Official Journal of the Spanish Society of Anatomy

European Journal of Anatomy

September, 1999
Vol. 3 - Supplement 1

XVIII CONGRESS OF THE SPANISH SOCIETY OF ANATOMY

ABSTRACTS

Valladolid, 27 - 30 September, 1999

European Journal of Anatomy

AIMS AND SCOPE The journal aims at covering all aspects of Anatomy and Morphology. Along with primary original contributions, about: Morphological research, applied Anatomy, morphological techniques and methods, the Journal will publish review articles on morphological sciences and Anatomy teaching, and position papers requested by the Editorial Board, conference proceedings and invited lectures in the following fields: Neuroanatomy, Endocrinology, Embryology, Splanchnology, and Locomotor system.

SUBSCRIPTIONS The European Journal of Anatomy publishes 3 issues per annum. Subscription prices for 1999 are: 15000 sp ptas for personal subscription, 5000 sp ptas for members of "Sociedad Anatómica Española" or 25000 sp ptas for institutional subscriptions (VAT, TVA/BTW, MWST or IVA are not included). Orders for current subscriptions and back issues should be sent to Sociedad Anatómica Española, Avda. Campo Charro s/n, E-37007, Salamanca, Spain (Telefax: +34-923-294559; E-Mail: ejasae@gugu.usal.es). Information on this journal is available on the Sociedad Anatómica Española homepage at http: //www.med.ub.es/sae/sae.htm

ISSN: 1136-4890
Depósito Legal: S. 665-1996

Imprime:
Gráficas VARONA
Polígono «El Montalvo», parcela 49
Teléf. (923) 190036. Fax (923) 190027
37008 Salamanca

XVIII CONGRESS OF THE SPANISH SOCIETY OF ANATOMY

September 27, 28, 29 y 30, 1999

ABSTRACTS

VALLADOLID

PRESIDENTE DE HONOR

S.M. LA REINA DE ESPAÑA

COMITÉ DE HONOR

Excmo. Sr. D. Juan José Lucas Giménez
Presidente de la Junta de Castilla y León

Excmo. Sr. D. Isaías García Monge
Delegado del Gobierno en Castilla y León

Excmo. Sr. D. Tomás Villanueva Rodríguez
Consejero de Educacón y Cultura

Ilmo. Sr. D. Francisco Javier León de la Riva
Alcalde del Excmo. Ayuntamiento de Valladolid

Ilmo. Sr. D. Ramiro Ruiz Medrano
Presidente de la Diputación de Valladolid

Ilmo. Sr. D. Alfredo Blanco Quirós
Decano de la Facultad de Medicina de la Universidad de Valladolid

Organizing Committee

Presidents

Prof. Dr. D. Enrique Barbosa Ayúcar
Prof. Dr. D. Pedro Gómez Bosque

Secretary

Prof. Dr. D. José Antonio Gil Verona

Technical Secretary

Dra. D.ª Sagrario Callejo Alonso

Vocals

Dra. D.ª María Isabel Alonso Revuelta
Dra. D.º Mercedes Barbosa Cachorro
Prof. Dr. D. José Carreres Quevedo
Prof. Dra. D.ª Natividad García Atarés
Prof. Dr. D. Ángel Gato Casado
Prof. Dr. D. José Antonio Moro Balbás
Prof. Dr. D. Juan Francisco Pastor Vázquez
Dr. D. Félix J. de Paz Fernández
Prof. Dr. D. Juan Represa de la Guerra
Dra. D.ª María Luisa Rodríguez Andrés
Dra. D.ª Isabel San José Crespo
Prof. Dr. D. Santiago Rodríguez García

PLENARY LECTURES

DELIVERY AND LOCOMOTION IN HUMAN EVOLUTION

J.L. Arsuaga

Department of Paleontology, Faculty of Geology. Complutense University, Madrid, Spain

Bipedal locomotion and birth are two intimately related aspects of human physiology. The first known bipedal hominids had substantially altered postcranial skeletons. The oldest fossil remains of a bipedal hominid is a fairly complete tibia that came from the Kenyan bed at Kanapoi. It is assigned to the species *Australopithecus anamensis* and has been dated at 4.2 million years old.

There is a much more abundant fossil record of a slightly later hominid, called *Australopithecus afarensis*, whose remains have been found in Ethiopia and Tanzania and have been dated as being between 3.7 and 2.9 million years old. From this species we even have a female skeleton in fairly good condition; it conserves the sacrum and the left coxal bone, although the latter is slightly deformed, hindering reconstruction of the pelvis. Its proper reference is A.L. 288-1, although it is more popularly known simply as "Lucy", and has been dated at 3.2 million years.

There are other later species of primitive hominids that have also afforded postcranial remains, including several loose subcoxal bones and a couple of reasonably complete pelvises. The most complete fossils correspond to the species *Australopithecus africanus*, which has been found in three African caves.

With all these fossils it is possible to garner some idea of what the pelvis of *Australopithecus* was like. First, the fully lateral orientation of its iliac crests indicates that the species were fully able to perform abduction of the hip and that they therefore practised bipedal locomotion identical to that of present day humans. The fossil footprints of the 3.6 million-year old bed at Laetoli, in Tanzania, confirm this notion. However, some important differences can be seen between the pelvis of *Australopithecus* and the the present human pelvis. The bicrestal width is very large with respect to the very small stature of the individuals and hence the neck of the femur is also very long.

The female known as Lucy, in particular, shows a marked degree of platypellia, which has been the subject of much debate. It should not be ruled out that this could be a characteristic of the individual rather than one of the species as a whole. Based on body weight and head volume, it has been calculated that an australopithecine female would deliver a term fetus with a brain the size of that of one of today´s chimpanzees. It is therefore possible to speculate on what birth would be like in *Australopithecus*.

Some authors, such as Lovejoy and Tague, are of the opinion that it would essentially be similar to that of a chimpanzee; that is, with a linear trajectory, without rotation and with posterior exit, but with the difference that the birth would be transversal; i.e., the greatest diameter of the fetal head (sagittal) would be accommodated to the maximum diameter of the superior narrowing, which in a platypellic pelvis such as that of Lucy would be the transversal diameter. By contrast, I agree with Berge that in these first hominids birth would have had the essential characteristics of modern human birth:

A curved trajectory, rotation and ventral exit of the fetus, with the head oriented sagittally and not transversally. For this conclusion we base ourselves on the site at which we locate the vulva; from the morphology of the subpubic angle of the fossil pelvis, we believe that the vagina opened towards the front and not towards the rear.

After *Australopithecus* there are no more fossil pelvises until the one we have recently described -from the Sima de los Huesos bed in the Sierra de Atapuerca- dated at 300,000 years. This is a male pelvis that is extraordinarily robust with respect to current morphology, although not with respect to the individuals peers. The same bed has also yielded other (not as complete) pelvises that are equally robust. The bicrestal width of the pelvis from the Sima de los Huesos is still extremely broad, even for an individual with a modern structure: that individual's height has been estimated at some 177 cm. With such a body structure, the individual must have been very strong and must have weighed about 100 Kg.

The pelvis from the Sima de los Huesos has a very wide pelvic cavity, above all transversally, and especially at the height of the sciatic spines, which represent the main narrowing and obstacle at the time of birth. It would have been perfectly possible for a modern term fetus to pass through a male pelvis of these characteristics.

The pelvis of the Neanderthals essentially conserves the structure and characteristics of that from the Sima de los Huesos, although it is possible that the robustness of the skeleton and the muscle mass of the individual may have been reduced. There is a fairly complete male Neanderthal pelvis from the Israeli bed at Kebara, dated at some 60,000 years. Although several authors, such as Rak and Tague, have reported in this specimen a strong narrowing at the exit of the pelvic cavity -which has even led another author (Abitol) to state that one of the reasons for the extinction of the Neanderthals could have been difficulties at birth- my own interpretation of this fossil is that the narrowing referred to is a result of post-mortem deformation in the fossil bed.

The first pelvises like current ones are found in the Israeli beds of Skhul and Qafzeh, dated at around 100,000 years. These beds have yielded the oldest complete skeletons of our own species: *Homo sapiens*. In them it is possible to recognize the main factor that has modelled the modern pelvis: a narrowing of the hip that has led the acetabula to approach each other, thus decreasing the arm of resistance in hip abduction, increasing its biomechanical efficiency. As an undesired result of this, we now have a narrower birth canal and a harder birth.

MORPHOGENESIS IN VERTEBRATE EVOLUTION

A. García-Bellido

Molecular Biology Center "Severo Ochoa", CSIC, Madrid

Comparative analysis, at molecular level, of many genes involved in developmental processes is uncovering their strong conservation along evolution. Interactions among proteins and between these and the (Cis) regulatory regions of genes point to a high degree of molecular recognition. This molecular recognition represents inertia towards mutational changes during evolution. Escape from this limitation has consisted of the repetition of genes and the combination of coding regions of active protein domains. Thus, the enormous morphological diversity among metazoans has been achieved with only a small increase in the number of genes. Moreover, not only

genes but also sets of them related functionally by molecular recognition (syntagms) specifying discrete morphogenetic operations are conserved from metazoan ancestors in the Precambrian. The molecular basis of these variations lies in combinatorial changes in the regulatory sequences of genes, which determine when and where they are expressed in protein coding regions. Evolutionary variations have thus resulted from combinatorial changes in morphogenetic operations in different cell lines and times of development along phylogenia. Morphogenesis thus begins to become understood as the result of the activity of genes and their syntagms defining conserved morphogenetic operations that, in a combinatorial way, specify the different morphologies seen in the embryonic and adult forms of different metazoans.

ANOIKIS

J.L. Ojeda

Department of Anatomy and Cell Biology, University of Cantabria, Santander, Spain

Cell death (apoptosis) has long been recognized to play a significant role in a variety of biological processes such as embryonic morphogenesis and carcinogenesis. The morphological signs of apoptosis are the final results of a complex biochemical cascade of events. There is increasing evidence that the family of intracellular cysteine proteases caspases plays a pivotal role in this process.

The extracellular matrix regulates cell growth and differentiation and acts as a survival factor for many cell types. Local disruption of the extracellular matrix by pharmacological or genetic means results in selective apoptosis in adjacent cells. The term "anoikis", from the Greek for "homelessness" was coined to denote this type of apoptosis. Anoikis appears to be limited to epithelial and endothelial cells and does not occur in mesenchymal cells. Anoikis has been assumed to be mediated by changes in integrin signalling and can be prevented by inhibiting tyrosine phosphatases.

There is also increasing evidence that specific developmental strategies are repeated many times during embryogenesis. Anoikis appears to be a basic strategy in the cavitation process of solid structures and in the regression of embryonic organs. Anoikis could also serve as a physiological tumour control mechanism by deleting cells which escape positional control. Further investigation into the mechanisms of the process is likely to yield insight into a number of developmental events and, possibly, tumour genesis.

RADIOLOGICAL ANATOMY OF THE CENTRAL NERVOUS SYSTEM AND THE RETROPERITONEUM. TWO AREAS FOR STUDY BY COMPUTERIZED IMAGING

V. Pedraza Muriel and J. Fernández Mena

Department of Radiology and Physical Medicine. School of Medicine, University of Granada, Spain

As a window onto the next millennium, the last quarter of the present century has brought with it a revolution in the morphological and functional analysis of the human body. COMPUTERIZED AXIAL TOMOGRAPHY, from G.N. Hounsfield and McCormack, and SPECTROSCOPIC and MAGNETIC RESONANCE IMAGING (MRI) from Bloch, Purcell and Lauterbure, are the most important manifestations of the study of the conditions -both normal and pathological- of human anatomy.

CAT reveals the anatomical sections of Flechsig through study of the frequency distribution of the different coefficients of attenuation to X-ray irradiation of tissues. Thus, bone, the grey matter, the white matter, the ventricular systems, the CSF and other elements appear clearly differentiated. The intracranial vascular tree, until now "monopolized" by angiography, has now become one of the axes of modern CAT techniques and its possibilities of three-dimensional spatial reconstruction.

ULTRASONOGRAPHY offers two particular possibilities for study of the CNS. The first and most important is transfontanellar study of the brain in newborns while the second is the verification of cerebral lesions and their surgical treatment through an artificial window to the brain by trepanation. Ultrasound, through the differentiation of the behaviour of each tissue stratum when subjected to electromagnetic waves, reveals gyrus and cisternae, cortex and subcortex, diencephalic structures and CSF systems and involves the use of a non-ionizing energy source. This affords great spatial resolution in the study of neonatal pathologies.

More recently, MAGNETIC RESONANCE has opened important doors into the recognition of anatomical structures, the characterisation of tissues and lesions, the study of the extra- and intracranial vascular axes, the dynamics and behaviour of the CSF, and cerebral biochemistry via spectroscopic data.

Finally, POSITRON EMISSION TOMOGRAPHY (PET) represents a diagnostic tool of incalculable value in the detection and assessment of functional damage. The metabolic activity of tissues is measured through standardized values of the uptake of 18-fluoro-2-deoxy-glucose (FDG) and its detection is carried out by positron emission radionuclide activity.

Computerized tomography, Ultrasonography and MRI have allowed such areas as the cranial cavity or the retroperitoneum, held within bony and muscular environments, to open up their doors and show the structures they contain. The whole of the head, with its complex structuring in the form of nuclei and radiating fibres, its vascular networks and their organization, can now be inspected in vivo in a bloodless fashion with the new methods of medical diagnostic imaging. The kidney and the adrenal glands, the large vascular and lymphatic networks of the central retroperitoneum, its fascia and compartments can be visualized on all spatial planes and this now permits a precise visualization of their structures, their relationships, their routes of communication and the anatomical changes that occur during pathological circumstances.

Anatomy, physiology, electromagnetic radiations, mathematics, physics and geometry now throw light and colour onto our current view - 500 years after Vesalius and Leonardo da Vinci- of the different spaces, compartments and organs of human anatomy and allow us to direct modern medicine and its applications to caring for others and to scientific knowledge in general.

THE ANATOMY OF DREAMING

F. Reinoso Suárez

Dpto. de Ciencias Morfológicas. Universidad Autónoma de Madrid. Spain

It is my belief that the best dreams are those that we dream when we are awake. However, today most neuroscientists agree that the richest and best structured dreams occur during the REM sleep. In fact, the structures responsible for REM sleep, whose anatomy is at present under discussion, are necessary for normal dreams. The pontine tegmentum structures with a definite role in the control of different events characterising REM sleep (EEG activation, atonia, PGO activity and rapid eye movements) are precisely located. However, the exact site of the structure that can simultaneously trigger all the manifestations of REM sleep, with all its bioelectrical and behavioural manifestations, is still controversial. We have demonstrated that the ventral part of the oral pontine reticular nucleus is the nodal link, acting like an orchestra conductor, of the extense neuronal network that plays harmoniously, generating and maintaining REM sleep. A systematic multidisciplinary study of the ventral part of the oral pontine reticular nucleus borders, connectivity, neuronal and synaptic morphology, chemical structure, at light and electron microscopic level, as well as functional studies of unitary recordings, electrical and chemical stimulation in vitro and in vivo preparations have been made. All our data demonstrate the complex morphofunctional organisation of the neuronal network responsible for the generation and maintenance of REM sleep. The final part presents the results of current literature on formal dream features and PET imaging studies of REM sleep, which agree well with our functional anatomic results.

BORBON REFORMISM AND MODERN ANATOMY IN SPAIN (1700-1808)

J. Riera Palmero

Dept. of Psychiatry, Radiology and Medicine's History, Faculty of Medicine, University of Valladolid, Spain

The Borbon "Reformism" begun in Spain with the crowning of Philip V (1700) lent a European "flavour" and modernizing trend to Spanish scientific culture. The project involved a pronounced increase in anatomical and surgical knowledge, areas in which eighteenth century Spanish science made some of its most outstanding contributions. Oriented towards topographic investigation, morphology became an essential discipline for the practice of surgery. In this sense, the founding of the Royal Colleges of Surgery (Cádiz, 1748; Barcelona, 1760, and Madrid, 1788) marked the upgrading, as it were, of our anatomists and surgeons to European level. Anatomy, which was completely absent from Spanish universities in the seventeenth century, began to be recovered first in the General Hospital of Madrid and, shortly after, in institutions devoted to the training of Army and Navy surgeons. Thanks to the auspicious support of the Borbons, the work of Pedro Virgili (1699-1776) ended by promoting a Spanish School of Anatomy and Surgery, first in Cádiz and years later in Barcelona.

The influence from abroad, mainly France, led to the distribution and diffusion of books about and instruments for anatomy and surgery. To such novelties should be added reams of texts and manuals translated into Spanish, chiefly of French origin. Attracted by Borbon policies, many anatomists and surgeons visited Spain, adding their efforts to the renovating trend supported by Philip V, Ferdinand VI and Charles III. The modernizing project, financed by the Secretary for War and the Naval Affairs, granted travel and accommodation allowances to anatomists and surgeons so that they could perfect their techniques abroad at institutions such as those of Leyden, Bolonia, Paris and London.

Spanish anatomists contributed with their endeavours not only to diffusing our morphology across Europe, as witnessed in the "Anatomy Course" of Jaime Bonelis and Ignacio Lacaba, but also made highly original contributions, such as that of Antonio de Gimbernat, responsible for the description of the ligament named after him. Even more important was the contribution of the Navy surgeon Domingo Russi, who in 1760 described in practical autopsy to the Marquis of Las Amarillas a case of dextrocardia with situs inversus.

ORAL COMMUNICATIONS

A. 1 NORMAL OPTICAL DENSITOMETRIC PARAMETERS IN EXFOLIATIVE CYTOLOGY FROM DIFFERENT ZONES OF THE ORAL MUCOSA

M.T. Castaño Oreja, Darío Pose Nieto, Teresa Jorge Mora, Juan Suárez Quintanilla, Maximino Quintáns Rodríguez

Departamento de Ciencias Morfológicas. Facultad de Medicina. Universidad de Santiago

INTRODUCTION: Exfoliative cytology is a quick, inexpensive and non-invasive diagnostic tool, which makes it highly useful in clinical practice. The purpose of this study was to examine the behaviour of optical densitometric parameters of normality in five different zones of the oral mucosa free of pathology. Methods: Thirty three patients, ranging between five and seventy five years of age, free of general and oral pathology, were studied. The smear was carried out with the Cytobrush(R) and fixation with Labofix(R). The smears were stained with Hematoxylin Eosin and the analysis carried out with a semiautomatic image analyser (Microimage 3.0). The following parameters were studied: luminic density of the cytoplasm, luminic density of the nucleus, red density of the cytoplasm, blue density of the cytoplasm and green density of the cytoplasm. We used SPSS 7.5 in the statistical analysis. We carried out a variance analysis with the Bonferroni correction for multiple comparison of means, which was considered statistically significant $p<0.01$. Conclusion: Significant differences were found in the parameters studied (optical density) between different zones of the oral mucosa.

A. 2 NORMAL MORPHOMETRIC PARAMETERS IN EXFOLIATIVE CYTOLOGY FROM DIFFERENT ZONES OF THE ORAL MUCOSA

A. Crespo Abelleira, Teresa Jorge Mora, Darío Pose Nieto, Javier Jorge Barreiro, Juan Suárez Quintanilla

Departamento de Ciencias Morfológicas. Facultad de Medicina. Universidad de Santiago

INTRODUCTION: Exfoliative cytology is a quick, inexpensive and non-invasive method of diagnosis, which makes it very useful in clinical practice. The morphofunctional alterations of cell atypia or any other cell dysfunction may be quantified with a quantitative analysis through exfoliative cytology. Morphological alterations (alteration of the nuclear and cytoplasmatic areas and nuclear pleomorphism) are quantified with a morphometric analysis. Great disparity exists in values of normality from different authors in morphometry. Therefore, we have examined these values and have attempted to relate them to different zones of the oral mucosa.

METHODS: Oral smears were taken of thirty-three individuals ranging between five and seventy five years old, from five sites of the oral mucosa, free of general and buccal pathology. The smear was carried out with the Cytobrush(R) and fixation with Labofix(R). The smears were stained with Hematoxylin Eosin and the analysis was done with a semiautomatic image analyser (Microimage 3.0). The following parameters were studied: cytoplasmic area, nuclear area, ratio cytoplasm/nucleus, cellular roundness and nuclear roundness. SPSS 7.5 was used in the statistical analysis. We carried out a variance analysis with the Bonferroni correction for the multiple comparison of means, which was considered to be statistically significant $p<0.01$.

CONCLUSIONS: We have found significant differences among all the zones of the oral mucosa related to all the parameters except nuclear roundness. This would indicate that when doing exfoliative cytology studies of the oral cavity, we should take into account the area studied with its characteristic values.

A. 3 OPTICAL DENSITOMETRIC PARAMETERS IN THE EXFOLIATIVE CYTOLOGY OF NORMAL ORAL MUCOSA RELATED TO AGE

F.J. Jorge Barreiro, Juan Suárez Quintanilla, Darío Pose Nieto, Teresa Jorge Mora and Isaac Fuentes Boquete

Departamento de Ciencias Morfológicas. Facultad de Medicina. Universidad de Santiago

INTRODUCTION: Exfoliative cytology is a quick, inexpensive and non-invasive diagnostic tool, which makes it highly useful in clinical practice. The morphofunctional alterations of cell atypia or any other cell dysfunction may be quantified with a quantitative analysis through exfoliative cytology. Functional alterations (increased keratinization, nuclear hyperchromatism, chromatin clumping) can be quantified with a densitometric analysis.

OBJETIVE: The purpose of this study was to examine the behaviour of optical densitometric parameters of normality related to age.

METHODS: Thirty three patients, ranging between five and seventy five years of age, free of general and oral pathology, were studied. The smear was carried out with the Cytobrush(R) and fixation with Labofix(R). The smearrs were stained with Hematoxylin Eosin and the analysis carried out with a semiautomatic image analyser (Microimage 3.0). The following parameters were studied: optical density of the cytoplasm, optical density of the nucleus, red density of the cytoplasm, blue density of the cytoplasm and green density of the cytoplasm. We used SPSS 7.5 in the statistical analysis. The correlation of the variables was determined by estimating Pearson's (normal distribution) or Spearman's (abnormal distribution) correlation coefficient. It was found to be statistically significant $p<0.01$.

RESULTS: The mean value of the optical density of the cytoplasm is 143,68. The mean value of the optical density of the nucleus is 74,91. The correlation of optical densitometric parameters studied with age is very poor (correlation coefficient Rho of Spearman=0,147 for the optical density of the cytoplasm, and correlation of Pearson=0,195 for the optical density of the nucleus) although it has a high level of significance in this study (p=0,001).

CONCLUSION: There was a very low, but statistically significant correlation, between the parameters studied and age.

A. 4 MORPHOMETRIC PARAMETERS IN EXFOLIATIVE CYTOLOGY OF THE ORAL MUCOSA RELATED TO AGE

M. Quintáns Rodríguez, Darío Pose Nieto, Teresa Jorge Mora, Juan Suárez Quintanilla, Isaac Fuentes Boquete, Javier Jorge Barreiro

Departamento de Ciencias Morfológicas. Facultad de Medicina. Universidad de Santiago

INTRODUCTION: Exfoliative cytology is a quick, inexpensive and non-invasive method of diagnosis, which makes it very useful in clinical practice. Great disparity exists in values of normality in morphometry. Therefore we have examined these values and have attempted to relate them to age.

METHODS: The study was carried out on thirty three patients, free of general and buccal pathology, ranging in age between five and seventy five years. The smear was carried out with the Cytobrush(R) and fixation with Labofix(R). The smears were stained with Hematoxylin Eosin and the analysis was done with a semiautomatic image analyser (Microimage 3.0). The following parameters were studied: cellular area, cytoplasmic area, nuclear area, ratio cytoplasm/nucleus, maximum cellular diameter, minimum cellular diameter, mean diameters of the cell, cellular perimeter, maximum, minimum and mean diameters of the nucleus, nuclear perimeter, cellular roundness, cellular appearance, roundness and appearance of the nucleus. SPSS 7.5 was used in the statistical analysis. The correlation of the variables was determined by estimating Pearson's (normal distribution) or Spearman's (abnormal distribution) correlation coefficient. Our findings were compared with the previous results.

CONCLUSION: There was a very low correlation; which is statistically significant ($p<0,01$), between cellular roundness, mean nucleus diameter and age. We also found a very low, but statistically significant ($p<0,05$) correlation between the area, maximum diameter, perimeter and age of the nucleus.

A. 5 COMPARATIVE STUDY OF THE GUSTATORY PAPILLAE OF HUMANS AND RATS

C.A. Chamorro, A. Bengoa, B. Peláez, C. García and L. Anel

Dto. De Anatomía y Biología Celular. Facultad de Veterinaria de León

The mucosa of the tongues of mammals displays a highly differentiated papillary system. The papillae are disseminated over the surface of the tongue and have two specific functions: gustatory and mechanical. The gustatory papillae show pores and taste buds, whereas mechanical papillae play a role in protecting the surface of the tongue, food prehension, chewing, etc. In this work we analyse the gustatory papillae of the tongue of humans and rats, exploring their morphological characteristics by means of scanning electron microscopy (SEM). The most characteristic features of the papillae are described and their respective analogies, differences and possible morphofunctional correlations are discussed. This is of interest because the rat is frequently used as a model in animal experimentation.

The vallate papillae of humans show differences with respect to those of the rat as regards number (8-10 *vs* 1, respectively); diameter (1-2 mm *vs* 0.6-0.35 mm, respectively) and the presence of a complete pad surrounding them and the existence of round pseudopapillae on their surface. In both species, gustatory pores have been found in the papillary sulcus. At high magnification, numerous micropits can be seen on the papillary surface in humans (keratinization) and microplicae on the papillary surface in the rat.

Humans have three types of fungiform papillae: barrel-shaped, dome-shaped and club-shaped. Their dimensions vary between 100 and 250 μm in diameter (sometimes reaching 800 μm). In both species it is possible to observe a papillary base and an upper portion featuring a varying number of gustatory pores. At high magnification, the base shows a pattern of microplicae (only slight keratinization) whereas the upper part displays micropits. The fungiform papillae of the rat are located on the anterior portion and lateral parts of the tongue. They show a body (100-150 μm) and a base. At the surface is a 15-20 μm diameter circular zone which may or may not have a single gustatory pore. At high magnification a keratinization pattern similar to that found in humans can be observed.

The foliate papillae of humans appear as elongated projections arranged as parallel plicae or papillary leaves measuring 500-700 μm in width and varying in length and separated by sulci 50-100 μm wide. The gustatory pores open into the papillary sulci. At high magnification microplicae can be seen. In the rat these papillae appear as sulci measuring 200-300 μm in length, inside which the gustatory pores open. At high magnification microplicae and micropits can be seen.

The vallate papillae have a gustatory function in both species, although they are larger in humans, where they also show a more keratinized epithelium, more exposed to the friction of food. The fungiform papillae are similar in humans and rats but gustatory functionality is more pronounced in humans where many gustatory pores can be seen that may not be present in the rat. Also, the foliate papillae show a characteristic morphology in humans and are more rudimentary in the rat.

A. 6 COMPARATIVE STUDY OF THE MECHANICAL PAPILLAE OF THE TONGUE OF HUMANS AND RATS

C. García, A. Bengoa, C.A. Chamorro, B. Peláez and L. Anel

Dto. De Anatomía y Biología Celular.Facultad de Veterinaria de León

The mechanical papillae are widely distributed across the surface of the tongue and play a protective role and also help in forming the food bolus. In certain species, they also help in food prehension. Although they are generally known under the term filiform papillae, studies with scanning electron microscopy (SEM) have revealed the existence of a considerable degree of morphological variations in some species.

In the present work, we performed a comparative analysis of the mechanical papillae of the tongue of humans and rats by SEM with a view to discerning morphological differences and similarities and possible functional correlations, considering that the rat is frequently used as a model in animal experimentation.

The filiform papillae of the human tongue have a papillary body ranging between 200 and 500 μm in diameter out of which emerge 6-10 papillary hairs of 260-360 μm in length and 30-60 μm in width. The ends of these projections display an epithelium covered by micropits, responding to a keratinization pattern brought about by greater contact and stronger erosion by the food bolus.

On the rat tongue it is possible to discern three types of mechanical papillae on the basis of their size and morphological characteristics: the small conical type (100-150 μm in height), which are located on the anterior third of the tongue and have a gently flattened conical shape, arched caudally; large conical papillae (200-250 μm), present on the intermolar eminence and showing a strong horny tip, and true filiform papillae, which are located at the back of the tongue and out of whose body (50-100 μm in diameter) emerge 2-6 thin backwards-curving hairs (5-6 μm in width and 60-100 μm in length). In some zones of the intermolar eminence, papillae similar to the large conical variety can be seen although their morphology is intermediate to that of the true filiform variety. At high magnification, the epithelial cells of these papillae show a keratinization pattern that varies as a function of their position on the papilla itself: the farther back or inferior the position of the epithelium, the lower the degree of keratinization and the greater the number of microplicae. By contrast, the more anterior and superior the papilla, the greater the keratinization and hence the greater the number of micropits.

On comparing the mechanical papillae in humans and rats, we have confirmed the morphological differences between both species. However, both human filiform papillae and the small conical papillae of the rat have a similar distribution pattern and in both species surround the fungiform papillae, suggesting a protective role. In the other hand, morphologically a similarity can be seen between human filiform papillae and the true filiform papillae in the rat. The projections or papillary hairs of human filiform papillae spread across the back of the tongue, creating a surface that facilitates mastication, bolus production and later swallowing. In the rat, swallowing is facilitated by the slight posterior inclination of the true filiform papillae while the small conical papillae participate in the capture and prehension of food. The large conical papillae located on the intermolar eminence of the rat tongue participate in chewing and bolus production since they help to further degrade the food already ground by the teeth.

A. 7 ANALYSIS OF THE EXPRESSION OF HUMAN CD38 AND OF ITS LIGAND CD31 IN GASTRO-INTESTINAL AND BRONCHO-ALVEOLAR TRACTS

J.E. Fernández[1], A.E. Aránega[4], J.A. Marchal[2], J.C. Prados[3], M.A. Vélez[3], C. Melguizo[3], E. Carrillo[1], H. Boulaiz[1], L. Álvarez[1] and A. Aránega[1]

[1] Departamento de Ciencias Morfológicas, Facultad de Medicina, Univ. Granada. [2] Departamento de Ciencias de la Salud, Univ. de Jaén. [3] Departamento de Ciencias de la Salud, Univ. Almería. [4] Departamento de Biología Experimental, Univ. Jaén

INTRODUCTION: Human CD38 is a 45 kD ectoenzyme endowed with ADP-ribosyl cyclase and hydrolase activities. The molecule plays a central role in lymphocyte activation, proliferation and selectin-type adhesion with endothelial cells (HEC). A HEC surface molecule displaying all features of a CD38L has been identified by means of a mAb (Moon-1), able to block CD-38 mediated adhesion processes. The 130 kD molecule recognised by Moon-1 is CD31, a member of the Ig superfamily.

MATERIALS AND METHODS

Specimen preparation. The study included 60 specimens derived from gastro-intestinal and bronchi-alveolar tracts obtained following ablative surgery of various malignancies.

Cell preparation. LPL were isolated from normal or diseased (inflammatory bowel disease, IBD) mucosa obtained from patients undergoing bowel resection according to a modified methods of Bull and Bookman. Broncho alveolar lavage (BAL) cells were obtained by fiberoptic brochoscopy. T cells were purified by negative selection procedures. The mAbs used in this study were IB4 (anti-CD38) and Moon-1, an IgG1 recognizing surface molecule acting a ligand for CD38 and further characterized as CD31. Another mAbs were included as isotype-matched controls.

Immunophenotyping of LPL mand lymphocyte subsets in BAL, LPL o LP T cells (105/sample) were stained by means of an indirect immunofluorescence assay and analyzed using a FACSort equipment.

Alkaline immunophosphatase and immunoperoxidase techniques. The tissue sample blocks were prepared as previously by the authors.

RESULTS: The analysis of the digestive tract included samples from the stomach, and from the small and large intestine. The mucus-secreting epithelial cells of the stomach and columnar epithelial cells of the small and large intestine are CD38-, as well as the mucus-secreting goblet cells in the intestinal lining and secretory epithelial cells forming the mucosa glands. On the contrary, the underlining lamina propria layer expresses the CD38 molecule at high epitope density, the reactivity pattern of the Moon-1 mAb is confined to vascular and lymphatic HEC. On LPL, results indicate that CD38 expression is increased as compared to circulating lymphocytes purified from the same patient, the molecule being present on the majority of T and B lymphocytes.

The analysis of the lung and bronchi show that CD38 displays a small spot distribution, likely attributable to normal leukocutes present in the interalveolar spaces. Apart from HEC cells located in the inter-alveolar space, the CD31 molecule is also expressed by some of the cells forming the alveolar ducts and alveoli. The anti-CD38 mAb IB4 clearly stains bronchial cells and rare submucosal cells of unclear origin, while the Moon-1 mAb reactivity is limited to the vascular structures. On BAL lymphocytes, CD38 expression is considerably lower than in any other lymphatic population examined, while CD31 is present mostly same percentage observed for circulating mononuclear cells.

DISCUSSION: The aim of this paper is to draw information on the biological relevance of the CD38/CD31 system in vivo by completing the studies on their distribution throughout the human body. In the lymphatic district, the relevant observation is that CD38 is expressed at high density by the cells of the lamina propria in the digestive tracts This envisage a role for CD38 in the immunology of stomach, small and large intestine, whose relevance as an immunological organ is increasing rapidly.

Finally, anti-CD38 mAbs displayed a small spot reactivity when incubated with lung tissue, likely referable to lymphatic elements normally present in the respiratory

system. As in the case of intestine, this was confirmed by the analysis of CD38 expression on leukocytes obtained from BAL The conclusions of this work indicate that definite T cell subsets, as well as B and myeloid cells co-express CD38 and its putative ligand. What remains to be elucidated is the significance or the function of the two molecules when co-expressed by the same cell; in this respect, the regulation of the catalytic activities of CD38 by means of aggregation phenomena is a suggestive clue.

A. 8 ANATOMY OF THE PERITONEAL CAVITY

M.C. Mias, R. Sola, A. Dealbert, R. Casals

Facultad de Medicina, LLeida

The anatomical observation of the peritoneal cavity in the cadaver has always presented difficulties for the alteration of the anatomical structures due to the conservation procedures. The intraabdominal viscera not only lost their elasticity, their capacity of movement and their colouring but rather it also exists a real loss of the spaces an alteration of the compartments, a retraction of the epiplon with a distortion of the real vision of the peritoneal cavity.

The apparition of new surgical techniques as the laparoscopy permit us the observation "in vivo" of the peritoneal cavity without conservation procedures.

The authors present comparative images of the peritoneal cavity coming from anatomical dissection in cadaver and other obtained by means of laparoscopy during the surgery of the peritoneal cavity.

An easy and understanding anatomical situation of the real abdominal organs with its relations, its movements and its real colouring is allowed.

The laparoscopy is a new technical procedure that could become very important in the teaching of the anatomy because it offers many advantages, as the anatomy in alive, specially in a difficult anatomical region as the peritoneal cavity.

A. 9 ANATOMICAL ROUTES IN THE TREATMENT OF THE INGUINAL HERNIA

R. Sola, M.C. Mias, A. Dealbert, R. Casals

Facultad de Medicina, LLeida

Among the hernias (protrusion of intestinal content for an anatomical hole) the inguinal hernia is one of the most frequent pathology of the inguinal region in the human being and its treatment has always been discussed

The surgical treatment of the inguinal hernia has always been based on the concept of correct and reinforce the defect of the posterior wall of the inguinal region and a lot of different techniques have been used along the years. The anatomical ways of access used are different depending of the procedure. So we can distinguish anterior and posterior techniques.

The laparoscopy is a new surgical technique that permit us to consent directly to the posterior wall of the inguinal region by a posterior access at distance of the pathological area without increasing the destruction of the anatomical structures in order to find the preperitoneal space.

The two current laparoscopical hernioplasties are different because one of them reach the preperitoneal space traversing the peritoneum, is the transabdominopreperitoneal hernioplasty and the other one enters directly in this preperitoneal space locating you between the fascia transversalis and the peritoneum.

The authors describe thanks to their experience as surgeons the different anatomical access way in the surgical treatment of the inguinal hernia reflecting the anatomical differences among all them.

The anatomical routes in the treatment of the inguinal hernia are different but the objective is always the same:to reach the preperitoneal space in order to repair the posterior wall of the inguinal region.

A. 10 ARTERIOGRAPHIC ANATOMY OF TIROCERVICAL TRUNK

Alfonso López-Muñiz[1], Mercedes Villar López[2], Luis Carlos Hernández[1], Florentino Vega[2], Esperanza Bengoechea[1]

[1] Morphology and Cellular Biology Department. Faculty of Medicine. University of Oviedo. [2] Radiology Department. Central Hospital of Asturias. Spain

INTRODUCTION: The tirocervical trunk of Farabeuf is a branch of the subclavian artery. This arterial trunk is a wide and short trunk that arises from the front of the first part of subclavian artery, distal to the vertebral artery, at the medial border of the scalenus anterior muscle. Classically, it gives off three branches: the inferior thyroid (generally with the superior cervical artery), the superficial cervical and the suprascapular arteries. However, numerous variants in the morphology (the existence of an infundibular dilation) and in the number of branches in the tirocervical trunk were observed.

The perfect knowledge of these variants is fundamentally, now that this region has a great clinical interest.

MATERIAL AND METHODS: 635 arteriographic studies of the thyrocervical trunk were revised. In total 880 subclavian arteries were evaluated, since in 161 patients the arteriographies were not rateable and in 68 patients a side could only be valued (44 the right arteries and 24 the left arteries).

The range of ages was between 15 and 80 years, being 356 men and 119 women.

All the arteriographies has been carried out with technique of digital subtraction, in a CGR team, DG 200 model, with 512x512 reconstruction.

RESULTS: Our results confirm the high anatomical variability of the thyrocervical trunk, being the similar frequencies in those that were observed: a trunk with three branches, a trunk with two branches or the trunk absence:

1.- The classical morphology with three branches has only been observed in 36% of the cases in the right side and in 35,5% of the arteries in the left one.

2.- The presence of a trunk giving off two branches was founded in 39% of the right subclavian arteries and in 29,5% of the left subclavian arteries.

3.- The existence of independent branches, beginning directly in the subclavian arteries (with absence of arterial trunk), was demonstrated in 25% of the right subclavian arteries and in 35% of the left arteries.

The presence of diverticulum in 2.6% of the patients was observed.

DISCUSSION: The tirocervicalscapular trunk is the branch of the subclavian artery with more anatomical variants. In previous dissection studies, the trunk tirocer-

vical in less than half of the bodies were observed: 46.5% (Daseler and Anson, 1959). Similar percentages are described by means of radiographic techniques (Abrams and Jönsson, 1983; Kadir, 1991).

Our study ratifies the high variability in this branch of the subclavian artery.

A. 11 IDENTIFICATION OF BENDING MODES OF HUMAN RADIUS AND ULNA. INFLUENCE OF SOFT TISSUES AND JOINTS

M. Moreno Cascales, M.A. Fdez-Villacañas Marín, G. Doménech Ratto and P.L. Martínez García

Dpto. C. C. Morfológicas y Psicobiología. Facultad de Medicina. Universidad de Murcia

In order to stablish the resonance frequency of the human radius and ulna as well as the mode shapes we applied the impulse frequency response technique on dried bone and embalmed cadaver.

For the present work we used 4 dry radius, 5 dry ulnae and 2 embalmed forearm. On the forearm we did a progressive dissection doing five measurements (intact forearm, removed skin, removed muscles, removed interosseous membrane and exarticulation of the proximal radioulnar joint). With modal analysis on each bone we obtained the frequency response, the damping and the associated shape modes.

The obtained mean resonance frequencies for dry ulnae were 429 and 550 Hz; and for dry radius were 655 and 1000 Hz. The single bending modes were obtained from the medio-lateral and anterior-posterior planes for ulnae and only from the medio-lateral plane for radius.

The obtained mean resonance frequencies for ulna of the embalmed forearm were as follows: Intact forearm 375 Hz, removed skin 410 Hz, removed muscles 275 Hz, removed interosseous membrane 220 Hz and exarticulation of the proximal radioulnar joint 410 Hz. The shape modes associated for that frequencies were a single bending mode at medio-lateral as well as another at the anterior-posterior planes.

Our results are in agree with that obtained by Jurist, Petersen and Steele in relation with the frequencies obtained and the shape modes.

A. 12 INFLUENCE OF ISIKINETIC VELOCITY ON THE KNEE JOINT PEAK TORQUE RATIOS

M.E. Del Valle, M.B. Calzada, B. Méndez and G. Sirgo

Escuela de Medicina del Deporte. Dpto. Morfología y Biol. Cel. Universidad de Oviedo

INTRODUCTION: Muscles surrounding joints, beside being active organs of movement, complete a second important function, that is the stabilisation.

The sportsman's muscles behaviour in connection with force and work balances is a great important topic in prevention and treatment of many lesions. These relationships or muscular balances are usually established in form of ratios.

By means of biomechanical studies, correlations among several muscular groups in the lower limb have been provided, and, particularly, a coactivation between agonist and antagonist muscles of a joint, but the findings present marked variability in function, possibly, of the movement speed carried out during the evaluation. Speed can influence on the flexion/extension strength ratios, although the studies carried out until the present time isn't clear.

The objective of this study is to analyse if the speed carries out during a isokinetic evaluation may influence on maximal torque ratios.

METHODOLOGICAL PROCEDURES: We have carried out isokinetic evaluations in three moments of the season, about the flexor-extensor apparatus of both knee joints at 60°/seg. and 300°/seg. in 30 senior athletes of national category (10sprinters, 10 javelin throwers, and 10 long jumpers). The concentric and eccentric contractions were evaluated. The evaluations were carried out in the following moments: final of generic training (1ª), at the beginning of competitive phase (2ª) and final of season. An isokinetic dynamometer Kin-Com (Chattanooga Group Inc.) was used. Informed consent was obtained from all subjects included in the study.

In the statistical analysis non parametric tests were used.

RESULTS

1. Concentric quadriceps to eccentric hamstrings maximal torque ratios (EX).

They are lower at 300°/seg., existing in all the cases significant differences. The means values of ratios including to all the sportsmen and the three evaluations carried out at 300°/s is 0.7705 ± 0.1458 in the right knee and 0.7752 ± 0.1593 in the left knee, and in evaluations carried out at 60°/seg., 1.2886 ± 0.2802 (right knee) and 1.2921 ± 0.2436 (left knee).

2. Concentric hamstrings to eccentric quadriceps maximal torque ratios (FL).

Except for an isolated value (third evaluation in sprinters) ratios are higher at slow speed, only existing in some cases statistical significance. The means values of ratios including all the sportsmen and the three evaluations carried out at 300°/seg. are 0.3806 ± 0.1064 (right knee) and 0.3861 ± 0.1047 (left knee); and evaluations carried out at 60°/seg., 0.4476 ± 0.1067 (right knee) and 0.4215 ± 0.1049 (left knee).

In conclusion, the evaluation speed influences clearly in maximal torque functional ratios (flexion and extension), for what this parameter should be considered when valuing these ratios.

A. 13 ANASTOMOSIS BETWEEN THE EXTERNAL AND THE INFERIOR OR RECURRENT LARYNGEAL NERVES

J.R. Sañudo, E. Maranillo, X. León[1], R.M. Mirapeix and M. Quer[1]

Unidad de Anatomía y Embriología. Universidad Autónoma de Barcelona. [1]Departamento de Otorrinolaringología. Hospital de Sant Pau. Barcelona

It has recently 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 con-

sidered as an extension of the sensory innervation to the subglottic area. This neural anastomosis has been reported in small samples with a prevalence of 6% to 44%. Due to important role that anastomosis can play in the knowledge of the neurophysiology of the larynx, the goal of this work is to establish its prevalence and morphological details in a large sample.

A total of 90 human larynges obtained from necropsies (57 males and 33 females, age range from 41 to 95 years) were examined by careful dissection using a surgical microscope.

The anastomosis appeared in 88.8% of larynges (bilateral in 58.8% and unilateral in 41.2%). The anastomosis appeared as one (74.8%), two (18.9%) or three branches (4.7%). The joining point of the anastomotical branch/s with the RN was very variable, spanning from the point in which it gave off the muscular branch to the arytenoid muscle to the point in which it pierces the thyroarytenoid muscle. The anastomotical branch gave off the articular branch to the cricothyroid joint (47%) and a sensory branch to the anterior surface of the subglottic area (50.3%).

The different prevalence and morphology of this complex anastomotical pattern suggest functional differences in relation to the sensory and motor innervation of individual subjects as has been pointed out by other authors in experimental studies.

A. 14 ROLE OF CADMIUM IN FIBRONECTIN PROTEIN ON A CULTURED SMOOTH MUSCLE LAYER

A. Aranega[1], J.A. Marchal[2], C. Vélez[3], J. Prados[3], C. Melguizo[3], A.E. Aránega[4], E. Carrillo[1], J.E. Fernández[1], H. Boulaiz[1], L. Álvarez[1] and A. Aránega[1]

[1] Departamento de Ciencias Morfológicas, Facultad de Medicina, Univ. Granada. [2] Departamento de Ciencias de la Salud, Univ. de Jaén. [3] Departamento de Ciencias de la Salud, Univ. Almería, [4]Departamento de Biología Experimental, Univ. Jaén

INTRODUCTION: After long-term culture, smooth muscle cells (SMCs) in the arterial media are modified from a contractile to a sympathetic phenotype. This process includes a prominent structural reorganization and makes the cells able to migrate into the intima, divide, and secrete extracellular matrix components. A similar change occurs in culture and then in vitro system has been established as a useful model in which to study the control of SMC differentiation. The purpose of this study was to analyze the expression of fibronectin in vascular SMCs during the 6 weeks in culture and related whith phenotype.

MATERIAL AND METHODS: SMCs were enzymatically isolated from chick aorta and cultured during long-term.The cells after different times of culture was analyzed.The expression of fibronectin was explored by FAS-can, Western blotting and indirect immunofluorescence microscopy.

RESULTS AND DISCUSSION: The results indicate that the fibronectin was strongly expressed for a few days, after two weeks fibronectin was decreased, at the end of the test period, the corresponding protein were deposited around the cells in a fibrillar pattern. Long-term culture led to alterations in total protein levels, as well as changes in protein levels, in the cytoplasmic and cytoskeletal fractions of cultured cells. Fibronectin was decreased in the cytoskeletal fraction. These findings suggest that the alterations caused by long-term culture in fibronectin protein content may be related with the change the phenotype. These result suggest that the alterations are secondary effects to long-term culture the smc.

A. 15 TRASPLANTE DE CONDROCITOS: "realidad o mito". ESTUDIO EXPERIMENTAL

L.C. Hernández, J.A. De la Fuente[2], A. López Muñiz, J. Iglesias[3]; P. Riera, M. Ojanguren[1], and M.A. del Brío

Dpto. Morfología y Biología Celular. Facultad de Medicina. Universidad de Oviedo.
[1]Servicio de Traumatología. Hospital Central. Asturias. [2]Servicios Médicos Sporting SAD. [3]Banco de Tejidos de Castilla y León

INTRODUCTION: The main problem in the treatment of articular lesions involves articular cartilage. The strategies used in order to solve this trouble include growth factor, artificial plastics, heterologous and autologous grafts. Nevertheless the articular cartilage obtained was morphological, biochemical and mechanical abnormal. The objective of the present study was to analyse whether transplantation of cultured chondrcoytes is able to rescue damaged articular surfaces.

MATHERIAL AND METHODS: The study was carried out in pig. Three experimental groups were considered: control animals (lesion induced without treatment; periositum grafted animals (the induced lesion is covered with perioustium alone); periostium + chondrocytes grafted animals (the induced lesion is treated with periostium graft plus chondrocytes). Animals were allowed to survive 3, 6 and 9 months. Samples from the articular cartilage were analysed by means of Magnetic resonance, surface observation microscopy, structural and ultrastrustructural techniques.

RESULTS: The time-related changes in the articular induced lesions with or without treatment are presented. The structural findings show that all articular cartilage layers are present at 9 months, and the ultrastructural analysis reveals that chondrocytes are active. Both surface microscopy and MR confirm the histological data.

A. 16 A STUDY OF SOMATOTYPES IN SUBJECTS WHO ARE DEPENDENT ON DRUGS AND WHO ARE IN THERAPEUTIC COMMUNITIES IN GALICIA

C. Lalín Novoa, M. Pimentel González, A. Pérez Caaveiro, M.P. Martínez Pereira, M.A. Saavedra García, M.T. Castaño Oreja, M.A. Giráldez García

INEF Pontevedra. Universidad de Santiago de Compostela

INTRODUCTION: A series of internal and external manifestations are associated with the consumption of drugs, all of which have a direct bearing on the subject's state of health. One of the effects which is evident is the physical deterioration of the person, not only as regards their internal organs, but also their external appearance, their perceptive capacity and their motor functions. Attempts to

improve their bodily appearance during rehabilitation are reflected in the preoccupation of the drug dependents with weight gain.

Recovery of physical appearance constitutes an objective for the subject in treatment, and it is also an indication of good progress. A valid instrument for quantifying these parameters is the determination of the somatotype. This study is of interest because there are no references in literature about the three components which determine the morphology of the subjects. Knowledge about this would permit the implementation of an appropriate programme of physical activity.

OBJECTIVES: 1) To establish the somatotype of the people in the therapeutic communities of the Autonomous Community of Galicia. 2) To define the influence of age and sex on the corporal morphology. 3) To determine the progress or modifications of the somatotype during the subject's stay in the therapeutic community. 4) To estimate the influence of the physical activity carried out in the centre, and it's bearing on the typology of the individuals.

MATHERIAL AND METHODS: 140 people were studied in the Therapeutic Communities of the Autonomous Community of Galicia. These people belonged to the Autonomous Drug Plan and Project Man: 123 men and 17 women (87.8% and 12.1%, respectively), whose average age was from 28.2 ± 4.5 years (28.4 ± 4.5 in men and 26.5 ± 4 in women). The details were obtained by means of an interview, a questionnaire, and the anthrompometic measurements recommended by the International World Group of Kinanthropometry and the Spanish Group of Kinanthropometry (weight, size, bone diameter, cutaneous folds and perimeters).

The Heath-Carter (1967) method was used to calculate the components of the somatotype. A statistics package, SPSS V. 8.0 for Windows was used for the treatment of the data, with the permission of the University of Coruña, thus carrying out a descriptive analysis of the variability study. We used Pearson's coefficient of lineal correlation for the calculations of the lineal association measurements, the T-test for the comparison between the averages of our independent samples.

RESULTS: An average value of somatotype of 2.9-4.2-2.1 (σ: 1.1-1.2-1) was found. In the men, it was 2.7-4.2-2.2 (σ: 0.9-1.3-1) and in the women 4.7-3.6-1.5 (σ: 0.9-0.8-06). A very low and statistically meaningless correlation was found with respect to age in both sexes. There is a low correlation (R-2.5, $p<0.05$) between the time of the stay in the centre and the mesomorphy in men. In women there is a greater correlation (R=0.55, $p<0.05$) with the endomorphy. As regards the practice of physical activity during the stay in the centre a coefficient of lineal correlation of 0.28 ($p<0.05$) was found with the mesomorphic component and of -0.25 ($p<0.05$) with the ectomorphy in the men. The latter component has a high correlation with the endomorphy and mesomorphy (R=-0.5 and -0.6, both with $p<0.001$). In women who didn't practice physical activity a coefficient of lineal correlation of -0.97 ($p<0.05$) was found between the time of the stay and the ectomorphy, which at the same time has a high correlation with the endomorphy (-0.8, $p<0.05$).

DISCUSSION: After an exhaustive examination we have not been able to find studies of this nature in literature. In our case, the values of the somatotype indicate that the individual drug dependents studied show an endo-mesmorphic typology (of an average value). Bearing in mind the extent of physical deterioration which they have when they are first admitted to the rehabilitation programmes, the values found constitute a good recovery of their morphology. The mesomorphic predominance could be due, among other factors, to the type of active life developed in a natural medium. As in the general population, the mesomorphic component predominates in men and the endomorphic component in women. Age did not constitute significant differences in the somatotype when we take the small range (18.5-39.9) into account. We have demonstrated that during the time of their stay in the community, the men increase their mesomorphy and the women their endomorphy. When physical activity is carried out, the men, besides increasing their mesomorphy, present a decrease in their ectomorphic component. This decrease is related to an increase of the other components. In the women who didn't carry out physical activity during their stay, the ectomorphic decreases and the endomorphic increases. This means that in general lineality decreased in the males and the mesomorphic and endomorphic components (muscle and fat) increased. The women also had a decrease in the ectomorphic but their endomorphic component (fat) only increased. This data indicates that physical activity has certain positive effects on the men, even if they are lower than those expected. In our study, the women didn't experience any benefit derived from the practice of the physical activity. This may well be due to the low number of cases studied.

CONCLUSIONS: The subjects, drug dependents who were admitted to the Galician therapeutic communities, have an endo-mesomorphic somatotype, with a predominance of the mesomorphic component in the males and the endomorphic in the females. The values don't vary with age but they do vary depending on the time of the stay at the centre, and the practice of physical activity. The ectomorphy decreases in both sexes and the mesomorphy increases in the men and the endomorphy in the women.

We would like to extend our thanks and gratitude to the management and the therapeutic team of the therapeutic communities (Aclad, Male Proyect, Alborada, Asfedro).

A. 17 THYROID GLAND SURGICAL ANATOMY

A. Bernardes, S. Monte-Arroio, J. Dionisio and J. Patricio

To perform a thyroidectomy it is necessary a careful cervical dissection because of the gland pathological condition and in order to preserve: the parathyroid glands, the recurrent laryngeal nerve and the external branch of the superior laryngeal nerve.

This Video presents some images from four corpses dissection and some aspects related with three surgical thyroidectomies.

First, we describe the situation, relation and blood supply of thyroid glands previously injected with coloured gelatine through the superior and inferior thyroid arteries.

Identification and section of the pre thyroid fascia namely: the superficial cervical fascia, infrahyoid muscles fascia and the pre tracheal lamina (that is not the "real gland capsule"). The Video presents also the following thyroidectomy fundamental aspects:

– dissection very close to the gland (between the pre tracheal lamina and the real thyroid capsule);

– visualisation of the recurrent laryngeal nerve mainly near its penetration into the larynx, the site of greatest vulnerability at operation;

– identification and preservation of all the superior parathyroid glands situated midway along the posterior thyroid border, sometimes within the fascial glandular sheath, and the inferior parathyroid glands below and near the inferior lobar poles; individual branch vessels at the upper pole are ligated rather than by mass ligature minimising the risk of injury to the external branch of the superior laryngeal nerve.

In selected cases, after thyroidectomy we use fibrin sealant to prevent seroma formation.

A. 18 APPLICATIONS OF THE HELICAL TC IN COMBINATION WITH A WORKING STATION IN THE STUDY OF HUMAN ANATOMY

Tomás Sempere, Jové Montserrat, Miguel Torrente, Alejandro Castillo, Pedro Cobos and Verónica Piera

Unitat D'Anatomia Humana i Embriologia. Departament de Ciències Mèdiques Bàsiques. Facultat de Medicina I Ciències de la Salut. Universitat Rovira I Virgili

INTRODUCTION: The helical TC has signified an important advance in the images diagnosis. That can be applied not only in the differentiation human pathologies but as an excellent method for the teaching study of human anatomy.

OBJECTIVE: The aim of the present work is to report the experience in this field of the Human Anatomy and Embryology Unit of School of Medicine of Rovira i Virgili University, in collaboration with the helical TC unit of the Radiodiagnosis Service of the Joan XXIII University Hospital in Tarragona.

MATERIAL AND METHODS: In the last four years more than fifty thousand explorations with this method in conjunction whit the working station of Maximum Projection Intensity (MPI), Multiplane Reconstruction (MPR), and their virtual representation in 3D enable to obtain more than 2000 anatomic reconstruction.

RESULTS: The different anatomy images (non pathologic) of several organs and systems as well as congenital malformations have been applied in the training of pregraduate and postgraduate medical students and resident physicians.

A. 19 CONFOCAL LASER AND SCANNING ELECTRON MICROSCOPY STUDY OF THE ARTERIAL VESSELS IN HUMAN CEREBRAL CORTEX

F. Reina de la Torre[1], J. Sahuquillo[2] and A. Rodríguez Baeza[1]

[1]Departament de Ciències Morfològiques (Unitat d'Anatomia i Embriologia). Facultat de Medicina. Universitat Autònoma de Barcelona. Bellaterra. Barcelona. [2]Unitat de Neurotraumatologia. Hospitals Vall d'Hebron. Barcelona

INTRODUCTION: The vascular system plays an important role in different physiological and pathological processes of the central nervous system. Because of many of studies on cerebral circulation have been performed in experimental animals, both the microangioarchitecture and the anatomical structures involved in cerebral blood flow regulation in humans are not fully understood. By using scanning electron microscopy (SEM) of microcorrosion casts we have established the three-dimensional pattern of microcirculation in human cerebral cortex. By this technique and confocal laser scanning microscopy (CLSM) we have also studied the presence of perivascular structures and their possible role on blood flow regulation of the cerebral cortex.

MATERIAL AND METHODS: The present work was carried out on 20 fresh human brains obtained from patients from the Anatomical Forensic Institute of Barcelona. Their age ranged from 25 to 75 years. No patients with head injury or any other cerebrovascular pathology were included in this study. SEM of microvascular corrosion casts and CLSM of fluorescent-injected specimens were used. The polyester resin employed was undiluted Mercox®. In five cases the resin was previously stained with acridine orange. Thin sections (100 μm) from these specimens were obtained and further observed with a CLSM.

RESULTS: Four types of arterial vessels in the cerebral cortex were identified. They formed a cortical vascular network arranged in four vascular layers parallel to the pial surface. The greatest vascular density corresponded to the middle and deep vascular layers. Circular constrictions were found at the origin of cortical arteries. Also, arterial anastomoses were observed at their initial course.

Two perivascular structures associated with arteriolar and capillary vessels were found. Their morphology and distribution remember that of smooth muscle cells and pericytes. SEM showed that these structures were not tightly joined to the cast surface, but were connected to the vascular cast by narrow plastic bridges. CLSM demonstrated that the resin invaded the subendothelial space, thus originating these perivascular structures.

CONCLUSIONS: The blood supply to the human cerebral cortex depends on short, middle and long cortical arteries that occasionally show vascular connections. They give rise to a highly anastomosed capillary network that it is arranged in four vascular layers. Perivascular structures associated with arteriolar and capillary vessels appear to represent smooth muscle cells and pericytes. They are formed by the passage of the resin to the subendothelial space probably through weak endothelial cell junctions. The effusion of resin into this perivascular space may evidence the structural basis for myocyte and pericyte role in chemical cerebral blood flow autorregulation, that could be mediated by substances released locally or transported to these cells trough these junctions.

Supported by Fondo de Investigaciones Sanitarias (FIS 96/1783 and FIS 98/1385.

A. 20 MORPHOLOGICAL CHANGES AND ENDOTHELIAL CELL INJURY IN THE HUMAN CORTICAL BRAIN MICROVASCULARIZATION AFTER A SEVERE HEAD TRAUMA

A. Rodríguez Baeza[1], F. Reina de la Torre[1], M. Martí[2] and J. Sahuquillo[3]

Departament de Ciències Morfològiques (Unitat d'Anatomia i Embriologia). Universitat Autònoma de Barcelona. Bellaterra. Barcelona. Servei Microscopia Electrònica. Universitat Autònoma de Barcelona. Bellaterra. Barcelona. Unitat de Naurotraumatologia. Hospitals Vall d'Hebron. Barcelona

INTRODUCTION: Although mechanisms involved in the cerebral blood flow control are controversial it seems that their alterations could play an important role both in the increase of intracranial pressure found after a severe head injury and in loss of cortical brain ability to maintain the cerebral blood flow constant, specially when there is a brain swelling. The purpose of this study is to determine the morphological vascular changes of the cerebral cortex in patients who died after a severe head trauma, as well as to look deeply into those specific alterations of endothelial and periendothelial cells of microvessels that would justify the vascular injury observed in these patients.

MATERIAL AND METHODS: We have studied fifteen fresh human brains corresponding to patients died after a severe head injury and who had been previously monitored in the Neurotraumatology Intensive Care Unit of Vall d'Hebron University Hospitals. Morphological study of cortical microvessels has been performed both by scanning electron microscopy (SEM) of microcorrosion casts and confocal laser scanning microscopy (CLSM) after immunocytochemistry staining of endothelial cells. In order to detect cell injury and apoptotic cell death in the microvascularization, cortical brain sections (30-50 μm) of some specific areas were analysed using an in situ terminal deoxynucleotidyl transferase-mediated biotinylated deoxyuridine triphosphate nick end labeling (TUNEL), propidium iodide staining and immunocytochemistry of endothelial cell (by monoclonal antibody MAS-336).

RESULTS: The study by SEM showed significant alterations on the surface of vascular casts that affected both arteriolar and capillary vessels. Casts from cortical arterioles were characterised in many cases by the presence of evident longitudinal groovings and folds on their surface (corrugated arterioles) that reduced their diameter. This pattern did not affect the superficial traject of cortical arterioles but the intracortical, showing a transitional zone of morphological alterations. Cortical arterioles with a very eroded surface alternating with apparently undamaged zones were also present. In the capillary territory we have observed a great number of casts which showed a very irregular surface, with prominent folds and a decrease of the lumen diameter. They corresponded to capillaries mainly located at middle and deep capillary zones.

Immunocytochemistry showed important irregularities on the surface of endothelium of microvessels with enlargement of the vascular wall thickness and cytoplasmatic vacuolizations. TUNEL method and double staining with propidium iodide reveals specific apoptotic phenomena that affected some endothelial cells of these altered microvessels.

CONCLUSIONS: Morphological changes found by SEM on the surface of microcorrosion casts from patients who died after a severe head injury seem to reveal the important role of endothelium from arterioles (resistance vessels) and capillaries (interchange vessels) in the physiopathology of head injury. Morphological changes on microcorrosion casts look correspond with those alterations observed by CLSM. The cytoplasm of endothelial cells shows swelling and abundant vacuoles. TUNEL method has revealed the presence of apoptotic cell death in endothelium of these microvessels.

Supported by Fondo de Investigaciones Sanitarias (FIS 98/1385).

A. 21 THE EFFECT OF UNILATERAL MOLAR EXTRACTION ON THE CARTILAGE OF THE CENTRAL PORTION OF THE MANDIBULAR CONDYLES OF THE RAT

M.T. Castaño Oreja, Isaac Fuentes Bo-quete, Antonio Crespo Abelleira, Ángeles Rodríguez Cobos, Manuel Avelino Giráldez García, Miguel Saavedra García and Francisco Javier Jorge Barreiro

Department of Morphological Sciences. Faculty of Medicine and Dentistry. University of Santiago de Compostela

INTRODUCTION: It has been demonstrated that mechanical factors influence the development of the cartilage of the mandibular condyles. The purpose of this study is to evaluate the effect of unilateral molar extraction as a cause of biomechanical stress by checking the differences that exist in the histological zones of the cartilage of the mandibular condyles of adult rats whose teeth have been extracted and rats whose teeth have not been extracted.

MATERIAL AND METHODS: We have used 12 male Sprague-Dawley rats, who were five months old, distributed in two groups, one experimental (8 rats) and one control (4 rats). Both groups were subjected to the same living and feeding conditions. In the experimental group, the right upper and lower molars were extracted under deep anesthesia (chloral hydrate 4 %; 1 ml/100 g body weight).

After a survival period of 6 months all the animals were killed using an overdose of chloral hydrate and immediately afterwards the left and right temporomandibular joints were extracted in blocks. The samples were fixed in buffered formol-saline solution for a week and they were subsequently immersed in EDTA until demineralization had occurred. Following this, the samples were dehydrated and embedded in paraffin and then coronal cuts of 5 microns were made. The sections were stained with hematoxylin-eosin and Weighert's hematoxylin-fast green-safranin O. In the central portion of the condyle (area of maximum thinning of the meniscus), we compared the histological zones of the secondary cartilage of the right and left experimental condyles and the condyles of the experimental and control groups.

RESULTS: In the control group there is no secondary cartilage in the lateral and medial quarters of the condyle and the maximum thickness has a superlateral disposition. In the experimental group the secondary cartilage has a greater extension, the thickness is notably lower in the contralateral condyle and is lower or similar to the control in the ipsilateral condyle. The location of the maximum thickness is central in the ipsilateral condyle, while it is medial in the contralateral. The subchondral trabeculation is more intense and verticalized in the experimental group than in the control group.

There is greater cellularity in all of the layers of the experimental cartilage and this becomes more evident in the proliferative zone.

The zoning of the contralateral cartilage varies with respect to the cartilage of the control group and the ipsilaterals in that it is thicker in the proliferative area; the area of greater maturation is not as thick, it is less radialized and has a lower chondrochite differenciation; the thickness of the lower maturation zone is practically reduced by half.

The zoning of the secondary cartilage of the ispsilateral condyles varies according to the controls and the contralaterals as regards the fact that they demonstrate grea-

ter radialization and that this affects the proliferative zone, which could be called a transition zone. The thickness of both mature zones is greater in the contralaterals, and is more evident in the lower one where the values are almost doubled. In the deep portion of the mature inferior zone there are cells which are larger and are slightly hypertrophied.

CONCLUSIONS: There is a modification of the support points in both of the experimental condyles. The greatest biomechanical stress appears to affect the contralateral condyle thus causing a greater advancement of the subchondral.

A. 22 ANTHROPOMETRIC STUDY IN URBAN, SEDENTARY TEENAGERS BETWEEN 15 AND 19 YEARS OLD FROM BILBAO (BASQUE COUNTRY)

J. Gondra del Río[1], M.D. Cabañas Armesilla[2] and A. Angulo Gutiérrez[3]

[1]Instituto Médico Basurto, Fac. de Medicina y Odontología. Univ. del País Vasco. Euskal Herriko Univertsitatea. [2]Dpto. de Ciencias Morfológicas II. Fac. de Medicina. Univ. Complutense. Madrid. [3]Centro Médico Bailén. Bilbao

INTRODUCTION: The anthropometry is an uninvasive method (universally applied), to assess the proportions and composition of the human body, and to signs health as well as nutritional status. An anthropometric study in sedentary (less than 2,5 hours of physical exercise per week) students from the city of Bilbao (Basque Country), between 15 and 19 years old, was carried out.

MATERIAL AND METHODS: 25 males and 58 females with an age ranged between 15 and 19 years old were studied, and 17 direct anthropometric variables were analyzed. The Heath-Carter anthropometric somatotype, the Body Mass Index (B.M.I.), and the profile and sum of skinfolds, were studied and analyzed statistically, using the Excel programme.

RESUTS: The mean and standard desviation of Body Mass Index was 22.56 ± 3.98 for males and 21.47 ± 2.88 for females.

A grade I or grade II overweight was founded in the 24% of males subjects. These grade of overweight was founded in the 11 % of the females. A normal weight or even less than normal, was founded in the 76 % of boys and the 89 % of girls.

The mean of somatotype was (4.78, 4.27, 2.92) for males and (5.90, 3.11, 2.68) for females: In both (males and females) Endomorphy is dominant and Mesomorphy is greater than Ectomorphy (Mesomorphic - Endomorph).

The mean and standard deviation of sum of 6 skinfolds (in mm.) was 108.34 ± 45.13 for males and 141.53 ± 35.45 for females.

The profile of skinfolds is, according to skinfolds thickness, from major to minor: abdominal, front thigh, subescapular, supraspinale, medial calf and tricipital, in males, and front thigh, medial calf, abdominal, tricipital, supraspinale and subescapular in females.

DISCUSSION: According to Body Mass Index the male people of our study are 20% with overweight, and this percentage is different that the results obtains in the study of Seidell (1997) in European adult men between 35 and 64 years old (48.8 ± 4.1 in overweight, and 15.5 ± 4.2 in obesity). These changes can't be explained only for the age. In the female people, our study showed a 9% overweight, and 2% in obesity, very different and clearly minor that the range in European adult women between 35 and 64 years old, with 34.6 ± 4.5 overweight, and 21.7 ± 9.1 in obesity. The mean somatotype of males and females is Mesomorphic-Endomorph similar to the balance obtains in the study of Rebato, 1996.

A. 23 URETHRA STUDY OF THE RAT WITH RAPID EPOXY ADHESIVE AND DISSECTION UNDER MICROSCOPE

A. Bernardes, J. Dionisio, S. Cabrita, R. Álvarez and L. Furtado

Facultade de Medicina da Universidade de Coimbra. Portugal

INTRODUCTION: This study is realised because the characterization of the urethra of the rat is difficult in the male.

PURPOSE: To investigate the conformation and relation of the urethra of the rat for future works in carcinogenesis experimental of the bladder.

MATERIAL AND METHODS: 10 rats male and 10 rats female F344 were sacrificed and after the dissection the urethra was measured after injection the rapid epoxy adhesive "Plico – Ceys- Barcelona by puncture of the bladder with abocat Fr 14.

RESULTS: The video shows the relations of the urethra with other structures of the pelvis. Proves that the female urethra of the rat is easy for characterization when in the case of the male is very difficult and makes traumatise because structure and curvatures natural of the urethra.

CONCLUSION: The authors recommend only the female rats for characterization retrograde of the urethra for anatomic reasons.

E. 1 DEVELOPMENT OF THE HUMAN ELBOW JOINT

J.A. Mérida-Velasco, Indalecio Sánchez-Montesinos, Joaquín Espín Ferra, José F. Rodríguez Vázquez[1], José R. Mérida Velasco[1] and Juan Jiménez Collado[1]

Human and Experimental Embriology Research Group, Department of Morphological Sciences, University of Granada, Granada, Spain; and [1]Department of Morphological Sciences II, University Complutense of Madrid, Madrid, Spain

INTRODUCTION: Many studies have been published on the development of the human elbow joint, but different authors disagree on its morphogenetic time-table. Most discrepancies center on the cavitation of the elbow joint (including the humeroradial, humeroulnar and superior radioulnar joints), the organization of the tunnel of the ulnar nerve, and the elbow ossification.

MATERIAL AND METHODS: Forty-nine human embryos and fetuses from the Embryo Collections of the Departments of Morphological Sciences of the Universities of Granada and Madrid (Universidad Complutense) were used in the present study. Crown-rump (C-R) length, plane of section, and stage of development (O'Rahilly and Müller, 1987) are shown in Table 1. The usual laboratory

procedures were used to prepare 10-15 mm-thick transverse or sagittal serial sections, which were stained with hematoxylin-eosin (McManus and Mowry, 1968) for light microscopic study.

RESULTS:

1. *The skeletal elements of the elbow joint*

The tunnel of the ulnar nerve began to form at the posterior aspect of the medial epicondyle in O'Rahilly stage 20.

The skeletal elements of the elbow joint began to ossify during week 12 of development. The process began at the distal end of the humerus, spread to the trochlear fossa of the ulna, and concluded in the proximal end of the radius.

2. *The elbow joint cavity*

In our material the interzone of the humeroradial and humeroulnar joints were first found in O'Rahilly stage 18. In O'Rahilly stage 20, the humeroradial and humeroulnar interzones appeared as three-layered structures. At the end of O'Rahilly stage 21, cavitation began in these two interzones. Our observations show that cavitation of the elbow joint commenced in the part of the humeroradial interzone closest to the ulna, and in the part of the humeroulnar interzone closest to the radius.

In O'Rahilly stage 23, cavitation of the proximal radioulnar joint commenced. This process occurred independently from the rest of the elbow joint cavities, first becoming evident between the annular ligament and the head of the radius that appeared, and fused secondarily with the rest of the elbow joint cavity.

The deep aspect of the joint capsule and overlying superior rim of the annular ligament gave rise to a triangular protuberance with its apex pointing into the joint lumen toward the humeroradial joint cavity. This prominence appeared to fill the space between the opposed surfaces of the bones.

3. *The elbow joint ligaments*

The joint capsule first became identifiable in O'Rahilly stage 18, and was clearly visible by O'Rahilly stage 20.

We found the first signs of the annular ligament at the end of O'Rahilly stage 21, when it appeared as a condensation slightly separated from the deep surface of the joint capsule.

In O'Rahilly stage 22 the ligamentum quadratum of Denucé first appeared as an area of condensed mesenchyme at the floor of the proximal radioulnar joint. From O'Rahilly stage 23 on, this ligament and the annular ligament became clearly distinguishable. In addition, we found that at the beginning of the fetal period (week 9 of development), the joint capsule became anchored to the annular ligament, thus freeing the head of the radius. During week 12 of development the annular ligament and ligamentum quadratum of Denucé were both easily identifiable. The other ligaments were not well defined at this stage, appearing as areas of somewhat more condensed mesenchyme.

DISCUSSION: The interzone of the humeroradial and humeroulnar joints was first found in O'Rahilly stage 18. However, O'Rahilly and Gardner (1975, 1978) and Wadsworth (1982) observed the interzone, consisting of a homogeneous area of densification, in O'Rahilly stage 19.

In agreemet with Haines (1947), in O'Rahilly stage 20 the humeroradial and humeroulnar interzones appeared as three-layered structures. At the end of O'Rahilly stage 21, cavitation began in these two interzones. Our findings contrast with those of many authors who noted the first signs of cavitation in the elbow joint at different times of development. Andersen (1962) reported that cavitation of the elbow joint was first evident in human embryos between 28 and 33 mm (O'Rahilly stage 23); O'Rahilly and Gardner (1975, 1978), O'Rahilly et al. (1981) and O'Rahilly and Müller (1996) reported that cavitation commenced in stage 32 (O'Rahilly stage 23); Wadsworth (1982) found the first signs of this process in 31-mm specimens (O'Rahilly stage 23) in the humeroulnar joint, and in 34-mm fetuses (week 9) in the humeroradial joint. Haines (1947) noted that in 34-mm specimens (week 9) the humeroradial joint cavity was well formed, and that the joint cavity had also begun to form between the head of the radius and the annular ligament.

Our observations show that cavitation of the elbow joint commenced in the part of the humeroradial interzone closest to the ulna, and in the part of the humeroulnar interzone closest to the radius. This contrasts with findings reported by Davies (1950) and Andersen (1962), according to whom cavitation began in the central part of the humeroradial joint and spread thenceforth toward the edges. However, Mitrovic (1978) claimed that cavitation occurred simultaneously with the appearance of small grooves in the outer and central parts of the interzone, and Gray and Gardner (1951) noted that cavitation in the elbow started sometimes at the edges, and sometimes in the central part of the interzone.

In O'Rahilly stage 23, cavitation of the proximal radioulnar joint commenced. This process occurred independently from the rest of the elbow joint cavities (Gray and Gardner, 1951), first becoming evident between the annular ligament and the head of the radius, which appeared independently (Gray and Gardner, 1951) and fused secondarily with the rest of the elbow joint cavity. Wadsworth (1982) found that cavitation between the annular ligament and the head of the radius was not evident until the 34-mm stage of the fetal period (week 9), and Andersen (1962) found that the earliest signs of the process appeared even later, in 53-mm specimens (week 11).

The deep aspect of the joint capsule and overlying superior rim of the annular ligament gave rise to a triangular protuberance with its apex pointing into the joint lumen toward the humeroradial joint cavity. This prominence appeared to fill the space between the opposed surfaces of the bones, somewhat like a meniscus. In agreement with Retterer (1902), we believe this to be the mesenchymal condensation of the synovial membrane. Mitrovic (1978) observed a similar structure, noting that the humeroulnar cavity was occasionally interrupted in one or two places by areas of mesenchyme.

TABLE I. FEATURES OF THE SPECIMENS USED

Embryos C-R length mm		Plane of O'Rahilly Section stage		Fetuses C-R length mm		Plane of Weeks of Section development	
X-12	15	Transverse	18	CA-1	35	Transverse	9
GG-1	17	Transverse	18	MA-4	35	Transverse	9
BE-1	17	Transverse	19	RI-1	38	Transverse	9
BB-5	18	Transverse	19	H-19	39	Transverse	9
E-19	19	Transverse	20	BB-1	39	Transverse	9
JD-19	19	Transverse	20	GV-3	41	Transverse	10
JD-7	19	Transverse	20	AM-1	41	Transverse	10
PT-9	20	Transverse	20	PE-7	41	Transverse	10
JD-2	20	Transverse	20	ZO-1	42	Transverse	10
R-1	21	Transverse	21	SA-1	44	Transverse	10
MA-7	22	Transverse	21	GV-1	45	Transverse	10
X-6	22.5	Transverse	21	MA-3	46	Transverse	10
PE-8	23	Transverse	22	SA-3	48	Transverse	10
HA-2	23	Transverse	22	X-8	50	Sagittal	11
CH-1	24	Transverse	22	MA-2	50	Transverse	11
X-14	24	Transverse	22	MA-1	52	Transverse	11
EA-3	24.5	Sagittal	22	X-11	53	Transverse	11
BB-4	26	Transverse	22	BB-3	53	Transverse	11
GV-4	27	Transverse	22	H-4	62	Transverse	12
NA-2	27.5	Transverse	22	SA-4	63	Transverse	12
HE-1	28	Transverse	23	PE-3	70,5	Transverse	12
FA-5	28	Transverse	23				
NA-1	29	Transverse	23				
RI-4	29	Transverse	23				
X-18	30	Transverse	23				
BB-2	30	Transverse	23				
H-23	31	Transverse	23				
X-4	31	Transverse	23				

E. 2 A STUDY OF THE CARTILAGE CANALS OF THE MANDIBULAR CONDYLE DURING DEVELOPMENT (SPECIMENS FROM 9-17 WEEKS)

J.R. Mérida Velasco[1], J.F. Rodríguez Vázquez[1], J.A. Mérida Velasco[2], I. Sánchez Montesinos[2], E. Herrero González[1] and J.[3] Jiménez Collado

[1]Departamento de Ciencias Morfológicas II, Facultad de Medicina, Universidad Complutense, Madrid. [3]Instituto de Embriología, Universidad Complutense, Madrid. [2]Departamento de Ciencias Morfológicas, Facultad de Medicina, Universidad de Granada

INTRODUCTION: Cartilage plays an essential role in the morphogenesis of the skeletal system. The presence of the vessel inside the cartilage was first reported in the 18th century by W. Hunter (1743), although the term "cartilage canals" was proposed by Howship (1815). Most authors have studied the distribution of vascular canals in the epiphysis of long bones but few have focused on the development of these structures in human condylar cartilage.

MATERIAL AND METHODS: Serial sections of 50 human specimens between week 9 and week 17 of development were studied by optical microscopy. All specimens belonged to the Collection of the Institute of Embryology of the Complutense University of Madrid.

RESULTS: During the cavitation stage (week 9 and 11 of development) (Mérida Velasco et al, 1999) chondrification began in the centre of the condylar blastema. The condylar cartilage is a conocal structure surrounded by intramembranous ossification. The vertex of the cone is proximal to the future mandibular foramen. During week 11 of development an invagination of the mesenchyme was observed in the external portion of the condylar cartilage adjacent to the Joint disc. In the maturation stage (after week 12 of development) (Mérida Velasco et al 1999) an invagination of the vascular mesenchyme appeared in the condylar cartilage. During week 16 of development vascular canals were clearly evident.

DISCUSSION: In our specimens, chondrification of the mandibular condyle was observed to begin in week 9 of development, at the same time as the inferior joint cavity appears. By contrast, other studies have identified the beginning of chondrification as taking place in week 10 week 11 and even in week 12. During week 13 of development we observed invagination of vascular mesenchyme in the external portion of the condylar cartilage. During week 16 of development we observed the vascular canals as clearly evident. In contrast, other authors reported the appearance of these structures between week 15 and 19 of development. In the specimens studied here we did not observe any anastomoses between the vascular canals. Some authors have postulated that the function of this vascularization is to provide nutrition for the cartilage, enabling the mandible to grow more quickly in order to make room for the growth and eruption of the deciduous teeth.

REFERENCES:

HUNTER, W (1743). *Phil. Trans.* 42: 514-521.

HOWSHIP (1815). In: Watermann, R. (1961). Koln: Kolner Universitatstverlag.

MÉRIDA VELASCO et al. (1999). *Anat Rec*, 255: 20-33.

E. 3 CHRONOLOGY AND SEQUENCE OF THE DEVELOPMENT OF THE STAPEDIAL ARTERY IN HUMAN EMBRYOS

J.F. Rodríguez Vázquez[1], J.R. Mérida Velasco[1], J.A. Mérida Velasco[2], I. Sánchez-Montesinos[2], L.A. Arráez Aybar[1] and J. Jiménez Collado[3]

[1] Dpto. Ciencias Morfológicas II, Facultad de Medicina, Universidad Complutense, Madrid-28040. [2] Dpto. Ciencias Morfológicas, Facultad de Medicina, Universidad de Granada. [3] Instituto de Embriología, Universidad Complutense, Madrid

INTRODUCTION: According to Orts Llorca, 1932, in vertebrates the stapede artery, as denominated by Hyrtl in 1845 and described by Cuvier, 1840 is constantly present during mammalian embryonic development and is responsible for supplying blood to the facial structures and orbit (Padget, 1948). The aim of this work is to study the origin and arrangement of this artery during embryonic development and to chronologically sequence developmental events in order to understand the location of this vessel when it persists in the adult.

MATERIALS AND METHODS: Light microscopic studies were done on twenty human embryos from the Collection of the Institute of Embryology at the Complutense University of Madrid. The specimens ranged from 4.8 to 27 mm crown-rump (C-R) length.

RESULTS: In 6 mm C-R length specimens an arterial branch was clearly distinguished emerging from the lateral wall of the primitive internal carotid artery corresponding to the dorsal portion of the second arterial arch. This portion is attached to the artery whereas the remaining section is undergoing a process of involution. This arterial section corresponds to the stapedial artery.

Between 7-9 mm C-R length, the artery becomes shorter and its trajectory is limited to the mesenchyme of the second arch dorsal to the first pharyngeal pouch, ventral to the Andercht ganglion and the facial nerve runs along its lateral side.

In specimens from 10.75 to 12 mm C-R length, the artery originates in the internal carotid artery and then joins an artery that extends along the lateral walls to the fetal head. To do this, it crosses the precartilaginous anlage of the stapes and runs beneath the horizontal section of the facial nerve and the primitive cephalic vein.

Between 13 and 15 mm C-R length the artery reaches its maximum volume and runs through the future middle ear.

At 16 mm C-R length the stapedial artery begins to involute at the portion nearest to its site of emergence from the internal carotid artery. When the muscular blastema of the musculature of the first arch appear the maxillary artery has increased in calibre.

At 21 mm C-R length, complete atrophy of the stapedial artery is observed in the portion between its origin at the internal carotid artery to its passage through the stapedial orifice. The middle meningeal artery and its branches are now tributaries of the maxillary artery to which it becomes attached after 17 mm C-R length.

DISCUSSION: We consider the stapedial artery to be a branch of the internal carotid artery and to correspond to the non-involuted dorsal portion of the second arterial arch, and in this respect coincide with the opinion of Orts Llorca, 1932, 1934. We do not believe the stapedial artery to be a direct branch of the hyoid artery as suggested by Portela et al., 1959. Between 12 and 15 mm C-R length

(O'Rahilly's stages 17-18), as Congdon, 1922, also pointed out, the artery reaches its maximum development, providing the cephalic blood supply via its continuity with the supraorbitary branch (the future middle meningeal artery). In O'Rahilly's stage 20, the stapedial artery is obliterated in the portion most proximal to its origin at the internal carotid artery. As a consequence, the medial meningeal artery is a branch of the maxillary artery and the medial meningeal portion situated next to the future middle ear will form the tympanic branch of this artery.

E. 4 EXPRESSION OF SOME COMPONENTS OF THE BASAL MEMBRANE DURING OTOCYSTOGENESIS IN MAMMALS

M. Barbosa Cachorro, S. Callejo Alonso, J.A. Gil Verona, F. De Paz Fernández, J.F. Pastor Vázquez and E. Barbosa Ayúcar

Dpto. De Anatomía Humana. Facultad de Medicina. Universidad de Valladolid

Embryonic epithelia make movements of invagination and evagination that are common to a large number of anlages in which there is not well known the role the basal membrane that support them plays. In order to understand its participation in this morphogenetic process we have studied by inmunohistochemical and inmunocitochemical methods the expression of chondroitin-sulfate, laminin and collagen IV in the developing otic anlage of rat embryos between 10.3 and 12.7 days old.

Embryos were obtained from pregnant rats by cesarean and classified according to Brown and Fabro (1981). Some embryos were fixed in Carnoy and after submerged in Alcian Blue pH 5.8 and 0.4M. so that the chondroitin-sulfate was stained (Scott y Dorling, 1965). Another group of embryos were fixed in ethanol-acetic. The laminin as well as the collagen IV were detected using policlonal antilaminin and anticollagen IV antibodies respectively (a gift by Prof. Foidart).

The expression of chondroitin-sulfate proteoglycan, laminin and collagen IV during the otocystogenesis is not homogeneus, what can be entailed with the different morphogenetic mechanisms involved in this process (placode, fovea, vesicle otocyste and gangliogenesis). During the fovea phase we could observe differences in the localization of the different components of the basal membrane, being realy obvious the absence of chondroitin-sulfate at grade of the basal membrane which invests the central portion of the ventral wall of the fovea, what can be related to the proliferative phenomena that deepen the anlage and/or the incipient migratory phenomena.

In rat embryos between 11.3 and 12.7 days old both anlages, right and left, differ in the inmunostain's origin place for the laminin and the collagen IV, so that the right inner ear in its craneal extrem shows a positive signal in the lateral wall while the left otocyst shows it in the medial one. From the origin wall the signal spreads, up to down, towards the opposite wall vanishing in the initial one.

The positive inmunostain behaviour for these components of the basal membrane in the endolynfatic duct since the beginning (12.3 days) until the end (12.7 days), has been proved to be similar to its homolateral anlage.

The chondroitin-sulfate proteoglycan, laminin and collagen IV vanish or show a discontinue character in the emigration zone of the epithelial cells of the anlage for forming the statoacoustic ganglia, being closely related the disappearance of these components to the transition from epithelium to ganglia.

References

Brown NA and Fabro S (1981). Quantitation of rat embryonic development in vitro: A morphological scoring system. *Teratology,* 24: 65-78.

Scott JE and Dorling J (1965). Differential staining of acid glycosaminoglycans (mucopolysaccharides) by Alcian Blue in salt solutions. *Histochemie,* 5: 221-233.

E. 5 EARLY STAGES OF THE INNER EAR DEVELOPMENT IN MAMMALS

M. Barbosa Cachorro, F. De Paz Fernández, J.F. Pastor Vázquez, S. Callejo Alonso, J.A. Gil Verona and E. Barbosa Ayúcar

Dpto.de Anatomía Humana. Facultad de Medicina. Universidad de Valladolid

The inner ear is one of the former structures that appear during the embryonic development. In order to establish a clear description of its earliest stages of development in mammals, we tried an histologycal and ultrastructural study by light microscopy and scanning in rat embryos between 10.3 and 11.7 days old.

Embryos were obtained from pregnant rats by cesarean and classified according to Brown and Fabro (1981). A serie of embryons was fixed in Bouin's fluid and later stained with haemathoxylin-eosin what allowed us to make an histologycal study of the inner ear. Another group of specimens was fixed in glutaraldehide buffer cacodilate and below they were treated to be examinated into the electron microscopy.

The inner ear initiates its development as a thickness of the dorsal cephalic ectoderm nearby the rhombencephalon which receives the name of otic placode. It deepens in the underlying mesenchyme to constitute the fovea, which establishes in rat embryos because of an asymmetrical epithelial invagination process in the same way as it has been related by Meier (1978a y b) and Hilfer (1989) in avian embryos. The ectodermic cells modify their shape due to the rearrengement of their cytoskeleton changing from cubic-flat to cylindrical pseudostratified. In these early stages of development we have distinguish alredy two cell populations with different staining characters so as different superficial features. In embryos 11.3 days old the fovea's floor "rises" to form the lateral wall of the anlage so that it transforms into a vesicle that comunicates with the amniotic cavity through a pore that will close later. The vesicle isolated from the superficial ectoderm became deformed in dorso-ventral direction and loses gradually the uniformity of its walls. In these embryos 11.7 days old the endolynfatic duct initiates its development as an evagination in the dorso-medial portion at the craneal half of the anlage.

References

Brown NA and Fabro S (1981). Quantitation of rat embryonic development in vitro: A morphological scoring system. *Teratology,* 24: 65-78.

Meier S (1978). Development of the embryonic chick otic placode I. Light microscopic analysis. *Anat Rec,* 191: 447-458.

MEIER S (1978). Development of the embryonic chick otic placode II. Electron microscopic analysis. *Anat Rec,* 191: 459-478.

HILFER SR, ESTEVES RA and SANZO JF (1989). Invagination of the otic placode: normal development and experimental manipulation. *J Exp Zool,* 251: 253-264.

E. 6 CYTOCHEMICAL, SCANNING ELECTRON MICROSCOPY X-RAY MICROANALYSIS AND SPECTROPHOTOMETRIC STUDY OF Ca2+ CONTENTS AND DISTRIBUTION IN THE RAT PINEAL GLAND DURING ONTOGENETIC DEVELOPMENT

L. Muñoz Barragán, J.C. Carvajal, M.B. Gómez Esteban, M.L. Mosqueira, A.J. Álvarez-Morujo and S. Carbajo

Universidad de Salamanca

Ca2+ is a very abundant ion in the pineal gland and appears to be involved in the synthesis and release of melatonin from pinealocytes. In the present work we studied the content of Ca2+ in the pineal glands of rats during their ontogenetic development, combining spectrophotometric, X-Ray Microanalysis and cytochemical methods. For the ultracytochemical studies, the pineal glands of rats sacrificed in perinatal (fetuses of 19 and 20 days, newborns and animals of 10 days of age), intermediate (30 and 60 days of age) and adult (180 and 360 days of age) periods were incubated in potassium pyroantimonate; it was observed that Ca2+ is very scarce in rat fetuses, both in the intercellular spaces and inside the cells, while in newborns and animals of 10 days of postnatal life the ion was observed in the RER, the Golgi cisternae and mitochondria. Accumulations were also observed in the nucleus of a reduced number of pinealocytes. In animals of 30 and 60 days of age, Ca2+ appeared in the interstitial space, in the mitochondria of type pinealocytes and -in large accumulations- in the cytoplasm and nuclei of type 11 pinealocytes. In animals sacrificed at 180 and 360 days of age the Ca2+ content in type 11 pinealocytes was specially striking. A parallel study based on scanning electron microscopy-X-ray analysis revealed the existence of a strong Ca2+ peak (Ka 1+2 at 3.69 KeV) present in random areas of the pineal glands of rats of 30 and 60 days of age while this was very low in rats of 2 and 8 days of age. At the same time, the spectrophotometric study indicated the existence of a continued increase in pineal C a 2+ levels, rising from 0.157 + 0.04 ,umol/mg in 20-day old fetuses to 1.050 + 0.289, umol/mg at 180 days of postnatal life. The data suggest that pineal Ca2+ contents increase when the noradrenergic regulation of melatonin secretion is operating. We also observed that both types of pinealocytes are present very early on in the post-natal life of the rats studied.

E. 7 MYELIN OLIGODENDROCYTE SPECIFIC PROTEINS ARE EXPRESSED BY MULLER CELLS DURING DEVELOPMENT AND ADULT VERTEBRATE RETINA

F.A. Prada, A. Quesada, Y. Aguilera, A. Espinar, and C. Prada

Department of Morphological Science and Institute of Development Biology. University of Seville. School of Medicine. Seville, Spain

INTRODUCTION: In vertebrate retina Müller cells have been considered as modified astrocytes which adapt their morphology and functional role depending on the zone and strata of retina they where found. Previous studies (Meller and Glees, 1965; Magalhaes and Coimbra, 1972 and Prada et al., 1989, 1995, 1998) show these cells could have the role of ependymal cells, fibrous or protoplasmic astrocyte and radial glia cell for neuronal migration during development.

Although the appearance of the Müller cell inner portion during the process of myelination supports the idea that they must be functionally considered as oligodendrocytes (Prada, 1980; Ikeda, 1980 and Smith, 1982), no previously studies have been capable of demostrate this fact.

In present work, using different markers of myelin proteines, we show that inner portion of Müller cells may function in myelogenesis in a similar way as the oligodendroglia in the central nervous systen (CNS).

MATERIAL AND METHODS: Eye globes of white Leghorn chick embryos and freshy dissociated adult cells of chick, rabbit, turtle and lizard, were processed according to current immunocytochemical protocols using a monoclonal antibody against MOSP and A2B5. MOSP is a myelin oligodendrocyte specific protein reported to be specifically expressed by oligodendrocytes in CNS of many vertebrate species but not in the retina (Dyer et al. 1991). A2B5 is a specific protein expressed only during early development of oligodendrocytes.

Animal care protocols used in our laboratory are in conformity with the appropiate national legislation (Decres 223/1988, BOE n.° 67) and guidelines from the European Communities (Council Directive 86/609/EEC).

RESULTS AND DISCUSSION: MOSP appears in both Müller cells and axon terminals of horizontal cells in the dorsotemporal retina at E13, time at which these cells are still undergoing morphological differentiation. One day later E14 MOSP is found in these cells throughout the retina with the exception of the very peripheral retina. From E16, Müller cells concentrate this protein in the vitreal part to de ventricular one.

Between E 7ı/2-8, and E16 the most inner portion of Müller cells also express A2B5. Müller cells of the adult rabbit, turtle and lizard retina are also labeled in their inner portion by anti MOSP.

Our results suggest- a closer relationship between Müller cells and oligodendrocytes than presently thought, and also explain the origin of the intra-retinal chick myelin surrounding the large axons of ganglion cells.

E. 8 NEUROGENESIS OF THE INSULAR CLAUSTRUM OF LAGOMORPHA

C. Reblet, A. Alejo, I. Blanco-Santiago and J.L. Bueno-López

Departamento de Neurociencias, Facultad de Medicina y Odontologia, Universidad del Pais Vasco, Leioa

The claustrum is a subcortical centre that includes two parts. The dorsal one or insular claustrum (IC) is reciprocally connected with the neocortex and the ventral one or entopiriform nucleus is connected with the paleocortex and amigdala. In the rat, the neurones of the IC are generated from E15 to E16 (embryonic days) with a caudo-

rostral gradient. (*Bayer and Altman, 1991, Neurosci. 45: 391-412*). Our aim was to study the time of origin of the insular claustrum neurones in the rabbit because this animal has a more developed claustrum than the rat and looks more like the cat.

Pregnant rabbits from 13 to 18 GD (gestations days) were intraperitoneally injected with one pulse of BrdU (40 mg/ kg). Two to three animals of 22 postnatal days per litter were anaesthetised (25 mg/kg ketamine hydrochlorhidre and 2 mg/kg xylacine) and sacrificed through the heart with 4% paraformaldehyde in phosphate-buffer pH 7,4. Their brains were cut into 40 microns and immunocytochemically processed. We have found that the neurones of the IC were generated from E13 to E18 getting a peak between E15 and E16. We have observed no longitudinal gradient, either dorso-ventral or medio-lateral. This indicated that the claustrum is a uniform nucleus as their cytoarchitecture shows. In addition, more cells were generated, mainly at E16, for the rostral and dorsal part of the IC than for the caudal and ventral one, possibly because the IC is wider rostrally and dorsally than caudally and ventrally.

Supported by: FIS 94/1684, DGES 0624/97, DGES 0048/97, UPV EB179/96. I. B.-S. A.A. are doctoral fellows of the University of the Basque Country.

E. 9 IMMUNOCYTOCHEMICAL DEVELOPMENTAL PATTERNS OF THE THORACO-LUMBAR SYMPATHETIC CHAIN IN THE CHICK AND A COMPARISON WITH ITS ADRENAL COUNTERPART

I. Sánchez-Montesinos, Juan A. Mérida-Velasco, Joaquín Espín-Ferra, José F. Rodríguez Vázquez[1], José R. Mérida Velasco[1], Juan Jiménez Collado[1], and Lucio Scopsi[2]

Human and Experimental Embriology Research Group, Department of Morphological Sciences, University of Granada, Granada, Spain; [1] Department of Morphological Sciences II, University Complutense of Madrid, Madrid, Spain and [2] Cancer Genetics Service, Casa di Cura S. Pio X, Milan, Italy.

INTRODUCTION: Chromaffin cells synthesize, store and secrete a complex mixture containing amines, structural proteins, enzymes and neurohormonal polypeptides. The vast majority of studies have been performed on mammals and only a few recent ones have dealt with avian species, focusing mainly on somatostatin, neuropeptide Y, and vasoactive intestinal polypeptide. The blending of this cocktail, apparently could be modulated by neural and hormonal signals.

MATERIAL AND METHODS: White Leghorn chick embryos of 3.5-, 4-, 5-, 7-, 10-, and 12-day of incubation were used. Immediately after removal from the shell, the embryos were fixed in Bouin's solution for 3 to 10 hours, washed, dehydrated in graded ethanols, and embedded in paraffin. Serial 10 μm-thick sections were cut, deparaffinized, and hydrated in 0.05 M Tris-buffered saline (TBS), pH 7.4. Endogenous peroxidase activity was suppressed as detailed previously (Frigo et al., 1991). Incubation with the primary antibodies (Tables 1-3) was carried out overnight at 4°C and was followed by the peroxidase-labelled avidin-biotin complex (ABC) system, with development in plain or silver-enhanced diaminobenzidine (Frigo et al., 1991). Controls consisted of sequential deletion of the various immunoreagent layers and replacement of the primary polyclonal (serum) antibodies either with the flow-through of affinity-purified serum or with the sera preabsorbed with an excess of the respective immunogens. The reactivity of the monoclonal antibodies was checked by substitution with nonrelated isotype-matched immunoglobulins at the same concentration.

The immunocytochemical development of the thoraco-lumbar sympathetic ganglia in the chick and its adrenal counterpart, was studied using antibodies to 17 separate antigens, including antibodies to pan-neuroendocrine markers, catecholamine synthesizing enzymes, proprotein-processing enzymes, and neuropeptides.

RESULTS: Some of the antigens studied were heavily expressed from the first days of development, e.g., Go protein-α subunit, thyrosine hydroxylase, and galanin, while for others a strong heterogeneity both in number of immunoreactive cells and intensity of immunostaining was recorded at the different stages, e.g., chromogranin-A, chromogranin-B, 7B2 protein, proprotein convertase 2 and 3, secretogranin II, somatostatin, and dopamine-β-hydroxylase. The first immunoreactivities to appear at day 3.5 where those for HNK-1, tyrosine hydroxylase, chromogranin-A, and chromogranin-B. Two different patterns were found both in the developmental adrenal medullary and thoraco-lumbar sympathetic ganglia. In the first, concerning chromogranine-A and B, Go protein-α subunit, tyrosine hydroxylase, and galanin, virtually all medullary and sympathetic ganglia cells were strongly immunostained from day 4 onward. Except for HNK-1, chromogranine-A and B, cells immunoreactive for all the remaining antigens showed a steady increase up to the day 12. In the second, including 7B2 protein, proprotein convertase 2, and secretogranin II, full antigenic expression was reached in medullary and prevertebral sympathetic ganglia cells, within days 10. Finally, differences were observed between medullary and sympathetic ganglia cells immunoreactivities, with regard to proprotein convertase 3, somatostatin, dopamine-β-hydroxylase, and met-enkephalin.

DISCUSSION: The presumptive medullary cells of the chick adrenal gland, are derived from the sympathetic chains at the thoraco-lumbar level (Le Douarin, 1980; Le Douarin and Smith, 1983), which in turn originate from the neural crest (Hammond and Yntema, 1947; Weston, 1970; Le Douarin, 1982; Le Douarin and Smith, 1983).

The chromaffin cells of the adrenal medulla, within the neurons of the sympathetic ganglia integrate the sympathoadrenal lineage (Grillo, 1966; Eränkö, 1976; Landis and Patterson, 1981; Coupland, 1989; Unsicker et al., 1989; Unsicker, 1993; Stemple and Anderson, 1993). These cell types share several characteristics, including the expression of catecholamines. The results of Vogel and Weston (1990) are consistent with the hypothesis that components of the sympathoadrenal lineage arise in two steps from a subpopulation of crest-derived cells that initially express neuronal traits.

Presumptive catecholamine positive neurons were among the first cells to leave the neural crest. A delay in the dispersal of these cells could bias their fate away from the sympathoadrenal lineage. Hence, the late emigrating cells appeared to be restricted from catecholaminergic differentiation (Artinger and Bronner-Fraser, 1992; Stemple and Anderson, 1993). In the rat adrenal gland, from day 14 onward, DBH were detected in the anlage of the adrenal gland, as well as in the sympathetic ganglia (Vogel and Weston, 1990); however, we have observed that in the chick, DBH immunoreactivity increasing both in gland and ganglia in day 5, although from day 7 onward, the pattern differed: increasing in adrenal gland, and disappearing in prevertebral sympathetic ganglia.

Chromaffin cells (and their tumor counterparts) synthesised, stored and released a complex cocktail of peptides, most notably the chromogranins and a large number of neuropeptides including the enkephalins and neuropeptide Y. The blending of this cocktail, apparently could be modulated by neural and hormonal signals (Unsicker, 1993). Whit regard to chromogranins (A and B) and enkephalins, we have observed that, while all medullary cells and about sixty per cent were immunoreactive, respectively, for granins and met-enkephalin, at day 12; no CgA, CgB and M-ENK immunoreactivity were detected at this stage in the prevertebral sympathetic ganglia cells.

Vogel and Weston (1990a) established that cells in the adrenal glands of the avian embryo express TH and 160-kDa neurofilament proteins early in development and that the proportion of cells in the adrenal gland that express both a neuronal marker and TH immunoreactivity declines during embryogenesis. In the rat adrenal gland, from day 14 onward, TH was detected in the anlage of the adrenal gland, as well as in the sympathetic ganglia (Vogel and Weston, 1990). However, we have observed that in the chick, virtually all cells in the adrenal gland as well as in the prevertebral sympathetic ganglia, were immunoreactive to TH from day 4 to 12. Those data are not consistent with the existence of a precursor for pheochromocytes that initially exhibited neuronal traits and then lost them under the influence of environmental conditions within the adrenal gland (Vogel and Weston, 1990a).

In the rat adrenal gland PNMT is not expresses until tha day 17-18 of gestation, increasing between the day 17 and 21 the numbers of cells that display PNMT-immunoreactivity (Bohn et al., 1982; Seidl and Unsicker, 1989), and was restricted to those sympathoblasts that populate the adrenal gland (Vogel and Weston, 1990). In the chick, no PNMT immunoreactivity was detected in the prevertebral sympathetic ganglia fron day 4 to 12. Therefore, ontogenetic increase in and maintenance of PNMT activity in adrenal medullary cells would depend on glucocorticoids (Bohn et al., 1981; Seidl and Unsicker, 1989), although the initial expression of PNMT by embryonic rat pheochromocytes is not dependent on glucocorticoids or pituitary function (Bohn et al., 1981). This conclusion was derived from the finding that the expression of the adrenergic phenotype cannot be triggered prematurely by high doses of glucocorticoids (Bohn et al., 1981). In this way, Hofmann et al. (1989) emphasised the role of glucocorticoids in initiation, development and maintenance of the endocrine chromaffin phenotype, representing the decisive signal for the initial induction of endocrine differentiation; and moreover, high steroid hormone concentrations, as present in the adrenal medulla, and not in prevertebral sympathetic ganglia, could be a prerequisite for the maturation of chromaffin cells.

Drs. J.A. Mérida Velasco, I. Sánchez-Montesinos and J. Espín Ferra are supported by grants from the Consejería de Educación y Ciencia de la Junta de Andalucía (Spain). Dr. L. Scopsi is supported by grants from the Italian Association for Cancer Research (AIRC). The skillful technical assistance of Morena Gobbo and Barbara Vergani is gratefully acknowledged. We are indebted to the following colleagues for the generous gift of antibodies: T. Asano (Department of Biochemistry, Aichi Prefectural Colony, Japan), E. Comoy (Departement de Biologie Clinique, Institut Gustave Roussy, Villejuif, France), M. Pelagi (DIBIT, S. Raffaele Institute, Milan, Italy), B.A. Spruce (Department of Biochemistry, University of Dundee, UK), D.F. Steiner (Department of Biochemistry and Molecular Biology, The Howard Hughes Medical Institute, Chicago, IL, USA).

The work was carried out while Dr. Scopsi was Senior Researcher at the Endocrinology Unit, The National Tumor Institute, Milan, Italy.

Some of this work was supported by the Dirección General de Enseñanza Superior e Investigación Científica (Grant PM97-0172).

TABLE 1: CHARACTERISTICS OF THE ANTIBODIES TO PAN-NEUROENDOCRINE MARKERS

SERUM/CLONE DIRECTED SOURCE	AGAINST	IMMUNOGEN	RAISED IN
A11 Biogenesis, Bournemouth,	Chromogranin A (CgA)	Chromaffin granules from human pheochromocytoma	Mouse England (see also Pelagi et al., 1989)
B11 Biogenesis, Bournemouth,	Chromogranin B (CgB)	Chromaffin granules from human pheochromocytoma	Mouse England (see also Pelagi et al., 1989)
5A7	Secretogranin II (SgII) See Pelagi et al., 1992	SgII-enriched fraction from bovine anterior pituitary homogenates	Mouse
SA 1100 Affiniti Res. Prod. Ltd.,	Protein 7B2	Porcine 7B2 [23-39] coupled to BSA	Rabbit Ilkeston, UK
RAP Go	Alpha subunit of Go protein	Purified Go alpha subunit from bovine brain	Rabbit * see Kato et al., 1987
BBS/NC/VI-H14 Dakopatts A/S Glostrup, DK (NSE)	Neuron-specific enolase	Purified human brain NSE	Mouse
HNK-1 Becton & Dickinson, CA, USA	3-sulfuglucuronic acid	Membrane extract of human lymphoblastoid cell line	Mouse

* Affinity-purified immunoglobulins

TABLE 2: CHARACTERISTICS OF THE ANTIBODIES TO PROPROTEIN-PROCESSING AND CATECHOLAMINE-SYNTHESIZING

SERUM/CLONE DIRECTED SOURCE	AGAINST	IMMUNOGEN	RAISED IN
2/40/15 Boehringer Mannheim (D)	Ttyrosine hydroxylase (TH)	Purified TH from a rat pheochromocytoma	Mouse
DZ 1020	Dopamine beta-hydroxilase (DBH) Eugene Tech Intern. Inc.	Purified DBH from bovine adrenal	Rabbit
PZ 1040	Phenylethanolamine Eugene Tech Intern. Inc. N-methyltransferase (PNMT)	Purified PNMT from bovine adrenal	Rabbit
RS20	Proprotein convertase-3 (PC3) See Smeekens et al., 1992 coupled to KLH	PreproPC3 Cys [95-108] + preproPC3 ([110-122]	Rabbit
PC2-P4	Pproprotein convertase-2 (PC2) See Smeekens et al., 1992	PreproPC2 [611-638]	Mouse-Rabbit and human combined

TABLE 3: CHARACTERISTICS OF THE ANTIBODIES TO NEUROPEPTIDES

SERUM/CLONE DIRECTED SOURCE	AGAINST	IMMUNOGEN	RAISED IN
CA-08-325	Somatostain coupled to HSA	Cyclic somatostain-14	Rabbit
RAS 7141N	Galanin Peninsula Lab. Inc., CA, USA	Purified DBH from bovine	Rabbit
NPY02	Neuropeptide tyrosine (NPY) See Grouzman et al., 1989	h NPY [1-36] amide coupel to KLH	Mouse
PE-25	Proenkephalin See Spruce et al., 1990	h preproenkephalin [1-267] (beta-galactosidase fusion protein)	Mouse
i604/004	Vasoactive intestinal polypeptide UCB Viopro ducts, Belgium (VIP)	Synthetic VIP coupled to bovine thyroglobulin	Rabbit

E. 10 HUMAN FETAL HEART WITH VENTRICULAR SEPTAL DEFECTS MORPHOMETRIC STUDY

L. Álvarez[1], J.A. Marchal[2], E. Carrillo[1], C. Melguizo[3], J. Prados[3], A.E.Aránega[4], C. Vélez[3], H. Boulaiz[1], J. Fernández[1] and A. Aránega[1]

[1] Departamento de Ciencias Morfológicas, Facultad de Medicina, Univ. Granada, [2] Departamento de Ciencias de la Salud, Univ. de Jaén, [3] Departamento de Ciencias de la Salud, Univ. Almería, [4] Departamento de Biología Experimental, Univ. Jaén

INTRODUCTION: Fetal echocardiographs in an effective tool in assessing cardiac pump function an the status of the fetal circulatory system. The data for the sizes of the great arterial trunks and their branches in human fetuses and neonates are scarce. In this study we analyse 22 cardiopulmonary blocks from human fetuses and neonates weighing from 1 to 3.8 kg (mean 2.2+0.85) with perimembranous ventricular septal defects. We compared these data with patterns of normality.

MATERIALS AND METHODS: We used 22 human cardiopulmonary blocks (12 males, 10 females) preserved in formaldehyde with a body weight from 1-to 3.8 Kg and and ranged in age from 0 hours (17 cases) to 14 days. Three hearts were from neonates aged with 1 hour, 2 days and 5 days, respectively and two hearts were from 14 days old neonates. All specimens had a perimembranous ventricular septal defect (14 cases), associated with a defect in the oval fossa in 6 cases, and with bicuspid aortic valves in 2 cases. A set of linear measurements were taken in all hearts with a millimeter ruler and compass. Minimum squaer regression analyses were used to study the rela-

tionship between logarithm fetal and neonatal body weight (Kg) and the different cardiometric parameters.

RESULTS: We compared the mean values for the 13 parameters measured in the hearts with perimembranous ventricular septal defects against mean values obtained for 496 normal hearts from fetuses and neonates ranging in body weight from 60 to 5000 g. Five measurements were used: (P_1) the mean values of the internal circunference of the arterial duct at its origin near the bifurcation of the pulmonary trunk, (P_2) the arterial duct at a point equidistant from its origin and its anastomosis with the aorta, (P_5) the thoracic aorta at a point 1cm distal to the anastomosis of the arterial duct, (P_{11}) the ascending aorta at a point 0.5 cm anterior to the brachiocephalic arterial trunk and the pulmonary value (P_{13}). The analyses of this parameters showed that the five measurements were larger than in normal hearts. Mean values of the other eight parameters were smaller in the pathological than in the normal hearts.

DISCUSSION: We found that the ascending aorta is wider than tha thoracic aorta in both normal hearts and hearts with perimembranous ventricular septal defect. According to Rudolph (1) the ascending aorta is similar in diameter to the ascending aorta. Studies of the dimensions of the great arterial trunks and their branches in the human heart during the fetal and perinatal periods are scarce. In particular, very little data are avalaible for the dimension of the arch vessels (2). Van Meurs-Van Woezik y cols. (3) believe that is important know the size of the vessels and valvar orifices in relation to normal calibers in any region to be surgically repaired. Our findings support the observations of Van Meurs-Van Woezik and Krediet (4) who concluded that "the ascending aorta functioning as the stem vessel of the aortic tree, provides suffiecient blood flow through the aortic arch during body development. The stem of the aortic arch must therefore grow more in diameter than each of this branches".

We hope that these data will increase our understanding of human fetal circulation (5, 6, 7) and facilitate the diagnosis and surgical correction of congenital cardiac defects.

REFERENCES

1. RUDOLPH AM (1985). Distribution and regulation of blood flow in the fetal and neonatal lamb. *Circulation*, 57: 811-821.
2. ANGELINI A, ALLAN LD, ANDERSON R, GRAWFORD D, CHITA S and YEN HO S (1988). Measurements of the dimensions of the aortic and pulmonary pathways inthe human fetus: a correlative echocardiographic and morphometric study. *Br Heart J,* 60.
3. VAN MEURS-VAN WOEZIK H, DEBETS F and KLEIN HW (1989). Internal diameters of the ventriculo-arterial functions and great arteries of normal infants and children: a data base for evaluation of congenital cardiac malformations. *Int J Cardiol,* 23: 303-308.
4. VAN MEURS-VAN WOEZIK H and KREDIET P (1982). Measurements of the descending aorta in infants abd children: comparison with other aortic dimenssions. *J Anat,* 135: 273-279.
5. SAHN DJ (1994). Perspectives in fetal echocardiography. *Cardiol Young* , 4: 90-98.
6. HANLEY FL (1993). Fetal response to extracorporeal circulatory support. *Cardiol Young*, 3: 263-272.
7. ÁLVAREZ L, ARANEGA A, SAUCEDO R, LÓPEZ F, ARANEGA AE and MUROS MA (1991). Morphometric data on the arterial duct in the human fetal heart. *Int J Cardiol,* 31: 337-334.

E.11 CHANGES IN HAIRLESS RHINO-J MICE (hr-rh-j) COLON

I. San José, N. García, R. Cabo, S. de la Fuente, J.A. Vega[1] and J. Represa

Departamento de Anatomía Humana. Facultad de Medicina Universidad de Valladolid Valladolid Spain. [1] Departamento de Morfología y Biología Celular Facultad de Medicina Universidad de Oviedo. Oviedo Spain

A mutation in the hr gene is responsible for typical epithelium phenotype in hairless mice. As this gene is expressed at high level not only in the skin but also in the large intestine, the aim of the study was to clarify its role in the colon. We have analysed by morphological (haematoxiline-eosine and Pas stain) and immunohystochemical techniques (chromogranin A) the colon of a mutated mouse strain, the hairless (hr-rh-j) type carrying the homozygotous hr gene rhino mutation.

The colon was studied in young (3 months, n= 3) and adult (9 months, n=7) wild type and mutated mice.

Major structural changes were found in the adult-animal groups revealing a significant increase in the size of villi, as well as in the number of globe cells respect to their normal littermates.

Chromogranin A immunohystochemistry revealed a significant increase in the expression of this protein in certain parts of colon in older mutated animals in comparison with wild type.

CONCLUSION: These results suggest that hr gene is involved in the structural maintenance of the mature colon, rather than in development. Our findings may also be consistent with an accelerated aging of this animals which is also present in other body structures.

END. 1 EFFECTS OF HYDRATION WITH MINERAL WATER ON THE GRANULATION OF MYO-EPITHELIOID CELLS AND BLOOD PRESSURE

A. Caballero[1], E. del Prado[1], T. del Castillo[2], A. Rodríguez[3] and S. Rodríguez[1]

[1] Dpto. de Anatomía Humana de la Universidad de Valladolid. [2] Centro de Salud Soria Sur. INSALUD. [3] Dpto de Fisiología y Farmacología de la Universidad de Salamanca

Over the past decade, the popularity of bottled water has increased subtancially in North America and Europe. Sales of bottled waters have increased by 400%, and 1 in 5 households now use bottled drinking water. Since bottled water is benning to assume a promenent place in the North American diet, its health effects requice evaluation. In North America, hypertension affects 20% of the entire populatión. A persistent diastolic bloob pressure increase a 5 mmHg is associated with a 34% increase in the risk of stroke and a 21% increase in the risk of coronary heart disease. High salt intake is believed to be an important contributor to occurrence of hypertension. Sodium is the major cation found in most salts, and it is believed to be the ion responsible for hypertensive effect of the high salt intake.

In this paper it is presented a study about synthesis, storage and release of renin of the myoepithelioid or granular cells of juxtaglomerular apparatus and the blood pressure in adult male wistar rats. Two groups, ten rats

each, have been used. The first one was used as the control one and drinks ad libitum running tap water apported by the firm Ondagua SA and whose ionic composition is sodium 6±3%mg/l, potassium ≤2.0±8% mg/l, magnesium ≤2.0±1% mg/l, calcium 20±1% mg/l, clorures 14.0±2% mg/l, dry residue 118 mg/l, bicarbonate 59 mg/l. The second one drinks ad libitum commercial mineral water (Montepinos) whose ionic composition is sodium 1.8 mg/l, magnesium 3.4 mg/l, calcium 93.8 mg/l, cloruros 3.6 mg/l, dry residue 255 mg/l and bicarbonate 298 mg/l.0.

Blood pressure and heart rate are determined in a non-cruel way. As soon as the animals have been sacrificed, we measure concentrations in plasma of the main ions on which blood pressure depend, also including the osmolality and renin concentration. The kidneys were removed and cut in their longitudinal axis, and then they were fixed in Smith liquid and stained with the modified Bowie stain with the aim of staining the granulations, acumulation of renin of the myoepithelioid cells, so as to later calculate the juxtaglomerular index.

We have not found statistical significant differences among ions concentrations. However, we have observed statistical significant decrease of sistolic and diastolic blood pressure associated with an important decrease in the storages of renin.

END. 2 MODIFICATION OF THE TESTIS ANATOMY FOR STRESS FACTOR

J.L. Nieto and E. Nieto

Dpto. Ciencias Morfológicas. Facultad de Medicina de Zaragoza (España)

The study assess the possible influence of stress on testicular histology of Wistar rat. We study the results obtained with anatomosurgical, physiopathologic and environmental modification (environment with monochromatic blue and red lights) criteria. The experimental design have been done following the criteria established by the Department of Morphological Sciences of the University of Zaragoza. The results obtained were not homogeneous. Variations in the cases of surgical stress and inmovilisation depends on the time between the estimulus and the testicular exeresis. With monochromatic lights the most important stree was observed with red lights. The blue light was mildly stressing. Also in these cases the time influenced the histological results of the testicle.

END. 3 INTERPHASE BONE-IMPLANT: A STUDY IN OVARIECTOMIZED FEMALE RATS

C. Clemente[1], R. Rodríguez-Torres[1], J. Tabuenca[2], A. López-Alonso[1] and L. Gómez-Pellico[1]

[1] Morphologic Sciences and Surgery Department. Faculty of Medicine. U. Alcalá. Spain. [2] Móstoles Hospital, Spain

SUMMARY: The use of implants in the human body has been developed during the last years, and very good results have been obtained with some biomaterials like titanium with or without hydroxyapatite coating. However, the last result can be modified by the host response itself. One of the most frequent situations is the presence of osteoporosis in subjects who need implants. This is why our study was aimed to evaluate the modifications, which occur in bone tissues with osteoporotic illness, which must also bear the presence of implantologic material.

An experimental study was performed in adult rats, which were placed an implant of titanium with hydroxyapatite coating into the medullary cavity of their right femora. The left ones were not implanted. Next, one half of the animals were caused osteoporosis by means of bilateral ovariectomy. The other half remained as the control group. Then, the sample was divided into groups of five animals, which were sacrificed at different times (12 and 18 weeks). Once the femora were obtained, a dual energy x-ray absorptiometric analysis was performed in the superior bone area of the implant and histological sections of the implant with undecalcified bone were made. These sections were analysed with conventional optical microscope and stereological studies were performed later. Both methods permitted to get quantitative values, which were analysed with the appropriate statistical tests.

We could observe that the implant caused and increase of bone tissue in either the healthy or the osteoporotic groups, opposed to the contralateral femur without implant. Comparing the bone mass in the control group with the osteoporotic group, we could observe a tendency to a greater bone formation in the control group, although the values were not statistically significant. Likewise, the bone morphology of the new formed bone in the control group was trabecular and young type, while the bone in the osteoporotic group was more scarce and with the characteristics of the mature bone.

N. 1 METAMIZOL-INDUCED FOS-LIKE IMMUNOREACTIVITY IN THE RAT CENTRAL NUCLEUS OF THE AMYGDALA

A.A. Valverde-Navarro, Mª Carmen Blasco-Ausina, Cristina Soto-Sarrión, José Cabanes Vila, Francisco E. Olucha-Bordonau, Tomás Hernández-Gil, Amparo Ruiz-Torner and Francisco Martínez-Soriano

Departamento de Ciencias Morfológicas. Facultad de Medicina. Universidad de Valencia

INTRODUCTION: The amygdala is a subcortical complex of nuclei which is thought to play a role in the mediation of emotion, including fear-related processes. More recently, this structure has been implicated as a mediator of morphine antinociception, since microinjections of morphine into the amygdala have been reported to produce antinociception in some pain tests. Of the subdivisions of the amygdala thought to contribute to the production of morphine antinociception, the central nucleus (Ce) appears to be an important component. In order to report more data about this antinociceptive role of the Ce, we tried to activate neurons of this subnucleus of the amygdala by systemical administration of the analgesic metamizol.

MATERIAL AND METHODS: To demostrate the activated neurons we used Fos immunocytochemistry. Rats were subdivided in two groups: a metamizol-treated group (that received 500 mg/kg, i.p., 150 minutes before the sacrifice) and a control rats group.

RESULTS: The results showed strong Fos-like immunoreactivity in the Ce of the metamizol-treated rats in contrast with the control rats where Fos-like immunoreactive elements were absent.

DISCUSSION: These results suggest that the amygdala may be a component of endogenous antinociceptive circuitry. Analgesics may activate Ce via the spino-ponto(parabrachial)-amygdaloid pathway, which is thought to be involved in the affective-emotional responses to noxious stimulation.

N. 2 SUBCELLULAR LOCALIZATION OF THE METABOTROPIC GLUTAMATE RECEPTOR 1b IN THE RAT CEREBELLAR CORTEX

P. Grandes[1], J.M. Mateos[1], R. Benítez[1], I. Elezgarai[1], J. Azkue[1], E. Lázaro[1], A. Osorio[1], A. Bilbao[1], R. Sarría[1], F. Conquet[2], F. Ferraguti[3], R. Kuhn[4] and T. Knöpfel[5]

[1] Dept. Neurosciences, Faculty of Medicine and Dentistry, Basque Country University, 699-48080 Bilbao, Spain; [2] IBCM-Glaxo, Rue du Bugnon 9, CH-1005 Lausanne, Switzerland; [3] Cambridge Brain Bank Laboratory, Dept. Pathology, University of Cambridge, MRC Centre Hills Road, Cambridge CB2 2QH, United Kingdom; [4] Nervous System, Novartis Pharma Inc., CH-4002 Basel, Switzerland; [5] Laboratory for Neuronal Circuit Dynamics, Riken Brain Science Institute, 2-1 Hirosawa, Wako-Shi, Saitama 351-0198, Japan

We used a specific mGluR1b antiserum to investigate immunocytochemically the subcellular distribution of mGluR1b in the rat cerebellar cortex.

A pre-embedding immunoperoxidase method showed mGluR1b immunoreaction product in dendritic spines of Purkinje cells receiving parallel fibre boutons. With a pre-embedding immunogold method, immunoparticles were revealed in perisynaptic (25%) and extrasynaptic positions along intracellular sites of dendritic spine membranes of Purkinje cells. The density of extrasynaptic immunoparticles was relatively high for over a long distance from the edge of the synapse, in contrast with the distribution of mGluR1a. Parallel fibre synaptic terminals were mGluR1b immunonegative.

As conclusion, mGluR1b and mGluR1a are targeted to the same subcellular compartments in Purkinje cells, but the density of the perisynaptic pool and the values and gradient of decrease of the extrasynaptic localization were different for the two mGluR1 isoforms, as assessed by quantitative analysis. The compartmentalization of mGluR1b and mGluR1a might serve distinct cerebellar functions.

Supported by DGICYT (PB95-0347) and Gobierno Vasco (PI-1997-41). Fellowships are in receipt by J.M. Mateos, J. Azkue and A. Osorio from Gobierno Vasco (grants BFI 95. 154, BFI98. 18 and BFI98. 109, respectively) and by R. Benítez and I. Elezgarai from Universidad del País Vasco (1996, 1998, respectively).

N. 4 DIFFERENCES IN THE MORPHOLOGY AND LAMINAR DISTRIBUTION OF CORTICO-CLAUSTRAL CELLS IN THE RABBIT CEREBRAL CORTEX

J.L. Bueno-López, A. Acera-Osa, J.L. Mendizábal-Zubiaga, E. Arana-Arri, C. Reblet and I. Gutiérrez-Ibarluzea

Department of Neurosciences, School of Medicine and Dentistry, The University of the Basque Country, E-48940 Leioa (Spain)

This study examines the morphology and laminar distribution of the cortico-claustral projection cells in the rabbit. Single injections of biotinylated dextran amine (BDA) were made in the caudal two thirds of the insular claustrum of six New Zealand rabbits *(Lagomorpha; Oryctolagus cuniculus)*. The corresponding retrograde labelling in the cortex was analysed and the distribution across areas of cells with different morphologies was estimated. The retrogradely labelled cells were observed in visual (areas 17 and 18), auditory (A2) and cingulate cortices (areas 29b, c and d). The projection was mostly ipsilateral. Some retrograde labelled neurones were seen in the contralateral cingulate cortex. Most of these cortico-claustral cells were pyramidal-like cells and neurones with inverted morphology in any cortical area. Notably, the cortico-claustral inverted cells were mainly located in layer VIa and the cortico-claustral pyramidal neurones were situated in layer V in all areas. We observed differences in the percentage of these two different types of cortico-claustral cells among areas. The inverted morphology was overwhelming in area 17 of the visual cortex (85% of the retrogradely labelled cells) and the pyramidal shape in the other studied areas (area 18, A2 and cingulate cortex, 75%, 71% and 87.6%, respectively). The results show that the inverted neurone is the common type of cortico-claustral projection cell in rabbit area 17 and possibly in other mammals (not shown here).

Supported by DGES 0048/97, GV OI065/97 y UPV EB 179/96. I. G.-I. and J.L. M.-Z. were doctoral fellows of the Regional Government of the Basque Country.

N. 5 THE THALAMOSTRIATAL SYSTEM: NEW INSIGHTS INTO BASAL GANGLIA FUNCTION

J.M. Giménez-Amaya[1], Silvano de las Heras[2], Elena Erroa, Elisa Menguala and José Luis Lanciego

[1] Departamento de Anatomía, Facultad de Medicina, Universidad de Navarra, 31080 Pamplona. Navarra. Spain. [2] Departamento de Morfología, Facultad de Medicina, Universidad Autónoma de Madrid

The thalamostriatal projections are largely neglected in current views of the basal ganglia function. In the last years, however, several studies have re-evaluated these projections and have postulated their implication in more complex tasks within the basal ganglia organization. In this contribution, we try to review the morphological and functional importance of this system after neuroanatomical tract-tracing studies in the rat, cat and monkey. An overview of the anatomical organization of the thalamostriatal projections in these three species is summarized. Direct thalamic afferents to the striatum originate mainly in the intralaminar and midline thalamic nuclei. Other thalamic sources of these projections include the ventral, lateral and posterior thalamic groups. Moreover, special attention is paid to the thalamus as an important place for interaction between the input and the output systems of the basal ganglia through the thalamostriatal projections. Thus, we focus on the overlapping territories between the thalamic projection of the output nuclei of the basal gan-

glia and the pedunculopontine tegmental nucleus, and the thalamostriatal neurons, which support the hypothesis that the output of the basal ganglia might participate in feedback subcortical circuits through the thalamostriatal projections. These circuits, however, may be established either directly through the pallidothalamic and nigrothalamic projections, or indirectly through the pallidoreticular connections. In our opinion, it is particularly appealing the idea that the final outcome of the basal ganglia processing can be conveyed either directly to the cerebral cortex via thalamic relay nuclei, or sent back to the basal ganglia themselves at the striatal level through the thalamostriatal projection system. A third and very suggestive possibility is that this information already processed in the basal ganglia might be directed to both the cerebral cortex and the striatum using the thalamic cells which project to these two structures by means of collaterals.

Supported by CICYT, Fundación Marcelino Botín and Universidad de Navarra.

N. 6 THE EFFECTS OF INTRASTRIATAL NIGRAL GRAFTS IN THE 6-OHDA MODEL OF PARKINSON'S DISEASE ARE INDEPENDENT OF THE HOST DOPAMINERGIC SYSTEM

E. López-Martín, A.M. Muñoz, M.B. Ramos-Prieto, M.J. Guerra and J.L. Labandeira-García

Department of Morphological Sciences, Faculty of Medicine, University of Santiago de Compostela, Spain

The aim of this study was to evaluate whether the recovery observed after grafting of fetal nigral cells in 6-hydroxydopamine lesioned rats is due to the graft itself, and whether the participation of the remaining host dopaminergic system is necessary. The effects of unilateral 6-hydroxydopamine lesion on rotational behavior were not significantly affected by sham grafting or by sham grafting plus repeat ipsilateral lesion, but were suppressed by nigral grafting, and by contralateral lesion. Immunohistochemical and in situ hybridization study of right striata of rats subjected to right-side lesion then right-side sham-grafting, and of right and left striata from rats subjected to right-side lesion then right-side sham-grafting then repeat right-side lesion then left-side lesion, revealed a) no significant amphetamine-induced Fos activation, b) marked increases in preproenkephalin mRNA levels, and c) decreases in preprotachykinin levels, with no significant differences in any of these variables among these three types of striata. After nigral grafting, however, intense Fos expression was observed in the striatum, and preproenkephalin and preproenkephalin mRNA levels returned to normal. This recovery was maintained after subsequent repeat ipsilateral 6-hydroxydopamine lesion followed by contralateral lesion. These results demonstrate that, after dopaminergic denervation, the nigral graft itself is able to induce recovery in the assessed parameters, and that the effects of grafting into striata with maximal unilateral 6-hydroxydopamine lesion are due to graft function, and are not significantly influenced by the remaining ipsilateral or contralateral host dopaminergic system.

Supported CICYT and XUGA.

N. 7 IMPLICATIONS OF AROMATASE P-450 IN THE PATHOGENESIS OF SPONTANEOUS HYPOPHYSEAL ADENOMAS. IMMUNOHISTOCHEMICAL CORRELATIONS WITH PCNA, BCL-2, P53, IL-6, IL1β, NOS-I AND CLASSICAL HYPOPHYSIAL HORMONES

J. Carretero, G. Vázquez, A. Martín-Clavijo, E. Hernández, M. Angoso, R. Herrero, M.C. Montero and R. Vázquez

Dept. of Human Anatomy and Histology, Faculty of Medicine. Lab. of Neuroendocrinology, Institute of Neuroscience of Castilla y León. University of Salamanca, Spain

Ten percent of intracranial tumors are hypophyseal adenomas, and spontaneous adenomas are very frecuent in old age (ranging from 23 to 35% in humans and 47 to 90% in rats). However, the mechanisms involved in the pathogenesis of these tumors are not well determined. Among alterations involving RB, p53 or G-proteins genes, variations of expression of different trancription factors as Pit-1, TEF or LIM-3; could be included abnormal expression of several substances that are inolved in the auto-paracrine modulation of hypophysis as: growth factors, interleukins, neurpeptides as VIP or enzymes as NOS-I or aromatase. Moreover, in the pathogenesis of these adenomas it is not well established the presence of changes in cellular proliferation or apoptosis. The aim of the present study was to adress these questions using immunohistochemical typification of spontaneous hypophyseal adenomas from old rats. The follow table showed the results obtained:

	PRL	GH	LH	TSH	ACTH	IL-1β	IL-6	PCNA	Bcl-2	p-53	Aromatase	NOS-I
TOTAL	73.3%	66.7%	40%	44.4%	40%	33.3%	60%	33.3%	56.7%	56.7%	70%	94.7%
PURE	40%	0%	3.3%	0%	6.7%	....	....	....	....	....	....	....
MULTIPLE	33.3%	66.7%	36.7%	44.4%	33.3%	....	....	....	....	....	....	....

The following relation showed the most important intratumoral coexistence between different markers assayed:

1.- 100% of pure PRL-positive adenomas were Aromatase-positive.

2.- 0% of pure LH-positive adenomas were Aromatase-positive.

3.- 70.59% of p53-positive adenomas were Bcl-2-positive.

4.- 80% of PCNA-positive adenomas were p53-positive.

5.- 90% of PCNA-positive adenomas were Bcl-2-positive.

6.- 100% of pure ACTH-positive adenomas were IL1β-positive.

7.- 0% of pure ACTH-positive adenomas were IL6-, PCNA-, p53- or Bcl-2-positive.

Aromatase and NOS-I are not expressed in old non-tumoral rats and only described tumors were positive to both enzymes. Moreover, IL-1β-expression is very scarce in hypophyses of old rats. Because aromatase is involved in aromatization of testosterone to estradiol and chronic treatments with estradiol induces the genesis of PRL-positive adenomas, our results suggest an important role of aromatase in the genesis of these tumors. Moreover IL-1β is involved in the physiological regulation of hypothala-

mic-hypophysial-adrenal axis and the very close relation between NOS-I expression and pure ACTH-positive tumors suggest that NOS-I could be related in the appearance of the tumors.

Supported by FIS 99/1187 program, SA58/98 Social Founds European and JCyL program and CO3/196 JCyL program.

N. 8 EFFECTS OF GONADAL STEROIDS ON IMMUNOHISTOCHEMICAL EXPRESSION OF AROMATASE P450 IN THE RAT HYPOPHYSIS

R. Vázquez, G. Vázquez, M. Rubio, F. Sánchez, R.J. Vázquez, J.M. Riesco, J.A. Juanes, E. Blanco and J. Carretero

Dept. of Human Anatomy and Histology, Faculty of Medicine. Lab. of Neuroendocrinology, Institute of Neuroscience of Castilla y León. University of Salamanca, Spain

Of the two main metabolic pathways of androgens, reduction and aromatization, the latter depends on the presence of the enzyme aromatase P450. The presence of aromatase has been demonstrated in different regions of the central nervous system, including the hypothalamus. However, despite the important role played by the hypophysis in the gonadal sphere, it is not well determined if the enzyme is located in the anterior hypophysis. There are discrepancies not well explained about the presence of aromatase in the hypophysis and there is only one previous study from our laboratory analysing its immunohistochemical expression in the anterior hypophysis, it suggests gender-related differences in the expression. In the present study, an immunohistochemical study was performed on adult male and female rats to determine the existence or not of aromatase in the hypophysis of the adult rat and the effect of gonadal steroids on its presence. The study revealed that the adult rat hypophysis does contain aromatase, although its levels differ widely between the sexes. In this sense, the hypophysis of male rats had 34.40% of cells immunoreactive to the enzyme while in females there was little reaction, only 9.84% were immunoreactive cells. Ovariectomy elicited a considerable increase in the reaction to aromatase in females; thus, following surgery, 19.46% of the cells showed positive immunoreaction. In male rats, castration reduced the number of reactive cells to 17.63%, although the reaction persisted. Treatment with gonadal steroids after castration and ovariectomy modified aromatase expression in the sense that in testosterone-treated castrated males the percentage of reactive cells increased to 46.52% while in ovariectomized females treated with oestradiol only 0.52% of the cells were reactive. Our results demonstrate the immunohistochemical expression of aromatase in the hypophysis of adult rats and suggest that the expression of this enzyme is sex-dependent and can be modified by castration and gonadal steroid administration. This in turn suggests that aromatase may be involved in the regulation of adenohypophyseal cytology by gonadal steroids.

Supported by FIS 99/1187 program and SA58/98 Social Founds European and J C y L program.

N. 9 CHANGES IN THE RATS SUBCOMMISSURAL ORGAN OF INDUCED HYDROCEPHALUS

Luz María Fernández-González[1], Isabel Martínez de la Peña-Valenzuela[1], Emilia Carmona-Calero[1], Agustín Castañeyra-Perdomo[1], Romualdo Ferres-Torres[1], Herminia Pérez-González[1], María del Mar Pérez-Delgado[1], Manoli Castañeyra-Martín[3], Norberto Marrero-Gordillo[1] and Carlos Ormazábal-Ramos[2]

[1] Departamento de Anatomía Humana, Facultad de Medicina, Universidad de La Laguna, Tenerife, Spain. [2] Departamento de Pediatria, Facultad de Medicina, Universid de La Laguna, Tenerife, Spain. [3] Instituto de Investigacion y Ciencias de Puerto del Rosario, Fuerteventura., Canarias, Spain.

The subcommissural organ (SCO) is a circumventricular ependymal gland, lies below of the posterior commissure.The specialized ependymal cells produce a secretory material, apparently a complex containing different glycoproteins, that are released into the ventricular cerebrospinal fluid, where they form Reissner's fiber (6). The functions of the SCO has been related with the mechanisms to regulate the blood pressure (3), salt/water balance (1) and with postnatal induced hydrocephalus (4) or prenatal congenital hydrocephalus (2, 7). The aim of this to study the possible association between hydrocephalus and alterations of the SCO.

Five male control rats (Sprague-Dawley), and 5 Kaolin induced hydrocephalus rats (Sprague-Dawley) were sacrificed at the 25th weeks of life. The hydrocephalus was induced injecting, at the 10th weeks of life, 6mg of Kaolin in the cisterna magna. Before the kaolin injection, 100 μl of CSF was extracted. 15 week later, before sacrifice, also 200 μl of CSF, from lateral ventricle, was extracted. At the beginning and at the end of the experiment the body weight was taken. Brains were fixed by vascular perfusion with Bouin's fluid. Embedding was in paraffin. Frontal and sagital serial sections, 10 mm thick, through the region of the SCO, were obtained. One of the series was stained with the Klüver-Barrera method, while the other one was processed for the immunoperoxidase method with AFRU, (5) as primary antibody.

Not significant differences in the body weight were found between the control and hydrocephalic animals. The total amount of protein in the CSF was decreased in the hydrocephalus compared to the control animals $p<0.05$. Also three proteins bands (95, 47, 32 kDa) were found in the CSF of the control group that were not present in the hydrocephalus CSF.

In the SCO of the control rat most of the AFRU-ir material is located in the perinuclear and supranuclear cytoplasmic regions of the specialized ependymal cells. The SCO of the hydrocephalic rats group only showed a light, statistically not significant increase of the AFRU-ir material. We also see an AFRU-ir redistribution which now filled a little more than in the control group the supranuclear region or the basal pole of the SCO cells in the form of a coarse granular secretion product. The SCO showed a rostro caudal size of 720 μm, but the rostro caudal length of the SCO (440 μm) in the hydrocephalic brain is significantly decreased than the control group. These finding are partially similar to the described by Irigoin et al (4) and Pérez-Figárez (5) and support that the hydrocephalus is accompanied by changes in the morphology and secretions of the SCO.

REFERENCES

1.- CARMONA-CALERO E, PÉREZ-GONZALEZ H, PÉREZ-DELGADO MM, MARRERO-GORDILLO N, PUCHADES-COMPANY MJ, CASTAÑEYRA-PERDOMO A and FERRES-TORRES R (1996). Efectos de la administracion cronica de captoril sobre el organo subcomisural del raton. *Arch Esp Morfol*, 1: 109-112.

2.- CASTAÑEYRA-PERDOMO A, MEYER G, CARMONA-CALERO E, BAÑUELOS-PINEDA J, MENDEZ-MEDINA R, ORMAZABAL-RAMOS C and FERRES-TORRES R (1994). Alterations of the subcommissural organ in the hydrocephalic human fetal brain,. *Dev Brain Res*, 79: 316-320

3.- CASTAÑEYRA-PERDOMO A, CARMONA-CALERO E, MEYER G, PÉREZ-GONZÁLEZ H, PÉREZ-DELGADO MM, MARRERO-GORDILLO N, RODRÍGUEZ S, RODRÍGUEZ EM (1998). Changes in the secretory activity of the subcommissural organ of spontaneously hypertensive rats. *Neuroscience Letters*, 246: 133-136.

4.- IRIGOIN C, RODRÍGUEZ EM, HEINRICHS M, FRESE K, HERZOG S, Oksche A and Rott R (1990). Immunocytochemical study of the subcommissural organ of rats with induced postnatal hydrocephalus. *Exp Brain Res*, 82: 384-392.

5.- PÉREZ-FIGÁREZ JM, JIMÉNEZ AJ, PÉREZ-MARTÍN M, FERNÁNDEZ-LLEBREZ P, CIFUENTES M, RODRÍGUEZ S and RODRÍGUEZ E.M. (1998) Spontaneous congenital hydrocephalus in the mutant mouse HYH. Changes in the ventricular system and the subcommissural organ. *J Neuropathol Exp Neurol*, 57: 188-202.

6.- RODRÍGUEZ EM, OKSCHE A, HEIN S, RODRÍGUEZ S and YULIS R (1984). Spatial and structural interrelationships between secretory cells of the subcommissural organ and blood vessels. An immunocytochemical study. *Cell Tissue Res*, 237: 443-449.

7.- RODRÍGUEZ EM, OKSCHE A, HEIN S and YULIS CR (1992). Cell Biology of the Subcommissural Organ. *Int Rev Cytol*, 135: 39-121.

ACKNOWLEDGEMENTS

Supported by the Consejería de Educación del Gobierno de la Comunidad Autonoma de Canarias, Project N°1998/002, and Universidad de La Laguna, Project N° 48/98. We thank Prof.Dr. E.M. Rodriguez for the generous gift of his antibody (AFRU).

N. 10 HYPERTENSION EFFECTS ON THE RAT AREA POSTREMA. A MORFOMETRIC STUDY

Isabel Martínez de la Peña-Valenzuela, Luz María Fernández-González, Emilia Carmona-Calero, Herminia Pérez-González, Romualdo Ferres-Torres, María del Mar Pérez-Delgado, Agustín Castañeyra-Perdomo and Norberto Marrero-Gordillo

Depto. Anatomia, Universidad de La Laguna, La Laguna Tenerife Spain

The area postrema (AP) forms a single mound of tissue in the caudal margin of the fourth ventricle, overlaying the central canal (1). The AP is related to the effect of angiotensin II (AGII) and the vasopressin (VA) in cardiovascular regulation (2, 3, 6) The AP and adjacent tissue of the caudal medial portion of the nucleus tractus solitarii have been also involved in the general nausea and vomiting syndrome, and in regulation of the body weight (1). In the present study we analyzed the effects of hypertension on the function and morphology of a circumventricular organ related with the AGII and VA.

Forty male rats, fed with a standard diet, were sacrificed in groups of 5 at postnatal weeks 10 and 15. There were four groups: Group 1.- control Wistar-Kyoto rats (WKY), Group 2.- WKY treated with captopril (WKY-T), Group 3.- Spontaneously hypertensive rats (SHR) and Group 4.- SHR treated with captopril (SHR-T). The 5 members of each group shared the same cage and drank water and saline (0.3 molar) simultaneously from the beginning of the study. The captopril treatment was administered in the drinking fluid from 8 postnatal weeks at a dosage of 0.1 mg /ml (according to Thunhorst et al, 1987 (9) We divided the AP into 2 regions (rostral, and caudal). The neurons and ependymocytes of the 2 regions were analyzed using a "MAGISCAN" Image Analysis System (Program GENIAS). The parameters determined were nuclear area perimeter and form factor. For statistical evaluation, we compared the control and experimental groups using a two-way ANOVA and a post hoc test (Bonferroni) among animals of the same age.

The nuclear neuronal area (NA) seems to be an expression of neuronal activity [4]. We have found the values of the NA and the nuclear perimeter (NP) significantly increased in the rostral part of the AP of the SHR compared to those WKY rats, while no change was found in the caudal part of AP. Contrary to our finding, Nazarali et al. (1989) (7), reported only an increase of the number of AGII receptors in the SHR subfornical organ (OSF) but not in AP. Then we have seen, that the rostral part of AP is probably more implicated in the cardiovascular regulations, such as, it has been described by other authors (6, 8).

REFERENCES

1.- CASTAÑEYRA-PERDOMO A, MEYER G and HEYLINGS DJ (1992). Early development of the human area postrema and subfornical organ. *Anat Rec*, 232: 612-619

2.- CASTAÑEYRA-PERDOMO A, MEYER G, CARMONA-CALERO E, PÉREZ-GONZÁLEZ H, PEREZ-DELGADO MM, BAÑUELO-PINEDA J and FERRES-TORRES R (1993). The effect of chronic administration of captopril on the mouse subfornical organ and area postrema. *Exp Neurol*, 120: 145-148.

3.- CASTREN E and SAAVEDRA JM (1989). Angiotensin II receptors in the paraventricular nucleus, subfornical organ, and pituitary gland of hypophysectomized, adrenalectomized and vasopressin-deficient rats. *Proc Natl Acad Sci* USA, 86: 725-729.

4.- HILDEBRAND R (1980). Nuclear volume and cellular matabolism. *Adv Anat Embryol Cell Biol*, 60: 1-53.

5.- LI Z and FERGUSON AV (1993). Angiotensin II responsivenes of rat paraventricular and subfornical organ neurons in vitro. Neuroscience, 55: 197-207.

6.- MICHELINI LC, BARNES KL and FERRARIO CM (1986). Area postrema lesions augment the pressor activity of centrally administered vasopressin. *Clin Exp Theoric Practice*, A8: 1107-1125.

7.- NAZARALI AJ, GUTKIND JS, CORREA FM and SAAVEDRA JM (1989). Enalapril decrases angiotensin II receptors in subfornical organ of SHR. *Am J Physiol*, 256 (Heart Circ. Physiol. 25): H1609-H1614.

8.- SAAVEDRA JM, VORREA FMA, PLUNKETT LM, ISRAEL A, KURIARA M and SHIGEMATSU K (1986). Binding of angiotensin and atrial natriuretic peptide in brain of hypertensive rats. *Nature*, 320: 758-760.

9.- THUNHORST RL, FITTS DA and SIMPSON JB (1987). Separation of captopril effects on salt and water intake by subfornical organs lesions. *Am J Physiol*, 252 (Regulatory Integrative Comp. Physiol. 21): R409-R418.

ACKNOWLEDGEMENTS

Supported by the Consejería de Educación del Gobierno de la Comunidad Autonoma de Canarias. Project. Nº 1998/002, and Universidad de La Laguna Project Nº 48/98.

N. 11 AFFERENT PROJECTIONS TO THE MEDIODORSAL AND ANTERIOR THALAMIC NUCLEI (AN ANATOMIC EXPLANATION OF THE PATHOPHYSIOLOGY OF FATAL FAMILIAL INSOMNIA)

J.L. Velayos, F. Alfageme, L. Carrascal and J. M. Muñoz

Universidad Autónoma de Madrid

ABSTRACT: In fatal familial insomnia (FFI), a prion degenerative nervous disease, anatomopathological alterations in mediodorsal (MD) and anterior (A) nuclei of the thalamus (specially, the MD intermediate band and the anteroventral nucleus) (2) are constantly observed. After a time, the deep layers of the cerebral cortex are also affected. The most affected area is the cingular cortex (1).

As extrapolation is possible because the connectivity of MD and A in the cat is similar to the primate, we tried to supply anatomical data which help to the understanding of the pathophysiology of FFI: afferent projections to MD and A in the cat were studied by means of stereotaxic injections of neuronal tracers (horseradish peroxidase, HRP, and fluorochromes). HRP and NADPH-diaphorase (NADPH-d) colocation in neuronal bodies which send projections to MD and A was studied. Animals were always anaesthetized with nembutal before surgery and perfusion.

Tracer injections in A labelled the cingular cortex. MD injections labelled the cingular cortex but also more rostral areas. In both cases the cell labelling was always observed in the deeper layers of the cortex. The retrograde labelling in the basal prosencephalon was observed specially after injections in the MD intermediate band and in the anteroventral and anteromedial nuclei of the A complex. The suprachiasmatic nucleus was specially labelled after tracer injections in the intermediate band of MD. The reticular thalamic nucleus was aboundantly labelled after injections in the three bands of MD and scarcely after injections in the anteroventral nucleus. Retrograde cell labelling of the brainstem was more prominent after tracer injections in MD than in A.

The interruption of such connections could explain most of the symptomatology of FFI. The possibility that prion agents responsible for FFI spread from the MD and A (mainly the intermediate band of MD and the anteroventral nucleus of the A complex) through a retrograde pathway is discussed (3).

Retrograde labelled neuronal bodies around vessels in the basal prosencephalon and brainstem were observed mainly after tracer injections in the intermediate band of MD and in the anteroventral nucleus of the A complex. This finding, along with the fact of a HRP - NADPH-d colocation in such a type of neurons in the cases of MD and A tracer injections leads us to propose a possible pathophysiological implication of nitrergic systems in FFI.

ACKNOWLEDGEMENTS

This work was supported by grants CAICYT PM 95-0034, BMH4-CT96-856 (EU) and Rodríguez-Pascual Foundation (1999).

REFERENCES

1.- CORTELLI P, PERANI D, PARCHI P, GRASSI F, MONTAGNA P, DE MARTÍN M, CASTELLANI R, TINUPER P, GAMBETTI P, LUGARESI E and FAZIO F (1997). *Neurology,* 49: 126-133.

2.- GAMBETTI P, PARCHI P, PETERSEN RB, CHEN SG and LUGARESI E (1995). *Brain Pathology,* 5: 43-51.

3.- VELAYOS JL, OLIVA M and ALFAGEME F (1998). *Brain Path,* 8: 549-552.

N. 12 LOCATION OF SPINOTHALAMIC CELLS IN THE GUINEA PIG FOLLOWING INJECTIONS OF WGA-HRP AND FAST BLUE IN THE VENTROMEDIAL REGION OF THE THALAMUS

I. Fuentes Boquete, Ángeles Rodríguez Cobos, Luis Alberto Gómez Segade and Constantino Midón Martínez

Department of Morphological Sciences. Faculty of Medicine and Dentistry.University of Santiago de Compostela

INTRODUCTION: The spinothalamic tract (STT) of mammals has been classicaly involved in pain and temperature transmission. Cells of spinothalamic origin have been traced using several neuroanatomical tracers in rats, cats and monkeys. These studies have shown that a great number of spinothalamic fibres end in several nuclei of the ventromedial region of the thalamus. In spite of the peculiar anatomical and biochemical characteristics of the nervous system of the guinea pig, the somatosensory system and particularly the spinothalamic pathways are poorly understood. Thus, in the present study we determined the location of spinothalamic neurons in the guinea pig, which project to the ventromedial region of the thalamus with two tracers: wheat germ agglutinin conjugated with horseradish peroxidase (WGA-HRP) and Fast Blue (FB).

MATERIAL AND METHODS: In 6 adult guinea pigs, under deep anesthesia (sodium pentobarbital; 30-40 mg/kg body weight), the ventromedial region of the thalamus was injected with either WGA-HRP (Sigma) or FB (Dr. Illing, Germany). Following a postoperative survival period of 42 h (WGA-HRP) or 15 days (FB), the animals were killed and the blocks of brain and spinal cord were immediately removed and postfixed. Coronal sections were cut through the diencephalon and the spinal cord at 100 μm in a vibratome. The diencephalon and spinal cord sections of the animals injected with WGA-HRP were processed using o-tolidine/pyrocatechol and TMB methods, respectively.

RESULTS: On average, over 2,900 labelled STT cells were counted in all the spinal cord segments. Approximately 66.5% of the labelled neurons were found contralaterally to the injection site. The number of cells labelled with WGA-HRP was comparable to the number labelled with FB. Furthermore, the rostrocaudal distribution of labelled cells with both tracers was similar: 68% in the upper spinal cord, 10 % in the cervical enlargement and 4% in the lumbar enlargement.

Labelled cells were mainly found in laminae V (34%) and VII (29%). Lower percentages were observed in laminae I (16%), IV (8%) and VIII (4%), in the lateral cervical nucleus/lateral spinal nucleus (3%) and in the internal basilar nucleus (5%). Ipsilateral labelled neurons were

primarily located in lamina VII, while lower percentages were observed in laminae I and V. Contralateral labelled neurons predominated in laminae V and VII, but were also observed in laminae I, IV and VIII and in the internal basilar nucleus.

DISCUSSION: Our results demonstrated an important spinothalamic projection to the ventromedial region of the thalamus in the guinea pig. Compared to other species, the laminar origin of this projection has specific characteristics. The projection from lamina I, involved in nociception and thermoreception, is less abundant in the guinea pig that in the cat, while in the rat spinothalamic projection to the medial thalamus occurs from the deeper laminae. Furthermore, the participation of the two intumescentiae in the guinea pig is lower than in the rat, in the cat and in primates.

This research was supported by a grant-in-aid for scientific investigation from the Xunta de Galicia of Spain (XUGA20812B96).

N. 13 THE PERIGENICULATE COMPLEX OF THE THALAMUS AS A RELAY STATION FOR INTEGRATIVE VISCERAL INFORMATION TO THE AMYGDALA

F.E. Olucha-Bordonau, Eva Salvo-Marco, Alfonso A. Valverde-Navarro and Francisco Martínez-Soriano

Dpto. de Ciencias Morfológicas. Universidad de Valencia

INTRODUCTION: It has been largely viewed the medial nucleus of the medial geniculate body and surrounding perigeniculate nuclei (MGm/PG) as a relay station in progressing emmotionally relevant auditory information to the lateral nucleus of the amygdala and auditory cortex. Disruption of this pathway impairs the emotional association of a neutral conditioned stimulus (CS) to a fearful unconditioned stimulus (US). Fear conditioning elicited a set of autonomic and behavioral reactions which can also modify the conditioning process.

MATHERIAL AND METHODS: To study the source of visceral inputs to the perigeniculate complex retrograde tracer injections were made in the MGm/PG complex and anterograde tracer injections were made in the retrograde labeled hypothalamic and brainstem areas involved in visceral processing.

RESULTS: Cholera toxin subunit B (CTb) injections resulted in retrograde labeling in the zona incerta, medial preoptic area, perifornical nucleus, retrochiasmatic area, tuber cinereum, lateral hypothalamic area, tuberomammillary area and posterior hypothalamic area, peeriaqueductal grey, laterodorsal tegmentum, and lateral part of the parabrachial nucleus. Biotindextranamine injections in the zona incerta, lateral hypothalamic area, laterodorsal tegmentum, and parabrachial nucleus resulted in anterograde labeling in the marginal zone of the ventral medial geniculate body, deep dorsal and caudo dorsal nucleus of the doral medial geniculate body, medial nucleus of the medial geniculate body, posterior intralaminar, peripeduncular nucleus and nucleus subparafascicularis lateralis.

DISCUSSION: All these nuclei, that we have termed perigeniculate nuclei, project to the lateral nucleus of the amygdala and our results demonstrated the existence of a connection between visceral hypothalamic and brainstem areas and the MGm/PG complex.

N. 14 SOMATOTOPY IN DORSAL ROOT GANGLIA IN THE ADULT RAT

Alberto Prats Galino, Anna Puigdellívol Sánchez and Domingo Ruano Gil

Department of Morphological Sciences, Faculty of Medicine, University of Barcelona, 08036 Barcelona, Spain

The existence of an arrangement of sensory neurones in dorsal root ganglia has been a controversial matter. We have studied it by applying fluorescent tracers simultaneously in the main hindlimb nerves, in three branches of the sciatic nerve and in distal cutaneous area such as the distal phalanges of the toes.

All the animals were deeply anaesthetised by chloral hydrate (30mg/100 mg b.w.). In a first group, the sciatic and femoral nerves were transected and the proximal cut ends were introduced in a capsule containing Fast Blue (FB) and Fluoro-Gold (FG), respectively. In a second group the branches of the sciatic, the tibialis, the peroneal and sural nerves were labelled the same way with FB, FG and Diamidino Yellow. Finally, the distal phalanges of the toes were injected with the same tracers (3 toes per animal maximum). All rats were anaesthetised again after five days, perfused through the ascending aorta with fixative and the lumbar dorsal root ganglia were dissected and cut on a cryostat.

Sciatic neurones were found in L4-L6 while femoral neurones were found in L2-L4. Sciatic and femoral neurones were distributed separately in DRG L4, with a thin overlapping zone. Femoral neurones occupied the most dorsal part of the ganglion and remains restricted in a rostrolateral position when progressing ventrally, while sciatic neurones occupied the rest of the ganglion.

The different populations of the sciatic branches were found to be considerably intermixed, however, although neurones projecting to the tibialis predominate in L4 and L5, peroneal nerve have a major representation in L4 and the sural nerve is mostly distributed in L5, with just a few neurones in L4.

Finally, about 300 neurones per toe were found sparsely distributed among L3-L5 DRG in a rostrocaudal arrangement, so that rostral ganglia contained more neurones sending their axons towards medial toes while neurones of caudal ganglia tended to innervate the lateral toes. Although neurones of a determinate toe tended to leave an unlabelled zone in determinate ganglia, all the populations were found to be considerably intermixed. Only a consistent arrangement was found in L5 DRG, where toe 4 uses to be distributed rostrally regarding toe 5.

We conclude that a somatotopical organization may be described in DRG, which is clear when studying different nerves although the different represented populations of a single main nerve are considerably intermixed.

Work supported by grants of the Fundació Marató TV3 (003-97)

N. 15 SUPRASPINAL PROJECTIONS TO THE CAUDAL VENTROLATERAL MEDULLA IN THE RAT

A.R. Cobos[1], Isaura Tavares, Armando Almeida and Deolinda Lima

Institute of Histology and Embryology, Faculty of Medicine and IBMC, University of Oporto, 4200 Porto, Portugal and [1] Department of Morphological Sciences, University of Santiago de Compostela, Spain

INTRODUCTION: The caudal ventrolateral medulla (VLM) has been implicated in autonomic and analgesic functions. VLM neurons project to areas of the central nervous system involved in the control of homeostatic mechanism associated with behavioral and emotional responses, as well as to brainstem regions implicated in antinociceptive control. The lateral reticular formation of the VLM (VLMlat) was recently shown to be engaged in the production of potent antinociception and to mediate hypertension induced analgesia through the inhibition of pain transmission at the spinal dorsal horn. The aim of the present study was to establish the brain afferent system to the VLMlat in order to disclose the anatomical substrate of its role in integration of sensory, motor and autonomic effects.

MATERIAL AND METHODS: Male wistar rats, weighing 280-300 g were anaesthetised with halothane (4% for induction and 1.5-2% for maintenance) and stereotaxically injected in the left VLMlat with 0.3 μl. of the retrograde tracer cholera toxin subunit B (CTb). Ten days later the rats were reanaesthetized with 35% chloral hydrate, i.p., and perfused with paraformaldehyde in 0,1 M phosphate buffer, pH 7.4. Brains were removed and coronal 40 μm frozen sections were obtained. One of each three sections of the brain was immunostained for CTb by ABC method and counterstained with formol-thionin.

RESULTS: Retrogradely labelled neurons were widely distributed in the brain prevailing in areas involved in the production of autonomic responses and modulation of pain such as the nucleus tractus solitarius, parabrachial nuclei, A_5 noradrenergic cell group, periaqueductal gray and paraventricular nucleus. Retrograde labelling was also observed in areas which are known to participate in the production of somatic motor reactions, such as the lateral reticular nucleus, inferior olive, dentate cerebellar nucleus, red nucleus and motor cortex. CTb labelled cells occurred in brain regions involved in the production of emotional motor reactions such as the central amygdaloid and sublenticular nucleus and bed nucleus of the stria terminalis and in areas involved in motor (limbic cortex) and visceral sensory (insular cortex) responses.

CONCLUSIONS: The involvement of the areas with VLMlat afferents in autonomic, somatosensory, emotional and motor functions suggests that the VLMlat may play an important role in the complex defense reaction which is known to be triggered upon physical threatening.

ACKNOWLEDMENTS

Supported by the Biotech project n° BIO4-CT98-0076 of the European Commission. Professor Angeles R. Cobos acknowledges a grant from Xunta de Galicia.

N. 16 NUCLEAR SIZE VARIATIONS OF THE PINEALOCYTE IN DIFFERENT SEASONAL AND SYNODICAL CYCLES

F. Martínez-Soriano, A. Ruiz-Torner, T. Hernandez-Gil, F.E. Olucha-Bordonau, A.A. Valverde-Navarro and J. Cabanes-Vila

Morphological Sciences Department. Valencia University. Valencia (SPAIN)

INTRODUCTION: Morphological and physiological studies suggest a possible division of the pineal parenchyma into an external, or "cortical", and another central, or "medullar", layers. Determinations of the size of the pinealocytes in the peripheral and central regions at various times during a 24h. Period, in different seasons, in rats, have been made by different authors regarding regional and day-night differences in pinealocyte size. On the other hand, the possibility that biological circadian rhythms may be influenced by geophysical variations such as diurnal fluctuations in the intensity of environmental iluminations, has been suggested and studied. Also, the gravitatory lunary cycles have been relationed with the fluctuation of the tides, growing of the harvest or temperature variations. We have studied the possible influence of the lunary cycles over the nuclei size of the pinealocyte in the "cortical" and in the "medullar" layers.

MATERIAL AND METHODS: Forty male wistar rats (mean body weight 240±37 g.) subjected in the same nutritional and environmental conditions (temperature,18-20ºC, natural light) were studied.The rats were divided in 8 groups of 5 animals each. Four groups were sacrificied in Winter: 2 during the new-moon days (one group between 10:00 and 12:00 hour and the second between 00:00 and 02:00 hour) and the other two during the full moon day, in the same above mentioned conditions. The same procedure and conditions was followed for the sacrificied of the four spring groups. The karyometric indices were determined from sections stained with toluidin blue. Nuclear size measurements were made in two layers of the gland.Only clearly visible pinealocyte nuclei were considered. For each animal and region 25 x 4 nuclei from different sections were measured.The selection of subsequent sections was made so that each section was at least 15 μm away from the precceding one, so as to avoid including more than one section of each nucleus. The nuclear volume was calculated from the measured values using the Jacobj formula. The statistical evaluation of the data was made after a descriptive study. Simultaneously we compared the means of more than two variables, using the analysis of variance.

RESULTS: The result shown that in the the winter and during photophasic period of new-moon day, the nuclear size of cortical layer are greater as whose of the medullar layer. In the contrary, during the scotophasic period the result was reversed. But in the all remainder situations,the nuclear sizes of the medullar layer pinealocytes are greater that whose of the cortical layer. Only during the scotophase of the moon full day of the spring, nuclear size of the pinealocytes in both layers are similars. These photophasic, seasonal and synodic variations of the pinealocyte nuclear sizes observed between both layers of the pineal gland, are statistically significatives.

CONCLUSION: The conclusion is that exist a independent mophological variations of the pinealocyte in the central and peripheric zones of the pineal gland. Variations dependent, both, of the combination of photophases, season and synodic cycles.

POSTERS

P. 1 STUDY OF THE S-100 IMMUNOREACTIVITY OF THE UPPER EYELID OF THE SHEEP (OVIS ARIES)

J. Aísa, M. Lahoz, P.J. Serrano, M.C. Pérez-Castejón, C. Junquera, M.C. Martínez-Ciriano, J. Deus and A. Vera-Gil

Dpto. de Ciencias Morfológicas. Facultad de Medicina de Zaragoza

INTRODUCTION: The interest of this study is based on the specificity of S-100 immunoreactivity (-ir) for several cellular types. The identification of these cellular types is of interest for the diagnosis and treatment of the eyelid tumours.

METHODS: On 30 sheep upper eyelids, rabbit anti-S-100 protein was used in immunoperoxidase staining studies, to determine the distribution of S-100 protein in normal tissue.

RESULTS: In the flat stratified epithelium of the upper eyelid edge, S-100 immunoreactive cells were observed in the basal layer, showing an oval nucleus, prominent nucleolus and dendritic protuberances.

In the connective tissue, S-100-ir was found in the inner root sheath of the hair folicle, as well as in the small nervous trunks around the basement membrane.

In the Zeiss, Moll, Meibomian and lacrimal glands no S-100-ir was found, although positive nervous trunks were found in the conective septum.

Vessels inside the connective tissue are surrounded by S-100 immunoreactive small nervous trunks, which can be also observed in the adventitia layer. With more augmentation, S-100 positive fine nervous fibres can be identified in the muscular wall of the arteriolae.

We also found S-100-ir among the muscular bundles within the connective tissue. However, we did not find any S-100-ir in the conjunctive layer.

DISCUSSION: Our results indicate that the distribution pattern of the S-100-ir in the sheep upper eyelid is less developed than the AChE+ and FIF+ patterns previously studied by us.

P. 2 MICROVASCULAR CONTRIBUTIONS FROM EXPERIMENTAL STUDIES TO KNOWLEDGE AND REPAIR OF BONE FRACTURES AT INVOLUTIVE STAGES

M.P. Alonso Martínez, E.R. Meaños Melón, M.J. Muizulis Alonso, M.E. Padín Iruegas, A.M. Tarrazo Antelo, S. Pedreira Currás and F. Navarrina Gámez

2.ª Cátedra de Anatomía, Dep. Ciencias Morfológicas, Fac.Medicina, Univ. Santiago de Compostela, Serv. Rehabilitación Hospital Xeral Clinico-Universitario de Santiago de Compostela

INTRODUCTION: Prospective and evolution that osseous fracture presents will be conditioned by differents parametres, between which the age factor and metabolic status play an important roll. In the face of normal involutive process that the human organism suffer by aging, the research work on osseous system acquire more relevance by the projection of these involutive status to induce pathologic processes as result of gonadal hormonal secretion stoppage, it involves the stablishment of menopause and andropause periods in women and men respectively, with the involutive osteoporotic problems that the aging incretory status setting up. As consecuence appear bone mass loss, decrease of its functional capacity and lower competence in biomecanical function of loads support, so it involves apparition of pain mainly on load joints and traumatisms with low intensity can make a important fracture most of times with bad evolution (pseudoarthrosis or no-union). We emphasize the roll that vascular parameter plays on osseous tissue function, as arterial supply as veins drainage too, we point up the importance of venous system on prospective and future of bone. So in this study we have stablished experimentaly both a involutive status by means of bilateral gonadal removal, and after we achieve a fracture in long bone, to study the answer of osseous tissue in this involutive status induced, the modifications of microvascular patterns and its adaptation to new experimental status and its projection on evolution of osseous fracture repair and prospective about.

MATERIAL AND METHODS: We used as experimental animal adult albino rats (8 weeks) OFA Spragüe Dawley, both sexes. Divided in several experimental groups (10 specimens each one): Ia) Male Control Group, Ib) Female Control Group, IIa) Male tiblal Fracture Group, IIb) Female tibial Fracture Group, IIIa) Males with tibial fracture and testicular gonadal Gland Bilateral Ablation Group, IIIb) Females with tibial fracture and ovarian gonadal Gland Bilateral Ablation Group. Fractures were realiced on 1/3 uper of tibial bone with microsaw, and peging later with steel needles both osseous fractured zones. At last of the differents periods of experimental life (one, three, and twelve months) we proceeded to exitus of animals in the three groups, subjecting the animals to histological technics and others to vascular injection technics, with rx ray initial and final controls, and later to histological proceedings, microscopical observation and microphotographic series, to histological and microvascular studies.

RESULTS AND DISCUSSION: Exitus of the animals at differents moments of experimental life period, let us consider the evolution at distinct stages on results obtained in the experimental areas of tibial long bones in controls, fracture groups and fracture with gonadal removal groups (subdivided in male and female to each one) over the both fracture zones physary-callus and callus-diaphysary in presence of its microvascularization in each area up and down, to checking evolution to good results with consolidation of callus zone (Groups IIa and IIb), or no good evolution of callus by ostheoporosis acquired through gonadal ablation, with a microvascular support decreased in callus border zones physary and diaphysary. Experimental females (IIIb) show up an important changes in its microvascular and celular patterns in both border callus areas more marked than experimental males; we emphasize it at level of venous system with the stablishment of venous stay (estasys) with a slower drainage in peripheral callus zones, and few density in callus structure, marked osteoporotic component and speudoarthrosis tendence, than male experimental ones(IIIa) which shows better tendences to good results in drainage, callus evolution with less no-union over fracture consolidation and osteoporotic incidence lower. So involutive induced status involve a negative prospective more serious and marked in female especimens by means of facts that works like this can verify.

P. 3 EXPERIMENTRAL STUDY ON BONE: CONTRIBUTIONS TO KNOWLEDGE OF ITS MICROVASCULAR PATTERNS IN ELDERLY STAGES

M.P. Alonso Martínez, E.R. Meaños Melón, M.J. Muizulis Alonso, E. Padin Iruegas, A.M. Tarrazo Antelo, S. Pedreira Currás and F. Navarrina Gámez

2ª Cátedra de Anatomía, Departamento de Ciencias Morfológicas, Facultad de Medicina, Universidad de Santiago de Compostela. Servicio de Rehabilitación, Complejo Hospitalario Xeral de Galicia-Clínico Universitario de Santiago

INTRODUCTION: In the face of global problematic that human body suffer in its normal general involutive process, within diferent lines of research and study there are an important part dedicated to the locomotor system, granted that the importance of these biological changes are projected on pathology field. As result of gonadal hormonal secretion stoppage, it involves the stablishment of menopause and andropause periods in women and men respectively, with the involutive osteoporotic problems that the aging incretory status setting up, as consecuence appear bone mass loss, decrease of its functional capacity or competence in the loads support biomecanical function, so it involves the apparition of pain mainly on load joints, and traumatisms with low intensity can make a fracture. Inside the multifactorial and complex framework that operate on course of this process, is the vascular system with a important rol, through our works we have can to check that experimental modifications on osseous microvascular pattern so in arterial and venous sectors make important morphofunctional modifications on the bone prospective. According to expossed, the actual work try to stablish an experimental involutive status by means of gonadal ablation, to study the microvascular patterns and its answer by the changes induced, with the repercussion on the osseous tissue.

MATERIAL AND METHODS: We have used adult albin rats, both sexes, divided in experimental groups: I) Male Control Group (10 animals), II) Female Control Group (10), III) Bilateral Testicular Ablation Group (20), IV) Bilateral Ovary Ablation Group (20), the animals have been submitted to standards about upkeep and food all experimental life long . Exitus were in 3 periods, at 3, 6, 9 months, Rx controls initial and final ; the oseous pieces obtained have been submited to standard proceedings so for histological studies and microvascular techniques of study, to finaly achieve the systematic microphotographic series.

RESULTS AND DISCUSSION: Our study was centred mainly on long bone, and we had verified that the patterns of microvascular architecture of control animals, showing small differences between specimen controls males and females, considering the differet morpho-functional zones of long bones too (epiphyseal, physeal, methaphyseal and diaphyseal areas). However the microvascular results corresponding to experimental groups offer facts highly significativs, stablishing changes in its patterns that are increased all experimental period long, so in arterial dispositions as venous areas, which involve functional repercussions more importan and showys on bone in proportion of experimental time advances, with modifications in the differents morpho-functionals zones, but we remark that experimental facts are more serious in female specimens with instauration of generalizated severe osteoporotic status, much more intense that in experimental males.

P. 4 ANTHROPOMETRIC STUDY IN ADULTS OF AGE 50-65 FROM THE BASQUE COUNTRY

M.D. Cabañas Armesilla[1], J. Gondra del Río[2], A. Angulo Gutiérrez[3]

[1] Department Ciencias Morfologicas II, Faculty of Medicine, University Complutense. Madrid. [2] Department of Neuroscience, Faculty of Medicine and Odontology, University of Pais Vasco / Euskal Herriko Unibertsitatea. [3] Medical Center Bailen, Bilbao

INTRODUCTION: The anthropometry is the easiest, most universally apply, low price and uninvasive method to assess size proportion and composition of the human body. Anthropometry signs health as well as nutritional status and predicts performance, health and surviving.

Anthropometric study realized in individuals of medium social and economical status (public officials, professionals, merchants), sedentary (≤ 2.5 hours of exertion a week), from Basque Country, of 50-65 years of age.

MATERIAL AND METHODS: Studied 48 males and 22 females from Bilbao of age 50-65. 17 anthropometric items where evaluated directly and somatotype, Body Mass Index (B.M.I.), profile and sum of skinfold. The data has been processed by the Excel program.The experiment was carried with the knowledge and consent from the individuals.

RESULTS

1. B.M.I.

	Males		Females	
	Mean	Deviation	Mean	Deviation
Age (years)	55.63	3.42	55.18	4.33
Height (cm.)	172.19	5.88	159.16	4.60
Weight (Kgs.)	81.53	13.80	61.23	7.87
Body Mass Index (B.M.I.)	27.42	3.88	24.18	3.00

Classification of B.M.I. [Seidell, 1997]				
B.M.I.	Classification W.H.O.	Popular description	Males (%)	Females (%)
< 18.5kg/m2	Low weight	Thin	0	0
18.5-24.9 kg/m^2		Healthy weight Normal or acceptable	25%	59%
25.0-29.9 kg/m^2	Overweight I	Overweight	56%	36%
30.0-39.9 kg/m^2	Overweight II	Obese	17%	4%
>40 kg/m^2	Overweight III	Morbid obese	2%	0

2. Somatotype

Mean of studied somatotypes			
	Endomorphy	Mesomorphy	Ectomorphy
Males	5.80 ± 1.32	5.22 ± 1.61	0.99 ± 0.69
Females	7.02 ± 1.66	4.01 ± 1.08	1.33 ± 0.93

Classification of somatotypes [Carter, 1990]	Males (%)	Females (%)
Balanced endomorphy	2	9
Mesomorphic endomorphy	52	86
Meso - endomorphy	27	0
Endomorphic mesomorphy	17	0
Balanced mesomorphy	0	0
Ectomorphic mesomorphy	0	0
Meso- Ectomorphy	0	5
Mesomorphic ectomorphy	0	0
Balanced ectomorphy	0	0
Endomorphic ectomorphy	0	0
Endo-ectomorphy	0	0
Ectomorphic endomorphy	2	0
Central	0	0

3. Sum and profile of skinfolds

	Sum 6 skinfolds	Sum 4 skinfolds[1]
Males	127.00 ± 34.50	53.01 ± 20.36
Females	166.98 ± 45.69	82.61 ± 22.93

1 Sum of 4 skinfolds: triceps, subscapular, supraspinale and abdominal

Distribution of sum of 6 skinfold			
Sum 6 skinfold (mm.)	Males (%)	Sum 6 skinfold (mm.)	Females (%)
≤55	0	≤65	5
55-70	2	65-80	0
70-85	10	80-95	0
85-100	15	95-110	0
100-115	15	110-125	1
115-130	8	125-140	9
130-145	19	140-155	23
145-160	8	155-170	23
160-175	4	170-185	9
175-190	8	185-200	5
>190	2	>200	23

	Males		Females	
	Mean	Deviation	Mean	Deviation
Triceps skinfold	13.91	4.92	23.81	8.57
Subscapular skinfold	25.78	6.91	24.10	7.40
Abdominal skinfold	34.30	8.22	36.46	11.55
Supraspinal skinfold	21.93	8.66	26.39	10.91
Thigh skinfold	18.82	8.55	33.24	10.12
Calf skinfold	12.26	6.78	22.98	6.78

DISCUSSION

1.- Trough the B.M.I., males have 56% overweight, 17% obese and 2% morbid obese. Rate clearly above that those for European adult male population of 35-65 years: 48 ± 4.1 with overweight and 15.5 ± 4.2 obese. [Seidell, 1997].

2.- Trough the B.M.I., in women there are 36% with overweight and 4% obese. Percentages slightly higher for overweight and clearly lower for obesity, than of those for European adult women of 35-64 years: 34.6 ± 4.5 with overweight and 21.7 ± 9.1 obese. [Seidell, 1997].

3.- Mean somatotype in males is Mesomorphic Endomorphy. Different from [Bailey, 1982] (Endomorphy Mesomorphy).

4.- Mean female somatotype is Mesomorphic Endomorphy. Equal with [Bailey, 1982] (Mesomorphic Endomorphy).

5 - Profile of skinfolds outstands, in males, from higher to lower thickness: abdominal, subscapular, supraspinal thigh, triceps and finally medial calf.

6.- Profile of skinfolds outstands, in females, from higher to lower thickness: abdominal, thigh, supraspinal, subscapular, triceps and finally medial calf.

P. 5 ECOGRAPHIC VASCULAR ANATOMY OF THE HUMAN ADULT AND PREPUBESCENT TESTIS

A. Carmona Bono[1], E. Rangel[2] and R. Jiménez-Castellanos[2]

Departamento de Ciencias Morfológicas[1] y Servicio de Radiodiagnóstico del Hospital Universitario Virgen Macarena[2]. Universidad de Sevilla

INTRODUCTION: Doppler ecography allows the study of scrotal anatomy in different planes and the normal course of vessels with their individual variants. In spite of these possibilities, the vascular anatomy of testis and its functional importance still remains less known. The goal in our study was to analyze the vascularisation pattern of the normal testis using doppler ecography, both in adult and children subjects.

MATERIAL AND METHODS: One hundred of patients were studied by doppler ecography. From them, 59 cases corresponded to prepubescent children. The distribution of testicular and extratesticular vessels was achieved as a reference.

RESULTS: The testis has a low resistence arterial flux, similarly to other parenchymatous organs. Thus, on one hand the morphology of the waves detected from the vessels which irrigate the testicular paranchyma (testicular artery and its branches) will show relatively wide systolic peaks and high levels of diastolic flux. On the other hand, cremasteric and deferential arteries which vascularise peritesticular soft tissues of the scrotum and epididymis, had a high resistence flux pattern with sharper systolic peaks and reduced or absent dyastolic flux. The scrotal venous return through the pampiniform plexus showed an absent flux at rest, which was weak following Valsalva's manoeuvre.

DISCUSSION: Basically, the vascular map observed in our study using colour doppler ecography coincides with that described by Middleton in aduld testis. In newborns and prepubescent infants the exam of the testicular vascular anatomy with doppler is more difficult. This fact is due to the lower size of testis and of its normal blood vessels. Thus, detection of low flux in the thin intratesticular arteries represents a challenge to the resolution of modern equipments, even though when they were used by expert radiologists. This limitation emphasizes the importance of experience and the close collaboration which has to be established between radiologists and clinicians in order to evaluate the testicular vascularisation in children. In conclusion, the knowledge of vascular anatomy and the different normal flux patterns of testis through doppler ecography results a very valuable tool to the study of normal vascularisation and its physiologic variants, as soon as to vascular pathology and its therapeutic implications.

P. 6 THE INFERIOR EPIGASTRIC ARTERY AS ALTERNATIVE IN CORONARY BYPASS

A.I. Cisneros, J.R. Whyte, A. Torres, M.D. Alconchel and R. Sarrat

Department of Morphology. School of Medicine. University of Zaragoza. Spain. Trabajo Subvencionado Por D.G.A. Beca N° B130-98

INTRODUCTION: The ideal artery has been looked for for a long time to perform coronary bypass, as Murray proposed in 1940, and from 60th decade onward, thank to Lester and Sauvage (1963) and Johnson and Favaloro (1967) publications, it had been done with saphena vein. In the end of eighties it was confirmed that the grafts performed with these vessels, was closed by means of progressive intimal hypertrophy and atherosclerotic injuries (Barner, 1973; Lytle, 1985; Landymore, 1987; Vincent, 1990) and then it was neccesary resort to look for another vessels, leaved the way clear for use the arteries (Loop, 1986; Puig, 1984). It have been used splenic artery (Edwars, 1973), radial artery (Curtis, 1975), right gastroepiploic artery (Pyms, 1987) or left (Bufolo, 1987), as well as internal mammary artery (Barner, 1973), with different results, and as a consequence of that, investigations continue to find the artery with less patogenicity.

We are going to realize an estructural study about inferior epigastric artery, thinked it will be a good substitute or complementary artery to internal mammary artery, as proposed Puig in 1990.

MATERIAL AND METHODS: We have studied inferior epigastric arteries proceeding from medico-legal autopsies. The pieces were stained with Orceina and Verhoeff (elastin methods) for the structural study, and with the α-actin method.

RESULTS: The inferior epigastric artery has structure of muscle artery, which emphasizes its vigorous, unique and festooned internal limiting membrane. Its media is constituted for smooth muscle cells in concentric disposition with an average of 20 layers, and is poor in elastic material, which is distributed in its internal third. The adventicia is characterized for the great richness in elastic material. The inferior epigastric artery shows in our study a low incidence of pathological intimal thickenings, and if they exist, are eccentrics.

To study immunohistochemicaly the influence of age in smooth muscle cells, we have observed how the α-actine activity is decreasing with the time.

DISCUSSION: The inferior epigastric artery for its morphology, in accordance with this study, its easy access (Barner, 1991; Mills, 1991), its lenght and diameter (Vincent, 1990; Zhbanov, 1995), its blood flow quantity (Cremer, 1993), as well as its atherosclerosis low incidence (our results coincide with Milgater, 1992), is a good choice for the coronary bypass.

P. 7 DETECTION OF CIRCULATING MELANOMA CELLS IN PERIPHERAL BLOOD WITH REVERSE TRANSCRIPCION AND POLYMERASE CHAIN REACTION

E. Carrillo[1], J. Prados[2], J.A. Marchal[3], C. Melguizo[2], C. Vélez[2], J. Fernández[1], A.E. Aránega[4], H. Boulaiz[1], S. Serrano[5], L. Álvarez[1] and A. Aránega[1]

[1] Departamento de Ciencias Morfológicas, Facultad de Medicina, Univ. Granada, [2] Departamento de Ciencias de la Salud, Univ. Almería, [3] Departamento de Ciencias de la Salud, Univ. de Jaén, [4] Departamento de Biología Experimental, Univ. Jaén, [5] Departamento de Dermatología, Univ. Granada

INTRODUCTION: The incidence of malignant melanoma, a highly immunogenic tumor, has increased steadily during the last decade. Mortality from malignant melanoma is related with lymphatic and blood metastases, because these are the routes of tumor dissemination. A variety of methods have been used to detect malignant cells in peripheral blood, including immunological and microscopic methods.

It was recently suggested that reverse transcription polymerase chain reaction (RT-PCR)-based detection of

tyrosinase messenger RNA (mRNA) in peripheral blood is useful in the early detection of circulating tumor cells, since tyrosinase is thought to be a melanocyte-specific marker.

MATERIAL AND METHODS: 50 samples from as many patients with histologically confirmed malignant melanoma in different stages. Blood sample was collected in tubes containing edetic adic (EDTA), was centrifuged and the plasma was discarded of the serum. Total RNA was extracted from the PBLC (peripheral-blood lymphocytes cells) by the guanidinium thiocyanate /caesium chloride. Our primers ME-l (5' TTG GCA GAT TGT CTG TAG CC) and ME-2 (3' AGG CAT TGT GCA TGC TGC TT) amplify a 284 bp fragment of the tyrosinase. β-actin primers spanning an intron were devised from the gene sequence (Gray et al., 1982) and used to check the integrity of the RNA samples for RT-PCR. The primers were 5' ATG GAT GAT GAT ATC GCC GCG and 3' TCT CCA TGT CGT CCC AGT TG, which produce a 248 bp fragment of β-actin. We used RT-PCR asssay to *detect* tyrosinase mRNA in the patiens affected of melanoma.

RESULTS: We tested peripheral blood samples from 50 patients with malignant melanoma in different stages of disease for the presence of tyrosinase transcripts. All patients with localized primary melanoma (n = 13) tested negative for tyrosinase mRNA. We used as positive control a lymph node sample from a patient with progressive metastatic disease. Among 37 patients with *progressive* metastatic disease (stages III and IV), tyrosinase mRNA was detected by RT-PCR in all patients, PBLC samples from ten healthy doners all tested negative for tyrosinase transcripts. Also, we could not amplify tyrosinase mRNA in blood samples from fourth patients with other malignances. The β-actin RT-PCR positive and negative controls and the positive lymph node gave strong signals in all cases, thus both RNA preparation and cDNA synthesis were successful.

DISCUSSION: In contrast with others criteria used to evaluate the prognosis of malignant melanoma (histopathologic appearance and clinical presentation), RT-PCR-based detection of tyrosinase mRNA in peripheral blood may be useful in the early detection of circulating melanoma cells, as tyrosinase is thought to be a specific marker of melanocytes. In our group of 50 patients with stage IIIB and stage IV disease, the number of circulating tumor cells is correlated with the tumoral burden. Our findings also suggest that the detection of tyrosinase mRNA in circulating cells may be a marker of rapid tumoral progression and poor clinical outcome. We emphasize the importance of the technique used to process blood samples from patients with malignant melanoma for the detection of circulating melanocytes with RT-PCR methods.

P. 8 PULMONARY DISTENSIBILITY. MORPHOMETRIC STUDY OF TWO SAMPLE GROUPS OF RATS

J.D. Escolar, Carlos Tejero, Mª Asunción Escolar, Raúl Garisa and María Roques.

Department of Morphological Sciences, Faculty of Medicine, Zaragoza

For morphometric study, the lung should be fixed at a determined insuflation pressure, known as transpulmonary pressure (TPP). Different TPP proposals exist the morphometric study of the lung. All of them have as a reference "physiological" pulmonary volumes. We propose that independently of those considered "physiological" volumes, for a morphometric study the lung must be distended to the maximum without producing artefacts.

MATERIAL AND METHODS: 80 Wistar rats were used divided into two age groups: adults of 5 months and older rats of 18 months. These were respectively divided into four groups according to the TPP used in fixation: 20 cm; 25 cm; 30 cm and 35 cm of H_2O. The animals were anaesthetised with pentothal. The fixation of the split lungs was performed with formaline at 10% via the trachea. The sampling was done at three levels, two systematically and one at random. The lungs were processed for light microscopy. Incisions were studied with the OS computer system. The following variables were quantified: pulmonary volume (PV); tissue volume (TV); alveolar chord (AC) which is in relation to the alveolus, total number of alveoli of each lung (AN) and internal alveolar surface (IAS). A statistical study was performed.

RESULTS: In the adult animals the results stood in relation with the TPP modifications. PV increased significantly when the TPP was raised to 20 cm and 25 cm of H_2O, the TV when raised from 20 cm to 30 cm and from 30 cm to 35 cm of H_2O, the number of alveoli increased from 20 cm to 30 cm and from 30 cm to 35 cm of H_2O, and the IAS from 20 to 25 cm of H_2O. AC diminished significantly when the TPP was raised from 20 cm to 25 cm and from 30 cm to 35 cm of H_2O. In the older animals there was no correspondence to the results with the increase in TPP. A significant difference was noted only in the animals fixed at 20 cm and 25 cm of TPP.

CONCLUSION: In the adult animals, the ideal fixation is that of 25 cm of H_2O, as the lung distends, recruits the alveoli and does not present artefacts. The results obtained in older animals leads us to believe that the results obtained in fixing the four TPP used are similar.

Este estudio ha sido realizado gracias a la ayuda de investigación nº 213-38 de la D.G.E.S. (Diputación General de Aragón)

P. 9 COLLAGENS IN HUMAN UMBILICAL VEIN FROM SMOKING MOTHERS: AN IMMUNOHISTOCHEMICAL ANALYSIS

P. Díaz-Herrera[1], A. Torres[2], J. White[2], C. Hernández-Urquía[1], A. Cisneros[2] and R. Sarrat[2]

[1] Departamento de Morfología. Universidad de Las Palmas de Gran Canaria. [2] Departamento de Ciencias Morfológicas. Universidad de Zaragoza

INTRODUCTION: Smoking during pregnancy is harmful for the fetus and leads to an increased risk of abortion and stillbird, as well as death soon after delivery. The average weight of newborn children of smoking mothers is lower than those born to non-smoking mothers. It has been demonstrated that the tobacco damages umbilical vessels at an structural and ultrastructural level. Because of it, the umbilical vein has been chosen as a model for evaluating the vascular injury provoked by cigarette. In cardiovascular system from adults, the distribution of wall collagens is altered as consequence of the atherosclerosis due to tobacco-smoking. To the best of our knowledge, there are not previous immunohistochemical studies of collagens in smoking-mothers umbilical veins. The primary purpose of this study was to know if different types of collagens in umbilical veins behave like in adult arteries.

MATERIAL AND METHODS: Umbilical veins from 50 newborn children delivered at term from healthy mothers were distributed in five groups of ten each (0, 10, 20, 30 and 40 cigarettes/day). Samples were obtained with the understanding and the consent of the mothers. Sections were reacted with policlonal antibodies specific to collagens type I, III and V and reacted with monoclonal antibody specific to collagen type IV. Immunolocalization of collagen types was performed by streptavidin method.

RESULTS: The umbilical vein of new born babies delivered from non-smoking mothers showed type I and type IV collagens while type III and type V collagens were practically absent. Type I collagen, which was organized into fibrillar structures localized between smooth muscles cells, was the most abundant collagen. Type IV collagen was observed as a membranous structure surrounding the smooth muscle cells and as a linear structure in the subendothelium, presumably in the endothelial basement membrane.When mother were smoking, it could be seen an increase of type I and type IV collagens. In this case, it was also observed the presence of type III and type V collagens. These two types of collagen were organized into fibrillar structures placed into intercellular space in the subendothelium. This increase was higher in umbilical veins of new babies from mothers that smoked more than 20 cigarettes per day.

DISCUSSION: The same as it happens in the arteries from the postnatal life subjected to tobacco-smoking, also the umbilical veins developed intimal thickening. When this happened, an increase of type I, type III, type V and type IV collagens was detected. Type I and type III collagens, which are the most important collagens involved in platelet aggregartion, play a critical role in the formation and progress of atherosclerosis. Type V and type IV collagens have been involved in the progress of atherosclerotic lesion. The second one, has also been related with the process of calcification into the endothelial thickening. From our results it can be deducted that collagens into the umbilical veins behave as they do in postnatal arteries subjected to cigarette-smoking. The same as other authors, we suggest that umbilical vessels can be used as a model to the study of atherosclerosis.

P. 10 DEPTH OF INTERNAL JUGULAR VEIN DURING CENTRAL VENOUS CANNULATION

J.C. Diz[1,2], P. Otero[2], R. Vázquez[2], M.E. Padín[3], M.J. Muizulis[3] and E.R. Meaños[3]

[1] Dep. de Biología Funcional y Ciencias de la Salud, Universidad de Vigo. [2] Servicio de Anestesiología, Hospital General de Galicia. [3] 2ª Cátedra de Anatomía, Dep. de Ciencias Morfológicas, Universidad de Santiago

INTRODUCTION: Central venous cannulation is a common procedure for continuous monitoring of central venous pressure (CVP), parenteral nutrition and drug administration. Internal jugular vein (IJV) is one of the most used cannulation sites, since the technique has a high success rate with a low rate of complications (the most important being carotid artery puncture and pneumothorax). The incidence of pneumothorax can be decreased with a proper technique, mainly avoiding overinsertion of the needle on locating the vein. However, in the bibliography there is not a clear recommendation regarding depth of insertion of the needle for locating the vein. Thus we decided to study the depth of location of the IJV during central venous cannulation.

PATIENTS AND METHODS: Sixty-nine consecutive patients submitted to cardiac surgery were included in the study. CVP monitoring through the IJV is part of the routine monitoring in this type of surgery. Under asepsis and continuous electrocardiogram monitoring we cannulated the IJV with the Seldinger technique, using the central access (between the two heads of the sternocleidomastoid muscle) and the patient in horizontal position. Just on drawing venous blood we measured the length of needle inserted, and this allowed for estimation of the depth of the IJV. After introducing the double lumen catheter (Baxter Multimed) we measured CVP with a pressure transducer. We did a chest X-ray in every patient to rule out the existence of pneumothorax and to ascertain the position of the catheter. Statistical analysis included bivariate correlation and multiple linear regression analysis.

RESULTS: The IJV was at a depth of 25,1±5,61 mm. ($\bar{x}$±S.D.), (range 11-38 mm.) and we did not find significative differences regarding sex, age or height of the patient, the side of the IJV or the anesthesiologist who did the procedure. On correlating depth of insertion with other variables, the Pearson coefficient was higher with the weight of the patient (r=0.3564), followed by body surface area (BSA) (r=0.3238), body mass index (BMI) (r=0.3199) and CVP (r=-0.2404), being the only variables with significative correlation. The only variables which significatively predicted the depth of insertion in the multiple linear regression analysis were weight of the patient (beta=0.3839) and CVP (beta=-0.2783), with a R multiple of 0.4514. The only complications we found were carotid artery puncture (2.9%) without sequelae, and we had no cases of pneumothorax. We recorded benign self-limited arrhythmias during guidewire insertion in 4.3% of the patients.

DISCUSSION: The value of depth of insertion of the IJV with the technique of cannulation we used is not the same as the distance between the IJV and the skin, since the needle is introduced forming a 30º angle with the skin. Thus, the vertical distance is lower than the depth of insertion. However, the value of depth of location of the vein is more useful clinically, since the venous cannulation procedures in the neck are not performed with the needle perpendicular to the skin. We found that in 91.3% of the patients the vein was at less than 31 mm., and this distance can be used as a reference for maximum needle insertion during IJV cannulation through the central access. This reference is especially useful with inexperienced anesthesiologists, and may decrease the risk of pneumothorax. As obese patients have a thicker subcutaneous adipose tissue, it was logical the correlation between weight of the patients and depth of location of the vein. However, the height of the patient had no correlation, and two of the most common indexes of body size (BSA and BMI) did not improve the correlation and did not predict the depth of insertion in the multiple linear regression analysis. Although we found a significative negative correlation of the depth of location with the CVP, the value of Pearson coefficient is lower than we expected. A common recommendation to facilitate IJV cannulation is to put the patient in Trendelenburg position to increase CVP (through an increase in venous return). With our data, the increase in CVP in Trendelenburg position will not facilitate the cannulation of the vein because it will make the IJV to be much more superficial, although there is an increase in the

diameter of the vein which makes its location easier. However, our recommendation is not putting the patients systematically in Trendelenburg position for IJV cannulation, especially in those in which an abrupt increase in venous return may be potentially hazardous.

P. 11 HYOIDES DYMORPHISM IN HUMANS RELATED WITH AGE AND SEX

C. Domínguez Martín, B. Martínez Rafael, J.A. Gil Verona, F.J. Paz Fernández, M. Barbosa and J.F. Pastor Vázquez

Anatomical Department, Faculty of Medicine, University of Valladolid

The hyoids bone is an osseous structure strictly related with the larynx , and this differs related with age and sex, above all in the period between adolescence and adult age. Papadopoulus et al (1989) have described a few differences related with sex in the shape and direction of the hyoids' great horners. With the aim of study if there are significative differences related with sex and age in the hyoids body, we have studied 27 hyoids of sex and age well known of an homogeneous population, in which we have measure the proportion of the hyoids body (body index) and the depth of the posterior side of the hyoid's body (depth).

In our serie, in both parameters studied we haven't seen a correlation neither with age nor sex. We only want to point out that the body index is a little higher in males (x=41.61) than in females (x=37.05), but is not a significative difference. Also there is no similitude relating the body index and the depth among them, related with age and sex.

Perhaps it would be necessary a more numerous sample and with a younger population, (younger than 40) in order to see any significative difference.

P. 12 DIFFERENCES IN PULMONARY DISTENSIBILITY IN LUNGS FILLED WITH AIR AND LUNGS FILLED WITH GAS. MORPHOMETRIC STUDY IN THE RAT

M.A. Escolar, Juan de Dios Escolar and María Roques

Department of Morphological Sciences, Faculty of Medicine, Zaragoza

There is disagreement at present over what is the best technique for pulmonary fixation. Classically the fixer is administrated via the trachea with a transpulmonary pressure (TPP) of 25 cm of H_2O. This would correspond to a superior volume than the total pulmonary capacity (TPC). For some authors the result of pulmonary fixation should be similar to how it is found in a "physiological" state, therefor the lung should be insuflated with gas until reaching an insuflation similar to a light respiration, close to a residual volume.

OBJECTIVE: Discover which is the ideal TPP and the method for pulmonary fixation.

MATERIAL AND METHODS: 84 animals were used divided into six groups: In the first the lungs via the trachea with a TPP of 25 cm of H_2O (group 25). The rest of the animal groups were fixed by the pulmonary artery in as much as they maintained a different positive TPP. In the second group the TPP was 8 cm H_2O in insuflation (group 8.I), in the third group the TPP was 20 cm H_2O in insuflation (group 20.I), in the fourth group, 20 cm H_2O in deflation (group 20.D), in group five, 8 cm H_2O in deflation (group 8.D). Group six was fixed at 8 cm H_2O in inflation and with the lungs within the thorax (group 8.IT). In the rest of the cases the fixation was done in split lungs. The lungs were processed for a morphometric study. A statistical study of the results was done.

RESULTS: The pulmonary volume in group 25 showed significant increase compared to the rest of the groups and in group 20.I compared to group 8.I and group 8.IT The number of alveoli increased significantly in group 25 compared to the other groups. The width of the alveolar wall diminished significantly in group 25 compared to the rest of the groups. The size of the alveoli showed no significant differences in any of the groups.

Although the split lungs, full of liquid, and fixed at 25 cm of TPP did not correspond to any "physiological" situation, they were the best distended showing the alveolar walls unfolded and the greatest recruitment of alveoli.

Este estudio ha sido realizado gracias a la ayuda de investigación nº 95-0186 de la DGICYT.

P. 13 ANATOMICAL VARIATION IN THE INSERTION OF THE DELTOID MUSCLE: PARTIALLY COMMON TENDON TO THE DELTOID, PECTORALIS MAJOR AND BRACHIALIS

M.A. Giráldez García, M.T. Castaño Oreja, C. Lalín Novoa, A. Pérez Caaveiro, M.A. Saavedra García, A. Crespo Abelleira, I. Fuentes Boquete and F.J. Jorge Barreiro

I.N.E.F., Galicia

INTRODUCTION: Although many variations of the insertion of the distal of the deltoid muscle have been described, most authors coincide in outlining that the muscular fibres end, by means of other tendons, in the deltoid tuberosity of the lateral face of the humerus and that, in any case, the tendons are hidden under the muscular fibres, which cover them totally.

MATERIAL AND METHODS: Our findings occurred during a routine dissection carried out by the students on the body of a man, a caucasion of 67 years of age. The photographic images of the piece were taken *in situ* once it had been extirpated.

The bilateral presence of a tendon is found in the distal insertion of the anterior or clavicular portion of the deltoid muscle. It is an elongated tendon with a circular section, even though it is somewhat flattened in the antroposterior direction It measures 6 cm in length and 0.9 in width. It is situated on the surface of the muscle and it is perfectly visible without retracting the fleshy mass, and is parallel to and immediately outside the fleshy belly of the short head of the biceps brachii muscle. In its upper extreme it receives the fibres of the anterior portion of the deltoids bipenniformally.

RESULTS: Fibres of the middle or acromial portion of the same muscle end on the lateral or external face of the muscle and these invade the lateral portion of the deep face of the tendon. The most external clavicular fibres of the pectoralis major muscle end in the upper half of the internal muscle and on an anterior plane.

While the same continues at a deeper level, and in its entire length with the tendinous expansion which covers the internal face of the humerus that inserts itself at this level.

In its lower extreme, both the tendon and the tendinous expansion continue with the fibres of the brachialis, which cover the anterior medial face of the humerus. In the same way, in the aforementioned tendon, the most external fibres insert themselves in scarce quantities and the rest do so in the tendinuous expansion. In its deep face it is stuck to the anterior edge of the intertubercular sulcus (cresta subtroquiteriana)in the middle half, while its lateral half receives fibres of the middle portion of the deltoid muscle.

DISCUSSION: As all muscles are authoctonous to the upper member, the deltoid is dorsal and both the pectoralis major and the brachialis are ventral. The habitual termination of the three muscles takes place in an independent way on the different sides of the deltoid tuberosity. In our case, a tendinous formation appears which covers the whole anterior edge of the intertubercular sulcus (cresta subtoquiteriana), where some portions of these muscles end.

From a mechanical point of view, it is difficult to interpret the advantages and disadvantages of this formation. The fibres of the pectoralis major would come to constitute a physiological portion of the deltoid muscle, from which both muscles would carry out the flexion, the internal rotation and the adduction of the arm. As regards flexion, although both are situated in front of the axis of the flexo-extension, the pectoral has greater momentum. As regards internal rotation, the fibres of the anterior portion of the deltoid end on both sides of the upper extreme of the tendon, and given the longitudanal disposition of the same, parallel to the axis of rotation, they wouldn't have a relevant role in the movement. On the other hand, the disposition of the pectoral, with a more oblique termination in the tendon, is more perpendicular to the axis and, therefore, it is more effective.

The participation of this portion of the deltoid in the adduction depends on the degrees of movement, as the action of the same can invert itself once it passes a particular angle. It seems that it would be difficult for this to happen with the pectoralis major. Given its position it is improbable that its fibres could situate themselves above the axis of the anterior posterior turn.

The external rotation of the arm is not a function which is habitually attributed to the middle portion of the deltoid, nevertheless, in our case it could be a possiblity. The muscular fibres of this portion end in the extreme border of the deep face of the tendon which we described. We could consider, that from the mechanical point of view, they continue with the expansion which arises from the internal side of the same tendon. Should that be the case, if we consider the muscular fibres and the tendinous expansion as a common miotendinous unit, this portion would roll itself over the humeral diafisis as the supinator does on the diafisis of the radius, bringing about the external rotation.

Orts Llorca defined a muscular loop between the deltoid muscle and the brachialis. Although, in our case there is a intertendinous connection, in such a way that the deltoid and the pectoralis major join together by means of a fibrous formation with the brachialis. However, we don't believe that they can act on the joint of the elbow nor that this can act on the glenohumeral joint, due to the fact that the tendon is fixed at its deep face to the intratubercular sulcus, preventing any possibility of slippage.

For this reason, it is worth asking what is the role or the reason for an anatomical element with the morphology and typical structures of a tendon and its expansion, but which doesn't have the sliding capacity because it is joined to the bone in its entire length.

From a surgical perspective, the possible presence of this tendon is worth bearing in mind in order to avoid confusing it with the tendon in the long portion of the biceps brachii. Acting on a small operatory field, we would find a tendon in the same direction of the long portion of the "emerging" biceps, under the deltoid muscles and the pectoralis major, which could lead to error.

CONCLUSION: We find the bilateral presence of a tendon which has not been described until now and where the following end; in the upper part: the anterior and middle portions of the deltoid muscle and the clavicular fibres of the brachialis muscle; on the outside; the fibres of the middle portion of the deltoid muscle end. These also invade the deep face. On the inside, a tendinous expansion continues which covers and sticks to the anteromedial face of the humerus, finishing up near the internal border of the bone. The lower extreme of the tendon and the lower edge of the tendinous expansion receive fibres from the brachialis.

P. 14 ARTICULATIO HUMERI: ANATOMY, MAGNETIC RESONANCE, MAGNETIC RESONANCE-ARTROGRAPHY AND THREE-DIMENSIONAL MR ARTROGRAPHY OF THE SHOULDER

L.C. Hernández, A. López Muñiz, E. Bengoechea, V. Martínez[1], M. Recio[1] and J. Viaño[1]

Departamento de Morfología y Biología Celular. Facultad de Medicina. Universidad de Oviedo. [1] RM Nuestra Señora del Rosario. Madrid

INTRODUCTION: This work was designed to analyze the normal anatomy of the shoulder joint (capsule, glenoid labrum and gleno-humeral ligaments) by comparing cadaver sections with images obtained by Magnetic Resonance (MR), and virtual endoscopia. As far as we know no data are available in this topic. Furthermore, the conventional explorations of shoulder by MR do not consent the correct evaluation the capsular complex and of the glenoid labrum. From a surgical-medical point of view this is of capital importance the correct valuation of these structures in order to ascertain an appropriate therapeutic, especially in the dislocations of humeral head. The association of intraarticular contrast and MR (RM-Arthrography) has solved this problem. Thus, the correct identification of anatomical structures (i.e., capsule, glenoid labrum and gleno-humeral ligaments) is at the basis of a correct diagnosis.

MATERIAL AND METHODS: Cadaver sections were obtained from the shoulder in the oblique- sagittal, oblique-coronal, and axilal planes. Cases found to be free of pathology using conventional methods, were reevaluated by MR-Arthrogarphy, using a 1.5 Tesla units. Patients received an intraarticular puncture of 12-18 ml (1ml of gadopentetate dimeglumine diluted in 200 ml of saline). The used sequences were SE-T1 with fat suppression in oblique-sagittal, oblique-coronal and axial incidences (projections), or 3DSPGR fat suppression (which consent to perform a virtual endoscopia-three-dimensional MR-Arthrography- in association to the program "Navigator").

RESULTS: MR-Athrography of the shoulder with both assessed sequences, SE-T1 fat suppression and 3D SPGR, has provided accurate details of the capsule, glenoid labrum and glenohumeral ligaments, and demonstrated a perfect correlation between anatomical and radiological studies. On the other hand, the 3D virtual endoscopia consent by to obtain "anatomical" intraarticular images.

P. 15 EPITHELIAL MEMBRANE ANTIGEN IN ORAL PREMALIGNANT LESIONS

Alfonso López-Muñiz, Luis C. Hernández, María J. Anitua, Blanca E. Fernández and Esperanza Bengoechea

Morphology and Cellular Biology Department. Faculty of Medicine. University of Oviedo (Spain)

INTRODUCTION: The diagnostic and prognostic valuation of the buccal premalignant lesions shows serious troubles.

Immunocytochemical techniques have been used for the diagnosis of the oral premalignant lesions and of the oral carcinomas. *Epithelial Membrane Antigen* (EMA), also known as episialin, belongs to a heterogeneous group of glycosulated proteins with a molecular weight in the range 265 to 400 kD. One of those antibodies, EMA, clone E29, was subsequently screened against a range of normal and neoplastic human tissues. Early, EMA labeled a variety of normal and neoplastic cells in histological preparations. In normal tissues, EMA reacted especially with *secretory epithelia*. The strongest staining is shown at the apical portion of the ductal lining cells of breast epithelium. A similar pattern of staining is observed in other glandular epithelia (sweat glands, salivary glands), while *squamous epithelium* showed an uneven pattern of expression. Its ubiquity on non-squamous epithelial surfaces suggests that it may have an important, possibly protective role.

MATERIAL AND METHODS: A series of 51 normal mucosas, 51 oral non-dysplastic leukoplakias and 44 oral dysplastic lesions were evaluated in paraffin-embedded sections using E29 Epithelial Membrane Antigen, EMA, monoclonal antibody. Quantitative analysis of immunoreactivity was assessed.

RESULTS: In normal mucosa, non-dysplastic leukoplakia and dysplasia, percentages of cases of more than 20% EMA-positive cells were 0%; 0% and 37.5% respectively. EMA had high ability for to establish the difference between benign and malignant cells of squamous epithelia. Its distribution was detected with typical patterns for each type of mucosa: normal, non-dysplastic lesion and dysplasia. The quantitative immunohistoanalysis found 1.6%±1.1; 4.4%±1.9 and 18.0%±11.1 EMA-positive surfaces, respectively.

DISCUSSION: The premalignant lesions with the most high marked cellular percentage had the highest proliferating grades, and, accordingly, EMA tumour expression must be considered a poor prognosis indicator. The EMA expression within squamous epithelial cells has to be investigated by quantitative immunohistochemical methods and used as a diagnostic, even prognostic indicator.

P. 16 ANATOMY STUDY ABOUT FREQUENCY OF LALOBUET PYRAMID AT ALTITUDE ABOVE 2.300 m

M. Coáguila Héctor Juan

Medical School - National University of San Agustín, Arequipa Peru (Mailing address: Urbanización Monterrey H-8 del distrito de José Luis Bustamante Y Rivero del departamento de Arequipa, Perú)

The pyramidal lobe of Lalobuet is an inconstant part of the thyroid gland. It is located between the left side, superior border of the isthmus and the hyoideus bone. It is important to recognize these relationships in surgery in order to avoid risks and complications.

The present study was carried out to evaluate the frequency of the Lalobuet pyramid in men of high altitude (above 2.300 m) in Arequipa, Peru. Autopsy studies were performed in 130 cadavers, mestizo race, mostly male and ranging in age from 50 to 55 years. These cases were from the Human Anatomy service, San Agustín University, School of Medicine, Arequipa, and the Central Morgue of Arequipa. We used the descriptive and comparative method in classical dissection. Our results showed that the Lalobuet pyramid was present in 23.07% of individuals at high altitude (above 2300 m). It is located in the left side of the isthmus (66.66%) a strip-like shape (100%) and in a oblique position (66.66%). We reviewed the literature findings (Testut & Latarjet, Lucas-Batista, Soja, Sinelnikov ...) in order to compare with our results.

CONCLUSIONS: The presence and frequency (23.07%) of the Lalobuet pyramid in men at high altitude, Arequipa Peru.

P. 17 THE MICROVASCULAR PATTERN OF TESTE GONAD ITS CHANGES IN MAJOR SALIVARY GLANDS MODIFICATIONS OF ITS BALANCE I: (SUBMAXILLARY GL. SUPPRESION)

E.R. Meaños Melón, M.P. Alonso Martínez, M.E. Padín Iruegas, M.J. Muizulis Alonso, S. Pedreira Currás, A.M. Tarrazo Antelo and F. Navarrina Gámez

2.ª Cátedra de Anatomía, Dep. Ciencias Morfológicas, Fac. Medicina, Univ. Santiago de Compostela, Servicio de Rehabilitación Hospital Xeral-Clínico Universitario de Santiago de Compostela

INTRODUCTION: The Major Salivary glands Parotid and Submaxillar are well known structures from an exocrine point of view; but more over each one it has a not so well know aspect and however perfectly reflected in world bibligraphy, from first publications of Japanese School in 40th and 50th decades (Ito and Ogata) widen by other groups and schools later, over the roll of these glands inside incretory system, as makers within its glands structure of specific incretory substances for each one (Parotin and Submaxillarin or so called too Parotin-S). In works of our own Anatomical Compostelan School we find out that endogenous balance modifications of this substances in mayor salivary glands by surgical ablation of one of them (Parotid or Submaxillary Gland), it makes importants modifications over other organics systems like long bone, in its microvascular architectural pattern on its severals morpho-functional zones; also at long bone fracture we

can see prospectivs changes in its evolution at show up this experimental status of mayor salivary gland ablation, with changes in microvasculature disposition at the fracture callus border. Also within endocrine system we had value repercussions, esteemed on microvascular point of view in thyroid gland, beside functional proyection at this level, when we get the parotid or submaxillary gland ablation. In this experimental study we want get supression of incretory activity of submaxillary gland by total and bilateral surgical ablation. Our target organ will be the testicle gonad and its microvascular pattern the parametre to study.

MATERIAL AND METHODS: The experimental animal is male adult albino rat (8 weeks) OFA Spragüe Dawley, weigh 200 grs. Divided in three groups (10 specimens each one): I Control Group, II Submaxillary Gland Bilateral Ablation Group, III Sham-operation Group. At last of three months period of experimental life, we proceeded to exitus of animals in the three groups, subjecting testes to technics of vascular injection, and later to histological proceedings, microscopical observation and photographic series.

RESULTS AND DISCUSSION: Results obtained in both control and sham-operation groups have not variations in campimetric microvascular pattern on arterial and venous fields. The microvascular pictures that shows experimental teste after parotine action without the submaxillarine action combinated, are significativs compared with controls, presenting a global decrease in all testicle microvascular territories. Intertubular arteries show lower calibre to control ones; we remark the diminution in number and anastomotic disposition of branches finded in peritubular network. This microvascular decrease is more evidenced when we study venous microvascular pattern evolution so in calibre lower of collectors and its branches decreased too, with a fine and more simple anastomotic structure of disposition. These changes in architectural disposition of arterial and venous patterns in peritubular and intertubular zones of teste, at this experimental status created and established will be projected with repercussion too over functionalism of this gonad.

P. 18 THE MICROVASCULAR PATTERN OF TESTE GONAD ITS CHANGES IN MAJOR SALIVARY GLANDS MODIFICATIONS OF ITS BALANCE II: (PAROTID SUPPRESSION)

E.R. Meaños Melón, M.P. Alonso Martínez, M.J. Muizulis Alonso, M.E. Padín Iruegas, A.M. Tarrazo Antelo, S. Pedreira Currás and F. Navarrina Gámez

2ª Cátedra de Anatomía, Dep. Ciencias Morfológicas, Fac. Medicina, Univ. Santiago de Compostela

INTRODUCTION: The Major Salivary glands Parotid and Submaxillar are well known structures from an exocrine point of view; but more over each one it has a not so well know aspect and however perfectly reflected in world bibligraphy, from first publications of Japanese School in 40th and 50th decades (Ito and Ogata) widen by other groups and schools later, over the roll of these glands inside incretory system, as makers within its glands structure of specific incretory substances for each one (Parotin and Submaxillarin or so called too Parotin-S). In works of our own Anatomical Compostelan School we find out that endogenous balance modifications of this substances in mayor salivary glands by surgical ablation of one of them (Parotid or Submaxillary Gland), it makes importants modifications over other organics systems like long bone, in its microvascular architectural pattern on its severals morpho-functional zones; also at long bone fracture we can see prospectivs changes in its evolution at show up this experimental status of mayor salivary gland ablation, with changes in microvasculature disposition at the fracture callus border. Also within endocrine system we had value repercussions, esteemed on microvascular point of view in thyroid gland, beside functional proyection at this level, when we get the parotid or submaxillary gland ablation. In this experimental study we want get supression of incretory activity of Parotid gland by total and bilateral surgical ablation. Our target organ will be the testicle gonad and its microvascular pattern the parametre to study.

MATERIAL AND METHODS: The experimental animal is male adult albino rat (8 weeks) OFA Spragüe Dawley, weigh 200 grs. Divided in three groups (10 specimens each one): I Control Group, II Parotid Gland Bilateral Ablation Group, III Sham-operation Group. At last of three months period of experimental life, we proceeded to exitus of animals in the three groups, subjecting testes to technics of vascular injection, and later to histological proceedings, microscopical observation and photographic series.

RESULTS AND DISCUSSION: Results obtained in both control and sham-operation groups have not variations in campimetric microvascular pattern on arterial and venous fields. The microvascular pictures that shows experimental teste after the Submaxillarine action without the Parotine action combinated, are significativs compared with controls, presenting a global increase in all testicle microvascular territories. Intertubular arteries show greats calibre to control ones; we remark the appreciation and magnification in number and anastomotic disposition of branches finded in peritubular network. This microvascular increase is more evidenced when we study venous microvascular pattern evolution so in calibre wider of collectors and its branches increased too, with a more complex anastomotic framework structural of disposition. These changes in architectural disposition of arterial and venous patterns in peritubular and intertubular zones of teste, at this experimental status created and established will be projected with repercussion too over functionalism of this gonad as hormonal as celularity.

P. 19 INFLUENCE OF TRAINING ON ELITE SPORTSMEN'S BODY COMPOSITION

B. Méndez, J. Egocheaga, G. Sirgo and M.E. Del Valle

Escuela de Medicina del Deporte. Dpto. Morfología y Biol. Cel. Universidad de Oviedo

INTRODUCTION: The anthropometric variables have a great importance to determine the sportsmen's body composition characteristics. They allow analysing differences among different sport modalities, as well as to compare different aspects of oneself sport. However, how training influences on body composition has not been studied with rigour.

Our objective is to determine the anthropometric and body composition differences among three groups of athletes of different sport modalities: long jump, javelin throwing and sprint Although we want to see possible differences in each group along the season.

MATERIAL AND METHODS: 30 male senior athletes have been evaluated: 10 jumpers, 10 throwers and 10 sprinters, about three moments of the season: end of generic training (volume), first moments of competition and final of season. Informed consent was obtained from all subjects included in the study.

The following measures were taken: height, weight, skinfold thicknesses (subscapular, triceps, suprailiac, abdominal, thigh, medial leg), bony diameters (bi-styloideo and femoral bi-condiliac).

For these measurements, precision scale of 100 mgr., hightmeter (1 mm. of precision), caliper pressure with increments of 0,2 mm., and bonecompas were used.

The study of body composition was carried out in turn for doubly indirect methods that are derivative equations, of some indirect method. Fat percentage and fat, fat free, muscular, residual and bony weights were determined.

RESULTS:

1.- Differences among groups:

– There were not significant differences in the size and bi-styloideo diameter among the three populations.

– Regarding the skinfolds, excepting the thigh one, significant differences between throwers and the other two groups were found, being bigger in the first ones; in the thigh skinfold, differences only appear between throwers and jumpers in the two first evaluations, disappearing in the last one. There are not significant differences between jumpers and sprinters, although in these ones, the values are bigger.

– As for fat percentage and weights, there are also differences between and the other two groups, being bigger in throwers; but here, differences appear between sprinters and jumpers in fatty and muscular weight in the first two evaluations, with greater values for the sprinters

2.- Differences along the season:

– A decrease in skinfolds values, fat percentage and fatty weight and an increase of the fat free and muscular weights were found in the three groups. The differences are more accused between the first one and the second evaluation. Although the decrease in the skinfolds values is generalised, the significant differences only affect to certain skinfolds, depending on the evaluated group.

– No significant differences in size, diameters and bony weight were found

CONCLUSIONS:

– Sport type, keeping in mind that in more similar populations as sprinters and jumpers don't exist significant differences, influences the anthropometric characteristics.

– The differences among populations reside fundamentally in the fatty component.

– In the same way, the training influences fundamentally in the fatty component of those corporal segments participating in the technical expression.

The most different moment in the body composition corresponds to the first moments of competition.

P. 20 INFLUENCES OF THE ALCOHOL IN INTESTINAL STRUCTURES

J.M. Morales, E. Rivada, V. Smith, F. Rius and P. Salinas

Dpto. Anatomía Humana. Facultad de Medicina. Universidad de Málaga

INTRODUCTION: Alcohol is a toxic that is absorbed in the gut. The alcoholic sick persons present a malabsorption syndrom. In this work the morphological lesions caused by the alcohol in intestinal structures have been studied with diverse types of experimental alcoholic models.

MATERIAL AND METHODS: We have used Wistar rats divided in groups of acute alcoholism (15 days), acute with normalization, chronic (5 months), chronic with normalization and extremely chronic (18 months).

The used alcoholic pattern has been alcohol to 20% in water. Inclusion in paraffin, staining with thricromic of Masson-Goldner and morphometrical study with a station SPARC STATION 20 DE SUN, with statistical exhaustive analysis of the results looking for significant differences for $p<0.05$.

They were measured the total height of the intestinal wall, height of the villus, width of the muscular wall, depth of the Lieberkühn crypts, width of the villus, distances among villus, and area, perimeter, maximum and minimum width of the neurons of the plexus of Meissner. The statistical analysis of the obtained data was carried out checking the normality in each one of the groups as well as its homogeneity with the test of Commogorof-Smirnoff and test of Levene. Later on it was carried out an analysis of the variance. The statistical significance of $p<0.05$ gave significant results. Lastly, to check significant differences among specific groups it was applied the test of Tukey.

RESULTS AND DISCUSSION: The groups of chronic alcoholism present alterations of the depth of the crypts that are responsible of regeneration of the villus in case of dysfunction.

A decrease of the width of the villus exists in the acute and chronic groups regarding the control group and it produces a loss of material between villus. In the chronic there is an increase of the distance among villus. The continuous alcoholic damage produces losses of villus and therefore decreasing of the surface of absorption. The muscular layers present a decrease of its width. It could explain the alterations of the intestinal motility.

The submucous plexus of Meissner presents alterations differed in the chronic pattern of alcoholism.

The retreat of the ethanol produces a tendency to the normality unless in the groups of extremely chronic, where the lesions at the crypts being damaged are irreversible and atrophy of villus takes place.

The morphological lesions described can be the origin of the Syndrome of Malabsorption that the alcoholic individuals present. The deterioration at the level of the enteric nervous system and of the intestinal muscular walls could explain the peristaltic alterations implied in the Malabsorption.

P. 21 ANTERIOR CHAMBER MORPHOMETRY OF EYE IN YOUNG POPULATION

M.A. Muñoz[1], J.C. Sanz[2] and F. González[2]

[1] Dpto. CC. Morfológicas I. E.U. Optica. Univ. Complutense de Madrid. [2] Dep. Optica. E.U. Optica. Univ. Complutense de Madrid

INTRODUCTION: The anterior chamber depth is the distance, from de posterior vertex of the cornea to the ante-

rior surface of the crystalline lens.The referring studies to the ocular biométrics characteristics in people with some refractive anomaly usually are centered in cornea, crystalline lens and axial length. The data are few on the depth of the anterior chamber. In this work it is tried to know the behavior the anterior chamber in young individuals and their relation with other visual parameters.

MATERIAL AND METHODS: The study was made in a population of 240 young people of ages between the 18 and 22 years. One quantified his refractive error by means autorrefractometer, its ocular morphometrics values was measured by means A-scan ultrasonography biometer, between which it was the depth of the anterior chamber. Each measure was recorded as the average of a minimum of three readings.

RESULTS AND DISCUSSION: The refractives characteristics of the population indicate to us that he is clearly myopic. Being the women more myopic than the men. The morphometrics results show us that the anterior chamber depth presents in the young people values for the right eye (3.27 ± 0.34 mm), superior in myopic (3.39 ± 0.35 mm) that in emmetropes (3.22 ± 0.32 mm) and that in hiperopes (3.02 ± 0.20 mm). These differences are significatives when comparing the myopic values and emmetropics. On the other hand, when observing the referring data men and women the same model has been verified who the measures of the men are always superior to those of the women, following that in the general values, myopes>emmetropes>hiperopes.

P .22 VARIATIONS ON THE USUAL COURSES OF THE RADIAL ARTERY. A CLINICAL CASE

M.E. Padín Iruegas, J.C. Diz Gómez, A.M. Tarrazo Antelo, E.R. Meaños Melón, M.P. Alonso Martínez, M. Muizulis Alonso, S.D. Pedreira Currás and F. Navarrina Gámez

2ª Cátedra de Anatomía, Departamento de Ciencias Morfológicas, University of Santiago de Compostela, España. Servicio de Rehabilitación del Hospital General de Galicia-Clínico Universitario, Santiago de Compostela, España

INTRODUCTION: Radial artery cannulation has become a common procedure for continuous measurement of blood pressure as well as frequent blood sampling for the determination of arterial blood gases.

The most useful site for cannulation of this artery is at the wrist, in a groove formed by the tendons of the supinator longus and flexor carpi radialis. Then the artery courses externally, covered by the extensor tendons of the thumb (abductor pollicis longus and extensor pollicis brevis), to enter the anatomical snuffbox. It then passes between the metacarpal bones through the first dorsal interosseous muscle into the palm, where it ends in the deep volar arterial arch. Although variations in the origin and the proximal course of this artery are common, specially a high origin of the radial artery, the finding of a radial artery or one of its branches, passing superficial to the lateral tendons of the snuffbox is rare, appearing in about 1% of the wrists; and it is named superficial radial artery.

MATERIAL AND METHODS: We report a case of bilateral superficial artery in a 69-year-old male patient scheduled for coronary artery bypass graft surgery.

During monitoring of the patient we were not able to feel the radial pulse in its typical position in both wrists. Exploring the forearm more proximally, we could outline the course of the artery over the lateral tendons in the anatomical snuffbox, about five centimeters proximal to the usual site of cannulation, in both forearms.

We could cannulate this artery in the right hand about three centimeters proximally to the anatomical snuffbox.

The patient had no ischemic complications in the hand in the postoperative period, and the catheter was withdrawn 48 hours later.

RESULTS AND DISCUSSION: This course of the radial artery is an uncommon anatomical variation, which appears in about 1% of the cases, and in 60% of these the finding is bilateral. The superficial radial artery courses over the lateral tendons in the anatomical snuffbox, before terminating in the deep palmar arch. This variation of the radial artery corresponds to the normal course and distribution of the radial artery described in primates. There is not a definite explanation of the embryological development of this artery. Normally, the superficial radial artery of the fetus divides into two terminal branches, superficial and deep; the superficial branch regresses, whereas the deep branch enlarges and becomes the radial artery of the adult. Several authors propose that the superficial radial artery is the result of the persistence of the superficial branch. When this variation appears, the radial artery can be subdivided into two branches: the superficial radial artery, and a "deep" radial artery, which is more slender and descends along the normal course of the true radial artery.

The unusual location of the artery may make finding the arterial pulse difficult, and it is then advisable to search for the pulse more proximally.

P. 23 MORPHOMETRY OF THE OPTO-LENTICULAR FORMATION

M.D. Peces, M.D. Merindano, M.A. Palomar, R. Linares and M.A. Capo

E.U. de Óptica, Madrid

The evaginations at the level of the prosencephalon give rise to the origen of the optic vesicles. The facing surface ectoderm thickens sligtly thus producing the lens pla-code. The optic vesicle and the placode lens invaginate to form both the optic cup and the lens vesicle.

In this research project we have attempted to analyse and describe the different phases in the development of the neural retina and the lens in human embryos comprised between stages XII and XVIII of O Rahilly.

The purpose of the study was to confirm that the development of the lens is de-termined to a large extent by the development of the retina.

Sixteen human embryos obtained from the Bank of the Institute of Embryology (U.C.M) were studied by means of the application of tridimensional reconstruction. The progamme used was the VIDS IV since it offers the possibility of analyzing images and thus obtaining different measurements of the retina and the lens. Of the different types of drawings available in the programme, the general drawing was preferred and 16 different measures were obtained of the retina and the lens of each of the embryos and each of the histological sections.

Due to the volume of the numerically codified data obtained, a statistical study was performed. The first step

consisted in defining which of the 16 different measurements of the retina and lens might be taken as being the most representative variables of both stuctures.

We then proceeded to examine the correlations between the different measure-ments or variables, their factorial analysis and multiple regression with the objetive of confirming and quantifying the evolution of the lens and the retina in human embryos.

The results obtained from the factorial analysis, correlation and multiple regre-ssion indicate that the variables of the lens, area and form factor and the variables of the retina related to the geometric description together with the size of the embryo are the factors which determine that the development of the lens is not directly related to the size of the embryo but rather to the variables which characterise the development of the retina thus confirming analytically the influence that the diferentiation of the retina has on that of the lens.

P. 24 ANATOMIC VARIANTS OF THE HUMAN SINONASAL REGION STUDIED BY CT

I. Pérez-Piñas[1], A. Carmona[2] and J. Jiménez-Castellanos[2]

Servicio de Radiodiagnóstico del H.U. Virgen Macarena[1] y Departamento de Ciencias Morfológicas[2]. Universidad de Sevilla

INTRODUCTION: A precise knowledge of nasosinusal anatomy is essential for the clinician. Conventional radiology does not allow a detailed study of the nasal cavity and paranasal sinuses, and is now largely replaced by computed tomography (CT). This gives an applied anatomic view of the region and the anatomic variants that are very often found there. The detection of these variants is essential for the use of current of nasosinusal endoscopic surgery.

MATERIAL AND METHODS: In the present work, we study the anatomic variants observed in the nasal fossae and paranasal sinuses in 110 Spanish subjects, using computed tomography in coronal scans, complemented by transversal ones. We have concentrated on the variants at the level of the nasal septum, middle concha, ethmoidal unciform process and ethmoidal bulla, together with other of lesser frequency.

RESULTS: The population studied showed great anatomic variability, and a high percentage (67%) presented one or more anatomic variants. Discounting agger nasi air cells and asymmetry of the sphenoidal sinuses, which were present in all our cases, the variations most often observed were, in order, deviation of the nasal septum, and the presence of a great ethmoidal bulla, Onodi air cells, and bony spurs of the nasal septum.

DISCUSSION: Our results are compared with those from other populations. Although *a priori* these variants should be considered normal, the frequent association of certain of them to nasosinusal disorders, especially inflammatory pathology, seems to suggest they could be a predisposing factor

P. 25 VASCULAR AND TUBULAR IMMUNOREACTIVITY FOR NEUROPEPTIDE Y IN THE MOUSE KIDNEY

A. Prada, A. Romero, C. Verástegui, R. Marrufo, M. Aparcero Fdez. del Campo and F.J. Fernández-Trujillo

Departamento de Ciencias Morfológicas. Facultad de Medicina. Universidad de Cádiz

INTRODUCTION: Neuropeptide Y (NPY) is a neuromodulator which has been reported at level of both central and peripheral nervous system. Its functional meaning as an important blood flow controller has been related with the coexistence with sympathetic neurotransmitter. Many reports have explored NPY presence at several organs including kidney, ureter and bladder.

At the kidney, the vasoconstrictor role of NPY was proposed by NPY-immunoreactive presence and receptor existence at the arterial vessels, not only in renal artery but also in its intrarenal accessories. Moreover NPY-immunoreactivity (NPY-IR) have been reported in efferent arterioles of juxtaglomerular system in several species. In this sense, NPY has been proposed as a mediator in the renin-angiotensin-aldosterone system by controlling the blood flow to the glomeruli.

In the other hand, NPY-IR tubular cells have been described in the rabbit kidney. These cells could be related to sodium reabsortion in those tubuli.

MATERIAL AND METHODS: Ten adult male mice (*Mus Musculus*, Swiss OF-1) were anaesthetised with ether inhalation and perfused with 0.9% saline solution, followed by 4% phosphate-buffered paraformaldehyde pH 7.4 prefixation. Postfixation and dehydration were made by usual hystological methods.

The 7-10 μm sections were incubated with rabbit polyclonal antiserum against NPY diluted 1/100, in a moisture chamber 72 hours at 4ºC. We used the indirect method of biotin-streptavidin complex. A revealed solution of 10 mg/15 ml DAB in presence of 20 μl/10 ml H_2O_2 was used for 20 min under microscopic control. The negative control procedures were carried out by omitting one or more steps of the method and by using sections incubated with inactivated antiserum with added excess antigen.

RESULTS: Our results were similar to those reported in other species. We found a wide distribution of NPY-IR both in the renal artery as in collateral intrarenal arteries. This NPY-IR was present in perivascular fibers around middle and little calibre arterioles and both in peri- and intravascular fibers of large calibre arteries. This immunoreactivity was usually related to the arterial element of juxtaglomerular apparatus too.

We showed the presence of NPY-IR interstitial cell closed to the tubule at the renal parenchyma. These findings were present not only in renal medulla but also in cortex. Some immunoreactive cells appeared in the interstices near to the glomerulus.

Sparse NPY-IR cells were seen at level of collecting tubule at inner medulla. The typology of these cells resembled dark cells.

DISCUSSION: No authors had reported these findings in the mouse. We think that NPY must have an important role in the renal function. Thus the presence at muscular layers in arterial vessels as in juxtaglomerular apparatus must be a mediator in the control of the blood flow and therefore in the urine filtration. NPY could be a related aspect within sympathetic system for the control of blood volume and arterial hypertension.

About IR cell in the parenchyma, we propose a possible role in the reabsortion of ions at the tubules. In this sense, NPY-IR cells could act as osmoreceptor by controlling the sodium reabsortion or by renin secretion.

P. 26 INFLUENCE OF EXERCISE IN THE BODY COMPARTMENTS OF ELDERLY PEOPLE

R. Rodríguez, J.M. Calvillo, M.C. Aponte, S. Aguado and L. Gómez Pellico

Dpto. Ciencias Morfológicas y Cirugía. Universidad de Alcalá. Facultad de Medicina

It is known that the proportion in the organic compartments (mineralised tissue, fat and muscular mass) varies with physical training both in young people and sportsman (Morris & cols. 1997; Jones & Dwyer 1998; Rodríguez & cols. 1997). However there are few investigations that analyse these variations in elderly people.

The purpose of this work is to verify the influence of a specific strength- training programme on the body composition of elderly people. A sample was taken of 60 individuals in order to evaluate the different body compositions by means of dual-energy x-ray absorptiometry both before and after an exercise programme. After analysing the average values of males and females as well as the Student t test for paired samples it could not be proven that there was any significant statistical difference in any of the analysed variables.

Due to the findings the conclusion is that the programme used with a total duration of 6 months at a rate of 2 hours weekly does not impose noticeable changes by means of densitometric analysis in this group of the population.

P. 27 KINEANTHROPOMETRIC STUDY OF AMATEUR SOCCER PLAYERS

M. Santos, F. Alburquerque, M. Rubio, F. Sánchez, L.F. Valero[1], J. Carretero and R. Vázquez

Dpto. de Anatomía e Histología Humanas and [1] Dpto. de Medicina Preventiva y Salud Pública. Facultad de Medicina. Universidad de Salamanca

A total of 58 Spanish amateur soccer players belonging to two categories - Third Division and the "Youth Honours" Division were studied. Sixteen anthropometric variables, the percentage of fat (Carter), and body composition (four-compartments model) were analysed. A study based on to sports categories and to the players' position on the field was performed. Analysis of Variance (ANOVA) was used to interpret the data obtained, thus permitting comparison among groups ($p<0.05$).

According to sports categories, significant differences were observed as regards the age of the two groups (19.7±1.24 in the Third Division group and 17.6±0.68 in the "Youth Honours" group). However, measurements of fat folds, of the perimeters studied and of diameters (with the exception of biacromial diameter) did not uncover significant differences between either group. The percentage of fat (Carter) was higher in the younger group of players and, as regards body composition, this group also had higher fat weight and less muscle weight than the other groups, although the differences were not statistically significant.

In the analysis according to position on the field, it was observed that both the percentage of fat (10.23±1.24) and the sum of six folds (72.67±28.14) were higher in defence players, with significant differences between these and the rest of the team members. The measurements of waist and thigh perimeters were higher in the goalkeepers than in the other groups, the differences in waist values between goalkeepers and forwards and thigh values between goalkeepers and mid-field players being significant. Finally, body composition revealed greater fat weight in the goalkeepers, with significant differences between these and the mid-field players and forwards. There was also more muscle weight in the goalkeepers with respect to the forwards.

Although significant differences emerged between both groups studied as regards age in both groups composition results were very similar, indicating that the players of the "Youth Honours". Division have reached an optimum degree of development for playing soccer. However, the greater amount of fat and the lower muscle weight shown by these players ("Youth Honours" Division) suggests that they might need more intense physical preparation. Also, alone, the analysis of the characteristics according to the position of the players on the field points to the current absence of a specific profile for soccer players.

P. 28 IS THERE ANY INNOCUOUS VASECTOMY TECHNIQUE?

R. Sarrat, J.R. Whyte, R. Mazo[1], A. Torres, A.I. Cisneros, P. Díaz Herrera[2], A. Whyte[1]

Department of Morphology. School of Medicine.University of Zaragoza. Spain. [1] Department of Animal Surgery. School of Veterinary. University of Zaragoza. Spain. [2] Department of Morphology. School of Medicine. Universidad of Las Palmas de Gran Canaria. Spain

INTRODUCTION: Almost one century answer for the vasectomy technique as a male esterilization method, irreversible in principle.

Surgical modalities have changed with time, from the doble ligature (closed ended) to the occlusion only in distal segment, leaved the proximal segment of the testicle free (open ended).

From the beginning it was thought that closing the excretor duct of a exocrine gland produced risks in parenchima, and as a consequence of that, investigations about innocuousness or harmful action of this technique have been doing without interruption.

Oslund (1926), Nelson (1952), Amann (1962), Flickinger (1972), Hadley (1983) y McDonald (1988) hold a brief for the innocuousness of the technique, althought Laumas (1980), Alexander (1972), Ureña y Malavasi (1980), Kumar (1990), Sarrat y Whyte (1996), etc., describe several injuries in the testicle.

MATERIAL AND METHODS: We have used Wistar rats, dogs and rabbits, which were vasectomized according to open ended vasectomy, and processed with routine techniques for histology and electron microscopy.

RESULTS: Our results in rat, dog and rabbit show that in a follow up of 1 year postvasectomy, the testicle structure and ultrastructure is not altered.

We have observed an intense spermatogenesis, an increase of Sertoli's cell size as well as its phagocitary function, and an absolutely normal configuration of interstitial islands.

DISCUSSION: The open ended technique eliminate the intratubular hypertension factor, leaved the testicle structure undamaged.

This opinion is answered for the main clinic publications of Errey and Adams (1986), Moss (1992), Denniston and Kuhel (1994), who suggest that the technique of this publication is the ideal to minimize the adverse effects.

P. 29 ANATOMICAL VARIATIONS OF THE HELIX ROOT (CRUS HELICIS)

L.A. Vallejo Valdezate, L.M. Gil-Carcedo García, I. Díaz Suárez. and P. Pomar Blanco

Hospital Universitario "del Rio Hortega". Cátedra de ORL. Valladolid. Spain

INTRODUCTION: The classic anatomical description of the pavilion of the ear and its different anatomical variations in the human are very similar in the different anatomical texts consulted. However, there is no detailed description of the different projections forming the pavilion.

We have only found a systematic description of two variations in some of the texts revised: in the tragus (*Tragus*), the presence of the so-called Fis supratragal relief (*Tuberculum supratragicum*); and in the helix, the *darwinian tubercle* (*Tuberculum auriculare*) is described almost constantly.

The variations which affect the helix can be grouped into those which affect size, direction or morphology. Among the last –those which are found on the helix-some cause a lack of the helix channel (*Sulcus cruris helicis*) by defect of its fold, others are found with fusion of the helix and anthelix roots (*Crura antehelicis*) and, lastly, others consistent in multiple helix roots

PATENTS AND METHODS: We studied 576 external ears corresponding to 288 patients whose ages ranged from 10 months to 97 years. The patients were selected at random from all those who came to our ORL consultation between January and December, 1997. All patients received a careful exploration of the pavilion, with special attention to the contour variations of the helix root. We described, in the first place, if they possessed any special anatomical detail in this zone. Then we specified if this detail, if any, was bilateral or not.

RESULTS: Of the 288 patients studied, 183 (66.54%) did not present any anatomical detail in particular in the helix root. In these patients, the profile of the helix was uniform, without any outstanding protuberance or depression, appearing as normally described in the texts on anatomy and otology.

In the rest of the patients (36.45%), we found the following anatomical variations of the helix root:

– Superior helix tubercle
– Inferior helix tubercle
– Cleft or groove in helix root

CONCLUSIONS:

• Almost a third of the patients explored show some variation in the morphology of the helix root. We therefore feel that this constitutes an anatomical detail in its own right.

• The anatomical variation found most frequently is the one we have called inferior helix tubercle, followed in frequency by the helix cleft and the superior helix tubercle.

• All of these anatomical variations can appear in very marked form (*notable*) or as discrete elevations (*minimal*). This fact has no statistical relationship with sex, although it does relate to age.

P. 30 INCIDENCE OF 31 EPIGENETIC CRANIAL VARIANTS IN A POPULATION OF CASTILLA AND LION

R. García Fraile, M.B. Gregorio Crespo, J.A. Gil Verona, F.J. Paz Fernández and J.F. Pastor Vázquez

Anatomical Department, Faculty of Medicine. University of Valladolid

The epigenetic characters have been a valuable tool in order to study the identity between ethnic groups and the affinity between populations. The term epigenetic refers to the fact that the expression of this characters would be given by the genetic factors, to which will join the ambient factors. Although ambient factors would be propitious, the character would not be expressed if the genetic charge wouldn't get to a determinate threshold. Is not yet cleared if factors as age and sex influence and in which way on the expression of the epigenetic characters, existing contradictory works.

We have studied 153 skulls of the Anatomical Museum of the Valladolid University, In all of the is well known the age, sex, burial time and origin.

We have studied only the age decades between 40 and 89 years old, because the other decades have a short number of specimens in order to be statistically evaluated.

The variations incidence is similar to another European series with only a few punctual differences.

We have not observed significative differences related with sex in no variations, and we can only stand out: lambdoid bone present, absent mastoid foramen, and the frontal foramen or incissure present, in which there are a few differences.

We have not observed significative differences related to age in no of the five decades.

P. 31 IMMUNOCYTOCHEMICAL DISTRIBUTION OF CALCITONIN GENE-RELATED PEPTIDE AND SUBSTANCE P IN THE MOUSE RENAL PELVIS

C. Verástegui, A. Romero, A. Prada, A. Camacho, I. López, R. Fernández and F.J. Fernández-Trujillo

Departamento de Ciencias Morfológicas. Facultad de Medicina. Universidad de Cádiz

INTRODUCTION: Calcitonin gene-related peptide (CGRP) and Substance P (SP) are two of the most widely distributed neuropeptides both in the central and peripheral nervous systems. They have been identified and characterised in the urogenital tract of several mammals.

CGRP has been found coexisting with SP within a subpopulation of primary sensory neurons in peripheral nerves in the guinea pig and rat.

Substance P is a peptide that has been implicated as a neurotransmitter in certain types of primary afferent neurons and it is present in varicose axons in the wall of the renal pelvis and around larger blood vessels in the kidney of some mammals such rats. In the mouse, SP-containing nerves represent an important component of the renal sensory innervation.

CGRP- and SP-immunoreactive nerve fibres have been described in the kidney of various mammals, but previously published data have indicated that in the mouse, unlike other species examined, the kidney is not supplied by sensory nerves containing nor SP neither CGRP.

The results that we have obtained contradict the published data, and indicate that, in the mouse, SP- and CGRP- containing nerves represent an important component of the renal pelvis innervation.

MATERIALS AND METHODS: Ten adult male mice (*Mus musculus*, Swiss OF-1) were anesthetized with ether inhalation and perfused transcardially with 0.9% saline, followed by 4% phosphate-buffered paraformaldehyde, pH 7.4. The kidneys were removed and 7-10 μm sections were cut on a microtome.

Serial sections were processed by streptavidin-biotin immunoperoxidase method. Sections were exposed to a rabbit anti-CGRP and anti-SP polyclonal commercial antisera diluted 1/100 and 1/200 respectively in a moisture chamber 72 hours at 4°C.The next steps were developed by using the avidin-biotin-peroxidase complex diluted 1:50 for 20 min at room temperature. The sections were revealed by rising in a 0.05% Diaminobenzidine solution in presence of 0.01% H_2O_2 in the washing buffer under microscopic control. The negative control procedures were carried out by omitting one or more steps of the method and by using sections incubated with inactivated antisera with added excess antigen.

RESULTS: Our results showed that, after staining of sections for either SP and CGRP immunoreactivity, staining axons were commonly observed in the renal pelvis but were never seen in the cortex or medulla.

Many fine, varicose SP-immunoreactive nerve fibres were found in the mouse renal pelvis, and around the larger renal blood vessels.

The presence of CGRP immunoreactivity had been shown mainly surrounding large blood vessels and in the renal pelvis. The immunoreactive fibres ran paralel to the long axis of each of the circular and longitudinal muscle layers. Some varicose axons were present in the subepithelial regions and further extended into the epithelium.

Nor SP- neither CGRP-Immunopositive fibres were seen in the renal cortex.

CGRP-immunoreactive fibres have been shown to be much more abundant in the mouse renal pelvis than SP-immunoreactive elements.

DISCUSSION: Few reports are available concerning CGRP-or SP-containing nerves in the mammal renal pelvis in contrast to the studies on other parts of the urinary tract such bladder and ureter.

To the best of our knowledge, this is the first time that SP and GRP have been immunocytochemically demonstrated in nerve fibres of the mouse renal pelvis.

It has been reported that CGRP and SP in the pyeloureteral system may play an important role not only in sensory but also in motor function.

Our findings suggest that sensory nerves containig SP may carry sensory information of several types from the mouse kidney.

This work contributes to establish the morphological basis of the mouse renal innervation for the selected neuroregulators, and the point of the scope of light microscopic studies is to establish the coexistence of these substances in the same nerve fibres by double-labelling techniques.

P. 32 CARDIAC MORPHOMETRIC AND 3D RECONSTRUCTION IN O'RAHILLY'S STAGE 16

L.A. Arráez-Aybar[1], H. Herrero-González[1] and J. Jiménez-Collado[2]

[1] Dpto de Ciencias Morfológicas II, Facultad de Medicina, Universidad Complutense, Madrid. [2] Instituto de Embriología, Universidad Complutense, Madrid

KEY WORDS: Cardiac growth, Computer reconstruction, Human embryos.

INTRODUCTION: Morphogenic processes underlying cardiac development and differentiation have been widely described and experimentally studied (1). However, in the quantitative works there is a remarkably high prevalence of these in the fetal period compared to a scarcity in the embryonic period (2). In an attempt to collect data and quantitative analyses in the embryonic phase of the period of cardiac organogenesis to establish specific patterns in the different O'Rahilly stages (3), firstly we present a morphometric study and 3D reconstruction of a heart in human embryos of O'Rahillys stage 16.

Computerised image analysis was applied using a methodology elaborated by us (4) for the impartial detection and extraction of functionally homologous regions in each of the consecutive sections of a specimen cut in serial sections. This was followed by quantitative analysis of size and form and the subsequent 3D reconstruction of these regions.

According to Orts Llorca, 1932, in vertebrates the stapede artery, as denominated by Hyrtl in 1845 and described by Cuvier, 1840 is constantly present during mammalian embryonic development and is responsible for supplying blood to the facial structures and orbit (Padget, 1948). The aim of this work is to study the origin and arrangement of this artery during embryonic development and to chronologically sequence developmental events in order to understand the location of this vessel when it persists in the adult.

MATERIAL AND METHODS: Light microscopic studies were done on twenty human embryos from the Collection of the Institute of Embryology at the Complutense University of Madrid. The specimens ranged from 4.8 to 27 mm crown-rump (C-R) length.

RESULTS: In 6 mm C-R length specimens an arterial branch was clearly distinguished emerging from the lateral wall of the primitive internal carotid artery corresponding to the dorsal portion of the second arterial arch. This portion is attached to the artery whereas the remaining section is undergoing a process of involution. This arterial section corresponds to the stapedial artery.

Between 7-9 mm C-R length, the artery becomes shorter and its trajectory is limited to the mesenchyme of the second arch dorsal to the first pharyngeal pouch, ventral to the Andercht ganglion and the facial nerve runs along its lateral side.

In specimens from 10.75 to 12 mm C-R length, the artery originates in the internal carotid artery and then joins an artery that extends along the lateral walls to the fetal head. To do this, it crosses the precartilaginous anlage of the stapes and runs beneath the horizontal section of the facial nerve and the primitive cephalic vein.

Between 13 and 15 mm C-R length the artery reaches its maximum volume and runs through the future middle ear.

At 16 mm C-R length the stapedial artery begins to involute at the portion nearest to its site of emergence from the internal carotid artery. When the muscular blastema of the musculature of the first arch appear the maxillary artery has increased in calibre.

At 21 mm C-R length, complete atrophy of the stapedial artery is observed in the portion between its origin at the internal carotid artery to its passage through the stapedial orifice. The middle meningeal artery and its branches are now tributaries of the maxillary artery to which it becomes attached after 17 mm C-R length.

DISCUSSION: We consider the stapedial artery to be a branch of the internal carotid artery and to correspond to

the non-involuted dorsal portion of the second arterial arch, and in this respect coincide with the opinion of Orts Llorca, 1932, 1934. We do not believe the stapedial artery to be a direct branch of the hyoid artery as suggested by Portela et al., 1959. Between 12 and 15 mm C-R length (O'Rahilly's stages 17-18), as Congdon, 1922, also pointed out, the artery reaches its maximum development, providing the cephalic blood supply via its continuity with the supraorbitary branch (the future middle meningeal artery). In O'Rahilly's stage 20, the stapedial artery is obliterated in the portion most proximal to its origin at the internal carotid artery. As a consequence, the medial meningeal artery is a branch of the maxillary artery and the medial meningeal portion situated next to the future middle ear will form the tympanic branch of this artery.

REFERENCES

1.- OST'ÁDAL (1997): The Developing Heart. Lippincott. Philadelphia.

2.- MANDARIN-DE-LACERDA (1995): *Ann Anat,* 177: 179-184.

3.- O'RAHILLY et al (1981): *Bull Ass Anat,* 65: 141-234.

4.- ARRÁEZ-AYBAR et al (1994): *Surg Radiol Anat,* 16: 419-422.

Supported by a grant from Universidad Complutense PR 49/98-7753.

P. 33 CELL PROFILERATION AT THE POSTERIOR LENS CAPSULE IN HUMAN EYES AFTER INTRAOCULAR LENS IMPLANTATION

M. Canals, J.M. Potau, J. Costa and D. Ruano

Departamento de Ciencias Morfológicas. Facultad de Medicina. Universidad de Barcelona

INTRODUCTION: In the present study changes occurring in the posterior lens capsule after intraocular lens implantation are shown in human donor eyes.

MATERIAL AND METHODS: A casual finding revealed the presence of intraocular lenses implanted bilaterally in an 81-year-old female that had been donated to our Department. Both eyes were enucleated, the corneoscleral discs removed and the remaining bulbi prepared for both SEM and optic microscopy.

RESULTS: In both eyes rigid intraocular lenses were implanted in the posterior chamber inside the capsular bag. Scanning electron microscopy revealed that the posterior capsule was intact and had a smooth posterior surface. Cumuli of confluent flat cells with an endothelial appearance were detected at the central and paracentral area of the anterior face of the posterior lens capsule. At the equatorial area of the capsular bag, hexagonally-shaped cells that were in close contact were observed. The arrangement of these hexagonal cells showed a morphological pattern that resembled the arrangement of crystalline lens fibres.

Optical microscopy of histological sections of the specimens studied revealed the existence of cuboidal epithelial cells filling the space between the anterior and posterior capsules in the equatorial area, except for the space filled by the lens haptics. These cells were densely arranged and seemed to keep both capsular layers in contact. The paracentral area of the posterior capsule showed a thin layer of flat enlarged cells at both its anterior and posterior surfaces.

DISCUSSION: Optic microscopy and SEM have shown the existence of cell proliferation in the capsular bag after intraocular lens implantation. These cells show a more intense proliferation at the equator, with a higher density of cells that afterwards seem to migrate centrewards. These cells show typical epithelial features and occasionally seem to recreate the morphology of primary lens fibres. Our findings suggest that lens epithelial cells remaining after extracapsular cataract extraction proliferate mainly at the equator of the capsular bag and then spread covering the anterior surface of the posterior capsule.

P. 34 ANALYSIS OF TLX-2 EXPRESSION IN EMBRYONIC HAEMATOPOYESIS

J. Carretero, M. Rubio, R. Vázquez, R.E. Rodríguez, D. Vega and R. González-Sarmiento

Dept. of Human Anatomy and Histology, Faculty of Medicine. Unity of Molecular Biology, Dept. of Medicine, Faculty of Medicine. Institute of Neuroscience of Castilla y León. University of Salamanca, Spain

Homeobox genes codify proteins, which act as transcription factors and interact with DNA by binding to homeodomains. These genes are presented grouped or dispersed in the mammalian genome. New Homeobox gene families, dispersed in the genome have been reported recently, among these, the Tlx genes family is. Tlx-family is characterized by a treonine residue placed in the 47 location of the homeodomain, including Tlx-1, Tlx-2 and Tlx-3 genes. We have cloned the Tlx-2 gene and we have analyzed its expression along the mouse embryonic development using in situ hybridization. The Tlx-2 gene is located in the chromosome 2 and it is constituted by 3 exons. In the present study, we show the time-sequential expression of this gene in haematopoietic cells. No hybridization expression was found at embryonic 8th day (E8d), initial expression was observed in the Yolk's sack at E11d. After E13d, Tlx-2 was observed to pass from Yolk's sack to haematopoyetic cells and, at E19d Tlx-2 expression was observed in nucleated peripheral blood, including umbilical vessels. Future experiments are necessary to further define the role of Tlx-2 in the hematopoietic system.

P. 35 DEVELOPMENT OF THE GOAT SUBCOMMISSURAL ORGAN. A MORPHOLOGY AND IMUNOHYSTOCHEMICAL STUDY

Manoli Castañeyra-Martín[2], Emilia Carmona-Calero[1], Romualdo Ferres-Torres[1], Herminia Pérez-González[1], Isabel Martínez de la Peña-Valenzuela[1], Luz María Fernández-González[1], María del Mar Pérez-Delgado[1], Norberto Marrero-Gordillo[1] and Agustín Castañeyra-Perdomo[1]

[1] Departamento de Anatomía Humana, Facultad de Medicina, Universidad de La Laguna, Tenerife, Spain. [2] Instituto de Investigacion y Ciencias de Puerto del Rosario, Fuerteventura, Canarias, Spain

The subcommissural organ (SCO) is an ependymal gland located at the entrance of the cerebral aqueduct, below the posterior commissure. The SCO secretes large molecular size glycoproteins into the ventricular cerebrospinal fluid, where they aggregate to form Reissner's fiber (RF) (1).

Although several and contradictory hypotheses have been advanced, the functional significance of the SCO is still unknown. One of the more explored hypothesis postulates the involvement of the SCO in water and electrolyte metabolism. Rodriguez et al. [3] and Severs et al. [6] have shown that aldosterone exerts an effect on the SCO, resulting in an increase in sodium excretion. Also worth mentioning is the presence of angiotensin II receptors in the SCO cells [2]. All this information led to the present investigation with the aim to detect possible changes in the immunoreactivity of the SCO of Fuerteventura Goat, an animal that lives in natural arid conditions, by using specific antibodies against the SCO secretory material.

Twelve-goat brains from Puerto del Rosario slaughterhouse were used, divided into four age groups (one month, three months, puberal and adult groups). The brain was fixed by immersion in Bouin's fluid. Embedding was in paraffin. Frontal and sagital serial sections, 10 μm thick, through the region of the SCO, were obtained. The sections were mounted in four parallel series. One of the series was stained with the Klüver-Barrera method, while the other one was processed for the immunoperoxidase method of Sternberger et al. [7]. An antibody against the glycoproteins of the bovine RF (AFRU, [3]) was used as primary antibody. The intensity of the immunoreaction of the SCO with AFRU was measured by recording the optical density of the immunostained sections of the SCO, using a Magiscan image analysis system, and the Genias program (Joyce Loebl, Newcastle, UK). Six sections of each goat, corresponding to the rostral, intermediate and caudal parts of the SCO were used for the analysis. The mean resulting from the values of the six sections of each goat was regarded as the animal's value. The values were plotted, and a statistical analysis (ANOVA) was performed.

During the postnatal development, the goat SCO presented an extraordinary expansion, covering all the posterior commissure, from the pineal to the post-commissural recess and showing both of two cellular (ependymal and hypendymal) stratums very well developed. The amount and location of AFRU-ir material in the ependymal and hypendymal cells of the SCO adult goat was different as compared with that of the SCO of perinatal goat. In the goat SCO, most of the AFRU-ir material is stored in the perinuclear and supranuclear cytoplasmic regions. As in others animals (4), about 60% of the immunoreactive material is contained in secretory granules located in the ventricular cell pole [3].

We think that the abnormal salt and water balance present in this kind of goat adapted to arid conditions might be the trigger for the changes occurring during the development of the SCO of this goat.

1.- Castañeyra-Perdomo A, Carmona-Calero E, Meyer G, Pérez-González H, Pérez-Delgado MM, Marrero-Gordillo N, Rodríguez S and Rodríguez EM (1998). Changes in the secretory activity of the subcommissural organ of spontaneously hypertensive rats. *Neurosci Lett,* 246: 133-136.

2.- Ghiani P, Uva, B, Vallarino M, Mandich A and Masini M (1988). Angiotensin II specific receptors in subcommissural organ, *Neurosci Lett*, 85: 212-216.

3.- Rodríguez EM, Oksche A, Hein S, Rodríguez S. and Yulis R (1984). Spatial and structural interrelationships between secretory cells of the subcommissural organ and blood vessels. An immunocytochemical study, *Cell Tissue Res*, 237: 443-449.

4.- Rodríguez EM, Herrera H, Peruzzo B, Rodríguez S, Hein S and Oksche A (1986). Ligt and electron-microscopic immunocytochemistry and lectin histochemistry of subcommissural organ: evidence for processing of the secretory material. *Cell Tissue Res.*, 243: 545-559.

5.- Rodríguez EM, Oksche A, Hein S and Yulis CR (1992). Cell Biology of the Subcommissural Organ. *Int Rev Cytol,* 135: 39-121.

6.- Severs WB, Balaban CD, Morrow BA, Snyder CL and Keil LC (1993). The subcommissural organ: Immunohistochemistry and potential relation to salt/water balance, In: A. Oksche, E.M. Rodriguez and P. Fernandez-Llebrez (Eds): *The subcommissural organ. An ependymal gland.* Springer-Verlag Berlin Heidelberg, pp 265-277.

7.- Sternberger LA, Hardy PH, Cicilis JJ and Meyer HG (1970). The unlabeled antibody enzyme method of immunohistochemistry; preparation and properties of soluble antigen-antibody complex (horseradish peroxidase-antiperoxidase) and its use in identification of spirochetes. *J Histochem Cytochem*, 18: 315-333.

Acknowledgements

Supported by the Consejería de Educación del Gobierno de la Comunidad Autonoma de Canarias. Project. Nº1998/002 and Universidad de La Laguna Project Nº 48/98. We thank Prof. Dr. E.M. Rodríguez for the generous gift of his antibody (AFRU)

P. 36 LOW FREQUENCY ELECTROMAGNETIC FIELDS INHIBIT NEURAL CREST CELLS DEVELOPMENT IN CHICK EMBRYO

S. de la Fuente, Rafael Ramos, Isabel San José, Santiago Rodríguez and Juan Represa

Departamento de Anatomía, Facultad de Medicina, Universidad de Valladolid

To date there is still no consensus concerning the effect of low frequency electromagnetic fields (ELMF) on developing embryos. Despite of this uncertainty, there is general agreement that ELMF can affect biological systems. Although cellular mechanisms are fairly unknown several reported experiments on chicken embryos indicate a significant increase in the number of malformations. As part of an ongoing study on biological responses to ELMF's, experiments were conducted to determine whether ELMF have effects on cell proliferation and -+cell migration in chick embryo. Such effects might lethal during early stages and therefore being largely ignored.

Chick embryos obtained from fertilised eggs (36-40 hour of incubation) were explanted for 24h on a solid media. Eventually primary cultures of Neural Crest Cells (NCC) were set up and grown in defined medium alone (M199) or in medium containing Nerve Growth Factor (NGF100ng/ml). Whole embryos explants and NCC cell cultures were exposed to ELMF (50 Hz), ranging from 0.01 to 5 Gauss of intensity for 24 h.; afterwards ELFM-exposed and control samples were fixed in 4% para-formaldehyde and processed for histology, immunostaining methods and morphometrical analysis.

The study has shown that in the whole embryos ELMF inhibited and perturb "in vitro" embryonic development and growth producing abnormal changes in Neural Crest Cells.

The morphological and morphometrical analysis demonstrated that low frequency ELMF inhibited cell growth of the whole embryo, which affects more intensively the neural tissues. The specific effect of ELMF on NCC growth and migration was further demonstrated by labelling the NCC with NCC-specific antibodies against HNK1 antigen. The incorporation of ^{3}H Thymidine revealed that DNA synthesis was reduced in the exposed embryos, suggesting direct effects of ELMF on cell division in the targeted tissues. This inhibitory effect on cell proliferation was already detectable at ELMF exposures of 0.01 Gauss with maximal growth inhibition occurring between 1 and 5 Gauss. Likewise in primary NCC cultures the exposure to ELMF produced intensity-dependent growth inhibition that was revealed by reduction in the rate of DNA synthesis. These results obtained in NCC primary cultures correlated well with the findings collected in whole embryos.

CONCLUSION: The present work indicates the existence of biological effects after ELMF exposure of embryonic tissues and therefore opening the possibility that ELMF may be potential teratology agents. The results also suggest that ELMF may act on embryonic cells by changing the cell cycle.

P. 37 HOMOCYSTEINE MIGHT ALTER THE DORSAL ROOT GANGLIA IN THE CHICK EMBRYO

M. Epeldegui, S. Navarro, C. Tudela, G. Varela-Moreiras[1], A. Peña, J. Puerta and J. Pérez-Miguelsanz

Dpto. CC. Morfológicas Fac. Med. UCM. [1] Universidad San Pablo CEU. Madrid. Spain

It has been questioned that an environmental factor, as elevated levels of Homocysteine (Hcys), is related with an incorrect development of the neural tube (Eskes, 1998). The segmental pattern of organization of the peripheral nervous system arises as a consequence of interactions taking place between neural crest, neural tube and somite cells (Kalcheim, 1989). Neural crest cells are the progenitors of dorsal root ganglia (DRG), simpathetic ganglia and Schwan cells (Kalcheim, 1989). The DRG are metameric series of structures that develop from neural crest cells within the dorsal somitic mesoderm (Geffen, 1996).

We have used chicken embryos (G.gallus) at stages (s) 3 to 10 HH, and after windowing the eggs and removing 1.5 ml of albumen, Hcys 20(M) was added directly on the embryo, they were reincubated until reached s 18-21, fixed in Bouin's solution, embedded in paraffin wax, and serially sectionated 8(m). The sections were stained with hematoxylin-eosin for morphological assessments. Also, the neural crest cells were studied using the monoclonal antibody HNK-1.

We have obtained 90% of survival, in which at the time of fixation there were macroscopically normal between 20 to 70% depending of the stage of experimentation.

The embryos treated with Hcys, show DRG apposed to the dorsolateral faces of the neural tube, in a continous and nonsegmented pattern. Also, these DRG have a different shape, they are smaller than in control embryos. These type of DRG are located on the whole cervical region, nevertheless the dorsal region is not affected completely and it is variable where these DRG become normal. This alterations appear on the 36% of the survival treated embryos. The lumbar and sacral region always have a normal fenotype for the DRG but other type of neural tube defect could be present. This could be another abnormality of the Hcys caused in the development of the nervous system.

ESKES, *Nutr. Rev.*, 56. 1998.

GEFFEN. *Dev. Biol.*,178. 1996.

KALCHEIM. *Development*, 106. 1989.

P. 38 NEUROPROTECTION BY MELATONIN FROM GLUTAMATE-INDUCED EXCITOTOXICITY DURING DEVELOPMENT OF THE CEREBELLUM IN THE CHICK EMBRYO

A. Espinar, A. Jiménez-Rubio, V. García-Lomas, J. Ambrosiani, A. Quesada and F.A. Prada

Department of Morphological Sciences and Institute of Developmental Biology. The University of Seville. School of Medicine. Sevilla. Spain

INTRODUCTION: The purpose of this study was to investigate the ability of melatonin to prevent cell damage in the cerebellar cortex of chick embryo caused by glutamate administration. Cell injury was evaluated estimating, at ultrastructural level, the phenomenon of cell death and the synaptogenesis of the Purkinje cells and the cerebellar glomerular synaptic complex.

MATERIAL AND METHODS: Fresh fertile white Leghorn Chick eggs *(Gallus domesticus)* were used. All injections were made into the vitelin sac (i. v.) according to standard techniques at two different times: before the initiation of Morphological differentiation, at 2 days of incubation (E-2; n=72) or before initiation of the process of synaptogenesis, at 6 days of incubation (E-6; n=72). Later, embryos were sacrificed at 13 (E-13; n=72) and 20 (E-20; n=72) days of incubation, respectively. In both series of experiments, three experimental groups were used. Melatonin group: injected i. v. with 100 µl 4 mM melatonin (n=36); glutamate group: injected i. v. with 100 µl of 1 mM monosodium L-glutamate (n=36); and melatonin plus glutamate group: injected i. v. with 100 µl 4 mM melatonin administrated 30 min before 100 µl 1 mM glutamate injection (n=36). Simultaneously, a control group was injected i. v. with the same volume of vehicle (0.5% ethanolic saline).

RESULTS AND DISCUSSION: Administration of glutamate during cerebellar development of the chick provokes excitotoxic neuronal degeneration characterized by a phenomenon of neuronal cell death that exhibited essentially the feature of a death pattern described as necrosis. The loss of synaptic afferentes are the cause of the progressive cell death at level of the Purkinje cells. Thus, the delay of dendritic trunk differentiation we have found in the embryos killed at E-13 is the initial stage of cellular damage that is followed by the lysis and cell death in the later stages of embryonic development (E-20). Our results show that melatonin has a neuroprotective effect againts glutamate-induced excitotoxicity. This effect is morphologically revealed by the lack of neural cell death in the embryos treated with melatonin prior to glutamate injection and also by the degree of a synaptogenesis similar to that exhibited by the control group. Likewise, we corroborate the absence of teratological effects of melatonin on chick cerebellar development. The possible mechanisms involved in the neuroprotective effect of melatonin, i.e.,

direct antioxidant effects, up-regulating endogenous antioxidant defenses, and inhibiting nitric oxide formation activated by glutamate, are discussed.

P. 39 SECRETORY ACTIVITY OF SCLEROBLASTS IN IHE BRACHIAL SCLEROTOME OF H.H. STAGE 19 CHICK EMBRYO

M. González-Santander Martinez and L. Gómez Pellico

Departamento de Ciencias Morfológicas y Cirugía. Facultad de Medicina. Universidad de Alcalá

INTRODUCTION: Solursh et al. (1) suggested the production of a hyaluronate-rich extracellular matrix by cells in the sclerotome, that would hydrate to facilitate scleroblast migration. 'in order to confirm this mechanism of migration, we have used the electron microscope to study the ultrastructure of the secretory function in the cytoplasm of the scleroblasts at the beginning of their migration towards the notochord.

MATERIAL AND METHODS: Hamburger and Hamilton (HH) stage 19 chick embryos were fixed in 2.5% glutaraldehyde, postfixed in 1% osmic acid and embedded in Araldite. The brachial sclerotome is then selected and semithin sections stained with toluidine blue. Ultrathin sections were stained with lead citrate and observed under the transmission electron microscope.

RESULTS: We have observed dictyosomes of the Golgi apparatus and the production of Golgian vesicles in the cytoplasm of the scleroblasts (Fig. 1). Several vesicles of different sizes, some electrolucent and some electrodense with a very osmophilic content (Fig. I a and b), are located around the dictyosomes. At this stage of differentiation, we have not been able to identify any exocytosis process of these vesicles towards the extracellular space through the cytoplasmic membrane of the scleroblasts.

DISCUSSION: The activity of the Golgi apparatus, with the production of vesicles, could be related to the early secretory activity of the scleroblasts in the synthesis of hyaluronic acid. The presence of hyaluronic acid in the extracellular space would be neccessary for the migration of the scleroblasts towards the notochord, according to Solursh et al (1) and Huang et al (2). We suggest that the secretion of an extracellular matrix rich in hyaluronates would start from HH stage 19, together with the expansion of the sclerotomal cellular mass The migration of the scleroblasts would disintegrate the sclerotome forming the sclerotomal mesenchyma that would originate the skeletal tissues (cartilage, bone and fibrous tissue) (Christ and Wilting) (3).

REFERENCES

1.- SOLURSH M et al. (1979). *J Embryol Exp Morphol,* 54: 75-98.

2.- HUANG R et al. (1996). *Acta Anat,* 155: 231-241.

3.- CHRIST B and WILTING J (1992). *Ann Anat,* 174: 23-32.

P. 40 CRANIAL ABNORMALITIES IN CHICK EMBRYO AFTER ADMINISTRATION OF ETHANOL

M. Guirao-Piñeyro, O. Roda Murillo, J.A. Roda Moreno, M.T. Pascual Morenilla, M. López Soler and F. Arrebola

Dpto. de Ciencias Morfológicas. Instituto de Neurociencias. Universidad de Granada

Since 1968, when LEMOINE first described a malformation syndrome in the offspring of alcoholic women, designated Fetal Alcohol Syndrome (FAS), numerous studies have been published both on the syndrome in general and on the existence of partial aspects (RANDALL CL, 1978).

The experimental teratologic study of ethanol on the development of the chick area has been demonstrated by different authors (BRODIE et al., 1990; SANDERS EJ et al., 1990; STEPHANIE R et al., 1998). There have also been reports of ethanol-induced brain abnormalities both in experimental animals and humans (KOPERA-FRYE K. et al., 1996).

Three hundred fertilized Gallus Domesticus White Leghorn chicken eggs were incubated in a humidity-controlled Masalles incubator, of which 85 eggs formed normal group, 95 experimental control group (vehicle) and the remaining 120 eggs were treated with 100 µl of 50% ethanol solution. The administration was via two 1 mm orifices in the shell at air chamber level.

The morphometric study of the skull used the method described by OLIVER S et al. (1987). The skull measurements were: transverse diameter, anteroposterior diameter and sagittal diameter.

All three diameters studied were significantly smaller in the ethanol-treated embryos

versus controls (normal and vehicle) after 17, 19 and 21 days of incubation.

Children of mothers with a history of alcohol abuse during the pregnancy present brain abnormalities (SOWELL ER et al., 1996). Brain abnormalities, facial dimorphism and microcephaly have been described in patients with FAS (SWAYC EVW et al., 1997).

We contribute the results of an experimental study that shows that ethanol affects the growth rates of the skull during embryonic development.

P. 41 HORIZONTAL CELLS EXPRESS A MYELIN OLIGODENDROCYTE SPECIFIC PROTEIN DURING DEVELOPMENT AND ADULT VERTEBRATE RETINA

A. Jiménez Rubio, V. García-Lomas, A. Espinar, M.S. Gūtiérrez, A. Quesada and F.A. Prada

Department of Morphological Science and Institute of Developmental Biology. University of Seville. School of Medicine. Seville. Spain

INTRODUCTION: Horizontal cells of the vertebrate retina are interneurons whose somas lie in the most outer zone of the inner nuclear layer. Each horizontal cells receives inputs from photoreceptors, and participates on information processing in the outer plexiform layer. These cells show special morphofunctional characteristics, such as the existence of electrical contacts between their axon terminals (Kaneko, 1970) which form a dense network of gap junctions through outer plexiform layer.

On the other hand, horizontal cells are unique because in addition to neurofilaments (characteristic of neurons), they also express molecules characteristic of glial cells, such as vimentin (Drager, 1983), R_4 and R_5 cytoskeletal antigens (Drager et al., 1984) and 3CB2 (Prada et al.,

1995). Perhaps, the above mentioned data are responsible of horizontal cells to be considered for some time as a kind of glial cell. Here we show that in general horizontal cells express a myelin oligodendrocyte specific protein (MOSP) during development and in adult vertebrate retina.

MATERIAL AND METHODS: Eye globes between E7-E20 and adult eye globes of white Leghorn chick embryos and freshly dissociated adult cells of chick, rabbit, turtle and lizard, were processed according to current immunocytochemical protocols using a monoclonal antibody against MOSP. MOSP is a myelin oligodendrocyte specific protein reported to be specifically expressed by oligodendrocytes in central nervous system of many vertebrate species but not in the retina (Dyer et al. 1991).

Animal care protocols used in our laboratory are in conformity with the appropiate national legislation (Decres 223/1988, BOE nº 67) and guidelines from the European Communities (Council Directive 86/609/EEC).

RESULTS AND DISCUSSION: MOSP appears in axon terminals of horizontal cells of the chick embryo retina at E14. At E13, horizontal cells begin the outgrowth their axon terminal, which is first detectable in the dorsotemporal retina. The early formation of the horizontal cell axon and the growth of the axon terminal is coincident to 3CB2 expression.

From E14 to E17, MOSP immunoreactivity expression increases. This fact is coinciding with differentiation and maduration of horizontal axon terminals. From E18 immunoreactivity pattern of MOSP in the outer plexiform layer is similar to the adult. Also these data have been controled in isolated cells during development and in other animals such as: chameleon, turtle, lizard and rabbit.

The presence of MOSP in horizontal cells axon terminals could be relationed with the extensive gap junctions located in horizontal cell axons.

P. 42 TROPIC INFLUENCE OF STRIATAL GDNF ON STRIATAL DOPAMINERGIC INNERVATION DURING NORMAL DEVELOPMENT AND IN DEVELOPING STRIATAL GRAFTS

J.L. Labandeira-García[1], E. López-Martín[1], J. Rodríguez-Pallares[1], M.J. Guerra[1] and H.J. Caruncho[2].

[1] Dept. of Morphological Sciences, and [2] Dept. of Fundamental Biology. University of Santiago de Compostela. Galicia. Spain

Glial cell line-derived neurotrophic factor (GDNF) has potent trophic action on fetal dopaminergic neurons. We have used a double immunocytochemical approach with antibodies that recognize GDNF and tyroxine hydroxylase (TH) or the phosphoprotein DARPP-32, to study the developmental pattern of their interactions in the rat striatum and in intrastriatal striatal transplants. Postnatally, at one day and also at one week, GDNF showed a patchy distribution in the striatum together with a high level of expression in the lateral striatal border, similar to that observed for the striatal marker DARPP-32 and also for TH. In the adult striatum, there was diffuse weak immunopositivity for GDNF, together with widespread expression of DARPP-32-positive neurons and TH-immunoreactive (TH-ir) fibers. In one-week-old intrastriatal striatal transplants, there were some GDNF immunopositive patches within the grafts and although there was not an abundance of TH positive fibers, the ones that were seen were located in GDNF-positive areas. This was clearly evident in two-week-old transplants, where TH-ir fibers appeared selectively concentrated in GDNF-positive patches. This pattern was repeated in three-week-old grafts. In cotransplants of mesencephalic and striatal fetal tissue (in a proportion of 1:4), TH-ir somata were located mainly at the borders of areas that were more strongly immunostained for GDNF, and TH-ir fibers were also abundant in these areas and were found in smaller numbers in regions that were weakly positive for GDNF.

These results demonstrate that GDNF-ir is coincident with that for TH and DARPP-32, and suggest that GDNF release by fetal striatal neurons both in normal development and in developing striatal grafts may have not only a trophic but also a tropic influence on TH-ir fibers and may be one of the factors that regulate dopaminergic innervation of the striatum.

Supported by CICYT and XUGA.

P. 43 INHIBITION OF APOPTOSIS BY OVEREXPRESSION OF bcl-2 GENE DISRUPS THE HAIR CELL PATTERNING AND INNERVATION ARRANGEMENT OF THE INNER EAR

J. Represa[1,2], Roberto Cabo[1], Isabel San José[1], Christine Layburn[2] and Thomas Van De Water[2]

[1] Departamento de Anatomía, Facultad de Medicina, Universidad de Valladolid, 47005 Valladolid, Spain. [2] Department of Neuroscience, Albert Einstein College of Medicine, New York, USA

In normal development Programmed Cell Death (PCD) is a widely used mechanism for generating the normal number of cells, but the role of PCD in the choice of cell fates is less understood. The specific aim of this work is to test whether PCD contributes to the final cell fates of neurons and hair cells in the inner ear. This was accomplished by over expression during normal development of the gene *bcl-2* that inhibits PCD. The *bcl-2* gene was introduced into the ear of normal embryos by using retroviral vectors, at the time of Cochleovestibular ganglion formation and during hair cell determination. The bcl-2 belongs to a group of genes that function as critical regulators of PCD and appear to be evolutionary conserved in fact human bcl-2 can substitute for a nematode gene (called *ced-9*). The *bcl*-2 gene has been shown to block a final common pathway for PCD. Nearly 100 chick embryos were injected in the right otocyst with the retroviral vector RCASBP (B)/*bcl-2* on E4 of incubation (Stage 21-22 of Hamilton & Hamburger). Out of 64 survivors to E16-E17 were processed for morphological analysis using standard histology and SEM. As negative control the opposite uninjected ear was also processed together with a series of injected embryos using ringer saline solution. A specific phenotype was seen in the ears injected with the retroviral vector while no abnormalities were seen in the controls. SEM results revealed an abnormal phenotype consisting in the presence of extensive blebbing of cells in a strip along most of the basillar papilla; these cells appeared to be in the process of extruding from the sensory epithelial sheet. In addition, the surface of epithelium appeared denuded of hair cells in some patches.

Immunostaining (with 160 kD neurophilament antibody) of the spiral ganglion revealed a considerable increase of neurons in certain areas while others contain only small-unstained cells like supporting cells. However there were no areas of sensory epithelium devoid of innervation. On the contrary the nerve fibers reaching the hair cells are more abundant and showed an increased sprouting.

CONCLUSION: Misexpression of *bcl-2* disrupts the receptor mosaic of the basilar papilla and the hair cells innervation. The results indicated that the altered phenotype is specifically caused by overexpression of *bcl-2* being unrelated to the injection maneuvers. The sensory epithelium of ears where bcl-2 has been overexpressed seem to have too many cells, since the requisite number fail to die either in the ganglion or in the sensory epithelium. Finally, our results also support the lateral inhibition theory to explain cell fate determination in the cochlear sensory epithelium.

P. 44 DEVELOPMENT OF OCULOMOTOR MUSCLES IN THE CHICK EMBRYO (*Gallus gallus*): IMMUNOHISTOCHEMICAL STUDY

C. Maestro de las Casas, C. Martínez-Álvarez, J. Pérez-Miguelsanz, A. Peña Melián and J. Puerta Fonollá

Departamento de Ciencias Morfológicas I. Facultad de Medicina. Universidad Complutense. Madrid. España

INTRODUCTION: For a long time there has been controversy over whether embryos heads present segmented structures, at least in the first stages of development. Following on from this and in relation to extrinsic ocular muscles, various possible metameric structures have been put forward: cephalic somites, by classic researchers (Tello, 1948; Gilbert, 1957), somitomeres (Meier, 1979; Noden, 1982, 1983) and cephalic cavities (Adelmann, 1926; Jacob et al., 1984; Wachtler et Jacob, 1986)- structures existing prior to the formation of mesenchyme condensation where muscular anlagen can be differentiated.

The role of motor innervation in the differentation of the muscles is still currently under debate. (Spencer et Porter,1988; Peirone et al., 1990; Fredette et Landmesser, 1991). The purpose of our study is to describe the ontogenesis of the oculomotor muscles from its earliest stages up to the appearance of the mesenchyme condensations and continuing as far as the innervation, differentation and segregation of the primordium muscles.

MATERIAL AND METHODS: We have used monoclonal antibody 13F4 which identifies a citoplasmatic antigene present in all muscle types (Rong et al., 1987) and the monoclonal antibody HNK-1 has affinity for embryonic chick neural crest cells (Tucker et al., 1984) in Gallus gallus embryos between the stages (E) 9 to 36 of HH (Hamburger-Hamilton, 1951).

RESULTS: The neural crest cells migrate in a ventrolaterally direction to the future region of cranial ganglia ubication, and to the branchial arches, the prechordal region still free of marked cells (E-17 to 22 HH).

The only cephalic cavity can be seen placed on the paraxial mesoderm (E-21 HH) precursor of the premandibular condensation (E-22 HH), some hours later this massis reached by oculomotor nerve (IIIrd) axons and its myoblasts are transformed into myocytes multinucleated by cell fusion (E-23 HH), laterly they are segregated in the different muscular blastemas. A dorsocaudal condensation of the optic vesicle (E-22 HH) is differentiated as a ventral oblique muscle.

DISCUSSION: Our results ratify the presence of an only cavity placed bilaterally in the bird embryo head, related to premandibular condensation, in can be differentiated the oculomotor muscles We other cavities haven't been found that would permit us to postulate a metameric pattern in the bird embryo head. With the antibody 13F4 we detected the first myoblasts in premandibular condensation being innerved mediocaudally with myocites being differenciated. A posteriori we can see the III craneal par branches and separation and differentation of the superior rectus, inferior rectus and medial rectus muscles. The oblique inferior muscle wouldn't be formed from the same condensation as it has been described but rather from an independent condensation

P. 45 DEVELOPMENT OF A IN VITRO EXPERIMENTAL MODEL TO STUDY THE DIFFERENTIATION IN CELLS OF MUSCULAR LINEAGE

J.A. Marchal[1], J.E. Fernández[2], J. Prados[3], A.E. Aránega[4], E. Carrillo[2], C. Melguizo[3], C. Vélez[3], L. Álvarez[2] and A. Aránega[2]

[1] Departamento de Ciencias de la Salud, Univ. de Jaén, [2] Departamento de Ciencias Morfológicas, Facultad de Medicina, Univ. Granada, [3] Departamento de Ciencias de la Salud, Univ. Almería, [4] Departamento de Biología Experimental, Univ. Jaén

INTRODUCTION: Rhabdomyosarcomas are tumors of the skeletal muscle that rarely demonstrate conclusive evidence of myogenic differentiation characteristic of mature myotubes. In fact, most rhabdomyosarcomas are poorly differentiated and hence very difficult to diagnose. Desmin and actin are proteins most frequently used as marker of muscle cell differentiation because its expression increases in multinuclear myotubes resulting from the fusion of myoblasts. Moreover, a combination of immunohistochemical staining using antimyoglobin and anti-CK mAbs is an useful marker in the diagnosis of childhood rhabdomyosarcomas. We report the establishment and characterization of a human rhabdomyosarcoma cell line, name RMS-GR, which provides an in vitro system for the study of the pathogenetic mechanism of rhabdomyosarcoma and the modulation of myogenic differentiation.

MATERIAL AND METHODS: The cell line RMS-GR was established from an embryonal rhabdomyosarcoma tumor biopsied in a 79-year-old man. Tumor tissue obtained from the primary lesion was stained with hematoxylin-eosin. Immunocytochemical staining was performed with a standard APAAP technique using the primary mAbs anti-vimentin, anti-desmin, anti-myoglobin and anti-pan-cytokeratin; PAP method was used to immunocytochemical staining with mAb anti-a-sarcomeric actin.Incubation omitting the primary mAb was used as a control. The RMS-GR cell line was established from the intraoperative biopsy specimen and was determined the cell growth and tumorigenicity into BALB/c nude mice. Moreover, morphologic study, FACScan, electrophoretic analysis of CK isoenzymes and karyotype were used.

RESULTS: The RMS-GR cells were polygonal, round or spindle-shaped. The RMS-GR cell line became stable with a doubling time of 42 h. Tumorigenicity of the cells

was confirmed by heterotransplantion into nude mice. Electron microscopic images showed typical cytoplasmic inclusion of aggregated intermediate filaments and myofibril-like thin filaments. The expression of desmin, vimentin, actin and human myoglobin was recognized by cytofluorometric analyses, and a large fraction of CK-MM and a small fraction of CK-BB and MCK-1 isoenzymes were found. Chromosomal analysis showed that the modal chromosome number was consistently near triploid with structural abnormalities mostly involving chromosomes 1, 3 and 8, and additional unidentified markers. No alteration of chromosome 2 was observed.

DISCUSSION: We established a new human rhabdomyosarcoma cell line from a primary tumor. Analyses of this cell line, named as RMS-GR, indicated that although several types of cells were seen no showed morphological features of differentiation under light microscope. Our cell line did not show the spontaneous cell fusion observed in the line established by Petkovi *et al.*, and in contrast with rhabdomyosarcoma cell line TS-RM-1, ultrastructural analysis of RMS-GR cells showed few lipid droplets and glycogen accumulations, features considered by Ezinger and Weiss as signs of differentiation. Markers, desmin and vimentin , together with myoglobin and sarcomeric actin expression, strongly suggested a moderate degree of myogenic differentiation. Throughout normal myogenesis, CK-MM and CK-MB isoenzymes increase steadily, substituting the CK-BB fraction more typical of embryonic tissues. Line RMS-GR showed a small fraction of CK-BB and a large fraction of CK-MM. The enzymatic pattern of the RMS-GR cell line suggested a moderate degree of myogenic differentiation in contrast with others rhabdomyosarcoma cell lines such as TE.32.7, RD and A-204. The most interesting cytogenetic features of our cell line were structural abnormalities involving chromosomes 1, 3 and 8, and additional unidentified markers. Monosomies of chromosomes 4, 14 and 18 were random losses, and no alteration in chromosome 2 was observed. Although no specific chromosomal alteration has been found in embryonal rhabdomyosarcoma, an abnormality in chromosome 1 is apparently a frequent finding in childhood malignancies.

CONCLUSSION: The RMS-GR cell line may provide a system to identify genes which are involved in the pathogenic mechanism of rhabdomyosarcomas, and to investigate the modulation of myogenic differentiation.

P. 46 THE ROLE OF FGF-2 IN INITIAL DIFFERENTIATION OF EYE-LENS EPITHELIUM CELLS IN CHICK

C. Martín[1], Mª Isabel Alonso[2]; José Antonio Moro[2]; Pedro Martín[1] and Ángel Gato[2]

[1] Departamento de Anatomía Humana. Facultad de Medicina. Universidad de Valladolid. [2] Instituto de Neurociencia de Castilla y León. Facultad de Medicina. Universidad de Valladolid

Following the vesicle stage, the eye-lens anlage develops in terms of migration, division and cell differentiation, which takes place in the aequator; consequently, the cells of the anterior epithelium are incorporated, as well as undergoing differentiation, into the posterior epithelium. This process is regulated by an FGF-2 anterior-posterior gradient generated by structures around the lens and the lens capsule itself. Although no knowledge exists concerning the mechanisms involved in the dramatic initial process of differentiation which simultaneously affects all the posterior epithelium cells shortly after formation of the lens vesicle, these presumably also play a role in FGF-2 differentiation.

In this paper we describe, by means of immunohistochemical techniques in chick embryos, the existence of two transitory sources of FGF-2 which coincide in space and time with en bloc differentiation of posterior epithelium cells in the eye-lens vesicle; one is the extracellular matrix situated inside the lens cavity itself and the other is a perfectly defined band of retinal tissue located near the lens vesicle. Given that chick embryo lens vesicle cells possess FGF receptors, these two FGF-2 sources might be responsible for producing a stimulus strong enough to bring about en bloc differentiation of all posterior epithelium cells.

Nowadays it is known that the action of FGF-2 is directly linked to the presence of heparan sulphate type sulphated proteoglycans; as a result, disrupting the synthesis of these compounds is a useful strategy for demonstrating the influence of both molecules on lens cell behaviour. In this study we show, by means of a synthesis inhibitor or by their digestion with specific glycosidases, that disruption of eye anlage sulphated proteoglycans at the optical vesicle stage halts en bloc differentiation of the posterior epithelium cells, some of which even maintain their capacity to replicate. This data shows that the coordinated action of FGF-2 and sulphated PGs is directly involved in the initial "en bloc" differentiation process of posterior epithelium cells in the chick embryo eye lens.

P. 47 CORRELATION BETWEEN MDR 1 AND C-MYC GENE EXPRESSION IN THE MYOGENIC DIFFERENTIATION: STUDY IN RHABDOMYOSARCOMA CELLS

C. Melguizo[1], José Prados[1], Juan Antonio Marchal[2], Celia Vélez[2], Amelia Aránega[3], Esmeralda Carrillo[4], Houria Boulaiz[4], Luis Álvarez[4] and Antonia Aránega[4]

[1] Department of Health Sciences, University of Almeria, Almería, [2] Department of Health Sciences and [3] Department of Experimental Science, University of Jaén and [4] Basic Cardiovascular Research Section, Department of Morphological Sciences, School of Medicine, University of Granada

INTRODUCTION: Classical multidrug resistance (MDR) is related to the expression of the *mdr 1* gene, whose product, P-glycoprotein, acts as a drug efflux pump that lowers intracellular drug concentrations. Studies in resistant cells showed that *mdr 1* is often amplified although the mechanism leading to overexpression of P-glycoprotein may involve amplification, increased stability of the protein, or increased transcription. The expression of *mdr 1* gene was found to correlate with the degree of celular differentiation, suggesting that genes related to MDR are modulated by factors involved in cellular proliferation and differentiation as *c-myc* and *N-myc*. The aim of this study was to investigate the possible correlation between mdr 1 gene and myogenic differentiation in striated muscle cells using a rhabdomyosarcoma (RMS) cell line as an experimental *in vitro* model.

MATERIAL AND METHODS: Cell lines. The RMS cell line RD (ATCC) was exposed continuously to actinomy-

cin D which was increased step-wise to a final concentration of 1.2 x 10-6 mM (RD-DAC).

PCR evaluation of mdr 1 mRNA levels. Reverse transcription of total RNA was done with *mdr 1 or* β-*actin* primers. PCR products were transferred to nylon membranes which were hybridized oligoprobes (*mdr 1* and β-*actin* sequences; cDNA 3027-3049 and 1874-1898 respectively). The intensity of the autoradiographic bands was determined by densitometry.

Northern and Southern blotting. RNA was transferred to nylon membranes which were hybridized with *c-myc* and *N-myc* probes. DNA was digested with EcoRI, electrophoresed and transferred to nylon membranes which was hybridized with *c-myc* - and probes and oligoprobes recognizing *mdr 1* and β-*actin* sequences.

Electron microscopy. Monolayers were fixed in situ with 2.5% glutaraldehyde and was postfixed with 1% osmium tetroxide, dehydrated in ethanol and then detached from the culture vessel by rapid treatment in propylene oxide and embedded in Epon 812. After polymerization ultrathin sections were cut and stained with uranyl acetate-lead citrate.

RESULTS: Determination of mdr 1 expression by PCR. Parental cell line RD yielded a weak PCR product for mdr 1, whereas the resistant line RD-DAC clearly showed greater *mdr 1* mRNA expression.

mdr 1 gene amplification. Parental cell line RD did not show gene amplification in comparison with DNA obtained from a biopsy of normal striated muscle. However, the number of copies of the *mdr 1* gene increased fivefold in lines grown with 20 x 10-9, 80 x 10-9 and 160 x 10-9 mM actinomycin D. Analyses of line RD-DAC revealed a tenfold amplification of the *mdr 1* gene in comparison with the control line and with normal striated muscle. β-*actin* was used as a control.

Light microscopic and electron microscopic observations. In the resistant cell line RD-DAC, the morphological findings (light microscopic) were similar to those in the parental cell line RD. The cells grew as irregular monolayers, forming confluent aggregates. Ultrastructural examination of cell line RD showed few features of myogenic differentiation in contrast with the resistant cell line RD-DAC in which were found: high proportion of lipid vesicles, myofilamentous material, myofilamentous component tended to be organized in well-defined bundles and some myotubes.

Northern and Southern blot analyses of c-myc and N-myc proto-oncogenes. Northern blot analysis showed no modifications in the levels of *c-myc* in the sublines obtained. However, down-regulation of *c-myc* was found in RD-DAC in relation to RD cells. *N-myc* mRNA was not modified. Southern blot analysis showed no modifications in the number of copies of either *c-myc* or *N-myc* genes in RD-DAC.

DISCUSSION: In this study we found that a minimum dose of the drug was necessary to increase the expression of *mdr 1* mRNA in the RD rhabdomyosarcoma cell line. The levels of mdr 1 were not necessarily related to *mdr 1* amplification. The overexpression of *mdr 1* mRNA in the actinomycin D-resistant rhabdomyosarcoma cell line RD-DAC paralleled a decrease in *c-myc* mRNA levels, while *N-myc* mRNA levels showed no modulation. These findings strongly suggest that *c-myc* modulates *mdr 1* gene expression, and plays an important role in the development of the myogenic differentiation programme activated in this resistant cell line.

P. 48 EXPRESSION OF INTERLEUKINES 1β, 2 AND 6 DURING THE DEVELOPMENT OF RAT EMBRYO ADENOHYPOPHYSIS

J.A. Moro[2], Mª Isabel Alonso[2], Ángel Gato[2], Pedro Martín[1], Aníbal de la Mano[1] and José Carretero

[1] Departamento de Anatomía Humana. Facultad de Medicina. Universidad de Valladolid. [2] Instituto de Neurociencia de Castilla y León. Facultad de Medicina. Universidad de Valladolid. Instituto de Neurociencia de Castilla y León. Facultad de Medicina. Universidad de Salamanca

In this study we have determined by immunomarking the expression of interleukines (IL) 1β, 2 and 6 during the development of adenohypophysis in 13.5 to 19.5 day-old rat embryos. An account was made of the number of cells marked with each of the ILs out of a total of 4,050 Rathke's pouch cells; the count was carried out in alternative sections, and for each section three computational zones, one central and two lateral, were selected. Earliest immunoreactivity appeared in 15.5-day-old embryos for interleukines IL-1β and IL-6, and especially for the latter. Positive IL-6 cells represented 25% of all adenohypophysis cells at 15.5 days, and with increased development there was a rise in the number of marked cells: at 19.5 days immunoreactivity to IL-6 was almost 50%. Cells marked with Ac. Anti-IL 1β represented around 6% after 15.5 days, and this increased to 40% in 19.5-day embryos. With both interleukines immunomarking was mixed, nuclear and cytoplasmic. The distribution of IL-6 was homogeneous throughout all the adenohypophysis, although marking intensity diminished from cranial to caudal. Nevertheless, IL-1β was more common in the more rostral and caudal parts of the adenohypophysis, the region corresponding to the future *pars anterior*.

At 13.5 days there was an increase in cell proliferation in the Rathke's pouch future *pars anterior*; this process increased significantly at 15.5 days and brought with it a progressive diminishing of the Rathke's pouch lumen, with the result that at 19-19.5 days the adenohypophysis cavity had almost disappeared. This proliferation overlapped in time and place with the expression of IL-1β, which leads us to believe that this interleukine might be involved in regulating cell proliferation in the *pars anterior* of the adenohypophysis during embryonal development.

After 15 days there was a limited increase in cell proliferation in the future *pars tuberalis* of the Rathke's pouch, which this continued until birth. This increase coincided chronologically and, in part, topographically with the adenohypophysis expression of IL-6; (although Ac. Anti-IL-6 marking was homogeneous, it appeared with greater intensity on the more cranial parts of the adenohypophysis). This suggests that IL-6 could play a role in *pars tuberalis* growth during the prenatal period.

Marking for IL-2 appeared somewhat later, after 16.5 days; at this time the number of marked cells represented 5.6% of all adenohypophysis cells. Immunoreactivity to IL-2 reached a maximum peak at 18.5 days (35% of marked cells), after which it descended significantly at 19.5 days (22% of marked cells). Marking for IL-2 was also mixed, nuclear and cytoplasmic, and was evident throughout the whole of the Rathke's pouch, with greater intensity, however, in the more rostral parts of the adenohypophysis. During the periods of IL-2 expression, the adenohypophysis was now functional, which suggests that this interleukine possibly intervenes in the initiation

and support of pituitary secretion. In addition, after 15.5 to 16 days intense vascular proliferation appeared in the future *pars anterior* of the adenohypophysis, a factor which coincided in part chronologically and topographically with the expression of IL-2 (although extended, Ac. Anti-IL-2 marking was predominantly evident in the Rathke's pouch rostral and caudal regions, which will form the *pars anterior* of the adenohypophysis); consequently, IL-2 might be involved in pituitary vascular production during the development of the embryo.

(Work supported by Junta de Castilla y León Grant CO3/296).

P. 49 IS THE TRACHEOESOPHAGEAL APPROXIMATION RELATED WITH THE ORIGIN OF THE TRACHEOESOPHAGEAL FISTULA?: A MORPHOMETRIC ANALYSIS IN THE HUMAN EMBRYONIC PERIOD

J. Nebot-Cegarra, Pere-Jordi Fàbregas and Silvia Ricart

Unitat d'Anatomia i d'Embriologia, Departament de Ciències Morfològiques, Facultat de Medicina, Universitat Autònoma de Barcelona

Traqueoesophageal fistula (TEF) arise in early stages of human embryo development, as the findings in a human embryo of stage 16 shows (1). Because of the most common type of TEF occurs at the level of the tracheal bifurcation, the closest between the tracheal bifurcation and esophagus has been suggest as a favorable condition to the production of a fistulous communication (2). This study is an attempt to answer this question by means of the morphometric analysis of the so called 'tracheoesophageal septum'.

MATERIAL AND METHODS: Serial cross sections of a graded series of normal human embryos (from Carnegie Stage (CS) 11 - Bellaterra Collection. Prof Domènech Mateu) were studied. The interval in the cross section selection was done in accordance with the crown-rump length of the embryo and the mean distance between the sections. In every specimen the first section was randomly chosen from the superior boundary of the septum. Distance between the lumen of the respiratory and the digestive tubes were calculated from the coordinates of the opposite points of apical surface of each endodermal layer. Coordinates were obtained by Visilog ® software. Calculations were done by Excel 7.0 Microsoft ®. In every embryo the minimum distances obtained from the selected sections were grouped in four portions: proximal portion (from the CS 13, it corresponded to the larynx), two middle portions and the distal portion (the end was the tracheal bifurcation), and the mean distance of every portion was calculated. This datum was used for comparative purpose with the other ones of the same specimen.

RESULTS: During the embryonic period the minimum distance between the lumen of the respiratory and digestive tubes was located at the following levels: from CS 11 to 16, at the proximal portion; at CS 17, at the middle-proximal portion; at the CS 18, at the middle-distal portion and from the CS 19 to 23, at the distal portion.

From the CS 13, the mesenchymal coat of both tubes was condensed. From the CSs 17 and 19, the muscular coat of the esophagus and the laryngeal cartilages were observed, respectively.

DISCUSSION: According to Zaw-Tun (1982) (3) the separation of the respiratory system from the foregut does not occur by septation but by caudal formation and growth of the respiratory diverticulum. Therefore separational anomalies would not stem from a separate defect by from the relative growth of one to the other derived from the foregut. In accordance with our results the distal portion of the respiratory tube (including the tracheal bifurcation) approaches to the esophagus during the embryonic period, but only from the CS 19 this segment is the closest. At this time both, trachea and esophagus, have an evident differentiation of the mesenchymal coat.

In conclusion, these data suggest us that the TEF must be produced before the tracheal bifurcation becomes the nearest portion to the esophagus. Thus, earlier alterations in the components of the 'tracheoesophageal septum', may determine anomalous tracheoesophageal approximation.

REFERENCES

SMITH EI (1957). *Contrib Embryol Carnegie Inst,* 36: 41-57

O'RAHILLY R and MÜLLER F (1984). *Ann Otol Rhinol Laryngol,* 93: 421-429;

ZAW-TUN HA (1982) *Acta Anat,* 114: 1-21.

P. 50 MORPHOMETRIC ABNORMALITIES IN EYEBALL OF CHICK EMBRYO INDUCED BY LOW FREQUENCY MAGNETIC FIELDS

M.T. Pascual Morenilla, O. Roda Murillo, J. Robles, J.A. Roda Moreno, M. López Soler, F. Arrebola, J.A. Casanova and M. Guirao Piñeyro

Dpto. de Ciencias Morfológicas. Instituto de Neurociencias. Universidad de Granada

Over the past 15 years, it has been detected that low frequency magnetic fields produce noxious effects on the human organism. Lankosz et al. (1983); Syromiatnikov, L. et al. (1990); Loomis, D. et al. (1994); Alfredson, L. et al. (1996); Feychting, M. et al. (1998), etc. reported a series of blood formula abnormalities and digestive, libido, melatonine secretion and behavior disorders, as well as a higher incidence of brain tumors, lymphomas, breast cancer, etc., after professional or residential exposure to magnetic fields. Current technology implies constant exposure to a series of sources of radiation, (¿radiant foci?) not only high tension cables but also transformers in different electrical equipment and a wide range of visualization screens, electrical appliances etc. We therefore considered of particular interest the study of the effects of these radiations on the eyeball, an organ of special significance due to the high dependence of its development on that of the brain.

Four hundred fertilized White Leghorn chicken eggs were incubated in a Masalles incubator at 37.8°C ± 0.4°C and relative humidity of 60-70%. The eggs were subjected to the action of magnetic fields by placing the incubation area between two Helmholtz coils in parallel. The eggs were divided into five groups of eighty eggs each; four of these groups were exposed to magnetic fields of 1μT, 0.1mT, 0.5mT and 1mT, respectively, at 50Hz. The fifth group was left as the control group. Extractions were performed on days 15 and 21 of incubation. Data was gathered on mortality and anteroposterior measurements of the whole eyeball, cornea, anterior chamber and

lens, using Hondex A/B SCAN IS-500 ultrasonography apparatus.

The above studies demonstrated that the magnetic fields significantly affected the development of the eyeball versus controls. After 21 days of incubation there was a reduction in the thickness of the cornea and in the anteroposterior diameter of the lens and the latter reduction was significantly greater at the higher intensities (0.5mT and 1mT). In contrast, the anterior chamber was more affected by the magnetic fields of lower intensity (1μT and 0.1mT). (*Cmsumfi.doc*)

P. 51 CEREBELLAR FISSURAL, FOLIAR AND CORTICAL DEVELOPMENT: A MORPHOMETRIC ANALYSIS

A.L. Peña-Melián, M.T. Vázquez-Osorio, F. Viejo-Tirado, E. Sánchez-Ortega and A.J. Puerta-Fonollá

Dpto.Ciencias Morfológicas I. Fac. Medicina. Universidad Complutense de Madrid

A morphometric analysis was made in chicken embryos (*Gallus gallus domesticus*) studying fissural, foliar and cortical development from 35 HH (Hamburger-Hamilton stages) until 43 HH. Sagital section was the most reliable so we selected it. In one hand we have considered the cerebellum centrifugal development in the extraventricular growth phase, with this aim we have measured the minimal distance, fissural bottom/ventricular surface, and between this area and the foliar top. In the other hand we have considered the evolution in each cortical layer thickness development, we have measured them in the bottom and slapes of fissures and in the foliar top.

We can resolve that fissural behaviour depends on the moment in which appears during the cerebellum expansive growth.

The more early remain in a constant distance from ventricular surfaces while the last to appear have a centrifugal movement in an exponencial way. The fisural depth for the expansive growth is not constant. Regarding thickness evolution in different cortical layers, we noticed that external granular layer has a constant growth until 40HH stage. In this phase, we can see superficial thickness maybe proliferative units. From 40HH stage, growth drops and superficial thickness in external granular layer disappear. At the same time we can see a distinct growth in the internal granular layer. Also we noticed that we can divide the cerebellar cortex into three sections limited between prima and prepiramidal fissures. Functional correlation is unknowned.

P. 52 GLIAL CELLS IN THE OPTIC DISC ZONE. EXPRESSION OF DIFFERENT ANTIGENS DURING CHICK DEVELOPMENT

A. Quesada, A. Carmona, J. Ambrosiani, Y. Aguilera and F.A. Prada

Department of Morphological Science and Institute of Developmental Biology. University of Seville. School of Medicine. Seville. Spain

INTRODUCTION: Müller cells constitute the most prominent glial cells of the vertebrate retina (Cajal, 1892) and have been considered as modified astrocytes (Polyak, 1957). In the optic disc margin, Müller cells adapts their morphology to structural changes of the retina (Prada et al. 1989). Glial cells in the optic disc zone are not well understood, including their nature and functional capacity.

In this report, using Golgi preparations and immunohistochemistry methods we analyzed the above mentioned aspects such as particular morphology and structural behaviour of these cells during development chick retina.

MATERIAL AND METHODS: Whole embryos of white Leghorn chick and eye globes of each were stage between E7-E20 an adult, were processed according to current immunohistochemical protocols. Sections were incubated with one of the following primary antibodies: 3CB2 (recognizes an intermediate filament associated protein), anti-glutamine syntetase (GS), MOSP (myelin oligodendrocyte specific protein), A2B5 (recognizes a specific protein expressed only during early development of oligodendrocytes)and 3BA8 (recognizes an antigen located in amacrine neurons of the retina). On the other hand, retinas of chick embrios between E7 and E20 stained by the Golgi method of Stensaas (1967) and adult retinas stained by the Golgi method Colonnier (1964), from our collection, were newly observed for this study.

Fertilized eggs and animals were obtained from our university vivaria. Animal care protocols used in our laboratory are in conformity with the apropiate national legislation (Decres 223/1988, BOE nº 67) and guidelines from the European Communities (Council Directive 86/609/EEC).

RESULTS AND DISCUSSION: We have observed in Golgi preparations that the optic disc zone show two types of glial cells which are located in the juxta-optic disc region and the optic disc. Near the optic disc, Müller cells show important variations respect to the morphological patter of the Müller cells of the central retina. In peripheral optic disc, Müller cells ondergone very important modifications suggesting a different population of glial cells. The morphology of these cells evokes typical protoplasmic astrocytes. Overall glia of the optic disc margin forms an arched network, which separates the retina from the optic nerve. Our immunohistochemistry study show that the optic disc glial cells express the following immunorreactivity: from E7 to adult retina 3CB2 (+); from E11 to adult retina MOSP (+); between E7 to E13 3BA8 (+). However, not expression of GS was found during development and adult retina. The results of our studies (Prada et al. 1989, 1995, 1998) and these of the present studies suggest that the glial cells of the retina show a great plasticity. In the optic disc, glial cells serve not only for protection and ordered conduction of the nerve fibres of the eye, but also for requirements of mechanical support during the normal movements of the eye. Moreover, the fact that optic disc glial cells show a myelin oligodendrocyte specific protein (MOSP) in developing and adult cells suggests a closer relationship between these cells and oligodendrocytes.

In conclusion, the optic disc glial cells are a special type of macroglia capable of carrying out functions described for the astrocytes and oligodendrocytes.

P. 53 BRAIN-DERIVED NEUROTROPHIC FACTOR (BDNF) GENE THERAPY PREVENTS APOPTOSIS AND VESTIBULAR GANGLION DEGENERATION

J. Represa[1,3], Alicia Carreres[2] Roberto Cabo[1], Christine Layburn[3] and Thomas Van De Water[3]

[1] Departamento de Anatomía, Facultad de Medicina, Universidad de Valladolid, 47005 Valladolid, Spain. [2] Servicio de Neurología Hospital Universitario de Valladolid. [3] Department of Neuroscience, Albert Einstein College of Medicine, New York, USA

Destruction of vestibular hair cells by a variety of insults results in the secondary degeneration of vestibular neurons of the VIII cranial nerve. This is due to the lost of neurotrophic factor support from the vestibular hair cells, which normally produced the neurotrophin BDNF. Moreover "in vitro" studies have shown that delivery of BDNF to vestibular neurons can replace the trophic support supplied by hair cells. To prevent the degeneration of vestibular neurons that occurs "in vivo" after neomycin destruction of vestibular hair cells, we used a replication defective herpes simplex-1 vector (HSVbdnflac-amplicon plasmid genome) to transfer the gene for BDNF into damaged vestibular ganglion. To deliver the virus carrying de BDNF gene we have used infusion into the scala timpani of adult CBA/6J mice with a miniature osmotic pump.

Three weeks after the HSV-1bdnflac gene therapy. After one or three weeks animals were anaesthetised and processed after necropsy for histology and immunostaining. Initial experiments indicated that the viral vector had successfully introduced external genes (LacZ gene and HSV-1bdnflac gene) into vestibular neurons and that transcription occurs over a period of 3 weeks. In later experiments we detected steady BDNF production of BDNF from vestibular neurons, and morphological analysis revealed that the BDNF trophic support prevented the lost by apoptosis of the vestibular neurons.

TABLE 1: SURVIVAL OF VESTIBULAR NEURONS AFTER NEOMYCIN INFUSION AND HSVBDNFLAC GENE THERAPY

CONDITION	NEURONS / VESTIBULAR GANGLION	*P* VALUE
Control (n=4)	6788 +/- 164.3	
Neomycin/HSVbdnflac (n=3)	6102 +/- 131.7	>0.06 (a) <0.001 (b)
Neomycine alone (n=3)	2045 +/- 246.1	<0.001 (a)

(a) Compared with untreated control mice
(b) Compared with treated with neomycin alone.

CONCLUSION: In this study we have demonstrated the effective use of gene therapy "in vivo" to produce BDNF by mouse inner ear neurons. The therapy prevents the neuronal degeneration observed in the neurons after neomycin treatment.

P. 54 ANTI-CYTOKERATIN 8 IMMUNOSTAINING REVEALS DIFFERENCES IN THE DIFFERENTIATION OF THE PALATAL EPITHELIUM OF TGF-β_3 NULL MUTANT MOUSE EMBRYOS

C. Tudela, J. Mena; R. Bonelli; J. Pérez-Miguelsanz; C. Maestro; A. Del Río; J. Puerta and C. Martínez-Alvarez.

Dpto. Ciencias Morfológicas I. Facultad de Medicina. Universidad Complutense de Madrid

Although observations have been reported suggesting that a failure in palatal shelves adhesion (Proetzel et al., 1995) or fusion (Kaartinen et al., 1997) causes cleft palate in the TGF-β_3 null mutant mice, an environmental scanning electron microscopic study demonstrated morphological alteration in the surface of the medial edge epithelium (MEE) of these mice prior to the contact of palatal shelves (Martínez-Alvarez et al., 1996). In an attempt to determine whether this epithelium has undergone a different differentiation pathway that could justify the failure of palatal fusion, we have analysed in the developing palate the expression pattern of cytokeratins 8, 18 and 19, that are known not to be expressed in the MEE of palatal epithelial sheets grown *in vitro* (Carette and Ferguson, 1991).

E14.5 CD1 and E15 TGF-β_3 null mutant mice were used in this work. Pregnant mice were killed by an overdose of chloroform and the embryos obtained by caesarian section. After removal of the tongue and jaw, the embryonary heads were fixed in buffered formalin (pH=7) and embbeded in paraffin. 5 μm thick sections were dewaxed and treated with 1mM heated EDTA (pH=8-8'2), in order to unmask the epitopes. Immunostaining was performed with three different monoclonal antibodies against cytokeratins 8, 18 and 19.

Unlike the observations reported by Carette and Ferguson (1991), our results demonstrate that citokeratin 19 does never express in the epithelium covering the palatal shelves of both the TGF-β_3 null and wild-type mice, either prior or during palatal fusion. Cytokeratin 18 is expressed in the prefusion and fusion oral, nasal and medial edge epithelia, both in the TGF-β_3 null and wild-type mice, although this cytokeratin expression is weaker in the nasal epithelium of TGF-β_3 null palates. Finally, cytokeratin 8 is weakly expressed in the surface of the prefusion nasal and medial edge epithelia of wild-type mice and in the nasal, oral and medial edge epithelia of TGF-β_3 null palates. However, as contact between the palatal shelves is established, cytokeratin 8 expression increases in the palate of the wild-type but not of the TGF-β_3 null mice, that remains weak.

We are therefore able to conclude that: 1) the *in vivo* expression of cytokeratins 8, 18 and 19 in the developing palate is different to its expression in cultured palatal epithelial sheets. 2) TGF-β_3 seems to induce the differentiation of the palatal epithelium to a prefusion phenotype characterized, amongst others, by an increased expression of cytokeratin 8.

This work has been supported by a grant from the Comunidad Autónoma de Madrid, Ref.: 08.6/0012/1998 and a grant from the Spanish Fondo de Investigación Sanitaria (Ministerio de Sanidad), Ref. 99/0939.

P. 55 EFFECTS INDUCED BY ALL-TRANSRA AND 13-CIS RA ON α-ACTIN AND α-ACTININ PROTEINS IN CARDIOMYOCYTES DURING DEVELOPMENT

C. Vélez[1], A.E. Aránega[2], J.A. Marchal[3], J.C. Prados[1], C. Melguizo[1], J.E. Fernández[4], E. Carrillo[4], Houria Boulaiz[4], L. Alvarez[4] and A. Aránega[4]

[1]Department of Health Sciences, University of Almería, 04002 Almería, Spain. 2Department of Experimental Biology, University of Jaén, 23071 Jaén,

Spain. [3]Department of Health Sciences, University of University of Jaén, 23071 Jaén, Spain and [4]Basic Cardiovascular Research Section, Department of Morphological Sciences, School of Medicine, University of Granada

Early heart development is known to be sensitive to retinoid concentrations. The molecular mechanism by which RA exerts its biological functions involves specific binding to intracellular proteins and interaction with nuclear receptor to modulate the transcription of specific genes. Although the influence of retinoids on cardiac morphogenesis has been described previously, the effect of retinoids on cardiomyocyte differentiation during development has not been characterized. The present study was designed to investigate the differentiating effects of all-trans RA and 13-cis RA on α-actin and α-actinin proteins at the subcellular level in cultures of chick embryo cardiomyocytes obtained from Hamburger and Hamilton's (HH) stage 22, 32 and 40 embryos.

Fertile white leghorn chicken eggs (Shaver Star cross 288) were incubated at 38.5°C in humid atmosphere. Hearts were ablated when the embryos had reached stages 22, 32 and 40 of Hamburger and Hamilton. Chick cardiomyocytes were isolated and treated during 24 and 48 h with all-trans RA and 13-cis RA to a final concentration of 10^{-6} *M* in the growth medium. Sodium dodecyl sulfate-gel electrophoresis, immunoblotting and fluorescense activated cell sorter analysis were used to quantify the effects of all-trans RA and 13-cis RA on cultures of chick cardiomyocytes during development.

The retinoids increased the concentration of α-actin and α-actinin in the cytoplasmic and cytoskeletal fractions of cells at all three stages of development. The effects was greatest in cardiomyocytes treated for 24 h with all-trans RA and in cells from HH22 embryos. The greatest increases in α-actin concentratioin ocurred in the cytoskeletal fraction of HH22 cells cultured for 24 h with all-trans RA or 13-cis RA, whereas the greatest increases in α-actinin were found in the cytoplasmic fraction of HH22 cells exposed to retinoids for 24 h.

We conclude that retinoic acid plays a role in the reorganization of the pattern of sarcomeric protein expression during cardiomyocyte differentiation.

P. 56 ROLE OF CALCIUM IONS IN THE EPITTELIAL MORPHOGENESIS

M. Cáceres, M. Barbosa, A. de la Mano, S. Callejo and E. Barbosa

Department of Human Anatomy, Faculty of Medicine, University of Valladolid

It is well know the role of microfilaments and calcium in the shape and locomotion of isolated cells.

During the first stages of development in many epithelial anlages there are changes of cellular shape and epithelial movements (invagination, evagination). Is also the epithelial association dependent of calcium? That is the question of our work.

In order to verify this hipothese we have studied as a model the early development in chick embryos of the nervous system and inner ear.

Chick embryos ranges between stages 8-11 of Hamburger and Hamilton (1951) were cultured (24 h in the medium of Britt-Hermann, 1959) in presence of Papaverine (blockade of membrane calcium channels).

After the culture the embryos were observed and classified, fixed in Bouin and Carnoy, embedded in Paraplast, sectioned at 8 microns and stain with Haematoxylin.-Eosin to histological study.

The observations of embryos shows that Papaverine (2.82 mgr/100)has a differential action upon the development of the neural tube according to the initial stages of treatment.

So the embryos of stages 8 and 8- the effect of Papaverine upon the neurulation affect the process of upfolding and the appearance of platineurias more or less extensive are the rule.

The alterations presents in stage 9 embryos disclose the fusion of the neural fold that rolling into the lumen of the neural tube. The neural crest cells remaining in the transition between the neuroepithelium and the ectoderm.

The neurulation is correctly accomplished by embryos treated at 10 stage.

The absence of calcium upon initial stages of otocystogenesis delayed the process of invagination and a otic placode flattened is present unilaterally.Our findings ratified the role of calcium upon the cranial neurulation observed by Lee and Nagele (1979).

The unilateral flattening of the otic placode show the role of calcium in the otocystogenesis in contrast with the observations of Hilfer et al. (1989).

Future experiments provide new insights about the role of calcium in the morphogenetic process of different epithelia.

REFERENCES

BRITT G and HERMANN JE. JEEM, 7: 66-72 (1959).

HAMBURGER V and HAMILTON HL. *J Morphol*, 88: 49-82 (1951).

HILFER SR, STEVES RA and SANZO JF. *J Exp Zool*, 251: 253-264 (1989).

LEE H and NAGELE RG. *Teratology*, 20: 321-332 (1979).

P. 57 Na, K-ATPase IN EMBRYONIC RAT GUT

A. de la Mano, S. Callejo, J.A. Moro, M. Cáceres, M. Barbosa and E. Barbosa

Department of Human Anatomy, Faculty of Medicine, University of Valladolid

The present work shows the initial results of an study that will be carried out about the appearance and distribution of Na, K-ATPase (sodium pump) in the gut anlage of early rat embryo.

Rat embryos at 10.5 days were obtained by caesarean of pregnant rat, dissected, fixed in formaldehyde, processed for paraffin, wax embedding and cut at 8 μ serial sections. Then they were stained with a monoclonal antibody against Na, K-ATPase alpha-subunit (DSHB) and a second antibody conjugated with fluorescein.

Our results showed a strong immunostain in the hidgut, including all cells and part of the material in the lumen of this structure, while another softer mark could be seen in the foregut, towards the luminal pole of cells, and without any signal in the lumen. Furthermore, the immunostaining spreads ventrally to the hidgut, towards the anterior wall of the embryo, looking like a cellular migration mechanism. This presumed route of migration is probably related to allantoid formation during this

period, since the presence of Na,K-ATPase in this structure is already described. The onset, distribution, evolution and functions of this cellular ATPase will be the matter of posterior studies.

P. 58 VARIATIONS OF THE CELLULAR PROLIFERATION OF PROLACTIN CELLS FROM LATE GESTATION TO LACTATION IN RATS

M. Angoso, J.L. Torres, M. Rubio, G. Vázquez, E. Hernández, J.M. Riesco, J.J. Herrero, R. Herrero and J. Carretero

Dept. of Human Anatomy and Histology, Faculty of Medicine. Lab. of Neuroendocrinology, Institute of Neuroscience of Castilla y León. University of Salamanca, Spain

Lactation is a physiological state associated to hyperactivity of hypophyseal prolactin-producing cells. It is know that the percentage of these cells is increased during lactation; however, discrepances about the mechanisms to increase the number of prolactin cells have been reported. In order to analyse if this increase is result of a previous proliferation, using immunohistochemical expression of PCNA as marker of cellular proliferation, variations of proliferation rate of prolactin-positive cells were determined from late gestation to lactation in adult female rats. During late gestation a very significant increase of the percentage of proliferating prolactin-cells was observed in relation with non-pregnant female in proestrus phase. As result of these proliferation the percentage of prolactin-positive cells after one week of lactation was higher than in non-lactating or in pregnant females, but the proliferation rate in these animals was lower than in the other groups studied. These results are showed in the following table:

	PCNA	Prolactin (PRL)	PCNA & PRL	PCNA & PRL/PRL
PROESTRUS FEMALES	0.74 ± 0.05	30.22 ± 0.28	0.44 ± 0.01	1.46 %
PREGNANT FEMALES	9.41 ± 0.79*	28.44 ± 0.90	4.00 ± 0.51*	14.06 %*
LACTATING FEMALES	0.55 ± 0.09	44.67 ± 1.38	0.22 ± 0.08**	0.49 %**

* p<0.01
** p<0.05

In sum, our results suggest that late gestation is a previous proliferative phase preparatory of the following lactation phase and that endocrine changes of late gestation involve cellular proliferation of hypophyseal prolactin-cells in order to arrange the gland for posterior necessities and to avoid proliferative changes during lactation.

Supported by FIS 99/1187 program and SA58/98 Social Founds European and JCyL program.

P. 59 INFLUENCE OF LATE PREGNANCY IN THE COEXISTENCE OF NADPH-DIAPHORASE AND TYROSINE HYDROXYLASE IN THE RAT HYPOTHALAMUS

E. Blanco[1,2], J.M. Riesco[1,2], J.A. Juanes[1], J. Carretero[1,2], M. Rubio[1] and R. Vázquez[1,2]

[1] Dpto. de Anatomía e Histología Humanas and [2] Instituto de Neurociencias de Castilla y León. Facultad de Medicina. Universidad de Salamanca

NADPH-diaphorase and tyrosine hydroxylase are two enzymes involved in the synthesis of nitric oxide and dopamine, respectively. Previous morphological studies have demonstrated their existence and distribution in the hypothalamus and the coexistence of both markers in the same cells in several hypothalamic nuclei. Both enzymes show modifications in the morphological expression and the numbers of hypothalamic neurons reactive to them when challenged by osmotic stimuli, such changes being related to the functional state of the animal. The aim of the present study was to analyze whether late gestation -the peripartum period in which many of the hypothalamic magnocellular neurons are in their phase of greatest activity- elicits changes in the number and morphological expression of diaphorase-positive, tyrosine hydroxylase-immunoreactive neurons and in the coexistence of both markers in the main hypothalamic nuclei.

Double histochemical and immunohistochemical labelling of the same brain sections as well as morphometric analysis were employed in a group of pregnant Wistar rats (sacrificed on day 22 of gestation by intracardiac perfusion after anaesthesia with Ketolar), using a groups of virgin rats as controls. All the hypothalamic nuclei analysed (supraoptic, paraventricular and accessory nuclei) showed diaphorase-positive and/or tyrosine hydroxylase- immunoreactive cells. The most significant findings in the gestating animals as regards the controls can be summarized thus: a) a strong reaction intensity in the diaphorase-active neurons, above all in the supraoptic nucleus, a similar number of neurons showing the coexistence of both enzymes; b) an increase in the number of neurons showing the coexistence of both enzymes in the paraventricular nucleus (especially in its posterior magnocellular and its rostral-most periventricular portions) and in the nucleus circularis; c) the presence of neurons showing the coexistence of both enzymes in other groups of neurons from the accessory nuclei such as the zona incerta or the ansa lenticularis.

As has been demonstrated to occur during periods of osmotic stress or following treatment that increases osmotic stimulation, the distribution and coexistence of neurons reactive to diaphorase or tyrosine hydroxylase in the hypothalamus are also modified during late gestation, possibly in relation to this physiological state, in which there is greater hypothalamic neuronal activity. These changes seem to depend on and be related to the functional state of the animal. Although the exact role of these substances at hypothalamic level remains obscure, a synergistic or reciprocal action of both enzymes may be important for certain aspects of neuroendocrine function.

P. 60 EFFECT OF HYPERTENSION AND CAPTOPRIL TREATMENT ON THE ANGIOTENSIN II IN THE RAT SUBFORNICAL ORGAN. AN IMMUNOHISTOCHEMICAL STUDY

E. Carmona-Calero, Herminia Pérez-González, María del M. Pérez-Delgado, Norberto Marrero-Gordillo, Isabel M. de la Peña-Valenzuela, Tomás González Hernández, Agustín Castañeyra-Perdomo and Romualdo Ferres-Torres

Department of Anatomy, Faculty of Medicine, University of La Laguna, Tenerife, Spain

The Subfornical Organ (SFO) is located in a key position at the confluence of the lateral and the third ventricles. The SFO is typically composed of a variety of ependymal cells, supraependymal neurons, glial cells, and macrophages on their ventricular surface. Glial cells, neuronal perikarya, dendrites, and axons comprise the nervous tissue that is permeated by a dense capillary plexus, that is connected with that of the choroid plexus.

This organ appears to play a key role in a number of important body functions, for example, that control of body fluid balance and arterial pressure is mediated by blood-borne angiotensin II via the SFO. Specific angiotensin II-sensitive neurons are present in the SFO, apparently in a superficial location (2,4,5), binding sites for angiotensin II (AII)(6), and type 1 receptors, located mainly in the SFO, have been said to play an important role in central cardiovascular control and their number is increased in genetic models of hypertension (1). In this work we study the immunohistochemical effects of treatment with a converting enzyme inhibitor (captopril) on the SFO in control and hypertensive rats.

We have studied 15 male rats, that were all sacrificed at the 15th week of life. Five control rats (Wistar-Kyoto rats, WKY) were divided in two groups: one control group of 5 animals (WKY). Another group of ten spontaneously hypertensive rats (SHR) (Leica S/A Barcelona, Spain), were also subdivided into a subgroup of 5 animals without treatment (SHR) and another 5 animals (SHR-T) that was treated with oral captopril from the 8th to the 15th week of life.

All animals received water and food ad libitum. Captopril was added to the drinking water of the treated group (SHR-T rats) after the 8th week of life until sacrifice (15th week), at a dosage of 0.1 mg/ml. We weekly measured the blood pressure by an indirect tail-cuff method, that allowed us to determine systolic and diastolic blood pressure. We also registered the daily fluid ingestion. Animals were fixed by perfusion with Bouin's fluid, postfixed during 24 hours in the same fixative, dehydrated and embedded in paraffin under standard conditions. Brains were cut in two alternative serial coronal sections. One of the serial sections was stained with the Klüver-Barrera method. In the other series, we selected the slides corresponding to the SFO region, that were processed with immunohistochemical method (avidin-biotin). As primary antibody, we used anti-AGII1-4, that was raised in rat as follow: angiotensin II (1-4) (ICN) after coupling to a carrier (thyroglobulin), was emulsified with complete Freund's adjuvant and injected subcutaneously into male rat back. Twenty one days later each rat received AGII1-4 emulsified with incomplete Freund's adjuvant in subcutaneus injections. And fourteen days later each rat received the AGII1-4 in an intraperitoneal injection without adjuvant. Seven days later the rat were killed by intracardiac exsanguination. The specificity of the antisera was evaluated by means of absorption test incubating the antisera overnight with the homologous antigen. The antigen was able to abolish the immunostaining.

Our results show that: 1) Blood pressure in WKR does not change with captopril treatment, whereas in treated-SHR blood pressure decreases to normal levels. 2) The highest concentration of AII-ir material was showed in the WKY and SHR-T group that is located mainly in the neuropil. The SHR group showed the AII-ir material into its neurons. These differences among the groups, could be related with a local blockade of the brain renin-angiotensin system (3).

References

Castren E and Saavedra JM (1989). Angiotensin II receptors in the paraventricular nucleus, subfornical organ, and pituitary gland of hypophysectomized, adrenalectomized and vasopressin-deficient rats. *Proc Natl Acad Sci USA*, 86: 725-729.

Dellmann HD (1986). The Subfornical Organ. Fine Structure. In P.M. Gross: *Circumventricular Organs and Body Fluids*, Vol. I, CRC Press, Boca Raton, Florida.

Fabris B, Chen B, Pipic V, Perich R and Johnston CL (1990). Inhibition of angiotensin-converting enzyme in plasma and tissue. *J Cardiovas Pharmacol*, 15, S6-S13

Félix D, Schelling P and Haas HL (1982). Angiotensin and single neurons. *Exp Brain Res, Suppl.* 4, 255.

Phillips MI and Félix D (1976). Specific angiotensin II receptive neurons in the cat subfornical organ. *Brain Res,* 109, 531.

Saavedra JM, Correa FM, Plunkett LM, Israel A, Kurihara M and Shigematsu K (1986). Binding of angiotensin and atrial natriuretic peptide in brain of hypertensive rats. *Nature,* 320: 758-60.

Acknowledgements: *This work was supported by the Universidad de La Laguna, project nº 202-48/98.*

P. 61 PRESENCE AND DISTRIBUTION OF NEUROENDOCRINE MARKERS IN THE MOUSE KIDNEY

R.M. Fernández, R. Marrufo, M. Aparcero, I. López, A. Camacho, A. Prada and C. Verástegui

Departamento de Ciencias Morfológicas. Facultad de Medicina. Universidad de Cádiz

Introduction: Neuroendocrine markers are a group of substances, which are specifically present in glial or neuronal elements. By using immunocytochemical techniques some neural structures have been identified in several systems related to the intrinsic and/or extrinsic control.

Our purpose was to determinate the morphological distribution of glial or neural structures in the mouse kidney. These are related with the diffuse neuroendocrine system and therefore with the intrinsic control of the renal function. Thus, we used a battery of antisera against neuronal (Enolase neuro-specific -ENS- and protein PGP 9.5) and glial markers (gliofibrilar acidic protein -GFAP-, protein S-100 and neurofilament-200).

To the best of our knowledge no authors have reported the existence of these neuroendocrine markers in the mouse kidney. In other mammals such rats, GFAP-immunoreactivity had been reported in podocytes and mesangial cells. We did not find works made in more species.

Materials and Methods: Ten adult male mice (*Mus musculus*, Swiss OF-1) were anaesthetised with ether inhalation and perfused with 0.9% saline solution, followed by Bouin liquid prefixation. Postfixation and dehydration were made by usual hystological methods. The 7-8 μm sections were obtained.

The sections were incubated with the antisera battery in a moisture chamber 72 hours at 4ºC. We used cow brain polyclonal antiserum anti-ENS (diluted1/500), rabbit polyclonal antiserum anti-GFAP (diluted 1/50), rabbit polyclonal antiserum anti-PGP 9.5 (diluted 1/800), rabbit

polyclonal antiserum anti-NF 200 (diluted 1/800), rabbit polyclonal antiserum anti-S 100 (diluted 1/100). We used the indirect method of biotin-streptavidin complex. A revealed solution of 10 mg/15 ml DAB in presence of 20 µl/10 ml H_2O_2 was used for 20 min under microscopic control. The negative control procedures were carried out by omitting one or more steps of the method and by using sections incubated with inactivated antiserum with added excess antigen.

RESULTS: The application of the immunocytochemical technique showed results for all the antisera. Immunoreactivity (IR) for glial neuroendocrine markers was seen in some large and medium calibre blood vessels located in the renal parenchyma. Immunoreactivity for GFAP-, S-100-protein- and NF-200- was seen in varicose fibre around the outer side of the arterioles, although in some cases this tinction was present at the level of muscular layer in large calibre arteries. We had seen a NF-200 positive structure that remind a neuronal cluster in the renal medulla closed to a papilla. We found similar results with the neural markers (Enolase and PGP 9.5). Many fine varicose nerve fibres were found laid around the arterial vessels. These immunoreactive fibres were present not only in large vessels but also in medium and little ones. They were not related to arterial components of glomeruli. Also, NSE immunoreactivity was found in the parenchyma between colector tubules.

DISCUSSION: Our work reports the presence both neural and glial structures in the mouse kidney. No authors had previously noticed these findings in the mouse kidney. According with the distribution of the positive nerve fibres, we think that the function of these must be related with the vasomotor control of the renal blood vessels.

P. 62 INFLUENCE OF THE PHOTOPHASIC, SEASONAL AND SYNODIC CYCLES ON THE "SYNAPTIC" BODIES NUMBER AND SERUM MELATONIN LEVEL

F. Martínez-Soriano, A. Ruiz-Torner, T. Hernández-Gil, F.E. Olucha-Bordonau, A.A. Valverde-Navarro, J. Cabanes-Vila and F. Tomás-Aguirre

Morphological Sciences Department. Valencia University. Valencia. (Spain)

INTRODUCTION: The pinealocytes of mammals contain structures generally known as synaptic bodies (SB). These bodies have an electron-dense, fine granular core surrounded by clear vesicles that vary in number along the 24-hours of the circadian cycle. In the pineal gland of the mammals, SB are a heterogeneus population. The more frequent are the synaptic ribbons (SR). The second more frequent SB are spherical (SS). In addition to the rod and the round spherical-shaped SB, also ovoid-, quadrangular- and triangular-shaped ones have been reported in certain species. These intermediate forms are present in significantly smaller number than the above mentioned forms. The Melatonin is the principal hormone secreted for the pineal gland and had a similar circadian fluctuations depending on the luminosity and the magnetic fields as SR. These considerations led us to study the evolution of SB and serum Melatonin levels during two photophasic, seasonal and synodic cycles.

MATERIAL AND METHODS: Forty male wistar rats (mean body weight 240±37 g.) subjected in the same nutritional and environmental conditions (temperature,18-20ºC, natural light) were studied.The rats were divided into 8 groups of 5 animals each. Animals of four groups were sacrificied in Winter: 2 during the new-moon days (one group between 10:00 and 12:00 hour, i.e. photophase, and the second between 00:00 and 02:00 hour, i.e. scotophase); and the other two during the full-moon days, in the same above mentioned conditions.The same procedure and conditions was followed for the sacrificied of the four spring groups. The number of SB was calculated by counting those observed in 8 grid squares (total area 33.800 μm^2, magnification x20.000) and expressed in 20.000 μm^2. Serum Melatonin concentrations were determined by radioimmunoassay techniques.

RESULTS: The results showed greater number of SR during the scotophase in both seasons, and in both synodic periods. During the new-moon photophase of Winter, number of SR was greater than in the full-moon photophase of the same season. In the scotophase of these season, the results were reversed. During the Spring the greater number of SR was observed in both photophase and scotophase periods. The full-moon days comparison between seasons, showed a greater SR number in Winter in all phases and periods, except in the photophase of full-moon days. In the case of the SS, its number was greater in the scotophase only during full-moon days of the Winter and in the new-moon day of the Spring. Although the number of intermediate synaptic bodies (ISB) was short, it was always greater in the scotophases of the same periods, except for the two phases of the Winter new-moon days, when the number of this ISB was similar. In these, the greater nocturnal values depended on the season studied: in Winter were observed in the full-moon days and in Spring in the new-moon days. The melatonin serum levels were always greater during scotophases of both seasons and lunary cycles. The highest values were observed during new-moon days of Winter and full-moon days of Spring.

CONCLUSIONS: The conclusions are that exist an individual and interactional influence of the external factors on the SR and ISB variations. In the case of the SS the influence of the synodic cycles is always depending on the other factors. The serum level of Melatonin is clearly influenced for the photophasic rhythms and the seasonal cycles but not for the lunary cycles.

P. 63 EFFECTS OF IN VITRO IMMUNOSUPPRESSION OF INTERLEUKIN-6 ON PROLIFERATION AND APOPTOSIS OF RAT HYPOPHYSEAL CELLS

E. Pérez, A. Martín-Clavijo, G. Vázquez, J. Somalo, M. Rubio, E. Hernández, M.C. Montero, R. Herrero and J. Carretero

Dept. of Surgery, Faculty of Medicine. Dept. of Human Anatomy and Histology, Faculty of Medicine. Lab. of Neuroendocrinology, Institute of Neuroscience of Castilla y León. University of Salamanca, Spain

Interleukins -ILs- are proteins involved in the immune system and have been related to the endocrine regulation of the hypothalamic-hypophyseal-adrenal axis as well as to the secretion of ACTH, prolactin, GH and, possibly, LH. ILs synthesis has been reported in the hypophysis and the action of these compounds is therefore believed to occur through paracrine mechanisms. IL-6 has been

implicated as regulatory factor involved in pituitary cellular proliferation. Some studies have reported a relationship between cellular proliferation and pituitary apoptosis, but it remains unknown whether IL-6 is a paracrine antiapoptotic and proliferative factor in the gland. The aim of the present work was to address these questions. An in vitro study, neutralising the possible paracrine effect of IL-6, by means of treatment with diluted (1:10) polyclonal antibody against IL-6 -Ab-IL-6- over 1, 3, 6 12 or 24 hours; and later determining the degree of apoptosis by hybridising BrdU to fragmented DNA by labelling of the 3'-DNA terminals, was carried out. In the control cultures, the percentage of apoptotic cells ranged between 1.06% and 1.38%, with a mean value of 1.12%, no significant variations were observed in the different time periods assayed. Immunosuppression with Ab-IL-6 induced significant increases in the percentages of apoptotic cells from the 3rd to the 12th hour of treatment ($p<0.05$). In the control dishes, percentages of PCNA-positive cells ranged from 43% to 50%, without significant differences in the different time periods assayed. Immunosuppression of IL6 induced significant decreases of percentages of PCNA-immunoreactive cells ($p<0.01$ after 3, 6 and 12 hours). The present study suggests a double role for IL-6 in the modulation of hypophyseal cells, by stimulating proliferation and inhibiting cellular apoptosis in the rat hypophysis.

Supported by CO3/196 J CyL program.

P. 64 EFFECT OF NICOTINE ON PARAVENTRICULAR AND SUPRAOPTICUS NUCLEUS OF THE MOUSE PREGNANT. AN INMUNOHISTOCHEMICAL STUDY

H. Pérez González, E. Carmona Calero, A. Castañeyra Perdomo, T. González Hernández, M.M. Pérez Delgado, N. Marrero Gordillo and R. Ferres Torres

Departamento de Anatomia. Facultad de Medicina. Universidad de La Laguna. La Laguna (Tenerife)

Cigarette smoking during pregnancy is well known to cause postnatal neurological and behavioral disturbances in the offspring, and animal models indicate that nicotine is a neurobehavioral teratogen (1). Several lines of evidence indicate that the Hypothalamus it self is likely to contain the site of action of nicotine (2).

The hypotalamic paraventricular nucleus (NPV) play an important role in the control of various endocrine, physiological and behavioral responses. Immunocytochemical data indicate that both angiotensin and vasopressin are found in the magnocellular and parvocellular neurons in the PVN and SPO of the hypothalamus, posibly in the same cells (3). Angiotensine is an integrative hormone/neurotransmitter which coordinates the activity of several physiological agents implicated in numerous brain functions (4).

In previous research we detected changes in the inmunohistochemical reaction for Arginine - Vasopressin in the NPV, in the nicotine treated animals (5)

The aim of the present work is to study the effects of nicotine treatment during gestacion on the NVP and supraopticus nucleus (NSO), with specifically antibody againt to Angiotensin II, to see the alterations in the production and/or release of this peptid.

We have used 15 pregnant female mice (Swiss) divided in three animals groups: Control group that given acces to standard chow and water, ad libitum. Experimental group I: treated with nicotine at a dosage 1 mg/ kg/day from seven days before sacrifised until the 18 th day of gestation, and experimental group II whit received nicotine (1 mg/Kg/day) from the 7 th to the 18 th day of gestation. On the 18 th day of gestation fetuses from the three grouos of animals were got by cesarean to the mother thercafter the mother was also sacrificed ant its brain was also fixed and processed for study. Before the sacrifice the body weight was taken. Brains were fixed by vascular perfusion with Bouin's fluid. Embedding was in paraffin. Frontal serial sections, 10 mm thick, through the region of the hypothalamus, were obtained. One of the series was stained with the Klüver-Barrera method, while the other one was processed for the immunoperoxidase method using as primary antibody anti-angiotensin II (1:500). The antisera was raised in rat similar as it was described in previous work (6) using a fraction 1-4 of angiotensin II (ICN), The specificity of the antisera was evaluated by means of absorption test incubating the antisera overnight with the homologus antigen. The antigen was able to abolish the immunostaining. The sections were incubated for two hours with second antibody (biotinylated anti-rat IgG 1:200). Inmunoreactions were visualized for DAB.

The NVP of the experimental group II, showed a statistically significant increase of the AGII-ir material compared to the control and experimental group I. While the NSO did not presented differences in the AGII-ir material among of control and experimental groups. No qualitative differences were observed among the different groups using the klüver-Berrera method. In a previous work, we described an increase in inmunoreactive material for arginine- vasopressin in Median Eminence after nicotine administration. Our results suggest that nicotine is an stimulant agent of the inmunohistochemical expression of Angiotensin II in mouse hypotalamic paraventricular nucleus.

References

1.- Dahlstrom A, Lundell B, Cuuvall M and Thapper 1: Nicotine and cotinine concentrations in the nursing mother and her infant. *Acta Paediatr Scand,* 1990; 79:142-144

2.- Clarke PBS, Schwartz RD, Paul SM, Pert CB, Pert A. Nicotinic binding in the rat brain: autoradiographic comparison of (3H) Acetylcholine, (3H) nicotine and (125 I)- Alfabungarotxin. 1985; 5:1307

3.- Imboden H and Félix D. An immunohistochemical comparison of the angiotensin and vasopressin hypothalamo-neurohypophysial systems in normotensive rats. *Regul Pept,* 36 (1991) 197-218.

4.- Nelson DO and Boulant JA. Altered neuroanatomical organization of spontaneously hypertensive (SHR) rats. *Brain Res.,* 226 (1981) 119-130.

5.- Pérez González H, Carmona Calero E, Castañeyra Perdomo A, Pérez Delgado MM and Ferres Torres R. Inmunohistochemistry of the CRF and AVP in the paraventricular nucleus of the mouse pregnant mother and fetuses after nicotine treatment during gestation. *Eur J Anat,* 1997; 1 supl: 48.

6.- Pérez Delgado MM, Pérez-Delgado MM, Carmona-Calero E, Marrero-Gordillo N, Pérez-González H and Castañeyra-Perdomo A. Effect of hypertension on the angiotensin ii fibres arriving to the posterior lobe of the hypophysis of the rat. an immunohistochemical study. *Histoly Histopathology,* (in press) 1999.

Acknowledgements: *This work was supported by the Universidad de La Laguna , project nº 202-81/97.*

P. 65 ULTRASTRUCTURAL CHARACTERISTICS OF THYROID APOPTOSIS INDUCED BY METHIMAZOLE WITHDRAWAL

J.M. Riesco-Lopez, J.M. Riesco, E. Pérez[1];J.A. Juanes, M.L. Diego-Lourenço, J. Carretero, G. Vázquez, M.A. Velasco and R. Vázquez

Departamento de Anatomía e Histología Humanas. [1]Departamento de Cirugía. Facultad de Medicina. Universidad de Salamanca

The morphological characteristics of apoptosis in epithelial cells are different from those seen in other tissues (Kerr and Searle, 1973). The aim of our work was to check whether the apoptosis occurring in the thyroid gland during the involution of experimental hyperplastic goiter has ultrastructural characteristics similar to those of other epithelia or, instead, shares the morphological characteristics of non-epithelial tissues. To check this, 10 adult male Sprague-Dawley rats housed under standard stabling conditions were used. Five of the animals received methimazole in their drinking water over 21 days. After this time, the goiter-inducing compound was withdrawn and the animals were given a survival time of 21 days, an ideal period for the appearance of apoptosis in thyrocytes (Riesco et al., 1998). The remaining five animals did not receive any type of treatment. All animals were sacrificed by decapitation under isofluorane anaesthesia and their thyroid glands were removed. The left lobe of the gland was processed for histochemical and immunohistochemical techniques (BrdU-ISEL) for visualization under the light microscope (following the protocol of Riesco et al., 1998) with a view to observing, as a control, the frequency and location of thyroid apoptosis. The right lobe was processed for transmission electron microscopy. For this, small blocks ($1mm^3$) of thyroid gland were fixed by immersion in Karnovsky's solution for 3 h and postfixed in osmium tetroxide at 1% (2 h at 4° C). Ultrathin sections (40 nm) of pieces embedded in araldite were contrasted with lead citrate and studied under a Zeiss EM-900 electron microscope at 80 kV.

The histochemical and immunohistochemical techniques revealed cells with features of apoptosis and apoptotic bodies in both the wall and follicular lumen, but only in the thyroid glands of the methimazole-treated animals. The ultrastructure of the thyroid follicles and thyrocytes of the animals with involution of hyperplastic goiter differed from those of the untreated animals. Ultrastructural images of necrosis were only seen occasionally; however, most cell death events displayed the typical features of apoptosis, with nuclear changes and an initial preservation of the cytoplasmic organelles, although with a generalized contraction of the thyrocyte and a loss of contact with neighbouring cells. Later changes followed two different morphological patterns depending on the direction followed by the thyrocytes in apoptotic development. When the apoptotic cells were directed towards the base of the follicle, cellular fragmentation occurred, accompanied by the formation of apoptotic bodies that were eventually phagocytosed by macrophages or intact neighbouring cells. By contrast, when the apoptotic cells were expelled from the wall and fell into the follicular lumen, they were not phagocytosed but rather underwent a process similar to necrosis known as secondary necrosis.

REFERENCES

KERR JF and SEARLE AN (1973): *Virchows Arch B. Cell Pathol*, 13: 89-102.

RIESCO JM et al. (1998). *Anat Embryol*, 198: 439-450.

P. 66 PROLIFERATION AND APOPTOSIS OF MAST CELLS DURING THE INVOLUTION OF EXPERIMENTAL NON-TUMORAL HYPERPLASTIC GOITER

J.M. Riesco, J.A. Juanes, J. Carretero; M.L. Diego-Lourenço, J.M. Riesco-López, E. Blanco, G. Vázquez, M. Rubio and R. Vázquez

Departamento de Anatomía e Histología Humanas. Facultad de Medicina. Universidad de Salamanca

The aim of the present report was to explore the hypertrophy and hyperplasia of thyroid mast cells from rats with hyperplastic goiter induced by methimazole administration and to determine whether the subsequent decrease that such cells undergo during the involution of the goiter due to withdrawal of the goitergenic substance was caused by apoptosis. Histochemical and immunocytochemical techniques for the detection of proliferation and apoptosis were used to assay the thyroid glands of 30 adult male Sprague-Dawley rats divided into 6 groups (n=5), 5 of which received methimazole in their drinking water over 21 days (0.2 g/l water). Survival times were 0, 7, 14, 21 and 44 days. The sixth group did not receive the goitergenic compound and was considered as the untreated group. All animals were killed under isofluorane anaesthesia by decapitation and the thyroid glands were removed and fixed in Bouin's fluid for 24 h and several transverse sections of 5 μm thickness were obtained and mounted on slides. For histochemical studies, paraffin sections of tissue were stained using the periodic acid/Schiff reaction (PAS). To study cell proliferation, PCNA immunoreactivity was detected by the enzymatic streptavidin-conjugated peroxidase technique (St-Av-Pox). Labelling of fragmented DNA by *in situ* end labelling with bromodeoxyuridine (BrdU-ISEL) was used to detect cells in apoptosis. Both immunocytochemical techniques were developed according to the protocol described in a previous paper (Riesco et al., 1998; *Anat. Embryol.* 198: 439-50).

In the untreated animals, mast cells were scarce and PCNA- and BrdU-ISEL-immunonegative, pointing to the absence of proliferation or apoptosis. In contrast, at the end of treatment with methimazole, mast cells were hypertrophied and hyperplastic, displaying an intense metachromatism when stained with PAS. These cells were observed among fibroblasts and in the proximities of the blood capillaries of extrafollicular tissue. Numerous mast cells were immunoreactive to PCNA but immunonegative as regards BrdU-ISEL, suggesting strong proliferative activity and little or no apoptosis in glands stimulated with methimazole. Depending on the survival time, when the compound was withdrawn the immunocytochemical techniques revealed a gradual decrease in PCNA immunolabelling of the mast cells and, by contrast, an increase in the number of cells reactive to BrdU-ISEL, disclosing a contrary effect to the phenomena of proliferation and apoptosis since as the survival time increased and the thyroid glands returned to normality, the number of proliferating cells decreased and the number of mast cells undergoing apoptosis increased.

P. 67 GENDER-RELATED MODIFICATIONS OF PROLIFERATION RATE OF PROLACTIN- AND VIPERGIC-CELLS INDUCED BY HYPOTHYROIDISM OR TRH

M. Rubio, G. Vázquez, E. Hernández, M. Angoso, R. Herrero, J.J. Herrero, M.C. Montero, E. Pérez, J.M. Riesco, J.M. Riesco-López and J. Carretero

Dept. of Human Anatomy and Histology, Faculty of Medicine. Lab. of Neuroendocrinology, Institute of Neuroscience of Castilla y León. University of Salamanca, Spain

Changes in the morphology and number of Prolactin- and VIP-immunoreactive cells have been described in the hypothyroidism. However, analysis of changes of the proliferation of these cells have not been assayed. In order to determine the proliferation rate of rat hypophyseal prolactin- and VIPergic-cells in hypothyroidism and whether changes observed are related to increases of TRH, an double immunohistochemical study for PCNA and prolactin or VIP was carried out in thyroidectomized rats and rats treated with TRH.

TRH but not thyroidectomy increased the percentage of prolactin-cells in both sexes ($p<0.01$). However, whereas cellular proliferation is not modified in females, it was increased significantly in males in both experimental desings. Both, TRH and thyroidectomy increased significantly the percentage of VIP-cells. However at time points assayed, only male rats showed significant variations in the proliferation rate of VIP-cells.

		PRL-cells	Prolif. PRL-cells	VIP-cells	Prolif. VIP-cells
Females	Untreated	30.22 ± 0.28	1.46 ± 0.03	19.842 ± 0.20	0.71 ± 0.01
	Thyroidectomized	21.13 ± 0.13	1.32 ± 0.02	31.50 ± 0.28	1.05 ± 0.02
	TRH	55.78 ± 0.48	1.01 ± 0.02	38.33 ± 0.58	0.76 ± 0.01
Females	Untreated	30.16 ± 0.34	1.86 ± 0.03	7.68 ± 0.18	0.91 ± 0.01
	Thyroidectomized	27.22 ± 0.91	6.10 ± 0.04	22.82 ± 0.35	6.57 ± 0.04
	TRH	51.43 ± 1.20	2.59 ± 0.03	23.47 ± 1.82	7.03 ± 0.05

Our results demonstrate that modifications of hypophyseal-thyroid axis induce changes in prolactin- and VIP-cells which are related with the proliferation rate of both cells, principally in male rats at time points assayed. These results suggest that TRH and, probably, thyroid hormones could be involved in the physiological regulation of proliferation and/or differentiation of prolactin- and VIP-cells.

Supported by FIS 99/1187 program and SA58/98 Social Founds European and JCyL program.

P. 68 IN VIVO AND IN VITRO MODULATION BY GNRH OF THE IMMUNOCYTOCHEMICAL EXPRESSION OF AROMATASE P450 IN RAT HYPOPHYSIS

G. Vázquez, J. Carretero, M.C. Montero, A. Martín-Clavijo, E. Hernández, M. Angoso, E. Blanco, J. Riesco-López and R. Vázquez

Dept. of Human Anatomy and Histology, Faculty of Medicine. Lab. of Neuroendocrinology, Institute of Neuroscience of Castilla y León. University of Salamanca, Spain

Aromatase is the enzyme responsible of transformation of testosterone to estradiol by aromatization. Recently we have reported a gender-related expression of the enzyme in the hypophyses of adult Wistar rats. Because these expression seem to have a close relation to gonadal steroids and GnRH is the central signal involved in the regulation of secretion of gonadotrophs hormones and gonadal steroids, could be hypothesized that GnRH could modulate the hypophysial expression of aromatase in rats. In order to determine whether GnRH is an hypothalamic modulator of hypophysial aromatase, an in vivo (adult GnRH treated rats) and in vitro (monolayer cultures treated with the peptide at different time points) immunocytochemical study was carried out. Variations of percentages of Aromatase-positive cells and their correlation with basal plasmatic levels of LH were analysed.

The follow table show the results obtained:

Sex of animals	In vivo treatment	LH plasma levels	Aromatase-ir cells
Females	Untreated	2.71 ± 0.03	0.84 ± 0.22
	GnRH	0.62 ± 0.27*	0.06 ± 0.01*
Females	Untreated	1.14 ± 0.03	34.40 ± 2.93
	GnRH	0.32 ± 0.05*	20.08 ± 1.01*
Culturales (males)	In vitro treatment		
Control	1 hour	---	43.49 ± 2.40
	3 hours	---	41.27 ± 2.06
	6 hours	---	44.05 ± 2.20
	12 hours	---	42.89 ± 2.14
	24 hours	---	44.44 ± 2.25
GnRH	1 hour	---	63.76 ± 3.19*
	3 hours	---	25.15 ± 1.26*
	6 hours	---	25.10 ± 1.25*
	12 hours	---	31.54 ± 1.58*
* p<0.01	24 hours	---	42.25 ± 2.11

Our in vitro results demonstrate a biphasic effect for GnRH on hypophysial aromatase expression: after an initial stimulation, GnRH inhibits the expression. In vivo, variations of aromatase expression correlated well with basal plasmatic LH levels. In sum our results suggest that GnRH could be a physiological modulator of hypophysial aromatase expression y a similar way that it regulates the LH secretion.

Supported by FIS 99/1187 program and SA58/98 Social Founds European and JCyL program.

P. 69 APOPTOSIS IS INVOLVED IN THE ANTIPROLIFERATIVE EFFECT OF CORTICOSTERONE IN NON-TUMORAL ACTH HYPOPHYSEAL CELLS

R.J. Vázquez, J. Carretero, M. Rubio, J. Somalo, F. Sánchez, E. Pérez, G. Vázquez and R. Vázquez

Dept. of Human Anatomy and Histology, Faculty of Medicine. Dept. of Surgery, Faculty of Medicine. Lab. of Neuroendocrinology, Institute of Neuroscience of Castilla y León. University of Salamanca, Spain

Glucocorticoids are the physiological negative feed back on ACTH secretion and could be involved in the regulation of maintenance of the population of hypophyseal ACTH-cells, because adrenalectomy induces an increase of these cells in the early stages following the surgical ablation. Glucocorticoids have been involved in the induction of apoptosis in several tissues. However, only recently they have been scarcely considered as a hormonal inductor of apoptosis in the anterior hypophysis. Apoptosis is considered as a physiological event to regulate different cell populations in endocrine glands inclu-

ding the anterior pituitary. Apoptotic regulation of ACTH-tumoral cells, AtT-20 cells, has been reported in response to inhibitory effect of bromocriptine, as well as response of other hypohyseal cells. The aim of the present study is analyse using a double immunohistochemical study for PCNA and ACTH and a double assay by ISEL and immunohistochemistry for ACTH whether or not corticosterone induces apoptosis and inhibits cellular proliferation to control the non-tumoral ACTH-cells in the anterior hypophysis. For these propose, untreated, sham-operated and bilaterally adrenalectomized rats, treated or not with corticosterone, were compared.

The follow table show the percentages of positive cells obtained in the present study.

TREATMENT		ACTH	ACTH & PCNA	ACTH & ISEL
Untreated		7.59 ± 0.27	0	0
Sham 2 days	*With out Cort.*	17.83 ± 1.03	7.57 ± 0.26	0
	Cort. 1st day	12.97 ± 0.45	0	0
Sham 4 days	*With out Cort.*	19.08 ± 1.01	5.02 ± 0.18	0
	Cort. 1st day	11.07 ± 0.39	0.81 ± 0.03	0
	Cort. 3rd day	17.61 ± 1.30	2.01 ± 0.07	15.90 ± 0.56
Sham 6 days	*With out Cort.*	8.06 ± 0.91	0	0
	Cort. 1st day	6.50 ± 0.18	0	0
	Cort. 3rd day	8.06 ± 0.91	0	1.49 ± 0.02
Adx 2 days	*With out Cort.*	18.43 ± 0.79	18.08 ± 0.63	0
	Cort. 1st day	7.11 ± 0.27	0	79.18 ± 2.77
Adx 4 days	*With out Cort.*	27.05 ± 2.01	20.98 ± 0.73	10.61 ± 0.37
	Cort. 1st day	10.87 ± 1.28	2.79 ± 0.16	24.10 ± 0.84
	Cort. 3rd day	16.92 ± 1.21	3.33 ± 0.14	32.86 ± 1.15
Adx 6 days	*With out Cort.*	31.30 ± 1.19	4.47 ± 0.16	1.21 ± 0.04
	Cort. 1st day	16.40 ± 2.01	2.44 ± 0.09	9.27 ± 0.32
	Cort. 3rd day	22.86 ± 1.27	3.01 ± 0.11	30.41 ± 1.06

In sum the results of the present study demonstrated a very important inhibition of corticosterone on proliferation of ACTH-cells and that apoptotis is involved in these anti-proliferative effect.

P. 70 CONNECTIVITY OF THE POSTERIOR PARIETAL CORTEX IN THE ALBINO RAT. I. ORIGIN OF CORTICAL AFFERENTS

A. Cardoso Muñoz, A. Martín Colmenero, C. Sastre Santos, M.A. Pérez de la Cruz, M.E. Santos Pérez, F. Collía Fernández and Carrascal Marino E.

Facultad de Medicina. Universidad de Salamanca

The aim of this study was to ellucidate the cortical areas of the cerebral cortex in the albino rat thas are connected with the parietal posterior cortex (PPC) in the rat. In ten Wistar rats, weighing 250-300 g, the retrograde tracer fluorogold was iontophoretically injected, in, stereotaxic coordinates correspondong to the PPC . After 7 days the animals were perfused with fixative, the brains were secctioned in different planes, and the tracer was demonstrated with an antibody system. The neurons retrogradely labelled in different cortical areas were represented in drawings in order to ascertain the different sources of afferent projections to the PPC. PPC receives projections from the secondary visual areas, the primary and secondary auditory cortex, the somatosensorial cortex, out of the barrel field, and the retrosplenial cortex.

P. 71 CHANGES IN THE PROTEINS OF THE CEREBROESPINAL FLUID OF DIFFERENTS TYPES OF HUMAN HYDROCEPHALUS

A. Castañeyra-Perdomo[1], Luz María Fernández-Gonzalez[1], Isabel Martínez de la Peña-Valenzuela[1], Emilia Carmona-Calero[1], Romualdo Ferres-Torres[1], Herminia Pérez-González[1], María del Mar Pérez-Delgado[1], Manoli Castañeyra-Martín[3],Norberto Marrero-Gordillo[1] and Carlos Ormazabal-Ramos[2]

[1] Departamento de Anatomía Humana, Facultad de Medicina, Universidad de La Laguna, Tenerife, Spain. [2] Departamento de Pediatria, Facultad de Medicina, Universid de La Laguna, Tenerife, Spain. [3] Instituto de Investigacion y Ciencias de Puerto del Rosario, Fuerteventura., Canarias, Spain

The cerebroespinal fluid (CSF) is a dynamic, multifunctional system (6) An understanding of the dynamics of the CSF is important in medicine. Alteration in CSF composition characterizes many pathologic processes of the central nervous system (6).The composition of the human CSF have been studied by several authors (6, 7) and it contains various proteins, with two of the main components being albumin (70 %) and gammaglobulin (10 to 15 %), and has an overall albumin: globulin ratio of about 5:1. The CSF absolute protein concentration is age-dependent, being the CSF mean protein concentrations ranging from 15-45 mg/dl(being greatest at birth: 45-120 mg/dl) (7).It has been demonstrated that the CSF protein composition is altered in the hydrocephalus (4). The subcommissural organ (SCO) is a cerebral gland related with the circulation and composition of the CSF, which secrets glycoprotein into CSF were its mayor part is condensed and form the RF, and other minor part become soluble into the CSF (3). On the other hand, it has been reported that the SCO is altered in the hydrocephalic human fetal brain (1). In the present work, we analyzed the proteins in the CSF of different kind of hydrocephalus and theirs possible relations with the secretions of the SCO.

We have used CSF from a total of seven human fetal and perinatal hydrocephalus(Dandy-Walker malformation, Posthemorragic hydrocephalus, mielomeningocele, 38 and 40 weeks human fetal hydrocephalus, and tetraventricular newborn hydrocephalus). Also we have used a CSF from a normal human newborn. The CSF were processed by electrophoresis according to (2) (sodium dodecyl sulfate-polyacrylamide gel electrophoresis SDS-PAGE, 5%-15% gradient).

The electrophoresis study showed that, the total amount of protein in the hydrocephalus CSF was bigger than in the normal newborn. We found a total of seven protein band of 24, 25, 31, 45, 91, 150 and 250 kDa, in the hydrocephalus CSF, that were not present in the normal. We must emphasize that the 31 and 150 kDa proteins band were present in six types of hydrocephalus and not in the normal CSF. A protein 150 kDa also was found by Rodriguez et al (1993) (3) in the CSF of two months old human hydrocephalus. These findings and the alterations of the SCO in hydrocephalus fetuses described in previous work (Castañeyra-Perdomo et al 1994) (1), support the possibility that in the human hydrocephalus (fetuses and infants) the secretory material released by the SCO into the CSF is altered.

REFERENCES

1.- CASTAÑEYRA-PERDOMO A, MEYER G, CARMONA-CALERO E, BAÑUELOS-PINEDA J, MÉNDEZ-MEDINA R, ORMAZABAL-

RAMOS C and FERRES-TORRES R (1994). Alterations of the subcommissural organ in the hydrocephalic human fetal brain. *Developmental Brain Research,* 79: 316-320.

2.- LAEMMLI UK (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage. Nature (Lond), 227: 680.

3.- RODRÍGUEZ EM, JARA P, RITCHER H, MONTECINOS H, FLÁNDEZ B, WIEGAND R and OKSCHE, A (1993). Evidence for the release of CSF-Soluble secretory material from the subcommissural organ, with particular reference to the situation in the human. In *The Subcommisural Organ, An Ependymal Brain Gland* (Oksche A, Rodríguez EM and Fernández-Llebrez P., eds.), pp 121-131. Springer-Verlag, Berlín.

4.- RODRÍGUEZ EM, OKSCHE A, HEIN S and YULIS R (1992). Cell biology of the subcommissural organ. *Int Rev Cytol,* 135: 39-121.

5.- TAKEUCHI IK, KIMURA R, MATSUDA M and SHOJI R (1987). Absence of SCO in the cerebral aqueduct of congenital hydrocephalus spontaneously occurring in MT/HokIdr mice. *Acta Neuropathol,* 73: 320-322.

6.- VESNA P (1988). Long-term recording of cerebrospinal fluid pressure in freely behaving rats. *Brain Research,* 462:112-117.

7.- WILLIAM A and BONADIO MD (1992). The cerebroespinal fluid: physiologic aspects and alterations associated with bacterial meningitis. *Pediatr Infect Dis J,* 11: 423-432.

P. 72 CHEMOARCHITECTURE OF THE HUMAN ENTORHINAL CORTEX WITH CALCIUM-BINDING PROTEINS DURING AGING

R. Ferres-Torres, F.J. González-Delgado, M.L. Suárez-Solá, M.M. Pérez-Delgado, H. Pérez-González, E. Carmona-Calero, N. Marrero-Gordillo and A.Castañeyra-Perdomo.

Departamento de Anatomía. Universidad de La Laguna. La Laguna (Tenerife). Spain

INTRODUCCIÓN, MATERIAL AND METHODS: The entorhinal cortex is considered an intermediate station between neocortex and hippocampus. It plays an important role in learning and declarative or explicit memory. We aimed in this work to systematically study the morphological and neurochemical parameters of the entorhinal neurons expressing the calcium-binding proteins calbindin, calretinin and parvalbumin during the whole adult life, in 34 human brains from 16 to 95 years. The brains came from donations to the Department of Anatomy and from autopsies in different hospitals of Tenerife. They were fixed in formalin 20%.

RESULTS: We used in this study the classification of the entorhinal cortex of von Economo and Koskinas (1925), which most closely resembled our own observations. In fields HA and HB we observed an initially weak and inconsistent labeling of the pyramidal cells in layer II, which are the origin of the perforant path. Calbindin expression was more constant in pyramidal cells of layer III.

Calretinin stained nonpyramidal neurons in all layers, modified pyramidal cells of layer VI, and islands of small neurons in layer II.

Parvalbumin was present in nonpyramidal neurons extending over layers II to V, but concentrated in layer III. They gave rise to an extensive axonal plexus which was particularly dense in layer III, and in the cell islands in layer II. Chandelier axons were observed in layers II and superficial III.

The basic pattern of the calcium-binding proteins did not change with aging. Calbindin-expression was more intense in middle age than in young people, and persisted into old age.

Calretinin-expression was remarkably preserved into old age; in the 10th decade of life, calretinin-positive cells were still present in the cell clusters in layer II and in pyramidal cells of layer VI.

Parvalbumin-expression was severely affected in old age, with an almost complete absence of immunoreactive neurons and axonal plexuses.

DISCUSSION: A comparison of the basic patterns of distribution of the calcium-binding proteins in different species shows important differences, which have to be taken into account before we attempt to draw conclusions on supposed homologies.

The extent of neuronal loss in the aging entorhinal cortex has been a matter of dispute. FALKAI and BOGERTS (1986), TRILLO and GONZALO (1992) and LIPPA et al. (1992) did not observe a significant decrease. By contrast, HEINSEN et al. (1994) found a cell loss of 26 to 39% in the entorhinal cortex of 22 subjects between 18 and 86 years; these results may, however, be contaminated with possible undetected Alzheimer cases. Our present data demonstrate changes in the immunoreactivity of the three calcium-binding proteins examined, most accentuated in the case of parvalbumin. However, we are unable to determine whether the decrease of immunoreactivity is produced by a disappearance of the neurons, or reflects a loss of protein-expression.

REFERENCES

ECONOMO C. VON and KOSKINAS. Die Cytoarchitektonik der Hirnrinde des erwachsenen Menschen. Springer, 1925.

FALKAI P and BOGERTS B (1986). *Eur Arch Psychiatr Neurol Sci,* 236: 154-161.

HEINSEN B, et al (1994). *Anat Embryol*, 190: 181-194.

LIPPA CF. et al (1992). *Neurobiol Aging,* 13: 405-411.

TRILLO L and GONZALO LM (1992). *Histol Histopathol*, 7: 17-22.

P. 73 DOPAMINERGIC MODULATION OF IMMUNOHISTOCHEMICAL EXPRESSION OF NOS-I IN THE PITUITARY GLAND OF MALE RAT

E. Hernández, G. Vázquez, J.R. Alonso, F. Sánchez, J.G. Briñón, E. Weruaga and J. Carretero

Dept. of Human Anatomy and Histology, Faculty of Medicine. Lab. of Neuroendocrinology, Institute of Neuroscience of Castilla y León. Dept. of Cell Biology and Pathology, Faculty of Biological Sciences. Lab. of Comparative Neurobiology, Institute of Neuroscience of Castilla y León. University of Salamanca, Spain

Nitric oxide is an unconventional transmitter because diffuses from its site of production. Its local production in the pituitary gland, gonadotroph and follicle-stellate cells, has been demonstrated. Inhibitory effects of nitric oxide on the prolactin release have been reported and could be an auto-paracrine modulator of the control of prolactin release. Prolactin release is controlled by multiple signals, among them, dopamine is the principal central inhibitory signal and, without rejecting other regulatory pathways, dopamine could exert its inhibitory effects by means of

stimulation of nitric oxide. However, effects of dopaminergic pituitary modulation on NOS-I producing pituitary cells have not been yet analysed. The aim of the present study was to analyse the effects of two dopamine antagonists, haloperidol and metoclopramide, on the hypophyseal immunohistochemical expression of constitutive neuronal oxide nitric sintase (NOS-I) on male adult rats. The hypophyses were embedded in paraffin and frontal serial sections of 5 μm were processed for immunohistochemistry using an highly specific antibody to NOS-I. Jointly to morphological study, variations of numerical density, percentage and size of NOS-I-positive cells were determined using and image analyser MIP-2 (IMCO-10). In untreated and control animals NOS-I-positive cells were very similar. Two types of NOS-I-positive cells appeared in all sections of the pars distalis analyzed: round or polygonal cells and stellate cells. Although some isolated cells were found, the NOS-I-immunoreactive cells commonly appeared grouped in clusters close to blood vessels. NOS-I-immunoreactive cells appeared as uniform and well stained cells and all their cytoplasmic surface, including cytoplasmic prolongations, showed immunoreactivity. Immunoreactivity for NOS-I in pituitary glands of Haloperidol- or Metoclopramide-treated males decreased considerably in relation with untreated or control animals. Variations of morpho-planimetric analysis observed in the study are shown in the following table.

	Cellular area m^2	Numerical Density n° cells/ m^3	Percentage
Untreated	131 ± 7.69	0.09 ± 0.016	1.34 ± 0.24
control	128 ± 6.65	0.10 ± 0.02	1.42 ± 0.31
metoclopramide	57 ± 1.92	0.09 ± 0.006	0.36 ± 0.09
haloperidol	76 ± 2.38	0.01 ± 0.004	0.25 ± 0.07

The results obtained demonstrate that dopamine antagonists induce an important decrease of the NOS-I-immunoreactive cells and suggest that dopamine could be a physiological stimulator of NOS-I expression in the hypophysis of the rat and that NOS-I could be involved in the dopaminergic regulation of hypophyseal prolactin.

P. 74 ANATOMY OF THE SURFACE OF THE ENCEFALO. MAGNETIC RESONANCE (MR)

L.C. Hernández, A. López Muñiz, E. Bengoechea, V. Martínez[1], M. Recio[1], M.A. López-Pino[1] and J. Viaño[1]

Departamento de Morfología y Biología Celular. Facultad de Medicina. Universidad de Oviedo. [1]RM Ntra. Sra. Del Rosario. Madrid

Introduction: The development of 3D sequences echo-gradient consent to obtain the total brain volumen in a short time from thin sections and three-dimensional reconstruction, as well as three-dimensional approaches to the cerebral surface. This type of approach improves functional RM and consent a better knowledge of the brain surface as studied using RM. This study was designed to establish the correlation between the cerebral surface observed directly form cadaver material, and that obtained by MR from voluntary normal subjects.

Material and Methods: The anatomical study of the cerebral surface was carried out in fixed or fresh-unfixed cadavers obtained from the Departamento de Morfología y Biologia Celular (Universidad de Oviedo, Spain). The RM images were obtained from healthy voluntary using a 1.5T equip. The sequence 3D-SPGR (TR/TE = 37/10 ms; flip = 35°; sections thickness: 1.3 mm) was used, in associated with an appropriate software.

Results: The surface anatomy of the brain, in both cadaver and radiological material (lobules, cerebral gyri, fissurae and sulci) is showed in 18 images.

P. 75 METAMIZOL-INDUCED FOS-LIKE IMMUNOREACTIVITY IN THE NUCLEUS OF THE TRACTUS SOLITARIUS OF RATS IS DOSE-DEPENDENT

T. Hernández-Gil-de-Tejada, Alfonso A. Valverde-Navarro, Mª Carmen Blasco-Ausina, Cristina Soto-Sarrión and Francisco Martínez-Soriano

Departamento de Ciencias Morfológicas. Universidad de Valencia

Introduction: The nucleus of the tractus solitarius (NTS), classically associated with regulation of autonomic and endocrine functions, has been recently involved in the modulation of nociception. Previous studies have reported that high doses of the analgesic metamizol induce strong Fos-like immunoreactivity in the NTS. In order to report more data about this antinociceptive role of the NTS, we studied the effect of different doses of metamizol on the expression of c-*fos* proto-oncogene along the NTS.

Material and Methods: Experiment was carried out in adult male Sprague-Dawley rats. Animals were subdivides in five groups. A control group of unstimulated rats and four groups of metamizol-treated rats. Each of the metamizol-treated group received an intraperitoneal dose of metamizol (50, 100, 250 or 500 mg/kg). To demostrate the activated neurons we used Fos immunocytochemistry.

Results: The results showed no-labeled cells in the NTS of the control rats. However in the metamizol-treated rats, the number of Fos-labeled neurons in the NTS increased progresively as the mg of metamizol injected did.

Discussion: These results support the suggested role of the NTS in the pain control. But, also, they contribute to a better knowledge about the mechanism of action at the central nervous system of the metamizol.

P. 76 RECOVERY OF NUCLEAR SIZE IN THE ENTORHINAL CORTEX OF HYPERAMMONEMIC RATS AFTER NORMAL DIET EXPOSURE REFLECTS MAIN CONNECTIVITY OF THE HIPPOCAMPUS

R. Insausti[1], A.M. Insausti[2], M.M. Arroyo[1] and V. Bajo[1], J.M. Gaztelu[3] and V Felipo[4]

[1] Dept. of Health Sciences, University of Castilla-La Mancha, School of Medicine. [2] Department of Mathematics, Statistics and Computation, University of Cantabria. [3] Department of Research, Ramón y Cajal Hospital. [4] FVIB Cytological Research Institute

Hepatic encephalopathy is a serious neurological disorder due mainly to inborn errors of metabolism or hepatic lesion. Glutamate neurotransmission impairment seems to be an important factor in the pathogenesis of the neurological alterations present both in clinical cases and experimental models. One of such models is the chronic administration of 20% w/w of sodium acetate in the animal diet. After 8 weeks of exposure, the neuronal nuclear size in layers II, III, V and VI of the entorhinal cortex are decreased (Insausti et al., 1997). We investigated the reversibility of such changes after 4 weeks of normal diet consumption.

Male Wistar rats (250-300g weight) were divided into 4 groups: 1) controls fed on a regular diet. 2) animals exposed to a diet containing ammonium acetate (20% w/w) in the chow pellets. 3) animals exposed to the ammonium diet for eight weeks. 4) animals exposed for eight weeks and returned to a regular diet for 4 weeks. After appropriate time, animals were sacrificed with an overdose of barbiturates, perfused transcardially with 4% paraformaldehyde and sectioned in the coronal plane at 50 μm in a freezing microtome. Sections every 250 μm were stained with thionin. Neuronal nuclear size in layers II-VI of the entorhinal cortex at intermediate (DIE, projecting to mid septo-temporal levels of the dentate gyrus) and lateral (DEL, projecting to the septal part of the dentate gyrus) portions were analyzed with a stereology program (C.A.S.T.- GRID) under a magnification of x1000. The analysis determined the neuronal nuclear size as an indicator of the functional activity of the neurons.

Both DIE and DEL layers II and V (origin of the projection to the dentate gyrus and to the neocortical mantle respectively), showed values comparable to controls in the group returned to normal diet. In contrast, neuronal nuclear size in layers III and VI did not return to control values.

Our study suggests a considerable, albeit selective for certain layers, capacity for recovery after withdrawal from the ammonium enriched diet. Moreover, this experimental model presents some parallelism with experimental models based on alcohol administration.

Supported by grants FIS 98/0062-02 and 98/0062-01.

P. 77 VOXEL MAN: AN INFORMATIC TOOL FOR TRIDIMENSIONAL GENERATION OF CEREBRAL STRUCTURES

J.A. Juanes[1], J.M. Riesco[1], E. Blanco[1], M.J. Velasco[2], F.J. Cabrero[1], M.J. García[3], J.J. Carmena[2], J. Marcos[4] and R. Vázquez[1]

[1] Facultad de Medicina. Universidad de Salamanca. [2] Hospital "del Río Hortega", Valladolid. [3] Hospital Clínico Universitario, Salamanca. [4] Hospital "12 de Octubre", Madrid

Voxel-Man is a space-filling brain model derived from a Magnetic Resonance Imaging (MRI) volume data set of a human head. All volume elements (voxels) have been labelled as regards their membership to a structural region, a functional region and a region supplied by a given blood vessel. The relationships between these regions, textural descriptions and histological images of the structural regions are stored in a knowledge base. Voxel-Man is the result of a research project aimed at investigating new computer-based techniques for representing the anatomy of the body. In contrast to currently available hypermedia systems, it is based on a space-filling "virtual body" which allows exploration in an unlimited number of possibilities. It is an ideal tool for teaching and studying anatomy and anatomical-radiological correlations. Ten million voxels (volume elements of 1 mm^3) provide the basis for three-dimensional images. The brain model is derived from Computer Tomography and MRI of a living subject and includes a knowledge base with information about the subject's morphology. A large window termed "services" is the main navigation tool. The lower portion of the window displays the basic operations of the atlas, such as remove, paint, add, make transparent or show exclusively.

The program runs under a MOTIF user interface presently on the following UNIX workstations: DEC 3000 AXP, SUN Sparcstation, Silicon Graphics, Hewlett-Packard, PC under LINUX. The following minimum configuration is required: colour display (with an 8-bit buffer for standard image quality); 100 Megabytes of main memory; installation medium: CD-ROM. Our aim is to offer some graphic possibilities of Voxel-Man in the generation of 3-D images from serial sections obtained from the "Visible Human" Project and from radiological images obtained with different techniques. The Voxel-Man program allows the user to work in relation to volume visualization, 3-D texture mapping, 3-D navigation, volume sculpting and surgical simulation, volume deformation, volume segmentation, multimodality registrations, image quality in volume visualization, and the symbolic modelling of human anatomy. At the same time we are currently using Voxel-Man in collaboration with workers at different hospitals for pilot applications in neurosurgical planning. The tool is therefore suitable not only for medical students but also for residents training in neuroradiology, neurology or neurosurgery.

Finally, we wish to emphasize that the use of powerful computers and information technology applications that facilitate the construction of three-dimensional anatomical images are now permitting the simulation of pathologies, enabling "virtual" surgical interventions. Moreover, from the point of view of teaching, these systems and applications are beginning to be considered excellent auxiliary tools for the teaching of anatomy.

P. 78 CONNECTIVITY OF THE POSTERIOR PARIETAL CORTEX IN THE ALBINO RAT. III. THALAMO-CORTICAL CONNECTIONS

A. Martín Colmenero, M.E. Santos Pérez, M.A. Pérez de la Cruz, C. Sastre Santos, A. Cardoso Muñoz, F. Collía Fernández and E. Carrascal Marino

Facultad de Medicina, Universidad de Salamanca

In order to ascertain the thalamo-cortical system of the parietal posterior cortex(PPC), the retrograde tracer Fluorogold(FG) and the anterograde tracer Biotynilated Dextran amine(BDA) were iontophoretically injected, in stereotaxically guided different points of PPC, in sixteen, weighin 250-300 g Wistar albino rats. After seven days the animals were perfused, and the brains were sectioned in a frontal plane.The tracers were displayed following the Lanciego and Wouterlood protocol. The neurons retro-

grade labelled with FG and the fibers anterograde shown with BDA were represented in thalamic schemes following the Paxinos Atlas.The thalamo-cortical system of the parietal posterior cortex includes projections to and from the laterodorsal nucleus,lateral posterior nucleus and to a lesser extent from ventro lateral nucleus, and from the nucleus reticularis.

P. 79 ANATOMICAL AND CLINICAL CORRELATION IN THE POSTRAUMATIC TEMPORAL LOBE EPILEPSY

M.J. Murillo, B. Mompeó and J.A. Ramírez

Department of Morphology. Las Palmas de Gran Canaria University

OBJECTIVE: To study the relationship between the traumatic temporal lobe lesions and the posterior epileptic seizures.

MATERIAL AND METHODS: A patient suffered, at three years of age, a small bullet wound in his right eye ball. At seven years of age he had partial simple seizures that were treated sucessfully with phenobarbital. The drug was suspended after an asymptomatic period of thirteen years. The antiepileptic treatment was restored because the same seizures reappeared two years later. At the present moment, the patient is free of symptoms.

Some radiography (Rx), computed tomography (CT) and electroencephalograms (EEG) were made.

RESULTS: The Rx and CT showed the small bullet in the cerebral tissues just over the temporal bone in the petrosal part. The EEG showed a right frontotemporal focus.

DISCUSSION: The expression "temporal lobe epilepsy" (TLE) is based on anatomical localizations and is applied to epileptic conditions characterized by repeated simple partial seizures and complex partial seizures which commencer principally in the mesial temporal region. Our patient had symptomatology of simple partial seizures, sometimes with secondary generalization. At the beginning of the seizures the patient had reported experiences having subjective qualities (named "experiential phenomena") of perceptual (eg: visual hallucinations) and mnemonic (eg: "deja vu" illusion) characteristics and also vegetative alterations. We want to know the correlation between the patient's symptomatology and the traumatic cerebral lesion. The small bullet followed a lineal direction from the right orbitary sphenoidal fissure, later it passed, outside the cavernous canal and the pituitary fosse, through the internal region of the anterior temporal lobe and, finally, it sttoped just over the vertex of the temporal bone in the petrosal part. Neither the Gasser ganglion nor the internal carotid artery were affected. The seizures symptomatology was only related to the injury produced by the course of small bullet inside the right temporal mesial region.

P. 80 MODIFICATION OF NEURONAL STRUCTURES CAUSED BY FREE RADICALS

M.D. Peces, M.A. Capo, M.T. Frejo, M.J. Anadon-Baselga and M.B. Sevil

E.U. de Óptica, Madrid

B-Aminopropionitrilo (BAPN) is the active metabolite of B-x glutamylaminopro-pionitrile, a peptide found in abundance in pulses leguminous plants and caracterized as a multifunctional aminonitrile acting on collagen, elastine and neurons.

We propose to evaluate the production of free radicals based on the oxidation of NADH mediated by the superoxide dismutasa (SOD), an enzyme that decrease the rate of oxidation of NADH. We will also confirm the protective role of sodium selenite in treated cultures due to its antioxidance capacity.

Primary neuronal cell cultures were prepared from cerebral hemispheres of 14-16 day old embryos (Rattus norvegicus) Wistar rats. The cells were sown in petri dishes with poli-L-Lys. After three days of cultivation BAPN was applied in differents concentrations and SOD activity was measured following the method based on the oxidation of NADH by means of radical superoxide and the results were valued with a spectofhotometer.

Our results demostrate that the concentration of SOD increase significantly as the concentration of used BAPN increase and the amount of SOD decrease significantly in the presence of sodium selenite.

Morphologically, axonal connections are diminished almost to the point of disappearing cultures treated with BAPN. The neuronal bodies are grouped, giving rise to neuronal accumulations, with a rugged irregular cross-section. When protectived by sodium selenite, the neurons show a larger number of synaptic contacs and less damage to the neuronal bodies.

The resuls indicate that the degenerative effects of BAPN on neurons in culture are a result of the production of free radicals and that sodium selenite has a protective role on such cultures.

P. 81 FOS EXPRESSION IN THE INTERGENICULATE LEAFLET MAY BLOCK THAT IN THE SUPRACHIASMATIC NUCLEUS IN THE RAT

A. Ruiz-Torner, A. Alfonso Valverde-Navarro and Francisco Martínez-Soriano

Dpto. de Ciencias Morfológicas. Universidad de Valencia

INTRODUCTION: Previous reports about photic expression of c-fos in both the suprachiasmatic nucleus (SCN) and intergeniculate leaflet (IGL) have suggested a different functional significance of Fos activation in these two structures. However, none of them have focussed specifically on finding out wether exist a relationship between Fos expression in the SCN and that in the IGL. For the moment, little is known about the mechanisms through which the IGL may influence the SCN circadian activity.

MATERIAL AND METHODS: Photic-induced Fos-like immunoreactivity (FLI) in was studied using immunocytochemistry. FLI in the SCN was compared with that in the IGL, both in control rats and photic-stimulated rats, entrainned to 24 hours light-dark cycles (LD) or constant dark (DD).

RESULTS: Results showed a very low number of Fos-positive cells in the SCN of LD control rats along the 24 hours of the cycle, except for the ZT2, whereas in the IGL a significant number of Fos labelled cells was kept during the light phase (ZT2 and ZT8) and the early dark phase

(ZT14), for going to SCN levels at the second half of the dark phase (ZT20). Light stimulus increased the number of FLI neurons in the SCN, but significantly more at the ZT20 than at the ZT14, whereas in the IGL FLI increased similarly in both ZT. FLI was absent in both the SCN and IGL of non-stimulated DD animals, regardless of the ZT. Both structures also displayed the same parallel behavior when rats from DD were light-stimulated, increasing substantially the number of FLI cells.

DISCUSSION: Our results demonstrate that light can induce c-fos expression in both the ventrolateral subdivision of the SCN and the IGL, but in different manner. The data support the idea that light reaches the SCN through the RHT before than via the GHT so light can induces Fos expression into the SCN without any interference. But if IGL was previously expressing Fos when light arrive to the SCN then it will make SCN-Fos expression difficult, suggesting that IGL may inhibite the expression of c-fos in the SCN. In conclusion, entrainment of circadian rhythms by light only would be possible when IGL neurons were not activated.

P. 82 SWIM STRESS AND ENVIRONMENTAL TEMPERATURE CHANGES ENHANCE NEURONAL NITRIC OXIDE SYNTHASE IN THE PARAVENTRICULAR NUCLEUS OF THE HYPOTHALAMUS

F. Sánchez, M.N. Moreno[1], M.J. López, P. Vacas, J.M. Molina and R. Vázquez

Dept of Human Anatomy and Histology. Faculty of Medicine. [1] Dept of Computing. Faculty of Sciences. University of Salamanca. 37007. Salamanca. Spain

Stress is associated with activation of the hypothalamic-pituitary-adrenocortical axis. Various stress related imputs converge upon the neurons located in the paraventricular nucleus of the hypothalamus (PVN). Neuronal nitric oxide synthase (NOS I) has been demonstrated to be present in the PVN by means of the NADPH-diaphorase histochemical method, immunohistochemistry and in situ hybridization. The present study was undertaken to determine whether or not different types of stress such us forced swim and exposure to low temperature promotes changes in the expression of the NADPH-activity in the PVN. Twenty-five adult male Wistar rats divided into six groups were used for the present study: normal animals (n=5), without receiving any treatment. Normal animals (n=5) sujected to a forced swim in a 36ºC water for 10 min. Normal animals (n=5) sujected to the same swim test for 30 min. Normal animals (n=5) exposed to low temperature for 3 hours. Normal animals (n=5) exposed to low temperature for 24 hours. Cryostat sections were cut and processed for the histochemical detection of the NADPH-diaphorase activity. A significant increase in the number of NADPH-diaphorase neurons was observed following swim stress especially after 30 min. Following exposure to low temperature for 24 hours statistical analysis displayed a marke increase in the number of nitrergic neurons. No significant differences following three hours of exposure to low temperature were found. These data confirm the involvement of NOS neurons of the PVN in the response to different types of stressors.

P. 83 STUDY WITH ANTEROGRADE TRACERS OF THE CALLOSAL PROJECTIONS OF THE TEMPORAL CORTEX. CORRELATIONS WITH THE IPSILATERAL PROJECTIONS

M.E. Santos Pérez, M.A. Pérez de la Cruz, A. Cardoso Muñoz, A. Martín Colmenero, C. Sastre Santos, F. Collía Fernández and E. Carrascal Marino

Faculty of Medicine, University of Salamanca

In this study ten Wistar albino male rats were used. They were anesthetized, placed in a stereotaxical support (Paxinos, 1997), and iontophoretically injected with the anterograde tracers BDA and PHA-L. After 7 days, the animals were perfused with a fixative (4% paraformaldehyde, 0.1% glutaraldehyde, 0.2% picric acid, in 0,4 M phosphate buffer pH 7,4. The brains were sectioned and the sections were processed in order to demonstrate BDA (BC method, DAB-Nickel) and PHA-L with the PAP method. (Lanciego, 1994).

The results obtained were studied and topographied as Zilles (1985) Te1 projects contralaterally to Te1, Te3 and Par2 and ipsilaterally to Te1, Te3, Par2 and Oc2L. Te3 projects ipsi- and contralaterally to Te1 and Te3. Always the contralateral projections were less developed than the ipsilateral ones.

LANCIEGO JL and WOUTERLOOD FG (1994). Dual anterograde axonal tracing with Phaseolus vulgaris-leucoagglutinin (PHA-L) and biotinylated dextran amine (BDA). *Neurosci Prot*, Mod. 4: 94-050-00-01.

PAXINOS G and WATSON C (1997). CD rom. *The rat brain in stereotaxic coordinates*. Academic Press, Sydney. Australia.

ZILLES K and WREE A (1985). Cortex: Areal and Laminar Structure. In *The Rat Nervous System*. Second Edition. Edited by George Paxinos. Academic Press. Sydney.

P. 84 CONNECTIVITY OF THE POSTERIOR PARIETAL CORTEX IN THE ALBINO RAT. II. CORTICO-CORTICAL EFFERENTS

C. Sastre Santos, A. Martín Colmenero, M.A. Pérez de la Cruz, M.E. Santos Pérez, A. Cardoso Muñoz, F. Collía Fernández and E. Carrascal Marino

Faculty of Medicine, University of Salamanca

The aim of this study was to investigate the fate of the cortical projections arising in parietal posterior cortex. Ten Wistar rats weighing 250-300g, were iontophoretically injected with the anterograde tracer biotynilated dextran amine (BDA), in the PPC area. After seven days the animals were perfused with fixative and the brains were sectioned in different planes. BDA labelling was shown with an ABC kit. The fibers anterogradely labeled were represented in schemes and localized in different cortical areas following the Paxinos estereotaxic Atlas. Posterior parietal cortex projects to ventrolateral and medial orbital areas, frontal cortex (Fr2), parietal cortex primary and secondary, primary auditory cortex, secondary visual areas and retroplenial cortex.

P. 85 CARPAL TUNNEL PRESSURE MEASUREMENT TECHNIQUE

F. Sánchez del Campo

Departamento de Anatomía Humana de la Facultad de Medicina de la Universidad Miguel Hernández de Elche- Alicante. Lizaur Adjunto COT. Hospital General de Elda. Alicante Utrilla.

The carpal tunnel is a closed space, which contains the nervus medianus and the mine flexor tendons of the fingers passing through.

It is forneed by four rigid walls. Three of them (two lateral and one inferior) depend on the carpal bones and the superior wall is the ligament which connects both lateral bone structures. (Ligamentum anulare carpii) the volume inside the carpal tunnel is exactly adjusted to the inner space and any pathological process causing a reduction in the tunnel walls may increase its tissular pressure.

Many authors have showed a preet interest in the pressure inside the carpal tunnel to reach a better knowledge of the physiopathological basic of the carpal tunnel syndrome.

The aim of this poster is to show the technique to measure the pressure of the carpal tunnel using a Quick Pressure Monitor System - S.T.I.C. (Stuker r) connected to a wick catheter.

P. 86 ERGONOMIC ANTHROPOMETRIC MODEL FOR A SPANISH WORKING POPULATION

F. Sánchez del Campo[1], A. Panchón[2] and J.R. Lobato[3]

[1] Departamento de Anatomía Humana. Facultad de Medicina. Campus de San Juan. Universidad Miguel Hernández de Elche. [2] Escuela Politécnica Superior. Universidad de Alicante. [3] Escuela Profesional de Medicina del Trabajo de la Universidad Miguel Hernández de Elche

This paper offers the anthropometric model, for men and women from the working population of Murcia (S-E of Spain), for industrial design.

There is a high contrast between the necessity to dispose of standardized anthropometric models for the Spanish working population and the lack of homogeneous data. Furthermore, there is a considerable dispersion in the methodology and measurements considered in the different works, which makes a comparison and integration difficult in a common database of Spanish population.

The randomized sample involves 327 individuals from the working population of the region of Murcia, 202 are men and 125 are women, both of ages between 16 and 64 years, the size of sample offers results of ±1% accuracy and 99% confidence interval.

All anthropometric variables have been defined and referred to the Norm ISO/DIS/7250.2 (1992) and EN/979 (1995). Measurements have been obtained by means of the anthropometer model Martin and trade mark Harpenden®.

The anthropometric profile giving in this paper shows variations in relation with the anthropometric data about the Spanish working population available until the date.

P. 87 ANTHROPOMETRIC PROFILE FOR ERGONOMIC DESIGN OF SITTING WORKPLACES

F. Sánchez del Campo[1], J.R. Lobato[2] and A. Panchón[3]

[1] Departamento de Anatomía Humana. Facultad de Medicina. Campus de San Juan. Universidad Miguel Hernández de Elche. [2] Escuela Profesional de Medicina del Trabajo de la Universidad Miguel Hernández de Elche. [3] Escuela Politécnica Superior. Universidad de Alicante

This paper offers the standardized anthropometric profile from the working population of Murcia (S-E of Spain), from an ergonomic point of view for design sitting workplaces.

The randomized sample involves 327 individuals from the working population of the region of Murcia, 202 are men and 125 are women, both of ages between 16 and 64 years, the size of sample offers results of ±1% accuracy and 99% confidence interval.

All anthropometric variables have been defined and referred to the Norm ISO/DIS/7250.2 (1992) and EN/979 (1995). Measurements have been obtained by means of the anthropometer model Martin and trade mark Harpenden®.

Anthropometric data constitute the standardized anthropometric profile of the working population of Murcia, it allows to update and perfect the anthropometric data about the working Spanish population available until the date for the ergonomic design of sitting workplaces.

P. 88 GLIAL CELLS DISTRIBUTION IN AN EPILEPTIC MODEL

F. Ortega, J. Gondra, J. Sendino, S. Mezquita, S. de Vega, M. de la Fuente and L. Hennequet

Departamento de Neurociencias. Facultad de Medicina y Odontología. Facultad de Farmacia. Unversidad del País Vasco / Euskal Herriko Unibertsitatea.

The well known role of glial cells in the liberation of activatory and inhibitory neuroactive substances in the CNS could be involved in the apparition of epileptic seizures and the posterior development of epilepsy if these descharges affect appropriate areas of the brain.

In this study we try to establish different epileptic models (audiogenic, neurotoxic) which lead us to advance in the knowledge of glial cells and the possible treatment of epilepsy.

We have use female rats (weight approx. 180 g.) of Sprague-Dawley strain.

These rats were injected:

– Pylocarpine Chlorhidrate (30 mg./kg.), subcutaneously (SC); previously (24h.) they were injected SC with Lithium Chloride (3 mEq.).

– Kainic acid, intraperitoneally (10 mg/kg).

After visual observation of epileptic seizures, salivar hipersecretion, mioclonic movements of the head and extremities, the animals were anaesthetised and transcardially perfused, at differents weeks after injection day. The brains were embedded in paraffin and sectioned at 7 μm. The sections were stained with the Kluver-Barrera and Phosphotungstic acid hematoxiline (PTHA) techniques.

We observed that the rats, 15-45 min. after injection, develop epileptic seizures. These seizures reappeared 15 d after injection, when the animals were placed under stress conditions (noise, manipulation, etc).

Macroscopically, the brains of the injected rats were smaller than the controls, having mainly affected the cerebral cortex. At microscopic level we observed damage in:

– The pyriform cortex: we found cystic lesions, decreasing in the number of neurons, neuropillar destruction and increasing of glial cells.

– The dorsal and lateral hippocampus: The neuronal degeneration was mainly observed in the older rats (9 to 14 weeks after injection).

– Finally, we appreciated degenerated neurons in the limbic cortex, and a glial increasing in the thalamus as well.

Grant: UPV 212.327-EB 128/98.

P. 89 S-ADENOSYL-L-METHIONINE PROTECTS FROM BRAIN DAMAGE IN AN *IN VIVO* ISCHEMIC-REPERFUSION MODEL IN THE RAT

M.A. Villalobos[1], J.P. De La Cruz[2], M.A. Cuerda[1], J.M. Smith-Agreda[1] and F. Sánchez de la Cuesta[2]

Departments of [1]Anatomy and [2]Pharmacology. School of Medicine. University of Málaga. Spain

INTRODUCTION: Oxygen-derived free radicals (ODFR) play an important role in several pathological process, mainly in tisular ischemia-reperfusion. The reduction in the blood flow in the ischemic process alters the vellular energetic metabolism which induces reversible or irreversible damage, depending on the intensity and nature of the ischemia.

In these events, production of ODFR take place in the physiopathological pathways, both in the ischemic period and in reperfusion, if any. These alterations may be in two ways: increasing oxidative systems (through activation of lipid peroxidation of membrane phospholipids) and/or depletion of the endogenous antioxidant defence (mainly the glutathione system).

Antioxidant drugs could prevent the production of lipid peroxidation (e.g. vitamin E) or increase glutathione system (e.g. N.acetyl-cysteine), ans they could prevent the ischemic lesions. In this way, S-adenosyl-L-methionine (SAMe) is an antioxidant drug that inhibits lipid peroxidation and increases glutathione levels, as was demonstrated by our group in human platelets and rat arteries, liver and kidney.

The aim of the present study is to evaluate the effect of SAMe in a model of combined focal and permanent ischemia with global ischemia-reperfusion in rat brain.

MATERIAL AND METHODS: The study was carried out in male Wistar rats, anesthetised with Droperidol (0.66 mg/Kg i.p.) and Fentanyl (0.012 mg/Kg i.p.). The experimental model was developped by electrocoagulation of the cortical branches of the middle cerebral artery and reversal obstruction of both common carotids (1 h ischemia and 1 h reperfusion). Rats were divided into four groups (n=10 rats per group):

– Group I. Sham operated rats (SOR)

– Group II. SOR treated with 50 mg/Kg/day i.p. (x 3 days) of SAMe

– Group III. Rats with the brain ischemia-reperfusion model (I-RR)

– Group IV. I-RR treated with SAMe

We determined brain lipid peroxidation as malondialdehyde production (MDA, nmol/mg protein), glutathione levels (GSH, mmol/g tissue) and mitochondrial ability to reduced tetraphenyltetrazolium (%TPTRed).

RESULTS: The results in cortex area were as follows:

	MDA		GSH		TPTRed	
	Ischemia	Reperfusion	Ischemia	Reperfusion	Ischemia	Reperfusion
Group I	121±0.11	1.28±0.66	6.59±0.63	6.17±0.38	100	88.78
Group II	1.15±0.12	1.25±0.10	9.30±0.51	8.17±0.42	100	92.15
Group III	2.99±0.20	1.90±0.14	3.35±0.29	4.19±0.22	72.12	54.46
Group IV	1.09±0.13	1.69±0.13	10.03±1.14	10.22±1.88	90.41	83.21

DISCUSSION AND CONCLUSION: This model of ischemia-reperfusion produced an imbalance in the cellular oxidative status, mainly by an increase of MDA and decrease in GSH, which produced a clear mitochondrial damage. The administration of SAMe modified this alterations: reduced MDA, increases GSH and prevented the failure in the TPT reduction capacity of the mitochondrias. These events are important because they confirm the effect of SAMe in other tissues, but it has not been reported previously in brain tissue. For that reason, we think that it seems a new way to prevent the ischemic lesions in the brain.

P. 90 DISTRIBUTION OF NEUROPEPTIDE Y IMMUNOREACTIVE VITAMIN D3 TARGET NEURONS IN THE RAT BRAIN

G.F. Jirikowski, Ch. Bolde and K. Prüfer

Institut für Anatomie II, Friedrich-Schiller-Universität Jena, Germany

With immunocytochemistry, we studied the distribution of Vitamin D3 receptor (VDR) in series of vibratome brain sections of intact rats. Immunofluorescence of these sections was employed to determine whether Vitamin D3 target neurons express Neuropeptide Y (NPY). Numerous VDR immunostained neurons were observed in various brain regions including the magnocellular hypothalamic nuclei, the hippocampus, the prefontal and cingulate cortex, the primary motor- and sensory cortices, the piriform cortex and the entorhinal cortex. VDR immunostaining was in most cases confined to the neuronal nuclei; however, some regions contained neurons with additional cytoplasmic VDR staining. Scattered VDR positive, NPY immunofluorescent neurons were observed in a fraction of the basket cells of the hippocampus and in some of the pyramidal cells of the motor and sensory cortex. Numerous interneurons in all cortical regions showed both immunoreactivities. While the hypothalamus contained abundant VDR and NPY immunoreactive neurons, a coexistence of the two antigens could not be observed. Our findings further emphasize that Vitamin D may be another important neurosteroid with distinct target areas throughout the brain. Steroids are known to be capable of crossing the blood brain barrier. They may be therefore be predestined peripheral

mediators of various neuroendocrine functions. NPY has been located mostly in interneurons, associated with modulatory functions. It is likely that NPY expression is in part controlled by peripheral Vitamin D through a direct genomic effect. This may contribute to the explanation of known Vitamin D actions on the brain including the mechanism of light therapy for treatment of certain forms of depression.

P. 91 KINANTHROPOMETRY ANALYSIS IN FOOTBALL PLAYERS OF THE NATIONAL Erd DIVISION FROM THE COMMUNITY OF MADRID

M.D. Cabañas Armesilla[1], A. Herrero de Lucas[2] and M. Catena Toledano

[1] Depto. Ciencias Morfológicas II. Fac. de Medicina. Univ. Complutense. Madrid. [2] Bachelor in Medicine and Surgery. [3] Chiropodist. Podomaster. Madrid

INTRODUCTION: Kinanthropometry alludes to health as nutritional status; predicting the performance, welfare and survival. Most sports pursue a morphotype esteemed ideal to reach success. Formerly football specialize fades the last years with the known as "total football", whereas players must roll in any position. Kinanthropometry is a method simple, inexpensive, not invasive and most universally applicable.

MATERIAL AND METHODS: Studied 50 football players of National 3rd Division from the Community of Madrid, age from 18 to 32 years, mean of 23.34 ± 3.79. Different categories were developed for the study, dividing sportsmen in goalkeepers, defenses, midfielders and forwards.

This analyses 49 anthropometric direct variables, adding somatotype, profile of sum up skinfolds and body mass index (B.M.I.) to it. Data has been processed by Microsoft EXCEL calculus sheet. The investigation and nature of measurement was explained to sportsmen, trainees and chairmen in order to be authorized by the club and obtain a signed consent from players.

RESULTS

1. B.M.I.

	Goalkeeper	Defense	Midfield	Forwards
	Mean	Mean	Mean	Mean
Age (years)	24.25 ± 5.30	23.00 ± 3.52	23.55 ±4.74	22.40 ± 1.52
Height (cm.)	182.4 ± 8.34	176.6 ± 4.76	173.5 ± 5.82	177.4 ± 7.20
Weight (Kg)	88.40 ± 8.90	78.45 ± 6.39	74.55 ± 7.97	77.06 ± 6.56
B.M.I.	26.53 ± 0.99	26.85 ± 0.86	24.61± 2.24	24.45 ± 0.99

CLASSIFICATION B.M.I. [Seidell, 1997]					
B.M.I.(Kg/m^2)	**Classification W.H.O**	**Goalkeeper (%)**	**Defense (%)**	**Midfield (%)**	**Forward (%)**
<18.5	Low weight	0	0	0	0
18.5 - 24.9	----	0	50	58.33	62.50
25.0 - 29.9	Overweight I	100	50	41.67	37.50
30.0 - 39.9	Overweight II	0	0	0	0
>40	Overweight III	0	0	0	0

2. SOMATOTYPE

SOMATOTYPE MEAN			
	Endomorphy	**Mesomorphy**	**Ectomorphy**
Goalkeeper	2.11 ± 0.23	3.67 ± 1.68	1.47 ± 0.48
Defense	1.98 ± 0.53	4.67 ± 0.98	1.80 ± 0.70
Midfield	2.17 ± 1.04	4.62 ± 1.08	1.76 ± 0.68
Forward	2.20 ± 0.51	4.68 ± o.91	1.94 ± 0.50

3. PROFILE AND SUMMING UP OF SKINFOLDS

	[1]Σ 6 skinfolds	[2]Σ 4 skinfold
Goalkeeper	57.75 ± 4.91	35.60 ± 3.48
Defense	52.78 ± 10.50	33.35 ± 7.68
Midfield	49.48 ± 21.29	32.46 ± 16.31
Forward	51.80 ± 10.04	34.45 ± 7.86

[1]Σ 6 skinfolds: Triceps, Subscapular, Supraspinal, Abdominal, Thigh and Calf.
[2]Σ 4 skinfolds: Triceps, Subscapular, Supraspinal and Abdominal

DISTRIBUTION OF Σ 6 SKINFOLDS (%)				
Σ 6 skinfold (mm.)	**Goalkeeper**	**Defense**	**Midfield**	**Forward**
<55	50	64	83	50
55 - 69	50	36	0	38
70 - 84	0	0	0	12
85 - 99	0	0	17	0
100 - 114	0	0	0	0
115 - 129	0	0	0	0
130 - 144	0	0	0	0
145 - 159	0	0	0	0
160 - 174	0	0	0	0
175 - 190	0	0	0	0
>190	0	0	0	0

	Goalkeeper	**Defense**	**Midfield**	**Forward**
	Mean	Mean	Mean	Mean
Triceps skinfold	6.95 ± 1.84	7.27 ± 1.94	6.98 ± 3.25	8.35 ± 2.16
Subscapular skinfold	11.05 ± 0.75	9.20 ± 2.97	9.53 ± 2.98	9.76 ± 2.52
Abdominal skinfold	12.45 ± 1.96	11.46 ± 3.43	10.33 ± 7.68	10.78 ± 3.40
Supraspinal skinfold	5.15 ± 0.57	4.92 ± 1.02	5.61 ± 3.10	5.52 ± 1.24
Thigh skinfold	14.45 ± 1.33	12.04 ± 2.07	11.00 ± 3.53	12.72 ± 3.15
Calf skinfold	7.70 ± 3.00	6.50 ± 2.13	5.92 ± 2.89	6.60 ±2.61

DISCUSSION: The B.M.I. exposes the mean value of 25.61 ± 1.26, whereas superior in goalkeepers, followed by defenders, forwards and midfielders. Studying Seidell's B.M.I. classification, 1997, is found that goalkeepers fit in overweight grade category, while the remainders accomplish weight limits considered normal, healthy or acceptable; arose by defenders post over midfielders and strikers.

Somatotype remarks Mesomorphy, very likely in most posts, grading dwarfly from forwards over defenders to midfielders, exposing greater values than goalkeepers.

The study of skinfolds express prevalence in goalkeepers over defenders forwards and midfielders in sum up of six skinfolders reverting the primacy of strikers to stoppers in the summing up of four skinfolds. The distribution is very homogeneous, with greater dispersion in forwards. Homogeneous thickness found in defenders, midfielders and forwards, with preeminence of skinfolds: thigh over abdominal, subscapular, triceps, calf and supraspinal; similar to goalkeepers in dominance of thigh over abdominal and subscapular, distinguishing bigger thickness of thigh to triceps and supraspinal.

P. 92 CORRELATION STATODYNAMIC BETWEEN THE LOWER LIMB AND THE PLANTAR PRINT

M.D. Cabañas Armesilla[1], M.I. Maestre López[2], J.L. Moreno de la Fuente[3] and M. Catena Toledano[3]

[1] Dpto. de Ciencias Morfológicas II. Fac. de Medicina. Univ. Complutense. Madrid. [2]Medical Service. School of Ntra. Sra. de las Maravillas. Madrid. [3]Podomaster. Madrid.

INTRODUCTION: The study of a population of 100 scholars, committed to regular practice of sports, ageing between 8 to 10 years and 15 to 16 years, by analyzing bilateral plantar prints and dynamic analysis of step in standardizing condition, to estimate the concordance of parameters found by both methods.

MATERIAL AND METHODS: The 100 scholars participated in the study by parental or tutorial authorization of signed consent bulletin, distributed to such effect. Analyzed individuals practice Basketball, Football, Handball and Judo, with regular training at school. Distribution was

made in 2 groups I and II, mean age of 91 and 151 years respectively.

Obtain plantar print by photopodogram, in radiographic paper AGFA for which analysis is applyed classic methods, measuring A. Clark, I. Chippaux and I. Staheli. Values treated statistically by Microsoft EXCEL calculus sheet.

Grade of heel deviation to varus or valgus measured with goniometer of Martin, one degree precision. Simultaneously has been realized studies of dynamic steps by recording with video camera of 6 cycles of march in ventral and dorsal vision, such image treated with computer program INFOFEET and image digitalizer ATI, evaluating qualitative and quantitative variables.

RESULTS AND DISCUSSION: 200 photopodograms, 100 right and 100 left, and 100 video recording were studied. The following statistic results, showed in tables I and II, have been obtained.

TABLE 1: MEAN STATISTIC VALUES

GROUPS	A.CLARK RIGHT	A.CLARK LEFT	I.CHIPPAUX RIGHT	I.CHIPPAUX LEFT	I.STAHELI RIGHT	I.STAHELI LEFT	DEVIATION R HEEL	DEVIATION L HEEL
GROUP I	33,03	32,61	41,10	41,39	0,7562	0,7473	3,214	3,081
GROUP II	44,42	47,42	36,19	33,76	0,6547	0,6123	3,033	1,333

TABLE 2: MEAN DYNAMIC VALUES. RMD: angle formed by mid-patella, center of intermaleolar space and base of 2nd interdigital space

GROUPS	PROPULSION DIGITAL R	PROPULSION DIGITAL L	ROTATION PATELLAR L	ROTATION PATELLER L	ANGLE RMD[I]	ANGLE RMD	DEVIATION R HEEL	DEVIATION L HEEL
GROUP I	0,38	0,50	0,10	0,14	23,76	18,90	8,292	7,704
GROUP II	0,35	0,35	0,043	-0,0434	28,72	19,46	5,230	5,769

It can be observed in group I a higher tendency to flat feet in both, while group II tends to bridge feet with bigger differences between both feet, due to growing factors and/or sport training.

Group II had a higher incidence in pronated feet than in group I, whereas incidence was practically null.

Significant statistic differences were found in both groups of population between thestatic and dynamic march analysis.

Classic methods of studies for plantar print does not value facts that affect in step that compensates with march and is analysed with dynamic evaluation of feet, as well as muscular tone. Study be complete with clinical evaluation for individuals in each foot, assesting a global information more reliable from a physiopathological point of view.

P. 93 THE DUPLICATION OF THE LONG SAPHENOUS VEIN AS A CAUSE OF VARICOSE VEINS RECURRENCE

B. Mompeó, F. Ortega and A. Centol

Departamento de Morfología de la U.L.P.G.C. Apartado 550, 35080 Las Palmas de Gran Canaria

The Long Saphenous vein (LSV) is a long vessel that is located at the inner face of the femoral and the tibial aspect of the lower leg. Venous insufficiency, as a consequence of a primary venous wall malfunction or as a weakness of the venous valves, is mainly located on the (LSV) territory.

On the other hand the incidence of recurrence of varicose veins is very high (7,2-8,7%). This can be as a consequence of both a bad surgical technique at the groin section and the ligation of the (LSV) or as a consequence of a incompetent perforating vein. However, one missing cause of recurrence can be the duplication of the saphenous trunk. The incidence of this anomalous situation has not been studied enough and some authors establish that it can be found in from 6-62% of the extremities.

MATERIAL AND METHODS: We combined an ecodoppler study of 126 lower extremities with varicose veins recurrence and an anatomical study of 10 LSV, in order to investigate how many recurrences can be the result of the presence of a double long saphenous vein trunk and to prove that anatomically this situation is true.

Both ecodoppler and anatomical explorations were developed as standard techniques.

RESULTS AND DISCUSSION: The ecodoppler shows that the LSV was the first point where the reflux could be detected (75.5%), followed by the ankle perforating veins (37.4%) and the crural perforating veins (31.9%). The high incidence of LSV insufficiency was related to both the crural and ankle insufficient perforating veins (28.8% and 33.5% respectively).

Anatomical studies show that the long Saphenous vein (LSV) can be duplicated in a higher or a lower extension, and we could found it in 60% of the studied legs. Perforating veins can join the femoral vein directly with the LSV, which can explain the high incidence of LSV reflux recurrence.

In conclusion, during duplex examination for varicose vein surgery, special attention must be paid to the duplications of the LSV. After surgery the remaining trunk can increase the diameter and also a perforating vein can develop a valve insufficiency with the result of the recurrence of the varicose veins.

P. 94 ACETYLCHOLINESTERASE CHEMOARCHITECTONIC SUBDIVISIONS IN THE RAT PERIAQUEDUCTAL GREY MATTER

A.A. Valverde-Navarro, F.E. Olucha-Bordonau, J. Cabanes-Vila, T. Hernández-Gil, A. Ruiz-Torner and F. Martínez-Soriano

Departamento de Ciencias Morfológicas, Facultad de Medicina, Universidad de Valencia, 46010 Valencia, Spain

INTRODUCTION: The periaqueductal grey matter (PAG), the area around the midbrain aqueduct in mammals, has been subdivided into different subregions based on their cytoarchitecture, connections, chemoarchitecture and functions. A more detailed study of the rostrocaudal organization of each subdivision of the PAG led a group of researchers to formulate the theory of longitudinal columnar organization of the PAG. However even when researchers agree on the number of subdivisions or columns, nomenclature used to refer them considerably varies. These variations between number of subregions or columns and the nomenclature among the cytoarchitectonic approached studies of the PAG make interesting to achieve new data on this field under a different point of

view, like the histochemistry. The aim of this work was to attempt a chemoarchitectonic characterization of the rat PAG by mapping histochemically the distribution of acetylcholinesterase (AChE).

MATERIAL AND METHODS: The identification of different anatomical regions of the periaqueductal grey matter of the rat was made in the present study by describing the histochemical staining of the activity of the enzyme AChE.

RESULTS: AChE histochemistry showed a different background staining into the columnar subdivisions of the rat PAG. More intense labelling was achieved along the entire dorsolateral and medial or periaqueductal columns. The rest of the columns defined into the PAG (dorsomedial, lateral and ventrolateral) displayed a weaker labelling, except for the external portion of the dorsomedial column where we could find a discrete area of strong AChE labelling.

DISCUSSION: The distribution pattern of the histochemically-studied AChE activity in the rat periaqueductal grey matter supports the idea of a rostrocaudally oriented columnar organization of this structure, introducing new data useful for the exact delineation of the columns.

AUTHOR INDEX

N

O

P

Q

R

S

T

V

W

Instructions to authors

General instructions

Manuscripts should be submitted in triplicate and typed double spaced with wide margins. They must be prepared carefully in the style of this journal. Figures should also be submitted together with the manuscript in triplicate. Unless originals are requested back within six weeks after publication of the manuscript, they will be destroyed. The text should be arranged in the following sections: Summary, Introduction, Materials and Methods, Results, Discussion and References. The text must be as concise as possible. Spelling and abbreviations should be consistent and the pages numbered consecutively. The desired position of figures and tables should be indicated in the margin. Changes in the proofs should be minimal; a charge will be made for changes introduced after the manuscript has been set in type. Only manuscripts written in English will be considered, authors not entirely familiar with English usage are advised to seek the assistance of an English-speaking colleague. Editorial assistance on English manucripts is available when needed; however, correct style is the responsibility of the authors. Manuscripts that have been extensively modified in the editorial process will be returned to the author for retyping. Once manuscripts have been accepted for publication, authors will be asked to send a soft copy (3.5 inch diskette) containing all the relevant information in either PC format (MS Word o Word Perfect) or in Macintosh format (any text processing application). In either case, the version of the application used (e.g. <Claris Works™ 3.0Ev1>) should be indicated.

The brief summary should not exceed one typewritten page and should be free of abbreviations and references. Immediately following the summary not more than five key words in English (taken from Index Medicus) should be supplied for subject indexing. The first page of the manuscript should give the following information: title of paper, names of authors, departamental and institutional affiliation, and address to which proofs are to be sent. To facilitate communication between the authors, editors and publisher, the author should provide a telex or fax number on the title page of the manuscript. Footnotes that do not refer to the title or authors should be numbered consecutively. Acknowledgements should be made in a separate paragraph following Discussion and before References. Citations, in the text, of names of authors must be followed by the year of publication. In cases in which there are more than two authors, only the first should be named, followed by "et al.". All papers cited in the text, and only these, should be listed under References. References should be listed at the end of the papers as follows: a) Single author-list alphabetically and then cronologically. b) Author and coauthor-list first alphabetically by coauthor, and then chronologically. c) First author and more than one coauthor-list chronologically (not alphabetically by the second author), because only the first author's name and "et al.", followed by the year of publication will be used in the text. d) In the event that more than one paper by the same author or team of authors published in the same year is cited, the letters a, b, c, etc., should follow the year in both the text and the reference list.

Examples

Berrebi AS and Mugnaini E (1991). Distribution and targets of the cartwheel cell axon in the dorsal cochlear nucleus of the guinea pig. *Anat Embryol,* 183: 427-454.

Shin SH (1978). Blockage of the ether-induced surge of prolactin by naloxone in male rats. *J Endrocrinol,* 79: 397-398.

Völker W, Schmidt A and Buddecke E (1989). Cytochemical changes in a human arterial proteoglycan related to atherosclerosis. *Atherosclerosis,* 77: 117-130.

Serafini-Fracassini A (1984). Elastogenesis in embryonic and postnatal development. In: Ruggeri A, Motta PM (eds). *Ultrastructure of the connective tissue matrix.* Nijhoff, Boston, pp 140-150.

Statement on ethics

Human experiments: papers describing experimental work on human subjects where there is some risk of harm must include: a) a statement that the experiments were conducted with the understanding and the consent of each subject; b) a statement that the ethical committee responsible has approved the experiments on living animals should provide: (a) a full description of any anaesthetic and surgical procedure used; (b) Evidence that adequate steps were taken to ensure tha animals did not suffer unnecessarily at any stage of the experiments.

Illustrations

The number and size of the illustrations must be consistent with the minimum requirement for clarification of the text. Previously published figures cannot be accested. Numerical data given in graphs or tables should not be repeated in the text. Because of the very high quality of reproduction, authors should pay special attention to the quality of their illustrations. The journal is not subsidized in any way and so when papers have an excessive number of halftone illustrations it is neccessary that authors and their institutions contribute to the cost of reproduction. Authors are required to bear the extra costs for color reproductions. All figures, whether layouts or micrographs, graphs, or diagrams, must be numbered consecutively and submitted on separate sheets. The publisher reserves the right to reduce or enlarge illustrations.

Halftone illustrations, i.e. micrographs, shaded drawings, and wash drawings earmarked for reproduction, should be submitted as sharp, glossy originals maximally trimmed at right angles to eliminate unnecessary areas and should be in the desired final size. For technical reasons, it is essential that the points detailed here be scrupulously observed. Inscriptions must be about 3 mm high. Magnification should be expressed numerically in the legend or by calibration bars in the micrographs. Wherever possible, individual micrographs should be mounted together in the form of economically arranged layouts, without necessarily taking their numerical order into consideration. Figures grouped together must be separated by spaces of no more than 1 mm. If at all possible, illustrations and legends should not be placed sideways. The layouts or single figures should match either the width of the column (8.6 cm) or the width column of the entire page (17.8 cm). The maximum length of the layout is 24 cm, including the legend printed at its foot. Large peripheral areas of figures with low information content should be avoided. If plates are submitted, the figures must be mounted on regular bond paper, not on cardboard. Instant lettering is recommended for lettering micrographs, including figure numbers. Line drawing should be submitted as good-quality glossy prints.

The letters should be at least 2 mm high in the printed version. The inscriptions should be clearly legible. If reduction is absolutely necessary, please state the scale desired. Legends should be concise and should provide a brief, self-sufficient explanation of the illustrations. Remarks such as: "For explanation see text" should be avoided. In the manuscript legends should be typed on a new page after the References.

Tables

Tables should be numbered consecutively in arabic numerals. Each table must be typed on a separate sheet and should have a brief, self-sufficient legend that explains its contents.

European Journal of Anatomy
September, 1999 • Volume 3 • Supplement 1

CONTENTS

ISSN: 1136-4890