal part of the medulla.

Infection of BSE takes place by oral intake of concentrate feed of animal origin (meat and bone meal). Infection via the gastrointestinal tract is likely to be also the route of scrapie infection in sheep. The new CJD variant in man is also most likely caused by oral infection. Experimental infection by scarification, injection or implantation has also been demonstrated.

Attention will be given to the route of the infectious agent from the gastrointestinal tract to the central nervous system and some species differences in this process.

33 Neuroanatomical evaluation of the innervation of neuroepithelial bodies in cat lung using neuronal tracing and denervation experiments. By I. Brouns, D. Adriaensen, J.-P. Timmermans and D. W. Scheuermann. *Laboratory of Cell Biology and Histology, University of Antwerp, Belgium.*

Neuroepithelial bodies (NEBs) in the airway epithelium are well-organised innervated groups of neuroendocrine cells. Electron microscopically, NEBs in cat lungs are supplied by nerve fibres that appear to loop through the corpuscle forming morphological afferent and efferent endings. Although not supported by conclusive neuroanatomical evidence, these nerve fibres are thought to derive from the nodose ganglion and to constitute the major nerve supply of NEBs. No data are available on the transmitter content of these nerve endings. This study aimed to elucidate the origin and chemical coding of the innervation of cat NEBs. The red-fluorescent tracer DiI was injected under surgical anaesthesia into the left nodose ganglion of 7-wk-old cats by means of a glass microelectrode. After allowing 3–5 wk for neuronal transport of the tracer to the nerve endings in peripheral organs, the animals were killed with an overdose of anaesthetic and the lungs were fixed and preserved in 4% paraformaldehyde. In addition, lungs and nodose ganglia of control (10–12 wk old) and of unilaterally infra- or supranodosally vagotomised cats were examined. All tissues were freeze-sectioned, immunostained for calcitonin gene-related peptide (CGRP), substance P (SP), vasoactive intestinal polypeptide (VIP), nitric oxide synthase (NOS), neuron specific enolase (NSE) and neuropeptide Y (NPY), and studied using confocal microscopy. Other sections were processed for NADPH diaphorase enzyme cytochemistry which appeared to label the ultrastructurally identified nerve terminals. The NADPH diaphorase staining appeared to be unaffected by vagotomy. In the traced animals some NSE-labelled NEBs in the ipsilateral lung received a DiI-traced nerve ending that showed an extensive arborisation at the basal pole of the NEBs, apparently not penetrating intraepithelially. At all airway levels in the controls a proportion of the NEBs received an extensive varicose CGRP/SP innervation basally. After infranodosal vagotomy this innervation, as well as the CGRP/SP fibres present in the bronchial epithelium and ganglia, largely disappeared from the ipsilateral lung but remained in the contralateral lung. No changes were noted in the NEB innervation after supranodosal vagotomy. In all specimens, the nodose ganglion was shown to contain a large number of CGRP/SP-immunoreactive neurons. All bronchi showed abundant SP-, VIP/(NOS)- and NPY-immunoreactive fibres, respectively, among others in close apposition to

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NEBs. In conclusion a subpopulation of cat NEBs is innervated basally by a population of CGRP/SP-containing sensory fibres originating in the vagal nodose ganglion, in addition to intracorpuscular endings the chemical coding and origin of which remain obscure. Supported by NFWO grant G.0302.95 to J.-P. T.

34 The effect of retinoic acid on the proportion of insulin cells in the developing chick pancreas. By B. Kramer and C. Penny. *Department of Anatomical Sciences, University of the Witwatersrand, Johannesburg, South Africa.*

Our overall objective is to identify factors important for the proliferation and differentiation of insulin cells. Retinoids are known to influence the maintenance and differentiation of many target tissues, but their effect on the proliferation of endocrine cells of the pancreas is not known. The aim of this experiment was thus to define the action of retinoic acid on the proportion of insulin cells of the developing chick pancreas. The endodermal component (including interstitial mesenchyme) of the dorsal pancreatic bud of 5-d chick embryos was transplanted onto Matrigel. Retinoic acid (10^{-6} M) was added to our standard serum-free medium, Ham's F12 containing insulin (5 μg/ml), transferrin (5 μg/ml) and selenium (10^{-10} M) (F12.ITS). Control grafts for this experiment were cultured in F12.ITS. After 7 d explants were freeze dried, vapour fixed and embedded in resin. 1 μm sections taken at intervals throughout the explants were subjected to immunocytochemistry to reveal insulin and glucagon containing cells. The number of insulin containing cells were expressed as a proportion of insulin plus glucagon cells. The proportion of insulin cells in explants in F12.ITS was 8.77 % (n = 7). When retinoic acid was added to this medium the proportion was 22.36 % (n = 7). Hence adding retinoic acid to the medium increased the proportion of insulin cells significantly ($P < 0.0001$).

35 Neuropeptides and neurotransmitters of the Islets of Langerhans of the Houbara bustard (*Chlamidotis undulata mcqueenii*). By E. P. K. Mensah-Brown, T. A. Bailey, P. A. Lawrence, D. J. Pallot and A. Garner. *Departments of Anatomy and Pharmacology, Faculty of Medicine and Health Sciences, UAE University, UAE.*

The innervation of the islet of Langerhans of injured and euthanised Houbara bustards (*Chlamydotis undulata mcqueenii*) by the neuropeptides cholecystokinin-8 (CCK-8), Galanin (GAL), neuropeptide Y (NPY) met-enkephalin (Met-EN), neurotensin (NT), vasoactive intestinal polypeptide (VIP), and neurotransmitter markers nitric oxide synthase (nNOS), the synthesising enzyme for nitric oxide, and tyrosine hydrolase (TH), the enzyme for synthesising noradrenaline, was studied by immunohistochemistry. Fatally injured captive-bred Houbara bustard held in captivity were euthanised and pancreases placed in Zamboni's fixative solution overnight. Specimens were routinely embedded in paraffin wax and sections immunostained by the indirect PAP- DAB method. Immunoreactivity to Met-EN, NPY and was discernible in neurons both around the periphery and centre of the islets. VIP was detectable in peripherally located islet cells and also in varicose nerve terminals situated at the periphery of the islet. Galanin was detectable in varicose nerve terminals mainly on the periphery but also in the central portion of islets. CCK-8 immunoreactivity was not observed within the pancreatic islet. Immunoreactivity to nNOS was discernible in both islet cells and neurons situated at the periphery and in nerve fibres some of which revealed varicose terminations. Tyrosine hydrolase immunoreactivity was discernible mainly in the walls of blood vessels around the islets but the occasional TH immunopositive neuron was observed within the islet. The roles of neuropeptides and neurotransmitters in the function of the avian pancreas is discussed.

36 Transformations and interrelations of cerebellar maps. By J. Voogd. *Department of Anatomy, Erasmus University Rotterdam, The Netherlands.*

Maps are used to summarise the often complex topography of elements in laminar structures. Cerebellar maps should account for the 3 major stages in its morphology. Early stages cover the development of the cerebellum from the ventricular neuroepithelium, through a process of determination, migration and settlement of its cells, into a more or less independent tissue mass. During intermediate stages the cerebellar cortex acquires its typical 3-layered structure, afferent, efferent and intrinsic connections develop and the transverse fissures which characterise the third, adult, stage appear. The transformations and the interrelations of these maps will be discussed, focusing on the poorly understood issues of the establishment of the lattice structure of parallel fibers and Purkinje cell dendrites, the change from the early multiple climbing fibre innervation of the Purkinje cells into the adult 1:1 relationship and the role of the ingrowing mossy fibres in the patterning, the fissuration and the plasticity of the cerebellar cortex.

POSTERS

P 1 The influence of muscular activity on local mineralisation patterns in fetal mouse metatarsals. By E. Tanck[1,2], L. Blankevoort[1], A. Haaijman[2], E. H. Burger[2] and R. Huiskes[1]. [1]*Orthopedic Research Laboratory, University of Nijmegen and* [2]*Department of Oral Cell Biology, Vrije Universiteit of Amsterdam, The Netherlands.*

In this study, we examined the theory that local mechanical loading may influence local development of fetal long bones. The influence of muscular activity on local mineralisation patterns in fetal cartilaginous metatarsals of the mouse was investigated. Information about the effects of muscle forces on mineralisation patterns in the embryo may be important for the prevention and treatment of musculoskeletal developmental deformities. For this purpose the mineralisation shapes of in vivo calcified metatarsals, i.e. calcification in the presence of muscle forces, were compared to the shapes of unloaded in vitro calcified metatarsals. A biphasic finite element (FE) analysis was performed to calculate the local distributions of mechanical variables at the mineralisation front during muscle loading. The results

showed that the mineralisation front in vivo was nearly straight, whereas in vitro it acquired a more or less convex shape. This was due to a slower mineralisation rate at the periphery of the mineralised cylinder compared to the centre. From the FE analysis it appeared that the distribution of fluid pressure could not explain this difference. The most likely candidate to explain the difference was the distortional strain, resulting from muscle contraction in vivo which is absent in vitro, as its value at the periphery was significantly higher than in the centre of the tissue. We hypothesise that without external loads the mineralisation process may be considered as a preprogrammed process, starting at the centre of the tissue and resulting in a spherical mineralisation front. In vivo, strain modulates the rate of the mineralisation process, resulting in the straight mineralisation front. These results suggest that disturbances in muscle development are likely to produce disturbed mineralisation patterns resulting in a disordered osteogenic process.

P 2 Distal femoral bone resorption after total knee replacement can be predicted by computer simulations. By G. H. van Lenthe, M. C. de Waal Malefijt and R. Huiskes. *Orthopaedic Research Laboratory University of Nijmegen, The Netherlands.*

A long-term threat to the durability of joint replacements is periprosthetic bone resorption. This will weaken the bone and promote fixation failure. It has been shown that the phenomenon of bone resorption around total hip replacement can be predicted with finite element computer simulation models, based on Wolff's Law of bone remodelling. As a result new prosthetic designs can be tested for long-term endurance, before animal or human experiments are started. It is a common finding now that total knee replacements promote bone resorption as well, just as do total hip replacements. The objective of this study was to test the hypothesis that the remodelling patterns found after total knee replacement can be predicted by the same computer simulation method.

A finite element model based on CT scans was constructed of an average femur with an implanted knee prosthesis. The long-term prediction of the apparent bone density was based on Wolff's Law. In the theory used bone cells are assumed to react to local deviations in strain energy density. The amount of bone remodelling was determined by calculating the gradual changes in bone mineral content from simulated DEXA-scans.

The remodelling simulations predicted dramatic bone loss in the distal femur. It was predicted that the maximum drop in bone mineral content was 94%, occurring in the most anterior distal part of the femur. In the posterior part of the condyles bone loss was much less severe, with a maximum net bone loss of 27%.

Comparison of the results with those from clinical data revealed that similar resorption patterns are found. It is concluded that long-term bone loss under the femoral knee component may very well be predicted by this computer model, suggesting that for the introduction of new knee prostheses animal experiments can be replaced, or at least preselected, and thereby limited. This work was sponsored by the Dutch Alternatives to Animal Experiments Platform.

P 3 In vivo analysis of trabecular bone architecture. By W. J. Niessen, A. M. Lopez, W. J. van Enk, K. Nicolay, P. M. van Roermund, B. M. ter Haar Romeny and M. A. Viergever. *Image Sciences Institute, Utrecht University Hospital, Utrecht, The Netherlands.*

Trabecular morphology has structural trends which are strongly correlated with physical function. In vivo analysis of the trabecular pattern has the potential to predict and treat malgrowth of bone owing to altered loading conditions in an early phase. Using multiscale texture analysis we determine the orientation trend of the trabecular network from high resolution CT and MR images. First studies show that the obtained orientations in healthy individuals agree with histomorphometric studies.

As a second step the study of trabecular morphology can be used to estimate bone strength. Several studies have already found strong correlations between the (anisotropic) mechanical properties of bone and the (anisotropic) trabecular network within the bone. It is our aim to test the developed techniques by comparing results with simulated and actual mechanical tests on specimens. The eventual goal is to be able to determine the risk of fracture directly from clinical imaging.

P 4 The influence of the perivascular innervation on wall shear stress induced arterial remodelling. By K. S. Han, G. Pasterkamp and B. Hillen. *Department of Functional Anatomy, Utrecht University, Utrecht, The Netherlands.*

The arterial diameter alters in response to changes in blood flow in order to restore local wall shear stress (WSS) to baseline values. This calibre response to changes in WSS occurs in both the acute stage by temporary dilation or constriction and in the chronic stage by adaptive remodelling.

While the autonomic nervous system (ANS) has a role in keeping the arterial tone balanced, the long term effect of the ANS on flow induced arterial remodelling remains undefined. The aim of the present study was to investigate the influence of denervation on WSS induced remodelling.

Under general anaesthesia a segment of the canine mesentery containing 3 adjacent principal arteries (PA 1, PA 2 and PA 3) was exposed. The blood flow and radius of these PAs were measured. We studied 3 groups. In group 1 (10 beagles), denervation of PAs 1 and 3 was achieved by local phenol application. In group 2 (7 beagles), flow increase in PA 2 was achieved by occlusion of PAs 1 and 3. In group 3 (5 beagles), flow increase as in group 2 was achieved and in addition phenol was applied on PA 2. Immediately after intervention, 4 wk after intervention and 8 wk after intervention, the blood flow and the radius of the PAs in the segment were measured and WSS were calculated.

The innervation of the phenol treated PAs had almost disappeared at 8 wk (group 1 and 3). The arterial wall of these vessels did not differ from non phenol treated arteries. In group 1, no changes in radius or flow were observed at any time points. In group 2, all parameters increased significantly at all time points compared to pre-intervention values. The WSS returned to baseline values at 8 wk. Group

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3 however, showed no arterial remodelling at 8 wk despite the increased values of flow and WSS at all time points. We conclude that the ANS has no effects on arterial flow and calibre in normal mesenteric PAs. However the results of the experiments indicate that an intact ANS is a prerequisite for normal WSS induced arterial remodelling in the mesenteric arteries.

	Flow ml/min	WSS N/m^2	Radius mm
Group 1 (n = 10)			
Pre-intervention	4.9 ± 1.4	1.57 ± 1.18	0.75 ± 0.19
Phenol	4.1 ± 1.2*	1.21 ± 0.68	0.75 ± 0.16
4 wk	5.0 ± 1.6	1.53 ± 0.99	0.73 ± 0.15
8 wk	4.4 ± 1.4	1.50 ± 0.88	0.79 ± 0.15
Group 2 (n = 7)			
Pre-intervention	4.8 ± 2.8	1.50 ± 0.45	0.67 ± 0.15
Occlusion	12.0 ± 7.2*	2.83 ± 1.19*	0.76 ± 0.16*
4 wk	13.5 ± 6.5*	2.98 ± 1.34*	0.78 ± 0.13*
8 wk	12.9 ± 4.0	2.13 ± 0.68	0.86 ± 0.14*
Group 3 (n = 5)			
Pre-intervention	4.1 ± 2.5	1.49 ± 0.82	0.65 ± 0.13
Occlusion/phenol	10.0 ± 4.2*	2.44 ± 1.31*	0.77 ± 0.15
4 wk	12.3 ± 7.4*	4.61 ± 2.57*	0.62 ± 0.06
8 wk	14.4 ± 10.4*	6.23 ± 5.13*	0.65 ± 0.14

Data expressed as mean ± S.D.; * $P < 0.05$ compared to pre-intervention.

P 5 Zinc sulphate-induced anosmia influences nerve densities of the basal cerebral arteries in rats. By M. W. Mundt, R. L. A. W. Bleys, J. W. de Groot and B. Hillen. *Department of Functional Anatomy, Rudolf Magnus Institute for Neurosciences, Utrecht University, The Netherlands.*

Detailed quantitative determination of nerve densities in the arteries at the base of the brain in rats and humans have demonstrated a topographical heterogeneity of nerve densities. The pattern of heterogeneity is not only specific for species but also for ageing and disease in humans and rats (Bleys et al. *J. Cereb. Blood Flow Metab.*, **16**, 1996). In our opinion these density differences reflect local neuronal control of segments of the basal cerebral arteries; high nerve densities may be necessary in regions where great flow fluctuations occur. For example, in rats the anterior cerebral artery which supplies the rhinencephalon is densely innervated, in humans the posterior cerebral artery which supplies the visual cortex receives an abundant nerve supply. We hypothesise that local patterns of innervation in cerebral arteries are influenced by changes in flow and/or pressure fluctuations and especially changes in the amplitude of flow fluctuations. According to our hypothesis anosmia would result in a reduction of flow fluctuations in vessels supplying the rhinencephalon as the consequence of a reduction of nerve densities in these arteries. For the present study a chemical anosmia model was made suitable for application in rats to investigate one direction of the presumed reciprocal relationship between flow fluctuations and nerve densities: the regulation of nerve densities by haemodynamic factors. A pilot experiment was carried out in 6 male Wistar rats. In 3 rats peripheral anosmia was induced by repeated intranasal application of 10% zinc sulphate, while the rats were anaesthetised by 0.01 ml Hypnorm per 100 g body weight. The animals were kept in an anosmic state for 8 wk. Three rats served as controls and received no treatment. Subsequently the rats were perfusion fixed (anaesthetised by 0.1 ml Nembutal per 100 g body weight) and whole-mount preparations of the basal cerebral arteries were immunohistochemically stained for the general neuronal marker PGP 9.5. The nerve densities of 16 arterial segments were quantified by image analysis and expressed as percentage area of the vessel wall. Statistical analysis demonstrated no left-right differences. For this reason left and right arteries combined in further analysis. It was found that nerve densities in anosmic animals were significantly lower for the internal ethmoidal artery (13.2 ± 1.2 versus 17.5 ± 0.6, mean ± S.D.) and the segment of the anterior cerebral artery between the origin of the internal ethmoidal artery and the fusion of left and right anterior cerebral arteries (15.7 ± 1.3 versus 17.8 ± 0.4), and significantly higher for the internal carotid artery (16.6 ± 0.6 versus 14.1 ± 1.5). Because the anterior cerebral and internal ethmoidal arteries supply the rhinencephalon these data support the presumed relationship between haemodynamic factors and nerve densities. The increase of nerve density in the internal carotid artery is unclear. Statistical significance obtained in small groups points to a relatively strong effect and warrants further investigation to the cascade of events that leads to these changes. The local production of neurotrophic factors may play an important role.

P 6 The effect of denervation on the expression of the myogenic regulatory factors in rat muscles of different phenotypes. By E. H. Walters, P. T. Loughna and N. C. Stickland. *Department of Veterinary Basic Sciences, Royal Veterinary College, London, UK.*

The myogenic regulatory factors (MRFs), which include MyoD and myogenin, have been shown to be key players in the regulation of muscle specific genes. Their expression during development and in the adult has been shown to be neurally regulated. Denervation has been shown to result in the accumulation of MyoD and myogenin transcripts (Eftimie et al. *Proc. Natl. Acad. Sci. USA* **88**, 1993) and when adult muscle is cross reinnervated, myogenin and MyoD have been shown to accumulate in slow and fast muscle fibres respectively (Hughes et al. *Development* **118**, 1993). In the present study we have compared the relative increases in MRF expression in muscles with different metabolic characteristics in response to denervation. The muscles of the lower hindlimb of 7 male and 7 female rats were denervated by sectioning the sciatic nerve in the midthigh region of one limb under halothane anaesthesia. After a recovery period of 48 h the rats were killed by cervical dislocation and the soleus, extensor digitorum longus, cranial (anterior) tibialis and plantaris muscles were removed. The same muscles from the contralateral limb were also removed, as were the muscles from 4 unoperated animals. These served as controls. The RNA was extracted and run on a formaldehyde gel, blotted overnight and probed with myogenin, MRF4, and myf 5 cDNAs. The present study shows that the fast EDL muscles react to

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denervation by increasing the levels of MRF 4, myogenin and Myf 5. MRF4 showed the greatest increase, followed by myogenin and Myf 5 which was present at the lowest levels in all 4 denervated muscles. The soleus which has slow contractile characteristics showed little change in the level of myogenin, MRF4 or myf 5 expression upon denervation, compared to the contralateral limb and control muscles. The tibialis anterior and plantaris muscles showed a moderate increase in the level of MRF4 and myogenin expression. Previous workers have also shown an increase in the level of MRF expression in various muscles in response to denervation, though the effect of disuse produced by passive immobilisation, where the nerve remains intact, results in a different response (Loughna & Brownson, *FEBS Lett*. **340**, 1996). These differences between atrophy produced by denervation and that produced by disuse alone cannot be easily explained by differences in mechanical environment as it has been shown that passive stretch produces a distinctly different pattern of MRF expression. The results presented here, together with the observations published previously, suggest that an as yet unidentified neurotrophic factor mediates the effects of the nerve in repressing the expression of the MRFs.

P 7 Evidence of an atypical fontanelle in the skull of the Savanna buffalo *Syncerus caffer* Sparrman 1779. By M. Hornsveld. *Department of Veterinary Anatomy, University of Pretoria, South Africa.*

A detailed study of the osteology of the cranial and facial bones of the buffalo of the southern African subregion was undertaken as part of another study. Initially 14 skulls obtained from animals of both genders and varying in age from 16 mo to very old at time of death, were used. These skulls revealed some osteological features which are more pronounced in this species than in the skulls of comparable species such as the domestic bovine for example. A defect was noticed in the lateral wall of the internal layer of the osseus cranium, which appeared to be of a maximum size at the age of approximately $2\frac{1}{2}$ y, measuring approximately $15 \times 10 \times 10$ mm. At that age, the defect then also involves the external surface of the parietal bone in the temporal fossa. The external component of the fontanelle then appears as a round defect of approximately 10 mm in diameter, in the previously complete osseus medial wall of the temporal fossa. Its position is on or near the suture of the external laminae of the frontal and parietal bones. However even though this fontanelle is associated with the frontoparietal suture, it cannot be described as a frontoparietal fontanelle (Fonticulus frontoparietalis) as such a structure is an unpaired midsagittal fontanelle which typically lies at the caudodorsal end of skulls. In some animals it may appear as if the external part of the fontanelle only involves the frontal bone and not the frontoparietal suture. To confirm earlier development of this defect, formalin fixed heads of 2 of the youngest available specimens were subsequently dissected; these were of animals of approximately 4 mo of age. The defect proved to begin as a large cartilaginous part of the wing of the presphenoid bone. It was concluded that when the structure is maximally developed, it should be considered as a full thickness sphenoidal fonticulus (Fonticulus sphenoidalis) although it differs in various aspects of that fontanelle in man. Both the internal and the external dimensions of this cartilaginous structure decrease in size after the age of $2\frac{1}{2}$ y. Internally, even in skulls obtained from some very old animals, a smaller part of this fontanelle always remains unossified and is the reason why in boiled skulls it may give the false impression of only a sphenofrontal fissure (Fissura sphenofrontalis). Externally the fontanelle ossifies completely, but evidence of it may also be seen in old animals. Gross anatomical features were described in detail and recorded by digital photography. Although of questionable direct scientific value at this stage detailed osteological recordings of the skull of the buffalo can be used in 'fingerprinting' individual skulls; this may be useful in the taxidermy trade in the identification of hunting trophies.

P 8 Virtual bronchoscopy using computerised tomography: a new method of investigating bronchial anatomy. By C. C. Dobson[1], and G. R. A. Avery[2]. [1]*Department of Radiology, Hull Royal Infirmary and* [2]*Department of Radiology, Castle Hill Hospital, Hull, UK.*

Recent advances in computerised tomography have allowed reconstruction of the bronchial anatomy of patients undergoing routine spiral CT of the thorax with intravenous contrast (100 mls of Ultravist 300). The data are obtained using 4 mm axial slices reconstructed at 2 mm with a pitch of 1.5 on a PQ 5000 Picker spiral CT. These slices are taken through the trachea and proximal bronchial tree. Static and video images were obtained by reconstruction using Voyager software, provided by Picker International Ltd, which allows for volume rendered imaging. We present images of normal bronchial anatomy from our first 5 patients scanned using this new technique and correlate it with conventional axial CT and bronchial cast anatomy. The patients had consented to undergo spiral CT of the thorax as part of the investigation of respiratory symptoms. The data collected underwent conventional and virtual reconstruction. We propose this technique as an alternative to conventional bronchoscopy which is an invasive procedure requiring sedation. It may also provide a useful teaching aid in the form of a 'drive through' interactive package.

P 9 An anatomical study of an English extended family from the post-medieval period. By J. Wakely[1], R. Richards[2] and J. N. James[1]. [1]*Department of Preclinical Sciences, University of Leicester and* [2]*Department of Medical Physics and Bio-engineering, University College London, UK.*

On historical and archaeological grounds a small group of skeletons excavated in 1972 from a rural parish church in Northamptonshire, England, were presumed to be members of an aristocratic extended family group, buried during the 16th and 17th centuries. In our study we have used a range of anatomical methods to test this hypothesis, concentrating on 2 well preserved skulls. These include comparison of cranial, postcranial and dental nonmetric variations, cranial metrics, comparison with extant monuments to named individuals, and computerised facial reconstruction. The latter method uses a laser scanner normally used clinically for digitising the facial contours of patients. The process uses a laser stripe to illuminate a profile on the face which is digitised by an acquisition system. The face is then rotated

to obtain a series of profiles. The resulting data set contains about 40000 3-dimensional points over the scanned surface accurate to better than 1 mm. In this work the scanner has been used to digitise 2 skulls and the faces of a number of living individuals of appropriate age, sex and physique, who had given informed consent. This reconstruction method relies upon published data of typical soft tissue thicknesses at a number of locations over the face as do sculpting reconstruction techniques. The skull database from the laser scan is displayed on a computer screen and landmarks placed appropriately for the published thickness data. The estimated position of the skin surface at each of these points is then derived. The donor face image is distorted like a thin rubber sheet over the skull. Cranial metrics partly confirmed visual inspection of the skulls in showing a general similarity in the proportions of the face, but nonmetric traits provided no useful information. Computerised facial reconstruction can only be an approximation to the living features of an individual but did show some familial likeness. It would therefore appear to be of some use in this case and would merit further applications in anatomical studies of ancient populations. Monuments from the 16th century do not show accurate portraits of the deceased, but increasing realism is portrayed in the following century. Blood grouping studies undertaken at the time of the original excavation were inconclusive. Further evidence of a family relationship between the skeletons might come from examination of DNA in the future, if preservation of the bones is adequate for this purpose.

P 10 Is there a loss in perivascular nerve fibres of the basal cerebral arteries in streptozotocin-induced diabetic rats? By I. E. THUNNISSEN[1], G. J. BIESSELS[2] and R. L. A. W. BLEYS[1]. [1]*Department of Functional Anatomy and* [2]*Department of Medical Pharmacology*, *Rudolf Magnus Institute for Neurosciences*, *Utrecht University*, *The Netherlands.*

Diabetic neuropathy can involve almost any peripheral nerve and is an important cause of morbidity among diabetic patients. Compared to the notorious complications in the peripheral nervous system, the long term diabetic complications in the central nervous system seem relatively subtle. Cerebral blood flow disturbances, impaired cerebrovascular reactivity, and damage to large and small extra- and intracranial cerebral vessels have been found in humans and animals with diabetes. Combinations of some or all of these factors may underlie the high incidence and worse outcome of stroke in patients with diabetes. The aim of the present study was to investigate whether cerebrovascular nerves are affected by diabetes mellitus. Adult male Wistar rats were rendered diabetic by a single injection of streptozotocin. Diabetes was verified by measuring glucose concentrations in blood samples taken from the tail vein. Untreated controls consisted of animals of the same initial weight range. After 6 mo the rats were perfused under deep anaesthesia (0.1 ml Nembutal per 100 g body weight) and whole-mount preparations of the basal cerebral arteries were immunocytochemically stained for PGP 9.5. Measurements of the diameter of the vessel showed no significant differences between the control and the diabetic groups. The density of the nerve fibres expressed as percentage area of the vessel wall and the intercept densities/mm (number of nerve bundles per mm) in all arteries was unchanged 6 mo after the induction of diabetes. However, it should be noted that the possibility of (ultra)structural changes in the perivascular nerves cannot be excluded. It may be that a longer period of exposure to the disease would result in nerve loss. On the other hand the apparent lack of effect of diabetes on PGP9.5 positive nerves may be the result of a decrease of a subpopulation of nerves balanced by an increase of another subpopulation.

P 11 Microwave-staining of enteric neurons, using Cuprolinic blue in combination with enzyme- and peroxidase immunohistochemistry. By C. J. VAN GINNEKEN[1], M. J. DE SMET[1], F. J. VAN MEIR[2] and A. A. WEYNS[1]. [1]*Laboratory of Veterinary Anatomy and Embryology and* [2]*Laboratory of Cell Biology*, *Faculty of Medicine*, *University of Antwerp*, *Belgium.*

In order to quantify subsets of enteric neurons, it is necessary to relate the number of neurons that express one or more neurochemical substance to the total number of enteric neurons. However methods that visualise subsets as well as the entire enteric neuron population are not readily available or have proved to be unreliable. Therefore we attempted to combine CGRP-immunohistochemistry, NADPH-diaphorase- and AChE- enzyme histochemistry—techniques that mark subsets of enteric neurons—with a technique that seemed to visualise the entire enteric neuron population, the Cuprolinic blue staining method.

For this study 2 fetal pigs from the second half of gestation and 2 neonatal cross-bred pigs (Piétrain × White Large) were used. The fetal pigs were obtained from a local slaughterhouse. The neonatal piglets were killed by severing the carotid arteries under deep barbiturate anaesthesia (Nembutal, Sanofi). Whole-mount preparations were made from small intestinal segments that were fixed in formaldehyde (4% w/v paraformaldehyde) for 2–4 h.

To guarantee representative staining results, the individual staining methods were modified by using microwaves and 2 extra incubations. Microwaves are thought to improve staining results because they induce molecular rotations, which in turn promote chemical interactions and facilitate penetration. Furthermore, the total time spent to perform the staining was strongly reduced. During the combination with AChE-enzyme histochemistry, the colour shifted in the Cuprolinic blue stained neurons from bluish to greenish, which was probably due to the use of the oxidative $K_3Fe(CN)_6$ solution. Because the colour shift brightened the stained structures and enhanced the contrast, the incubation in the $K_3Fe(CN)_6$ solution was routinely incorporated in the Cuprolinic blue staining method. The other additional incubation was performed prior to the incubation in the cuprolinic blue staining solution. The prestaining solution differed from the staining solution in that Cuprolinic blue was omitted from the solution but dimethyl sulphoxide and Triton X-100 were added to improve penetration. In addition during this incubation the whole-mount preparations were impregnated with $MgCl_2$. This guaranteed representative results when the staining solution was reused because the concentration of $MgCl_2$ became less critical.

By incorporating the above mentioned modifications, the characteristics of each of the individual techniques were preserved. The distributions of NADPH-diaphorase, AChE

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and CGRP immunoreactivity corresponded well with previous morphological and physiological reports. NADPH-diaphorase enzyme histochemistry revealed a rather small subset of the enteric neuron population. The distribution of NADPH-diaphorase expression accorded with the role of NO as a neuromodulator and as a mediator of the nonadrenergic, noncholinergic relaxation of the gut. AChE-enzyme histochemistry revealed the largest subset of enteric neurons. However, caution should be exercised when ascribing a functional role to AChE-expressing neurons since their relation to cholinergic neurons is not clear. Nevertheless AChE-enzyme histochemistry may prove its usefulness in classifying enteric neurons according to their chemical content. Even though the latter protocol was developed for the simultaneous visualisation of CGRP and Cuprolinic blue in enteric neurons, it should be possible to use it in combination with the immunoperoxidase visualisation of neurochemical substances other than CGRP. The distribution of CGRP immunoreactivity corresponded well with the role of CGRP in the regulation of gastrointestinal motility and circulation and with its role at the neuroneural junction.

The combination of Cuprolinic blue, NADPH-diaphorase enzyme histochemistry, AChE enzyme histochemistry and CGRP immunohistochemistry provides fast, useful and ready-to-use double labelling techniques. The main advantage of these techniques is that they permit evaluation, both qualitatively and quantitatively, of the total as well as subsets of the enteric neuron population.

P 12 Neuropeptides and neurotransmitters in the mucosa of the jejunum of the one-humped camel (*Camelus dromedarius*). By E. P. K. Mensah-Brown, P. A. Lawrence, D. J. Pallot and A. Garner. *Departments of Anatomy and Pharmacology, Faculty of Medicine and Health Sciences, UAE University, Al-Ain, UAE.*

The distribution of cholecystokinin-8 (CCK8), galanin (GAL), 5-hydroxytryptamine (5'HT),glucagon, neurotensin (NT), neuropeptide Y (NPY), somatostatin (SOM), substance P (SP), the synthesising enzyme for norepinephrine, tyrosine hydrolase (TH) and vasoactive intestinal polypeptide (VIP), has been studied in endocrine cells and lamina propria of the jejunal mucosa of the one-humped camel *Camelus dromedarius* by immunohistochemistry. Fresh camel jejunum was obtained at laparatomy from 2 adult males anaesthetised with 2 mg/ml propofol (Dipravin, Zeneca, Macclesfield, UK) after an intravenous dose of 50 mg xylazine and 50 mg of ketomine nitrochloride as premedication. Anaesthesia was maintained with propofol at a dose of 4 mg/kg. Specimens were placed in Zamboni's fixative solution and routinely embedded in paraffin wax. Sections were then immunostained using the indirect PAP-diaminobenzidene method. Immunoreactivity to 5-HT, CCK glucagon and NT were observed in endocrine cells only. NPY, SP, and SOM immunoreactivity was discernible in both endocrine cells and nerve fibres, some of which possess varicosities, and in the wall of blood vessels within the lamina propria of the mucosal layer and the mucularis mucosae. GAL, TH and VIP were detectable only in neural elements in the lamina propria and muscularis mucosae.

While CCK cells were uniformly distributed in the mucosa, NT cells occurred mainly in the villi and the other cells predominated in the crypts. Whilst 5-HT and CCK immunoreactive endocrine cells were either flask-shaped with apices opening into the lumen or basket-shaped with infranuclearly located granular material, NT and somatostatin cells were mainly flask-shaped. All the other cells were mainly basket-shaped and of the closed variety. Concerning the innervation of the mucosae nerve fibres immunoreactive to SP, NPY, Gal and VIP predominated at the upper portions of the villi but were of similar density as immunoreactivity to tyrosine hydrolase in the middle and lower portions. The distribution of these neuropeptides, neurotransmitters and endocrine cells revealed variations from other mammals, for example the high density of NPY immunoreactivity, that may have significance for the water absorption ability of the camel jejunum.

P 13 Morphological changes in the peripheral nerves of the rat. By A. K. Sharma, P. Lawrence, P. A. Samad and I. Ahmed (introduced by E. P. K. Mensah-Brown). *Department of Anatomy, Faculty of Medicine and Health Sciences, UAE University, Al-Ain, UAE.*

Rats are frequently chosen for experimental diabetes and toxic neuropathies for short term and long term experiments. There is clear evidence for continued growth in peripheral nerves up to at least 15 mo of age but little information is available as to what happens to these nerves after this time. We examined the ultrastructural changes in myelinated nerve fibres of the tibial nerve of male Wistar rats after 15 mo of age since in some experiments the period of observations can run up to 2 y. After 15 mo of age no appreciable changes were observed in the body weight and skeletal length of rats. The cross sectional fibre area, axon and myelin areas were not different between 15 and 27 mo of age. However there was a significant increase in the number of fibres with axonal and Schwann cell inclusions ($P < 0.05$) which is of pathophysiological significance. This study has revealed that there is no growth of myelinated fibres in tibial nerve of rats after 15 mo of age, but axonal abnormalities, especially axonal glycogenosomes are frequent. It is therefore concluded that these changes should be borne in mind before the extrapolation of structural changes in experimental neuropathy.

P 14 Immunocytochemical localisation of caveolin-1 in human term extra-embryonic membranes using confocal laser scanning microscopy. By S. Byrne, A. Cheent, J. Dimond, G. Fisher and C. D. Ockleford. *Department of Pre-clinical Sciences, Leicester University Medical School, Leicester, UK.*

Amongst coated vesicles in cells the best understood are the clathrin coated variety which undertake receptor mediated endocytosis and are implicated in transepithelial transport. A second group of vesicles involved in receptor mediated uptake and directed intracellular vesicular transport are characterised by a less massive coat with a different composition that typically contain 1 of 3 caveolin isoforms as the major component. We have used a polyclonal antibody raised in rabbit against the Type 1 isoform of

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caveolin (VIP-21) to define the protein's distribution within human placenta and fetal membranes. Tissue was obtained from Leicester Royal Infirmary following the natural full term delivery of 11 healthy babies. The patterns of specific indirect immunofluorescence distribution were inferred by comparison of experimental with control sections exposed to 1/100 dilution of the primary antibody solution and 20% nonimmune serum respectively. The specificity of the antibody was confirmed by immunoblotting. Immunoreactive sites were mapped in the chorionic villus tree, chorionic plate, basal plate, umbilical cord and amniochorion. The mesenchymal cells of the amnion and chorion were considerably more immunoreactive than the amniotic epithelium and clearly more so than the decidua basalis and parietalis. There was a pattern of cytological distribution observed in extended focus images of amniotic epithelial cells which showed strong apical labelling (punctate in places) and basolateral labelling particularly in the basal projections into the underlying basal lamina. The amniotic epithelium was more immunoreactive than the chorion laeve trophoblast epithelium which showed the lowest intensity of any cellular layer. The fluorescence intensity was low or undetectable in the trophoblast cells of the chorionic villi, but the endothelial cells of the chorionic villus core were strongly immunoreactive and the mesenchymal cells were also labelled but to a lesser extent than the endothelial cells. The chorionic plate was generally most immunoreactive in the mesenchymal layers, but the chorionic plate amniotic epithelium was also to some extent immunofluorescent. Stem villi containing thicker walled blood vessels exhibited immunoreactivity with the medial smooth muscle layer. The umbilical cord exhibited intense layers of immunofluorescence corresponding to the position of the cord amnion and intimal lining of umbilical vessels. A similar pattern of fluorescence was observed in the basal plate with generally low immunofluorescence but where the marginal layer to the intervillus space was evident an intense immunoreactivity was present. The characteristics of this layer are not well described in the literature but the cells are said to be trophoblastic. The pattern of immunoreactivity is therefore surprising as it is more typical of the endothelial cells in other parts of the tissue and differs from the typical low immunoreactivity of the trophoblast cells in chorionic villi. This layer of cells is located along the margins of an extremely extensive sinus and has the low morphological appearance of a pavement epi-/endo-thelium. The new immunological characteristic identified here and the morphology may be explained in at least two ways. Either (1) the cells have been misidentified in the past and are in fact endothelial cells or (2) the trophoblast at this site has differentiated to acquire more endothelial-like characteristics. Further phenotypic characterisation of these marginal sinus cells is warranted as caveolin-1 is not a unique endothelial marker.

P 15 Effect of inducing, maintaining, and recovery from general anaesthesia on the expression of the immediate early gene c-Fos in the inferior olive of the rat. By J. VAN DER MOER and T. J. H. RUIGROK. *Department of Anatomy, Erasmus University Rotterdam, The Netherlands.*

The cerebellum is a brain structure that is commonly associated with motor coordination and learning. Information on all types of sensory modalities is transmitted to the cerebellum by way of its afferent mossy fibre system, which reaches the Purkinje cells via the mossy fibre/parallel fibre/Purkinje cell synapses. The other main afferent system of the cerebellum, the climbing fibre system, originates from the inferior olive and terminates directly on the Purkinje cells. An attractive hypothesis on cerebellar functioning states that plastic changes in the synaptic weight between the parallel fibre terminals and Purkinje cells can be induced by near simultaneous activation of the climbing fibres, which in this context would be able to detect error signals. The changes in synapse efficacy would systematically alter the output of the cerebellum in a particular situation resulting in modification of the execution of those processes responsible for the occurrence of the error signal.

Periods of dizziness and/or disorientation experienced during recovery from general anaesthesia may be associated with the triggering of these error signals. In order to investigate if enhanced triggering of error signals related to a dysfunctioning vestibular system may occur during this recovery period we have investigated the effect of anaesthesia on the expression of the immediate early gene c-fos in the inferior olive of the rat. Activation of the c-fos gene, immunocytochemically detected by expression of the nuclear Fos protein, can be induced in neurons by a variety of behavioural, chemical or electrical stimuli. It is commonly seen as reflecting an enhanced electrical and/or metabolic activity of neurons expressing Fos-positive nuclei.

Rats ($n = 27$) were anaesthetised with either an i.p. injection of a ketamine/xylazine cocktail, with pentobarbital, or by continuous inhalation of a fluothane/NO_2/O_2 mixture. Experiments were performed in order to (1) establish the result of inducing general anaesthesia, (2) to establish the effect of maintaining general anaesthesia and (3) to establish the effect of recovery from general anaesthesia. Just before perfusion all rats received an overdose of pentobarbital. Control experiments showed that rats which only received an overdose of pentobarbital and which were perfused within 10 min did not show Fos labelling in the inferior olive.

Because expression of the Fos protein only starts 10–60 min after stimulation and has a half life of 2 h, interpretation of the results is not unequivocal. Nevertheless, we show that Fos expression is most dramatically enhanced 30 min after recovery from Fluothane or pentobarbital but after 2 hours when recovering from ketamine/xylazine anaesthesia. Time series of ketamine anaesthetics not only showed that the very process of inducing anaesthesia may already result in Fos expression but also that maintaining anaesthesia for prolonged times (up to 3 h) may reactivate enhanced expression of c-fos. In all cases expression of c-fos was most prominent in those areas of the inferior olive which are commonly associated with the vestibulo-

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cerebellum (i.e. nucleus beta and caudal half of the medial accessory olive).

These results indicate that general anaesthesia may indeed result in enhanced activity of cerebellar climbing fibres associated with control of vestibular processes. As such, these types of experiments may aid in evaluating the speed and ease of recovery processes.

P 16 Variation of tyrosine hydroxylase concentration in the human ofactory bulb By P. V. Hoogland and E. Huisman. *Department of Anatomy and Embryology, Vrije Universiteit Amsterdam, The Netherlands.*

In all vertebrates including humans a large number of tyrosine hydroxylase (TH) immunoreactive cells are present in the olfactory bulb. Studies with antibodies against dopamine have shown that most of the TH-containing cells in the olfactory bulb are dopaminergic neurons. The majority of the TH-containing cells belong to the group of periglomerular neurons. Solitary TH-immunoreactive neurons are present in the external plexiform layer, the granular cell layer and along the olfactory peduncle. In a previous study we found that an additional group of TH-positive neurons is present in the centrally located stratum album. In that study we described TH-positive structures in the human olfactory bulb as observed in cases that displayed the highest immunoreactivity. Now we focus on the variability of the staining of the TH-positive structures in the different cases we studied. It appears that only in a few cases all the known TH-positive structures are darkly stained. In spite of the fact that we used a standard staining procedure, the intensity of the TH staining varied considerably between different cases. This variation may concern the whole bulbus, but in some cases different parts of the same bulb showed varying staining intensities. Although the quality of the studied material was clearly related to the postmortem delay, we could not find a relation between TH staining intensity and the postmortem delay. Neither we could find a relation with age or gender. In a few cases, in which the tissue seemed to be well conserved, we could only find some weakly TH-positive debris at places where periglomerular dopamine-containing cells are supposed to be localised. It appeared that darkly stained structures are only present in material that was obtained from people that died during autumn and wintertime. Material obtained from people who died between April and October never showed darkly stained TH-positive neurons in the olfactory bulbs.

From experiments in rats and mice, it is well known that closure of the nares can induce in a couple of weeks a very prominent decrease of TH expression in the olfactory bulb. Since we have no detailed information about the patency of the nose during the last weeks before death in the cases we used in this study we cannot exclude the possibility that closure in one or the other way of the nose occurred. However we know one case in which a tube had been inserted in the left nasal opening during the last week. In this case no difference in TH-immunostaining was observed between the left and the right olfactory bulb.

The present study shows that it might well be possible that seasonal variations occur in the expression of TH in the human olfactory bulbs.

P 17 A suggested lateral surgical approach to the guttural pouch of the horse based on anatomical findings. By J. K. Niccol, N. Keller, M. Hornsveld and A. Guthrie. *Faculty of Veterinary Science, University of Pretoria, South Africa.*

There are 4 existing surgical approaches to the guttural pouch of the horse; each requires the skilful hand of a specialist surgeon to avoid sensitive blood vessels and nerves en route. When considering anatomical specimens, an area on the lateral aspect of the pouch could be defined where no major blood vessels or nerves are found. The aim of this project was to determine this site accurately by defining its anatomical borders precisely before testing it as an alternative surgical route. This proposed lateral percutaneous approach will cross muscle and bone only, but avoid the other structures, and therefore could have advantages above existing approaches. Heads for this study were obtained from 17 cadavers of different breeds, varying in age from approximately 6 mo to more than 20 y at death. Ages were estimated by considering the wear of the teeth. Additionally 3 formalin fixed specimens from the collection of the Anatomy department were also dissected to serve as reference material for the basic anatomy of the region concerned. Prior to dissection, lateral radiographs of all specimens were taken to verify pertinent bony structure and to exclude specimens with pathological conditions from the study. Using a checklist, various parameters (morphometric data, signalment, nerves and blood vessels) were recorded from the 20 specimens. The unfixed heads were subsequently boiled and skulls were prepared and correlated with the radiographs. The checklist data has been statistically analysed to verify that the site has the same anatomical location in horses of all ages, breed and gender, and to estimate the safety margins of the proposed site for clinical application. Ultimately it is aimed to develop not only a new surgical approach, but also a technique which will enable the general practitioners to utilise this proposed site for quick and easy access to the guttural pouch of horses.

P 18 The possibility of the deep peroneal nerve neurotisation by the superficial peroneal nerve: an anatomical approach. By M. Büyükmumcu[1], M. E. Üstün[2], M. Seker[1], Y. Kocaoggullari[2] and A. Sagmanligil[2]. [1]*Department of Anatomy and* [2]*Department of Neurosurgery, University of Selcuk, Turkey.*

Neurotisation is described as a transfer of the nerves for the reconstruction of nerve function in the region. Many spinal nerve roots injured due to stretch or other types of lesion are not repairable. Some spinal nerves might be repaired if they could be exposed in their intraforaminal course. A number of nerves have been used for neurotisation in different part of the peripheral nervous system such as the hypoglossal nerve, spinal accessory nerve or phrenic nerve anastomosis with facial nerve in facial paralysis. Lesions involving peripheral nerves that innervate antigravity muscles will produce wrist drop (radial nerve) and foot drop (common peroneal nerve). This study was undertaken to develop a practical and relatively safe surgical approach to the root lesion of L4. In this study, we aimed to examine the possibility of the deep peroneal nerve and its branches undergoing transposition by the superficial peroneal nerve

and their branches anatomically, its clinical effectiveness and safety. An injury in the region sufficient to interrupt conduction in the nerve leads to foot drop, in which the patient cannot dorsiflex the foot. 12 legs of finely dissected cadavers obtained for teaching purposes in the anatomy laboratory were used to display the common peroneal nerve and its branches. The fine dissection of nerves was continued until the branches of the nerves reached to the muscles. Each branch was then measured using a compass and analysed. The morphometric study was performed to determine the possibility of the transposition of the deep peroneal nerve with the superficial peroneal nerves. After the dissection was completed the thickness of the common peroneal nerve branches, the deep peroneal nerve and the superficial peroneal nerve including their muscular branches were measured and their results analysed statistically. The results of the measurements are given in the table.

It was observed that in the measured nerve branches, the transposition was possible between the peroneous longus and anterior tibial branches on the basis of their thickness and length. It is obvious that in recent decades advances in microsurgical reconstruction and understanding of the microanatomy have played major roles in improving the results of surgical treatment of the injuries. There is a need for further experimental research on the promotion of nerve regeneration and the refinement of surgical technique to see the possibility of this approach.

	Length of final branch to muscle	Nerve diameter	Distance of branch point from common peroneal nerve
SPN	–*	3.8 ± 0.11	–*
DPN	–*	3.5 ± 0.17	–*
PLB	**60 ± 0.19**	0.9 ± 0.03	9 ± 0.32
PLSB	38 ± 0.18	0.9 ± 0.03	9 ± 0.33
PBB	40 ± 0.30	0.8 ± 0.01	90 ± 0.22
AB	–*	0.8 ± 0.01	11 ± 0.22
ATB	**60 ± 0.22**	0.9 ± 0.02	13 ± 0.22
EDB	–*	0.7 ± 0.02	63 ± 0.46

Table. Measurements given in mm (mean ± standard deviation, n = 12). SPN: superficial peroneal nerve; DPN: deep peroneal nerve; PLB: peroneous longus branch; PLSB: peroneous longus second branch; PBB: peroneus brevis branch; AB: articular branch; ATB: anterior tibial branch; EDB: extensor digitorum branch. *These parameters were not possible to measure or not related to the study.

P 19 Anastomoses between the laryngeal nerves. By J. R. Sañudo*, E. Maranillo, X. Leon, R. M. Mirapeix and M. Quer. *Unit of Anatomy and Department of ENT, Autonomous University of Barcelona, Catalonia, Spain.*

Recently, it has been suggested that the thyroarytenoid muscle receives a dual nerve supply. One from the recurrent nerve (RN) and the other from the external laryngeal nerve (ELN) via a connection with the RN through the crycothyroid muscle. This connection has also been considered as an extension of the sensory innervation to the subglottic area. This neural connection or anastomosis initially described by Dilworth (1921) in human larynxes has been reported by other authors with a prevalence from 6% to 44%. However, besides the above-mentioned connection between the ELN and RN, other anastomoses between the laryngeal nerves have been described with variable disposition and frequency. Due the important role that the anastomoses can play in the knowledge of the neurophysiology of the larynx, the goal of this work is to establish their number, frequency, situation, distribution and relationships. A total of 90 human larynxes obtained from necropsies (57 males and 33 females, age range from 41 to 95 y) were examined by careful dissection with the help of a surgical microscope. Six different types of anastomosis were observed. They were classified according to the connecting nerves in 3 groups: (1) anastomoses between the internal laryngeal nerve (ILN) and the RN; (2) anastomoses between the ILN and the ELN; and (3) anastomoses between the ELN and the RN. The anastomoses of the first group appeared in 4 different patterns while the anastomoses of the second and third groups appeared with a unique pattern. The 4 patterns of the first group were: (a) Galen's anastomosis as the connection between the dorsal branches of both nerves (100%); (b) the arytenoid plexus as the connection between the arytenoid branches of both nerves in relation with the arytenoid muscle (100%); (c) the thyroarytenoid anastomosis as the connection of one descending branch of the ILN and an ascending branch of the RN (27% of cases); and (c) the crycoid anastomosis, previously only described in the cow, located in front of the crycoid lamina (8/10 cases). The second group of anastomoses appeared as the connection of ELN and RN throughout the crycothyroid muscle (80% of cases) and the third group as the connection of the ELN and ILN throughout the foramen thyroideum (31% of cases). The different prevalence of this complex anastomotic pattern between the laryngeal nerves suggests functional differences in relation with the sensory and motor innervation of individual subjects.

P 20 Developmental timing in the frog *Eleutherodactylus coqui*: implications for the evolution of direct development. By C. K. Osabutey[1,2], J. Hanken[3], R. P. Elinson[4], P. Bagley[1] and M. K. Richardson[1]. [1]*Department of Anatomy and Developmental Biology, St George's Hospital Medical School, London, UK,* [2]*School of Medical Sciences, Komfo Anokye Teaching Hospital, University of Science and Technology, Ghana,* [3]*Department of Environmental Population and Organismic Biology, University of Colorado, USA and* [4]*Department of Zoology, University of Toronto, Canada*

The embryology of *Eleutherodactylus coqui*, a direct developing neotropical anuran with no tadpole stage, is of considerable interest to evolutionary developmental biologists. *E. coqui* hatches as a miniature froglet, and its life cycle therefore differs considerably from that of the widely-studied indirect developer *Xenopus laevis*. This is reflected in some obvious differences in organogenesis. For example in *E. coqui* the limb buds develop much earlier than in *Xenopus*. We want to look systematically for other differences in organogenesis. However this is difficult because

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the standard stages for *E. coqui* have not been correlated with organ development. Furthermore these 2 species are staged according to different published staging tables. We overcame this problem by comparing developmental sequences. We describe here a histological study of organogenesis in *E. coqui* embryos from Townsend-Stewart stages 3 to 8. This corresponds to the organogenetic period when the principal features of the body plan are established. We found that stages 3 to 4 represent neurulation and the formation of primary organ rudiments. Stages 5 to 6 represent the beginning of cytodifferentiation in the organ systems. In stages 7 to 8 and beyond, there is progressive growth and differentiation in the organ systems. We have compared our data on organogenesis in *E. coqui* with published data for *Xenopus*. The comparison was made using an 'abacus' model which allows developmental sequences to be compared graphically. We found that differences in the life cycle of these species are reflected in a different pattern of organogenesis. In particular we note changes in developmental timing (heterochrony). These include the timing of skin pigmentation in the region of the head, stomodeal opening, mordification in the pharyngeal arteries, appearance of hepatic cords, and lens differentiation amongst other features. Many of these differences can be correlated with the need for indirect developers to adopt a free-living larval phase at an early stage of development.

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