

POSTERS

Anatomy Teaching**P-1 THE TEACHING OF ANATOMY IN FOUR IBEROAMERICAN DENTAL SCHOOLS.**

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The teaching of Human Anatomy has experienced an enormous evolution during the last years. The new teaching methods and technologies, the implementation of different Health professions requiring a specific formation, and the new international educational context have influenced very noticeably the way the Anatomy is taught and studied in our Faculties. Our Universities belong to four different geographic areas and differ in many aspects, both philosophical and organizational.

This first report offers a general view of the teaching of the Anatomy in four Iberoamerican dental schools, developed around some main points: a general view of the anatomy courses, both obligatory and compulsory. This description deals with the comparison of the general contents of the programs, the calendar, the courses structure, the evaluation methods, the number of the professors, and the material resources disponible for the students. This document has been prepared using the same methodology that permitted the redaction of the First and the Second "Report on the Teaching of the Anatomy in the Spanish Dental Schools" presented respectively in 1996 and 1998 by the Board nominated by the Spanish Anatomical Society (Sociedad Anatómica Española, SAE).

The main objective of our group is to discuss the existing problems in teaching Human Anatomy in Dentistry, to suggest some general recommendations regarding the teaching of Anatomy in the dentistry degree, and finally, to develop a common basis for the preparation and development of the different Anatomy courses for the students of the Iberoamerican dental faculties. The Iberoamerican dimension, in which the Spanish language and numerous cultural aspects are shared by a community of more than 400 million people, makes necessary an effort for strengthening the collaboration between professionals, both teachers and clinicians, dedicated to the Odontology.

We conclude that it is important to establish and maintain a strong relationship between the teachers of Anatomy in Dentistry in order to discuss programs and teaching or evaluating methods and especially to obtain updated information about all the different factors influencing the Teaching of both the Basic and the Clinical Sciences in Dentistry.

P-2 ADAPTING THE TEACHING OF HUMAN ANATOMY TO THE EUROPEAN HIGHER EDUCATION AREA (EHEA).

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The process of integration into the European Higher Education Area should be initiated with the intention to improve the teaching-learning process and teaching organization. In this paper we present one of the models for coping with this process in the teaching of human anatomy in the medical, physiotherapy and human nutrition and dietetic studies of the Universitat Rovira i Virgili.

Having defined the profile of education and competencies, and within the framework of the current curriculum, we used the following methodology: first, we selected the competencies in human anatomy required by the medical students; then we defined the learning objectives, related these objectives to the competencies to be evaluated and proposed the learning methodology; finally, we calculated the number of hours of work required by the students. This methodology was used in the three subjects of the medicine curriculum (Human Anatomy, Anatomy of the Locomotor Apparatus and Anatomical Techniques); two subjects on the physiotherapy curriculum, and in one subject on the human nutrition and dietetic curriculum.

P-3 PROBLEM-BASED LEARNING APPLIED TO LARGE GROUPS.

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As a result of the Bologna Declaration and the introduction of the European Credit-Transfer System, there is a pressing need to redesign the objectives of university-student education as well as the methodology applied in obtaining such objectives.

Problem-based learning (PBL), on the basis of articulating real-life problems, aims to assist student acquisition or development, in an on-going form, of a series of abilities: the integration of knowledge and skills; self-assessment; teamwork, oral discourse, argumentation and critical reasoning; the ability to relate and synthesise information, etc. That is, this system aims at teaching students to be more self-sufficient in the processes both of work and of learning.

Nevertheless, the main difficulty that PBL faces within Spanish universities is student overcrowding in the classrooms and lecture halls.

Lecturers teaching *Neuroanatomy and the Sense Organs* (in the Anatomy and Embryology Unit, UAB) put PBL into practice for one week with two student groups, each comprising 100-150 students. Each session lasted 1h, corresponding to the hour of theory usually given at this time. At the start of the class, the lecturer presented students with a clinical case to solve within 30 minutes. Students worked on this in groups of three, each group being asked to provide two copies of their solution: one for the lecturer and the other for subsequent correction and self-assessment of the clinical case. Each group was responsible for bringing to class the necessary bibliography with which to solve the problem. Once the version for the lecturer had been handed in, the whole class then assessed the various group solutions, with the lecturer then finally going on to comment on and discuss these, complementing his or her explanation with images or additional information.

P-4 OPINION OF ANATOMY STUDENTS ON THE NEWTECHNOLOGIES. OUR EXPERIENCE WITH THE ANILLO DIGITAL DOCENTE (DIGITALTEACHING RING).

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INTRODUCTION: Over four centuries have passed since Fray Luis de León delivered his classes from the pulpit. Over all this time, methodology and technology have been incorporated into the teaching process. Six years ago the University of Zaragoza put the Anillo Digital Docente (ADD) on its website for use in the educational process. The ADD enables teachers and students to access and exchange different kinds of information.

MATERIALANDMETHODS: By means of the ADD we are developing a kind of semi-attendance based teaching for the study of Anatomy, by placing the following documents on the website:

1. Schedule indicating each day's activity.
2. Sheet of concepts for each topic, with practical objectives and bibliography.
3. Subject syllabus.
4. Multiple-choice questions for self assessment.
5. Interactive atlas.

At the end of first and second year Anatomy in the studies for a Degree in Medicine, the students' level of satisfaction was assessed.

RESULTS: From the results, we would point out: that the ADD is a tool that the students were not keen on using. They do not consider the ADD essential for the study of Anatomy. Regarding the material placed in the ADD, the multiple-choice questions followed by the interactive atlas were the most commonly used and the schedule was not used at all. A considerable number of students find the access complicated. Most of them consider that the ADD has helped them to improve their study habits.

CONCLUSION: We propose that the use of the ADD for teaching Anatomy can help the teaching objectives of the subject to be achieved.

P-5 IMPACT OF ELECTRONIC MAILING ON TUTORIZATION OF ANATOMICAL PRACTICUM.

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The authors present their experience in the use of the electronic mailing to meliorate the answer of the students during the realisation of the clinical anatomical practices, in a subject named Clinical Anatomy in the third year of Medicine in our Faculty.

The students matriculated in this subject must made two clinical practices. The first one is in the operation room and the other one in the Trauma consultation in our Hospital in order to make anatomical observations. The students give a script that must be completed by them during the practice and later they could send it by mailing to the responsible teacher before the next fifteen days. The

teacher correct the practice and send another time it to the student for new corrections. So the students could reach the wished knowledge.

The authors have revised the total name of electronic mailing related with the two practices, and have measured the degree of participation of the students. They have observed that in a total of 106 students matriculated in the subject, only a 32% have send no mailing. The rest, 72 students, the 9,7% have send only one mailing, the 16% two mailings, the 22,2% five mailings and the 41,6% more than five mailings.

The first conclusion is that the use of the electronic mailing is a good instrument to control the work of the students during the practices, specially when there are a geographic difference between the Faculty and the Hospital. The second conclusion is about the time that represents to the teacher this control, but it has the advantage that could be made out of the Faculty. The third conclusion is related with the objectives of the practices that could be reached more satisfactorily after several corrections by the teacher. The last conclusion is to consider this control as a new valuation of the student's work, that must be considered in this moment of change in the anatomical learning.

P-6 SCIENTIFIC ILLUSTRATION AND MEDICALTEACHING.

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Most advances in Medicine have depended on the ability to visualize the structure and science of the body. Being able to clearly visualize the basic science has often made the difference between medical success and failure. It is not simply a matter of academic and scientific exercise. Data needs to be put into a form our senses understand.

Art and Medicine have been intertwined as far back as the Stone Age. Scientific illustration has been widely used, from ancient times, as a media to show information from one person to other, to show the anatomy and physiology of the human body, and also as a media to show different medical pathologies and congenital malformations to the observer, that can be either the physician, the patient, the student and/or the teacher.

The first known anatomical illustration is a Paleolithic drawing in the El Pindal cave in Spain, showing a leaf-shaped area locating the heart. Egyptian doctors, by 4000 BC utilized the first illustrations to accompany scientific text. In the Medieval Ages, artists and physicians often worked together, and this collaboration led to great advances in anatomical education during the Renaissance.

New technologies, the use of computers, have brought new possibilities and improvements in the use of scientific illustration as a media of communication between the teacher and the student and /or the physician and the patient.

Our study presents scientific representations of different stages of the human embryos development, and also scientific illustrations of the changes in the woman perine during the baby delivery. Both types of illustrations have been realized using the Photoshop Program in a computer, based on a previous obtained image. We describe the different steps in the process to get the final image.

We can appreciate in our results that this technique, the application of the Photoshop program on the initial drawing realized by the scientific illustrator, or on the image obtained from the scan of a previous photograph, offers us a big improvement in the final illustration, resulting in a great media for education and for communication between the medical and the patient.

P-7 THE INFLUENCE OF HISTOLOGY AND EMBRYOLOGY COURSES ON STUDENT ACHIEVEMENT IN GROSS HUMAN ANATOMY COURSES.

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In the School of Medicine of the Universidad Autónoma de Nuevo León, there is a clear division between pre-clinical and clinical subjects. Gross human anatomy is taught for one semester along with other subjects that include an introduction to medicine, art appreciation and creative thought, and the subjects of histology and embryology are taught during another semester, which also includes oral and written communication. Half of first year students are found registered in the gross human anatomy course, while the other students study histology and embryology. This study's objective is to establish the existence of a difference in achievement in the anatomy course after having taken other morphological science courses.

For the study, regular students were included who were registered in the anatomy course during the March-August 2004 semester. They agreed to participate in the same and they answered a survey, a pre-test and a post-test. Students who dropped out of the course and those who did not take the tests and/or answer the survey were eliminated from the study.

There is no relationship ($r=0.19$) between the type of higher middle education (public, private or technical preparatory school) and achievement in the human anatomy course.

For second semester students who took histology and embryology in the previous semester, it was observed that 89.64% of them credited at least one of the two subjects, and 67.24 % credited both of them, while those who did not credit either of them made up 10.34 %. A weak positive correlation ($r=0.43$) was found between the academic situation (passing-failing) in the subjects of histology and embryology and their achievement (grade) in the human gross anatomy course. Students who passed histology and embryology did not necessarily have higher grades in the anatomy course, while those who did not pass either of them tended to have lower achievement. For that reason, the academic situation in histology and embryology courses is not a factor for predicting achievement in the anatomy course.

P-8 EMBRYO-FETAL DEVELOPMENT BY ECHOGRAPHY.

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The embryology is an important subject not only in the student formation of Medicine and Surgery but in that of any student in relation to Health Sciences. However, the embryology is sometimes hard to understand for the pupil causing a formative lack which makes the comprehension difficult. The student feels unable to understand the main happenings which take place during the embryo development and also the base of the appearing of some congenital defects. In the last two decades have appeared considerable advances in obstetric ultrasonography. Nowadays, besides the bi-dimensional transvaginal echography it is possible to obtain three-dimensional images and even what it has been call 4D images or 3D images in movement. The clinical applications of ultrasound include confirmation of pregnancy and multiple gestation, estimation of gestational age, localization of placenta, monitoring of fetal wellbeing and is also useful in prenatal diagnosis and fetal therapy. The first-trimester fetal echography is accurate in detecting major structural abnormalities. Prenatal surgical therapy can also be offered to fetuses with simple anatomic defects that have predictably devastating developmental consequences. However, appropriate use of these new tools will not only provide information for better clinical assessment of fetal disease but also allows the student in an easier way to understand the more important anatomic modifications that take place in this development period and it is also useful for the knowledge of the abnormal changes which may cause pathologic alterations.

P-9 ANATOMICAL DISSECTION FOR PRE-GRADE STUDENTS: EXPERIENCE AFTER 12 YEARS OF PROGRAMMED COURSES.

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In our university, in general, not every student can practice anatomical dissection due to the low number of corpse donations. Thus, 12 years ago we began organizing standardized dissection and anatomical techniques courses during the month of July. This course is 50 hours long, distributed in 3 weeks, with classes during the morning, from 10:00 to 14:00. The number of students has varied from 20 to 32, depending on the number of the teachers collaborating each year. On the years on in which the number of applications exceeded the number of vacancies the selection has been based on the marks obtained during the year on the subjects related to Anatomy and Embryology. This courses were for free but nowadays it costs ≈ 180 (this prize includes a dissection kit, rubber gloves for every day and a diploma).

During this course the students either dissect an anatomical region or practice on different dissection techniques, as dyeing cerebral cuts, dyeing and transparentation on embryos or synthetic resin inclusion of anatomical preparations. The student is tutored all through the processes by a teacher who shows him or her how to use the medical instruments and all the steps he or she has to follow in order to correctly fulfil the work.

The purpose of these courses is multiple: 1) To introduce the interested students on the dissection techniques. 2) To provide them with dissection material for the annual practices. 3) To promote handling aptitudes, and to get used to the material and surgical techniques. 4) The compilation of photographic material for teaching and investigation purposes.

On the last day of the course the students answer a survey about the development, the contents and the experience during the course. The three most valued things are: 1) the relationship teacher-student, 2) the relationship among the students and 3) The increasing in knowledge.

The fact that the course did not have any other value than the personal experience and enrichment, on the first years, and the latter change to serve as official class hours on the last five years, has constituted a turning point, for on the first period the quality and interest of the students was obvious, while when the course implied the obtaining of recognizable hours of lessons, the attitude and quality of the projects has gone down.

A very difficult factor to value is how the experience on dissection can influence the latter tendency to choose surgical specialties or, on the contrary, to make the student realize that those are not the most suitable techniques for him or her.

P-10 GROSS-ANATOMY DISSECTIONS: A TOOL TOWARDS TO SELF-LEARNING.

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Traditionally, anatomical teaching is based on lectures and laboratory sessions, which include prosections, plastic models and radiological images. The students cope with the learning of Human Anatomy mostly by means of memoristic techniques. There are different opinions about the validity of dissections and/or prosections as good techniques for the learning of Anatomy. We think that the direct contact with the cadaver provides an excellent resource for both learning and teaching of Human Anatomy. For this reason, in both first and second years of the Medical curriculum of the UCLM we carry out a student based learning methodology, trying to increase time efficiency and the student's effort to learn. Students of Human Anatomy and Embryology disciplines have the possibility to perform two dissections in different anatomical regions without the teacher's help once finished their theoretical training. They must identify by themselves the anatomical structures pointed in a guide at the beginning of this activity, helped by appropriate books and atlases. When dissection is finished, the teacher evaluates it and answers the possible questions. We show here the results of a test answered by the students after the dissection procedure, in order to demonstrate the importance of such activities for Anatomy learning. In this work, we attempt to validate the efficiency of the student self-learning by means of these dissections, by comparing the results obtained for each dissection as well as the results obtained in first and second years.

P-11 GENERAL ANATOMY TEACHING PROCESS FOR ODONTOLOGY STUDENTS AT THE UNIVERSITY OF SEVILLE.

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Spanish Anatomical Society (SAE) has established general standards for the teaching of General Anatomy in Odontology. Here, we report our experience in the University of Seville where we made a study based on three anonymous surveys answered by a group of students of General Anatomy along the academic year 2003-04. We wanted to investigate several questions as: previous requirements with which the students arrive to our subject, their expectations before the beginning of the course, and the degree of satisfaction reached upon ending it, among others. The surveys were accomplished at the beginning, in the middle and, finally, at the end of the course and before the final exam. In good accordance with the answers given educational strategies were implemented to improve the assimilation of General Anatomy by the students. To conclude, we made a topic review taking into account the situation in other Spanish and European Universities in order to suggest some proposals to achieve that the content of our present academic program could be adapted to the new deals that implies the European credit system, and to reach the most complete training and academic formation of the future odontologists.

P-12 TEACHING CLINICAL ANATOMY TO SURGICAL INTERNS.

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We present our experience in the Department of Human Anatomy and Embryology of the University of Barcelona in teaching clinical anatomy for surgical interns.

Our teaching method consists in an introduction including a theoretical revision and a demonstration of prosection about the lesson to be given in each session. Most of the time of the course is dedicated to the anatomical dissection performed by the interns, and at the same time specialist doctors show them the relationship with the surgical approaches of the region. The interns are supplied with bibliography to induce them to look for information about surgical anatomy.

We also use complementary methods such as videos (*Acland's video atlas of human anatomy*) and videos that we have made ourselves.

The course lasts 250 hours: 100 theory and 150 practice.

P-13 THIEL'S METHOD AS SELECTED TECHNIQUE FOR TEACHING HUMAN SURGICAL ANATOMY.

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In the last few years, many selective surgical approaches, mainly supported by minimally invasive surgical

techniques, have been developed under the knowledge of a precise and detailed anatomy. Several studies on cadaveric specimens have shown to be very helpfully for surgery, and many others specialities (interventional radiology, endoscopy, oftalmology...); however, the traditional fixation of cadavers by means of formaline solutions does not allow the realization of different experimental surgical techniques, i.e. the gas injection into the peritoneal cavity during laparoscopic surgery. Attempting to get a better preservation of the anatomical structures in human cadavers, we have developed a technique based in a modification of Thiel's method. This technique showed some disadvantages, as deficient central nervous system fixation. The combination of intrarterial and intravenous fixation, as well as the combination with solutions of higher formaline concentrations, allow a better preservation of the human cadavers, mainly focussed for teaching in human surgical anatomy.

P-14 THE PROFESSIONAL PROFILE OF THE PROFESSOR OF HUMAN ANATOMY AND EMBRYOLOGY.

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The professional profiles of the teachers of Human Anatomy in the different degrees and diploma courses in which it appears as a syllabus subject vary widely.

The "Bolonia 2010" objective has made the restructuring of further education a subject of media debate. Some aspects of the changes are so far-reaching that they have not been allowed to transcend the walls of university.

An important part of the running of any project is the selection of the personnel who are going to start it up and implement it. This makes the criteria used in the selection of these personnel a key issue.

In this communication, we are going to present some ideas which we hope will lead to a debate within the SAE. We would particularly like to highlight these two ideas:

1.- Scientific societies, like the SAE, are the right forums in which to produce, through the process the members think fit, documents that set out the recommended professional profile for the different types of University teacher of Human Anatomy and Embryology envisaged in law. Any kind of guide to the career would be of great use to young people who are thinking about working in our field. It would provide those who are beginning work in the profession with, to coin a fashionable phrase, a "route map" of their future career. The Society could provide the criteria to define the "desirable profile" or the "minimum requirements" of a University teacher of Human Anatomy and Embryology.

2.- The configuration of Human Anatomy and Embryology as a professional speciality, a medical/non-clinical speciality like others in the Health field. To achieve this, the first thing would be to agree on a "speciality syllabus" which should contain the "list" of the things the "apprentice" would have to do, courses, training periods in laboratories and even in hospitals ("if they learn together, they will work together"). Access to an official training course could be made through an agreed series of preliminary studies. This training itinerary must be accredited and certified.

Systemic, Topographic and Clinical Anatomy

P-15 COMPARATIVE ANATOMY OF THE ANTERIOR PORTION (PARS NERVINA) OF THE JUGULAR FORAMEN IN HUMANS AND IN GREAT APES.

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In the present study the relation between the area of the anterior portion of the jugular foramina and the estimated size of the pharynx was compared in humans and in three species of great apes (orang-outang, gorilla and chimpanzee). It is our aim to find anatomic differences that may be related with the different phonatory capabilities in the groups studied. The jugular foramen, that is located between the occipital bone and the petrous portion of the temporal bone, is divided in an anterior and a posterior portion by a fibrous tract that runs between the intrajugular processes. The internal jugular vein runs through the posterior part as the continuation of the sigmoidal sinus. The glossopharyngeal, vagus and accessory nerves run through the anterior portion (pars nervina) together with the inferior petrosal sinus. As it is well known, the three mentioned nerves play a significant role in the innervation of the phonatory organs like the larynx, the pharynx and the soft palate. Our results show that humans have a larger area of the anterior portion of the jugular foramina in relation to the size of the pharynx compared to great apes. No significant differences were observed among the three species of great apes studied. This finding could imply a greater relative size of the glossopharyngeal, vagus and accessory nerves in humans, that could be related to the higher speed and precision of the muscles of the larynx, the pharynx and the soft palate that are needed for human speech. In addition, the relation between the area of the anterior portion of the jugular foramina and the size of the pharynx was studied in different species of hominids, showing that the members of the genus *Australopithecus* have values similar to great apes, while the members of the genus *Homo* have values similar to modern humans.

P-17 SECTIONAL ANATOMY OF THE EAR. INTRATEMPORAL SPACES. APPLICATION FOR THE UNDERSTANDING OF CAT AND NMR IMAGES.

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Understanding the elements forming the parts and the limits of the three portions of the ear within the temporal bone is difficult owing to their complexity, size, and orientation, and because they are deep structures surrounded by bone, are hard to dissect, and are not always approachable.

New technologies for the clinical exploration of the ear –nuclear magnetic resonance and computerised axial

tomography— allow these portions to be explored. The results obtained demand that clinicians must recycle their knowledge about structures that may have been forgotten, poorly understood or even overlooked. This implies considerable difficulties in the interpretation of images because some physicians may not have clear concepts as regards the spatial organisation of the ear.

With the objective of fostering a better understanding of the structures and spaces of the intratemporal ear in undergraduate students and offering a review of this issue in life-long training programs, we have performed an exhaustive study on the identification of the intratemporal structures and spaces of the ear, collecting multiple sagittal, horizontal and axial sections on the major axis of the petrous part. We complete these with oblique axial and horizontal sections, in many cases correlating them with CAT or NMR images similar to those used in clinical practice.

Part of this work is reflected in the poster, which is completed with a generous number of images summarizing the study.

P-18 INTERNAL EAR BY RM (3D FIESTA).

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The development of the new sequences in Magnetic Resonance (MR) allows establishing new protocols for the study of the internal ear and the internal auditory canal (IAC). All the studies were made in patients who had clinical symptomatology: tinnitus, hypoacusis... It was made in an equipment of MR Signa EXCITES 1.5TGE. The hi-res images strongly harnessed of the internal ear in T2 provide an optimal resistance between the nerves of the face acoustic package and the F.C.E. and between the bony and membranous labyrinth that contains perilympha. The importance of the sequence was shown 3D fiesta in the cribrate and valuation of congenital and inflammatory pathology. The obtaining of images 3D with high involution in T2 of the internal ear supposes a great advance in the study and evaluation of the pathology of the ear in this region. It allows a suitable valuation of the anatomical structures that compose it. It has the high sensitivity to discard and to diagnose pathology. It is of short duration and it does not need the administration of intravenous resistance. High-resolution MR imaging of the inner ear with a heavily T2-weighted 3D sequence: 3D FIESTA has been performed successfully at 1.5 T. Normally, the high signal from the perilymphatic and endolymphatic spaces is homogeneous and images of sufficient technical quality were obtained to enable the recognition of the and one-half turns of the cochlea, similarly, we could visualize the posterior labyrinth with the vestibule and the semicircular canals (lateral, superior, and posterior) joined by the crus commune. This approach allowed examine the fluid channels within the cochlea and separate demonstration of scala vestibuli and scala tympani, this is of particular relevance in the planning of cochlear implantation, in which detailed preoperative assessment of cochlear morphology is important. MR imaging also provides the ability to diagnose aplasia or hypoplasia of the vestibulocochlear and facial nerves. The cochlear nerve may be defined separately from the superior and inferior vestibular and

from the facial nerves within the IAC. 3D FIESTA is also useful in the correct diagnosis of congenital pathology such as malformation of the semicircular canals (as the lateral semicircular canal is the last to form embryologically, its malformation is one of the most common) isolated or in association with other inner ear anomalies: vestibular and utriculosaccular anomalies and cochlear dysplasias.

P-19 NASAL HOLE (*Foramina nasalia*) VARIABILITY IN A POPULATION FROM CASTILLA Y LEÓN.

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The nasal holes (*Foramina nasalia*) can allow vessels and/or nerves to go through. The holes of a vascular origin allow small veins or arteries to go through them. These are branches of the angular vessels, which connect the nasal cavities to the outside. If the holes have a nervous origin, they let terminal branches of the external nasal nerves, branches of the anterior ethmoidal nerves to go through, these nerves are going to innervate the skin on the back and sides of the nose up to the very tip. There can be one or more nasal nerves, and thus one or more nasal holes. When the holes are absent there is no vascular communication and the nerves have to pass between the inferior edge of the nasal bone and the lateral nose cartilage. The lack of references on osteology in the Spanish population makes it necessary for systematic studies to be carried out on these populations.

The nasal holes of 86 men and 55 women were studied. All of them with a known age (over 20 years) and coming from Palencia and Valladolid. They all belong to the osteological collection (665 crania) of the Museum of Anatomy of the University of Valladolid. From the 141 crania, a total of 268 nasal bones were studied. The deteriorated and pathological ones were rejected.

The mean was 2 holes present, the same for men and for women, being the range between 0 and 8 holes. The number of holes found more frequently is 1 for both sexes. The presence of only one hole is more frequently found in left bones, being especially significant in women. The presence of more than one hole predominates on the right side. The absence of holes has a similar frequency for both sexes (8.9%), having the bilateral absence a frequency of 2.1% (3 crania). No significant differences related to age have been found.

The number, arrangement and size of the nasal holes are intimately related to the degree of pre and postnatal development of certain soft structures, as the vessels and the nerves. The development of the vascular and nervous patterns is going to be influenced by external factors, mainly during the postnatal period, factors which are very difficult to control. Due to it, the variability of these nasal holes is considered to be a discontinuous, discrete, non metric, or epigenetic character.

There are not conspicuous sexual differences in the nasal region on humans; moreover, the nose has lost structural and functional importance over the millennia, the smell sense not being essential for us, the humans. On the other hand, the plastic and repairing surgery has been given a high profile during the last few years, being the

nose operations the most common ones. In these kinds of surgeries the surgeon has to know exactly the places where the vascular-nervous pedicles are located.

The obtained results are consistent with the ones Publisher for Danish and Japanese populations.

P-20 ANATOMIC DIFFERENCES IN CENTRAL CORNEAL THICKNESS, ANTERIOR CHAMBER DEPTH, LENS THICKNESS, VITREOUS CHAMBER DEPTH, AND AXIAL LENGTH BETWEEN NORMAL DRY EYES.

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OBJECTIVE: To quantify the anatomic differences in central corneal thickness, anterior ocular chamber depth, lens thickness, vitreous chamber depth, and ocular axial length between normal and dry eyes.

METHODS: The central corneal thickness, the ocular anterior chamber depth, the lens thickness, the vitreous chamber depth and the ocular axial length were measured in 70 normal subjects and in 58 subjects with dry eye. The central corneal thickness was measured with scanning-slit corneal topography while the ocular anterior chamber depth, the lens thickness, the vitreous chamber depth and the ocular axial length were measured with applanation ultrasound biometry.

RESULTS: The central corneal thickness was 0.558 ± 0.30 mm and 0.532 ± 0.34 mm in normal and dry eyes respectively ($p < 0.001$). The mean ocular anterior chamber depth was 3.17 ± 0.23 mm and 2.93 ± 0.35 mm in normal and dry eyes respectively ($p = 0.002$). The lens thickness was 4.49 ± 0.42 mm in the dry eye patients and 4.71 ± 0.32 mm in the normal subjects ($p = 0.022$). The vitreous chamber depth was 16.75 ± 1.75 mm and 15.54 ± 1.34 mm in normal and dry eyes respectively ($p = 0.001$). The ocular axial length was 24.58 ± 1.73 mm in normal subjects and 23.07 ± 1.48 mm in dry eye subjects ($p < 0.001$).

CONCLUSIONS: The quantitative ocular anatomy values are lower in dry eye subjects.

P-21 CORNEAL ANATOMICAL STUDY IN PATIENTS WITH BULLOUS KERATOPATHY SUBJECTED TO STRUMAL MICROPUNCTURE.

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The Bullous Keratopathy (BK) is one of the main indications for penetrating keratoplasty. However, a long waiting time before receiving this treatment beside that there is a group of patients in which this treatment is contraindicated has raised the development of alternative palliative therapies that treat the secondary effects of the epithelial damage observed in BK, like corneal persistent

erosions. One of these therapies is the anterior strumal micropuncture. The aim of our work was to study the anatomical changes induced by the anterior strumal micropuncture on the cornea of patients with BK and to research its mechanism of action.

We performed the anterior strumal micropuncture technique in 8 patients with BK: six of them were previously given a diagnosis of pseudophakic BK and 2 of them were phakic patients with secondary BK and endothelial dystrophy of Fuchs. Patients answer a questionnaire where they evaluated the severity of their ocular pain and they were subjected to penetrating keratoplasty in an average interval of eight months. In three of the cases we proceeded with a fixation of the receptor button with 10% glutaraldehyde, fixed in paraffin and dye with Hematoxyline-Eosine, then we studied subepithelial reaction and strumal neurovascularization in each of the epithelia.

Patients experienced a significant and stable improvement at least during the eight-months of pursuit: 7 of them presented a almost total reapplication of the corneal epithelium and one patient presented a reduction in the number of blisters. In this anatomopathological study of the serial slides of the corneal buttons we found different degrees of subepithelial fibrosis. In the places where microinjection was performed, we found ruptures on the Bowman's membrane and strumal fibrosis that affected the external third in each place of the puncture.

Strumal micropunctures have the advantage of decreasing the risk of bacteria keratitis and the appearance of corneal neurovascularization, which is a known risk factor of reject of penetrating keratoplasty.

The exact mechanism by which the epithelium sticks to the struma are still unknown.

P-22 ANALYTIC FUNCTION OF TRISOMIC CHILDREN'S PALATAL VAULT SURFACE.

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Metric studies have shown that lineal dimensions of palatal vaults are different in Down's syndrome (DS) individuals than in normal (N) controls. But, it is difficult to compare these numerical results because different points of reference were used in order to define their longitudinal parameters and their measuring techniques were different as well. So these conclusions were partially controversial with respect to palatal length and width. Also there are papers that show specific shapes for Down Syndrome patients, as the one called gothic palate and "u" or "w" palatal shapes in children.

In a former work adult's palate vault morphometry of patients with Down's syndrome was studied and compared to the normal population. The purpose of this study is to determine how the craniofacial morphology of individuals affected by the Down's syndrome diverges from healthy Spanish people in a child's population. In order to reach this purpose, the lineal dimensions (depth, width and maximum height) were obtained from dental casts and the morphometric shapes of the palatal vaults were determined. We used a moiré topography method which allows to found the lineal dimensions and the analytical function, $z=f(x,y)$, which is representative of palatal shape for Spanish patients of trisomy 21.

Moire topography is used for quantitative determination of palatal vaults shape of in Down's syndrome (DS) patients and control normal (N) groups from Spanish people. 40 patients with trisomy 21 (20 males and 20 females), aged from 9 to 11 years old, and 40 healthy controls (20 males and 20 females), aged from 9 to 11 years old, were studied. We found a characteristic palatal morphology associated to Down's syndrome, with different ratios for the three dimensions in relation to normal population.

P-23 **INFORMATIC APPLICATION FOR PROCESSING AND GEOMETRIC VISUALISATION OF ANATOMICAL-RADIOLOGICAL IMAGES OBTAINED USING DIFFERENT DIAGNOSTIC TECHNIQUES.**

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Amira is a powerful informatic tool for advanced visualisation, data analysis and the geometric reconstruction of anatomical structures, based on radiological images obtained using the customary diagnostic techniques, supporting DICOM (*Digital Imaging and Communications in Medicine*) formats.

Here we offer a three-dimensional reconstruction of the skull from Computed Tomography sections.

The processing of 3D images is supported by powerful automatic and interactive segmenting tools. The reconstruction of algorithms permits easy creation of polygonal models from segmented objects. It is possible to generate volumetric meshes that are appropriate for advanced simulations of finite elements.

The informatic application discussed here is able to execute, almost in real time, volumetric representations with large amounts of data: higher than 10 megabytes. The volumetric images thus generated can be combined with any type of polygonal representation.

Furthermore, Amira is able to compute anatomical-radiological sections of random orientation in 3D data, and it is possible to combine multiple sections.

Polygonal separation is another interesting feature of Amira and can be used to adaptively reduce the number of triangles in an exterior model.

Once the geometric image has been generated, it is possible to practice different user-specified sections on the plane and rotate the structure in any spatial plane. This means that it is possible to visualise the whole anatomical image in an integrated fashion.

The tool is available for the PC and UNIX environments and for PC stations based on Linux, permitting rapid visualisation and treatment of radiological images.

To conclude, Amira allows easy three-dimensional reconstruction, providing innovative and potent algorithms from the image processing and from the computational geometry. There is no doubt that this potent informatic application will allow the productivity of radiological images to be enhanced.

P-24 **CORRELATION BETWEEN THE RADIOLOGICAL ANATOMY FINDS AND CLINICAL EXPLORATION IN THE LUMBAR PAIN.**

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The low back pain is a problem of health today that originates a great number of consultations in primary care –is the second motive after the cold– and in urgencies. The family doctor has two methods to approach this problem. The first one is the correct physical exploration that east of a clear way to the reason that originates them, being able to discard of this form serious processes. The second one, as complementary exploration, is the study of anatomic parameters by X-ray photography, which though in the majority of the cases it is normal, in occasions there are detected alterations that orientate towards the reason of the clinic. These two explorations must allow the doctor to handle the backache and to value derivation to the specialized attention for the accomplishment of other tests.

We lead to end a patients' random systematical sampling that they came to the Service of Urgencies with lumbar pain, for four months. The number of quiet cases belonged to 30. We gathered the following variables: the age, the sex, the personal precedents, the time of evolution, the form of appearance and characteristics of the pain, the location and irradiation, and the activities that were aggravating it. We realize the physical exploration according to the guides of performance for primary care and, for the radiological exploration of the lumbosacral column; two projections were realized with the measurement of ten parameters.

The results of the present study showed than the 65-year-old major patients presented: osteoporosis ($p=0,002$), osteophytes ($p=0,018$; χ^2 test), and alterations in the articular process ($p=0,012$ test χ^2 test). Possibly more cases would present with lumbar spinal stenosis ($p=0,073$) and pedicle breaks ($p=0,059$). In them the radiological quality was the worst ($p=0,046$). The widespread pain might have minor presence of osteophytes ($p=0,096$). The low back pain of more than twelve weeks are associated with: osteoporosis ($p=0,016$), osteophytes ($p=0,06$) and process elements upset ($p=0,008$).

We conclude that though the accomplishment of X-ray photography's is not indispensable, we have found important relations between the clinical and the radiological information, which can be of great usefulness in the clinical daily practice.

P-25 **CLINICAL ANATOMY APPLIED TO REGIONAL ANESTHETIC TECHNIQUES.**

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Regional anaesthesia and plexus blockade are very useful in surgery on the extremities and have a lot of advantages for the patients. However, their use is not wide extended due to the high rate of failures because of the difficulty of the percutaneous approach of the nerves. Despite the use of the neurostimulation, which help to localize the nerves, these techniques require a good knowledge of the anatomical regions where the nerves pass through.

In our department we have made some specific preparations in order to organize courses of techniques on regional anaesthesia of plexus and peripheral nerves of the extremities. These preparations are focused in the areas where the anesthetic approaches are performed, so that, they permit to assay the different techniques and posteriorly verify where the tip of the needle is localized. They are specially designed to be able to remove, from superficial to profound, the different anatomical structures which the needle must pass through. The nerves and vessels keep their anatomical disposition and relations.

We have also made sections in special anatomical planes which help understanding the direction of the approaches of the nerves. They are also useful in the correlation with the ultrasound images, which have recently been introduced for the localization of the nerves.

During the course and to integrate the anatomical concepts with the functional and clinical aspects, there are anaesthesiologist and professors of anatomy who combine their knowledge.

P-26 DISTAL FOREARM TOURNIQUET WITH FOREARM BLOCK: A USEFUL TECHNIQUE FOR LONG-LASTING HAND SURGERY.

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INTRODUCTION AND OBJECTIVES: Tourniquet has been recently placed at the forearm for hand surgery, but when using local anesthesia or peripheral blocks, patients usually do not tolerate the tourniquet more than 20 minutes. To avoid this, and get a longer tourniquet tolerance, a technique which combines nerve blocks at the distal forearm with the tourniquet placed at the same level has been developed. The objective of this work is to test the usefulness of this technique in Dupuytren fasciectomy, an usual long-lasting hand surgery.

MATERIAL AND METHODS: A complete distal forearm block at 5 cms (3 finger breadths) proximal to the palmar wrist crease is performed with mepivacaine 2%. A 8-cm wide cuff is later set at two finger breadths proximal to the palmar wrist crease, and inflated 75 mmHg above systolic blood pressure. 13 patients scheduled to hand surgery lasting between 20 and 60 minutes underwent this techni-

que (Dupuytren fasciectomy in all cases). Pain during surgery, quality of ischemia and complications during surgery and 6 months after surgery were registered.

RESULTS: VAS pain (0-10) was rated as 0.89 at the surgical site and 0.61 at the tourniquet. A bloodless field was achieved in all cases. 2 cases required supplemental anesthesia at the beginning of surgery. No nervous or vascular complications were found at follow-up.

CONCLUSION: This is a simple, painless, safe and useful technique for hand surgery. It is mainly indicated for surgeries of the hand lasting between 20 and 60 minutes, when regional or general anesthesia are not suitable.

P-27 THE ANTEROLATERAL THIGH FLAP: ANATOMICAL VARIATIONS OF THE VASTUS LATERALIS NERVE.

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During the past 20 years, the neural anatomy of many flaps has been investigated, although no extensive studies have been reported yet on the anterolateral thigh (ALT) flap. The goal of this study was to describe the anatomical relation between the vastus lateralis nerve and the descending branch of lateral circumflex femoral artery supplying the anterolateral thigh flap. One hundred and fourteen anterolateral thigh flaps were elevated in 23 bilateral cadavers and 65 patients (three of them were bilateral) and the vastus lateralis nerve, descending branch of lateral circumflex femoral artery and the main perforator of the flap were dissected. For this purpose we proposed a classification of motor branches into three types. Type 1 : The nerve is medial to the descending branch of lateral circumflex femoral artery and cross it from medial to lateral distally to the connection of main perforator. Type 2 : The nerve is medial to the descending branch of lateral circumflex femoral artery (LCFA) and cross it from medial to lateral proximal and superficial to the connection of main perforator. Type 3 : The nerve is medial to the descending branch of LCFA and cross it from medial to lateral proximal and deep to the connection of main perforator.

MATERIAL AND METHODS: The flap dissections were made with a skin incision at 1 cm medial to the mark of intermuscular septum. The intermuscular septum was the line between the anterior superior iliac spine and the superolateral border of the patella. The perforating arteries were preoperative identified with a Doppler probe. The identification and dissection of perforating arteries was performed subfascially. The dissection of arteries was made under surgical loupe.

RESULTS: Our study shows that the type 1 was present in a 55%, the type 2 was present in a 35% and the type 3 was present in a 14% of the cases.

CONCLUSION: Variations between the localisation of the nerve to the perforating artery of the flap is high. Type 2 pedicular flap is necessary to sectioned the nerve in order to rotate the flap adequately.

P-28 ANATOMIC VARIATIONS OF CALCANEAN ARTICULAR FACETS.

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Knowledge of the anatomic-functional features of the different articulations forming the ankle joint is necessary to understand the fisiopathology, biomechanics and possible surgical procedures on the ankle and hindfoot. The existence of multiple morphological variations of the little articular facets which participate in these joints structure is particularly interesting in the composition of the talocalcaneonavicular articulation.

We have studied 1056 calcaneus belonging to the Hamann-Todd osteology collection at the Cleveland Museum of Natural History and 56 calcaneus belonging to the Anatomy Department at the Medical School of Murcia University, in order to find significant differences between different existing types of calcaneus, attending to the articular facets variations. "Bunning and Barnett classification of subtalar facet configuration" was used, distinguishing four types of joints, depending on the facets disposition: joined or separated (types A, Transitional, B and C).

We found significant differences between types B and Transitional of both collections. No significant differences were observed between the other types.

Significant differences found between Transitional and B may be explained by measurement subjective factors due to the erosion suffered by the bone along the time. Does the existence of these morphological variations question the enlargement surgery procedures of calcaneus by osteotomy?. In feet: 1.-Flat Foot in children. 2.-valgus and abducted in adults, secondary to an insufficiency of the posterior tibial-tendon.

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P-29 STATIC-DYNAMIC BEARING SYSTEM FOR LEG AND FOOT DISSECTION. A NEW DESIGN.

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At the Dissection Rooms of Human Anatomy University Departments there are a wide spread kind of bearing systems designed for lower limbs from cadaver. The purpose of such designs is fix, as well as possible, the sample

to be dissected in order to get the best accuracy and comfort during the dissection.

METHOD: We show the design and manufacturing process of a bearing-system for lower limb that was developed for the "I Curso Teórico-Práctico de Anatomía y Cirugía de Pie y Tobillo", hold in the "Universidad de Murcia" in January, 2005. A) The technical survey and the assemble of parts are showed. B) The practical performances are, also, showed.

CONCLUSIONS: The bearing system designed has been a great helpful tool to do the dissection and to get access to the surgical pathways of foot.

b) The bearing system has strength, ergonomical characteristics and versatility.

P-30 COMPARING FOUR DIFFERENT PROXIMAL SCIATIC APPROACHES FOR ANESTHESIA IN PEDIATRIC PATIENTS. A PRELIMINARY STUDY

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The sciatic nerve block provides optimal surgical anesthesia for foot and ankle surgery offering significant advantages in terms of postoperative analgesia. The proximal sciatic nerve approaches for anesthesia in pediatric patients are at the moment a strict impose of the approaches previously described in the adult patient. No specific sciatic approaches has been reported in the pediatric population. The aim of our study is to compare four different adult approaches in children undergoing foot and ankle surgery and over lower-extremity of three different new born cadavers.

METHODS: The data from 44 sciatic punctures of children between 6 months and 11 years, 6-52 kg of weight and 65-155 cm of height, scheduled for ankle or foot surgery are reported. All sciatic nerve blocks were performed using a nerve stimulator. After induction of general anesthesia the children was positioned in lateral modified Sims position and direction of the needle was inserted perpendicular to cutaneous plane in all cases. 12 Labat's classical approaches (L) were performed, based on the bony relationship of the posterior superior iliac spine and the greater trochanter; 12 Winnie modification of Labat's classical approaches (W), adding in an additional landmark, the sacral hiatus; 10 Casal's posterior sciatic approaches, based on the relationship of posterior iliac spine and the sacral hiatus; and 10 Rucci's sciatic approaches, adding to all of the forementioned references the ischial tuberosity. Presence or absence of muscular response, and details of the motor response, intensity of neurostimulation, depth of needle insertion, motor block and success of the technique were evaluated. We consider valid motor responses those distal over the foot, and invalid those proximal over the posterior aspect of the thigh and gluteus maximus. At the same time we evaluate the same approaches over 6 different new born cadavers in the university of our city. Distance from the needle to the nerve, and depth of the insertion were registered.

RESULTS: One tibial motor response was obtained in L, five in W, one in C and five in R. One peroneal response

in L, two in C and one in R. Only in one case combined motor response of tibial and peroneal was obtained in R. The rest of approaches obtained proximal motor responses or was absent. The depth of insertion of the needle was 5 ± 2 cm, 0,18 mA the minimal intensity of stimulation and 0,5 mA the maximum. Evaluating the same 4 different approaches in new born cadavers, only the Rucci's approach inserted the needle over the sciatic nerve in all cases, And Winnie's approach in all but one.

CONCLUSION: This study shows, in instance, that the Rucci's approach seems to be the most clinically successful approach and less needle depth must be required in the pediatric patient. The same results were confirmed in cadaver section. Perhaps, this approach uses a very large number of anatomical landmarks sometimes difficult to identify. The Labat's classical approach modified by Winnie shows similar success with bony landmarks easy to identify. The depth of insertion of the needle was 5 ± 2 cm, been necessary the use of 50-100 mm isolated needles for all approaches.

P-31 PEDICLED GRAFT OF THE NERVUS COMMUNICANS FIBULARIS. ANATOMICAL DESCRIPTION AND CLINICAL APPLICATION.

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End-to-end nerve repair using autografts is a standart technique in microsurgery. The environment around the nerve graft site is important. Scar tissue should be avoid as much as possible to provide a good blood supply to the graft site from healthy tissues, but this is not always possible. In some specific cases it is useful to employ vascularized nerve grafts. The technique is not easy and frequently is more aggressive than the standart free sural nerve graft. We present an anatomical description of the nervus comunicans fibularis around the posterolateral aspect of the knee and its microvascularization. Besides, we describe the surgical technique to use this nerve as a pedicled flap in order to improve the vascularization and regeneration of the nerve. Finally our clinical experience with this technique in fibular nerve lesions around the knee will be exposed.

P-32 SPECIFIC PATELLAR DISEASES. ANATOMOCLINICAL BASES.

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An in-depth study was first conducted on the anatomy of the patella and its relationship with the different structures that form the knee, as a basis for its clinical understanding. Diseases that solely involve the patella were then described, without taking into account involvement of the patella by secondary knee diseases.

After consulting the literature, these diseases were classified into four groups: patellar fracture, patellar

luxation, recurrent patellar luxation and patellar chondromalacia. For each group, an analysis was undertaken of the production mechanisms, predisposing factors, clinical symptoms and treatment, with special emphasis on physiotherapy.

The following conclusions were drawn from these analyses:

1.- The patella is a bone exposed to a large number of degenerative and traumatic diseases.

2.- There is a wide consensus among different authors except on the surgical approach to recurrent patellar luxation.

3.- All authors agree that a correct selection of surgical treatment is essential for a good recovery.

4.- A wide variety of techniques are available.

5.- The most important conclusion from the physiotherapeutic standpoint is that the intervention undergone by the patient must be known before manipulations are performed.

P-33 APPROXIMATION TO THE EVOLUTIVE PROCESS OF ANTERIOR CRUCIATE LIGAMENT AUTOGRAFT RECONSTRUCTION THROUGH EMG.

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INTRODUCTION AND OBJECTIVES: The electromyographer of surface is a device that gathers the electrical activity that is generated by the muscle. Its function is the neurophysiological evaluation of the activity neuromuscular.

The objective of our study is to value the increase of the potential of the action as a reflex of the improvement in the ability of contract of the muscle semitendinosus and intern vast, after the application of an early protocol of physiotherapy in patients which are operated of a torn ligament.

MATERIAL AND METHODS: The sample has been 8 sportsmen with torn anterior cruciated ligament. An electromyographic study was carried out in both inferior members of the muscle semitendinosus, registered in the one canal, and of the intern vast muscle in the 2 canal. On a first moment, in the injured member, we gather the activity of both muscles in the maximum isometric contraction of each one of them (stage 1). On a second moment, the activity of this muscles in function walking along a corridor of 12 metres was being gathered. Finally, we repeat the process with the healthy muscle. The study was carried out at 3 and 6 month after the operation.

RESULTS: The increase of the difference of potential, in microvolts, which is produced in the injured semitendinosus muscle at 3 and 6 month after intervention of the ligaments of anterior cruciated ligament, is of 12%. In the case of the intern vast muscle, the increase of activity is of 35%.

CONCLUSION: The rapid protocol of physiotherapy produces an increase of the muscular electrical activity which is measured in microvolts, objectived in the electromyographer of surface, therefore, it proves a recuperation in the semitendinosus muscle and in the intern vast muscle in patients which are operated of a ligament operation.

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P-34 AZYGOS LOBE. INCIDENCE AND CHEST X-RAY CORRELATION.

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Abnormal fissures and lobes of the lungs are common and usually insignificant. The azygos lobe consists of the upper right lung developing around the azygos vein. It begins during fetal life when the apical bronchus grows superiorly medial to the arch of the azygos vein instead of lateral to it. As a result, the azygos vein comes to finish at the bottom of a deep fissure in the superior lobe of the right lung, dragging along with it the parietal and visceral pleura; the vein remains in the substance of the lungs, but as the upper lobe develops inferiorly to superiorly, a double fold of visceral pleura (two layers of visceral pleura and two layers of parietal pleura) develops. The four layers of pleura are then known as the azygos fissure, and the lung tissue separated from the rest of the lung is known as the azygos lobe. The azygos vein may simulate a pulmonary mass that often alarms physicians not familiar with its appearance. We have reviewed 1,000 PA chest x-ray films founding 6 cases of azygos lobe. The chest x-ray films checked owned to inpatients and outpatients of the Ramón y Cajal Hospital in Madrid.

P-35 ANATOMIC VARIATIONS OF THE SUPRA AORTIC BODIES. STUDY VIA ANGIO-TC AND ANGIO-RM.

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In the current work we have achieved a valuation of the arrangement and morphology of the supra aortic bodies, using radiological methods of high technology, and comparing the results, with the ones observed in the classic anatomic literature, obtained by traditional anatomic dissection.

Tables and graphic representations of the frequency of apparition of the different variations of the supra aortic bodies in the radiological studies are included, and also a comparative study with the results of authors like Thomson Nizankosky or Sobota.

MATERIALAND METHOD:

Population:

900 individuals 400 females and 500 males studied by Angio-TC and Angio-Rm, valuating the disposition of the supra aortic bodies.

Machines used:

RN	Sonata	SIEMENS
	Signa 1,5 and LX	GE
TC Light speed ultra		GE

RESULTSAND CONCLUSIONS:

– The study achieved confirms that the results obtained by radiological techniques, are the same as the ones achieved by the classic dissection.

– The “typical” display of the supra aortic bodies is the one described classically as three bodies TBDC, ACI, ASI with a frequency superior to 80%.

– The mayor part of the variations respond to modifications of the left side of the aortic arch, being the TBDC the most stable.

– Variations can be observed depending on the number of bodies from 2 to 6, but there are also variations depending on the type of body studied.

P-36 COMPARATIVESTUDY OFTHE ANATOMIC VARIATIONS OF THE SUPRA AORTIC BODIES IN THE MASCULINE AND FEMENINE POPULATION EVALUATED VIAANGIO-TC AND ANGIO-RM.

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In the current work we have achieved a comparative study of the frequency of appearance of the anatomic variations of the supra aortic bodies in two populations, one of 500 men and another of 400 women, using radiological techniques of high technology like the Angio-Rm and the Angio-TC.

Tables of the frequency of apparition of the different variations of the supra aortic bodies in the radiological studies of both populations are included and also a comparative study and statistic valuation, by the T method of Student, of both populations.

MATERIALAND METHOD

Population:

900 individuals 400 females and 500 males studied by Angio-TC and Angio-Rm, valuating the disposition of the supra aortic bodies.

Machines used:

RN	Sonata	SIEMENS
	Signa 1,5 and LX	GE
TC Light speed ultra		GE

RESULTSAND CONCLUSIONS

The study achieved confirms by the application of the statistic T method of Student that “with a value >0.05, that there are no significant statistic differences between the results obtained in both populations.

P-37 ANATOMIC APPROACH TO THE BARO-REFLEX SYSTEM: CONTROL OF HIGH BLOOD PRESSURE.

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Since the work of Hering and De Castro in the beginning of the 20th century, there have been little advances in anatomy and physiology of the high blood pressure system which is the main cardiovascular risk factor. In this work we try to sintetize and update the knowledge about this complex system.

We have search in old references, phylsosal doctorate thesis and pubmed bibliography using the key words: sinus caroticum, hering nerve, high blood pressure, baroreflex.

The baroreflex arch begins at the sinus caroticum (mechanoreceptors) located in carotid bifurcation. It has two types of receptors. The afferent information runs up to the brain by the first extracranial root of glosopharyngeal nerve (nerve of Hering). These afferences are integrated at the nucleus of tractus solitarius at the dorsal medulla with the suprasegmentus information. There are connections with the C1 area of ventral rostral nuclei of the medulla (vascular tone pacemaker) just in front of nucleus ambiguus. From C1 leave efferences to the intermediolateral horn of spinal cord, from where there leave autonomic fibers to the blood vessels and suprarenal glands. There is also another efference from nucleus retrofacialis at the medulla to the heart via vagus nerve (Xcp).

This system works detecting high blood pressure at the baroreceptors of carotid bifurcation and buffering it by decreasing blood vessels tone and heart rate.

The baroreflex arch is a complex system which has a very important role in the control of high blood pressure. Further knowledge is needed to better understanding of its anatomy and physiology.

P-38 RELATION BETWEEN OCULAR MORPHOMETRY AND INTRAOCULAR PRESSURE.

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The measure of the intraocular pressure (IOP) is fundamental in the clinical practice and basing on her it is carried out decisive diagnoses in the valuation and the treatment of important ocular pathologies for what it is important a precise determination of their value. They have been carried out many studies in order to determine the factors or parameters oculars that could act like elements that influence in the variation of the values of the intraocular pressure. In this work we have studied the relationship between the intraocular pressure and the ocular morphometrics values such like the axial length, thickness of lens, depth of the anterior chamber, vitreous body and the corneal radius. So, we have analyzed the values of the intraocular pressure obtained by means no contact tonometry and the biometrics values obtained by means of an ultrascan biometer and in different refractive groups. This way we studied great part of the ocular parameters that are related, in order to determine the optical and physiological properties of the ocular system and to

determine the paper that they play in the variations of the intraocular pressure. We have checked in our results that, in groups refractive of middle and moderate myopia, the intraocular pressure is related with the increase of the corneal radius ($r=0.38$; $p=0.0001$). However, this relationship doesn't occur in the rest of the refractive considered groups (hyperopia, emmetropia and low myopia). Neither relationship between the pressure exists neither with the refractive error neither the axial length in spite of the fact that these last two parameters yes they are related to each other. Therefore, the relationship between the value of the ocular pressure and the corneal radius in the middle and moderate myopias it seems to be consequence of the structure biomechanics of the cornea. It doesn't seem that the rest of studied factors have a significant influence, such like the increase of the axial length or the refractive error. We believe therefore, that it would be necessary to keep in mind these results in the measures of the intraocular pressure.

P-39 CLINICAL SIGNIFICANCE OF OBTURATOR VESSEL VARIATIONS.

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Abdominal aortic bifurcation is found usually at L2-L3, originating more distally both internal and external iliac arteries. The obturator artery branches from the anterior stem of the internal iliac artery, but occasionally branches from another stem or from the external iliac artery.

The number of variations of Obturator artery origin, increases from 11.11% to 69%, according to different authors, and the origin from the external iliac artery is about 2.4%. There is no enough information about how the variant obturator artery divides after passing the obturator canal. Different studies have been done about terminal branches of external or internal iliac artery but there are a few data in the case of veins, that usually finished in the internal iliac vein. The existence of an abnormal obturator artery does not involve an abnormal obturator vein or vice versa.

In the case we show, both obturator artery as obturator vein branches, bilaterally, from iliac vessels. Obturator artery branches from the inferior epigastric artery and walk about 1/3 inner face of pubic arch and supplies the muscles obturator internus and rectus abdominis but after passing the obturator canal the obturator artery divides, as usual, into the anterior branch to the adductor muscles and the posterior branch to the hip joint.

Because of the frequency of obturator vessels variations, we think is very important to know its anatomical relations, not much referenced in textbooks of anatomy, respect to vessels or nerves such as inferior epigastric vessels, obturator nerve, inguinal ligament or femoral vessels. This anatomical relations are reviewed in several medical actuations on pubic or inguinal region, such as anterior surgical approach of the hip joint, pelvis fracture, repairing of femoral, inguinal and obturator hernias, lymfadenectomy of common iliac artery or external iliac artery, etc.

P-40 CONSEQUENCES OF REFLUX ON THE STRUCTURE AND THE CYTOSKELETAL PROTEINS OF VARICOSE SAPHE- NOUS VEINS.

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OBJECTIVES: To know if the presence of reflux in different segments of the great saphenous vein produces modifications in the structure, in the micro-vascularization or in the expression of cytoskeletal proteins of the venous wall.

MATERIAL AND METHODS: 42 sections of saphenous veins, obtained from 20 patients undergoing surgery for varicosities, were used in the study. Sectors with and without reflux were selected. To determine the sectors with reflux, the patients were under an ecodopler study before surgery. It was possible to locate the levels of venous sufficiency and insufficiency with the system. The sections obtained were processed for Weigert stain and immunohistological determination of von Willebrand factor, Actin and Desmin. The structure of the wall, the presence and distribution of cytoskeletal proteins and micro vessels were studied.

RESULTS: The sections with and without insufficiency did not show great differences in the same patient. The differences found were restricted to zones where there was a dilation of the venous wall. In those places of dilation there were decreased internal layers of smooth muscle cells when they were compared with other zones in the same section. The vasa-vasorum were located up to the circular smooth muscle cells in the media layer in the competent segments. When there was insufficiency, the vessels were up to the intimal layer. Only in the dilation zones there was a decrease in the desmin protein, which affected the smooth muscle cells of internal and circular layers.

CONCLUSIONS: In varicose veins there are structural modifications in the venous wall before reflux. After the reflux is produced, there are a lost of smooth muscle cells which belong to the layers nearer the venous lumen in the places with dilation.

P-41 THE RISK OF INJURY TO POSTERIOR CIRCUMFLEX HUMERAL ARTERY VARIATION. CLINICAL CONSEQUENCES.

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INTRODUCTION: The risk of injuring important anatomic structures of the proximal part of the humerus is possible in different techniques used. If these structures present an anatomic variation the risk could increase. In the shoulder the posterior circumflex humeral artery was an important artery. It supplied the posterior and middle parts of the deltoid muscle. Normally, this artery arises from the lateral border of the third part of axillary's artery. The aim of the study is present the variation of

posterior circumflex humeral artery for its clinical and surgical implications.

MATERIAL AND METHODS: In a woman's dissected cadaver we observed an unusual course of the posterior circumflex humeral artery.

RESULTS: In the woman's dissection the axillar nerve runs in the quadrilateral space but the posterior circumflex humeral right artery arises under the teres major muscle, medial to the long head of the triceps brachial muscle.

CONCLUSION: The course of the posterior circumflex humeral artery is important in order to prevent their damage in case of surgery involving this area or to correct others pathologies as for examples its compression.

Biomechanics

P-44 DEVELOPMENT AND ASSESSMENT OF A FINITE ELEMENT MODEL OF THE C3 ARTICULAR PROCESS.

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Articular processes (AP) of upper and middle cervical column have been involved in load weight-bearing functions. Currently, finite element (FE) analyses are used to assess the stress and strains upon bones. The aim of this study is to assess the role of cervical AP in supporting loads using a FE model to whom different load cases were applied. For this purpose, a highly-accurated FE model of the AP from the C3 vertebra was developed. The model was analysed under various load cases and boundary conditions simulating the load transmission in the C2/C3 joint in an effort to understand stress transmission in the trabecular bone tissue up to the cortical walls of the AP.

Anatomic data for the FE mesh geometry were acquired from a 25-years-old volunteer. C3 vertebra was scanned using CT scan at slice intervals of 1.5 mm; twenty-one consecutive scans were taken. The cross-sectional shapes of the transverse slices were digitised and stacked automatically to obtain a 3-D model of the C3 vertebra. AP was isolated and submitted to commercial FE software MSC. NASTRAN 2004 R2. The model used 4188 4-noded tetrahedra elements with 8210 nodes. Linear static materials were considered for bone. Young moduli values were 10000 and 450 MPa for cortical and trabecular bone respectively. Poisson's ratio was 0.41 for both.

A normal force was applied upon the whole articular surface. Even the boundary conditions designed, a zone of high stresses corresponding to the anterior wall of the superior articular process (SAP) was observed. When an uniaxial force was applied on the anterior one-third of the articular surface (AS), high peaks were recorded in the anterior wall of the SAP. On the other hand, uniaxial forces applied on the posterior one-third of the AS show a stress distribution in the posterior zone of SAP, inferior articular process and neural arch.

The results of the study give experimental support to a differential stress transmission within the C3 AP, depen-

ding of the joint movements executed. Some evidence is given that abnormal loads exerted upon the SAP could initiate the progression to osteoarthritis of the joint surfaces.

P-45 GAINS IN ISOKINETIC MUSCLE STRENGTH IN THE ELDERLY FOLLOWING SIX-MONTHS OF POWER TRAINING.

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Universidad de Alcalá.

The increase in the elderly population in recent decades has brought with it a significant increase in the economic and health care demands placed on the Spanish Health System. One of the most frequent conditions in this age group which is caused by the deterioration of the locomotor system is gonarthrosis. We thought that preventing an increase in this condition would improve the quality of life of the subjects and therefore decrease the need for medical assistance among this population.

We therefore conducted a study of the muscular strength provided by the flexor and extensor muscles of the knee joint with isokinetic muscular dynamometry in a healthy population of 14 men and 26 women between the ages of 52 and 75. The study was conducted before and after a six-month power training period. The variable in peak torque in the right knee was analysed at speeds of 60°/s and 120°/s. Our results showed significant increases in the peak torque in both sexes and at both speeds in both flexor and extensor muscles.

The conclusion reached is that muscular strength training leads to significant increases in the strength of the knee joint and therefore improves quality of life in the elderly.

P-46 THE BEHAVIOUR OF ISOKINETIC MUSCULAR STRENGTH IN THE ELBOW JOINT IN AN ELDERLY POPULATION SAMPLE FOLLOWING PHYSICAL TRAINING.

Carrascosa, J., Slocker, A., Gómez Martín, E., Aguado, S., Gómez Pellico, L.

Universidad de Alcalá.

Manual tasks among the elderly are indispensable from both a physical and psychological point of view to prevent disabilities which can deteriorate the quality of life and place a strain on the health care system. These manual tasks are only possible if the elbow muscle works properly. We conducted a study of the flexor-extensor muscles of the elbow both before and after a six-month training period of using isokinetic dynamometry (biodes). The sample was composed of a population of 40 healthy subjects (14 men and 26 women) between the ages of 52 and 75 on whom functional evaluations were conducted using isokinetic muscular dynamometry.

The study had a rigorous isokinetic protocol for the elbow joint, with shoulder alignment with 30° abduction and 45° flexion. The peak torque variables were analysed and the whole work at speeds of 60°/s and 120°/s for each extension and flexion movement.

The results showed increases in both peak torque and total work in the extensor muscle group of the elbow joint, although these results could not be demonstrated in the flexor muscle group. We concluded that power training exercises in the elderly are beneficial to this population, clearly improving both physical and mental aspects.

P-47 INCREASE IN THE ARTICULAR TRAJECTORY OF THE KNEE FOLLOWING A PERIOD OF PHYSICAL POWER TRAINING IN ELDERLY WOMEN.

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Universidad de Alcalá.

The debilitation of the knee joint with age is well known. Consequently, we thought that by specifically training the strength provided by the knee's flexor-extensor muscles would improve the articular position at the time of maximum mechanical solicitation, thus avoiding unnecessary overloading of certain articular points. We therefore conducted a study of the articular position at the time of maximum force on the knees of 26 healthy women between the ages of 52 and 75 using isokinetic dynamometry before and after a six-month period of physical power training.

This study statistically demonstrates that the peak torque angle is smaller 6 months after the power training, but only during extension. In other words, peak torque appears during the articular trajectory, which therefore translates into an improvement of the speed and quality of the muscular contraction of the knee in the extensor muscular group in women. These results could not be statistically demonstrated during flexion, perhaps because it is the weakest muscular group. In conclusion, the improvement of the articular position of the knee is accompanied by a significant increase in articular equilibrium and stability, decreasing reaction time and thus avoiding joint injuries and falls.

P-48 STRESS PATTERN IN CHILD'S FEET UNDER LOADING.

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In the bibliography there are not many studies about transmission of forces through child's feet that deal with the use of three-dimensional models in which the dimensions used are in line with a half-real model obtained from a children sample.

The true points of stress and the way the compression forces affect to the constituent elements of the plantar vault, in a global and a spatial point of view, are not well-known either. This question has a special importance because the footwear annexes the force transmission through the anatomic structures and because the shoe-manufacturers consider child and adult's footwear as homothetic images. Moreover, it is known that anthropometric mean measures of people change with the course of time, this makes necessary a periodic updating of the tables of variables that define child's feet.

The aim of this work is to define the nomogram of child's feet (right and left) for both genders, referred to three different ages: 5, 7 and 9 years-old.

In order to determinate the magnitudes that define the vault plantar morphometry it has been used an optic device of topographic moiré. With such device we obtain three-dimensional digital images and after we process the images obtained with a informatic program designed especially for this work, which permits to measure longitudinal and angular magnitudes, and the coordinates (x, y, z) of every point of foot surfaces. This permits to calculate the analytic function representatives of the former mentioned surfaces.

Later, using the mean values obtained, we make scale models to study their biomechanical behaviour with photoelastic interferometry. This way, systems of isoclinic fringes are employed to determine the orientations of principal stress axes. Also, singular points, including points of zero stress, are determined and we map injure risk areas of child's foot.

P-49 DISTAL PHALANX OF THE HALLUX: DISCRIMINANT FUNCTIONS AND UNIVARIATE METHODS FOR SEX DETERMINATION.

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Our aim was to obtain a reliable method for sex determination using the distal phalanx of the hallux when other useful bones, as the pelvic and cranial bones, are not available. Nine linear measurements and 3 angles of the distal phalanx of the hallux were taken in 47 amputated lower limbs. Demographic and pathological conditions of the sample were known by clinical history. With the univariate analysis we could detect statistically significant differences of means for 7 linear variables; values were higher in males. Fisher's stepwise discriminant analysis permitted to obtain an optimal discriminant function, which, with only 4 variables (total length, distal height, proximal transversal curved length and valgus deviation angle), correctly identified sex for 95.7% of the sample, either in matrix or jackknife classifications.

Musculoskeletal System: bone regeneration; arthritis; osteoporosis; muscular differentiation; muscular damage

P-50 BONE REGENERATION THROUGH THE APPLICATION OF P.R.P. AND BIO-OSS TO OSTEOPOROTIC RABBITS. A PILOT STUDY.

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In surgical treatments the need occasionally arises to regenerate bone in patients whose bone regeneration capacity is reduced, as occurs in osteoporosis. The object is either to correct an osseous defect or to obtain greater bone thickness to permit the placement of an implant. To do so, "biofill materials" are used, which often include bone growth stimulating factors. One of the most commonly used factors in odontostomatology due to the fact that it is easy to obtain and to use is P.R.P. (platelet-derived growth factor). In this study we assumed that filling an osseous cavity with an aloplastic biomaterial composed of bovine hydroxyapatite (Bio-Oss[®]) impregnated with P.R.P. would cause osseous growth similar to that obtained with the use of the biomaterial without the P.R.P.

To do so we used adult osteoporotic rabbits divided into two groups: (PRP+Bio-oss group and Bio-oss only group).

Through a sagittal incision, cavities measuring 1 cm in diameter were formed in the animals parietal bones which made it possible to stabilise two titanium cylinders, one on each side of the sagittal suture.

In the PRP+Bio-oss group the titanium cylinders were filled with P.R.P.+Bio-oss in the right parietal bone while that of the left was filled with PRP only. In the Bio-oss group, the titanium cylinder of the right parietal bone was filled with Bio-oss while the left side received no graft at all.

The animals were sacrificed four weeks alter the surgery and the study fragments were obtained. The samples were set in formol and included in methylmetacrilate.

Histomorphometry was performed, evaluating the bone area of the cavity created using MIP 4.1 image-analysing software.

The results showed that the combination of PRP+Bio-Oss achieves a higher percentage of osseous regeneration than when Bio-Oss is used alone, although the difference is not significant.

P-51 OSSEOUS RESPONSE TO DIFFERENT OSTEOPOROSIS TREATMENTS. A DENSITOMETRIC STUDY.

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We know that the loss of bone mineral density is associated with the advanced age and also with the most frequent and well-known alteration of osseous tissue: osteoporosis. There is a close relationship between the decrease in oestrogen which occurs in menopause and the higher frequency of osteoporosis in this group of women. Advanced age brings with it not only a decrease in oestrogen but also a decrease in the growth hormone (GH).

We assumed the variability of bone behaviour based on the influence of these factors. To evaluate it, we took a sample of Wistar female rats. We maintained a control group and ovariectomies were performed on the rest of the rats at three months of age. After 18 months, the ovariectomised rats were divided different groups: one group that received no treatment; another group to which subcutaneous GH was administered; a third group to which estradiol valerianate was administered subcutaneously and a fourth group which received a combination of the

two. To study the effects on the tibia, after 2.5 months of treatment the rats were sacrificed at the age of 24 months. The bone was extracted and dual photon x-ray absorptiometry was used to perform densitometry (DMO) on the proximal portion of the tibia including the metaphysary and proximal diaphysis region. Our results show DMO differences between the values of the control group and those of the untreated ovariectomised rats and between these and the ones that later received treatment. However, there are no statistically significant differences between the DMO of the three treated group. Finally, when comparing the values of the control group and the treated groups, the fact that no significant differences were found demonstrates that all of the treatments recovered the DMO in the previously ovariectomised rats to similar levels as those obtained in the control group of rats of the same age.

P-52 CLINICAL-HISTOLOGICAL ANALYSIS OF DIFFERENT MODELS OF GUIDED BONE REGENERATION PRIOR TO THE EMPLACEMENT OF DENTAL IMPLANTS. STUDY OF DIFFERENT BONE REGENERATION TECHNIQUES.

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Since the eighties, the successful replacement of lost natural teeth by means of analogous elements integrated into tissues (dental implants) has been one of the best clinical advances in dentistry. The introduction of dental implants as a therapeutic alternative to dental replacement has been documented in standard situations, with percentages of clinical success close to 100%. However, such success rates decrease in situations of bone deficit due to the development of periodontal disease and traumatic lesions, which lead to both tooth loss and loss of the alveolar bone and surrounding soft tissues, in turn eliciting a deficiency in the alveolar ridge and thereby hindering emplacement of the dental implant. In light of this, it has become necessary to obtain bone supports that will be sufficiently robust to guarantee the fixation of dental implants.

Different techniques have been implemented to achieve this, ranging from guided bone regeneration (GBR) to grafts of both autologous and allogenic bone (frozen, dehydrated and lyophilised).

More recently, evidence has appeared that GBR may be achieved, and even accelerated (Anitua, 2004), by using autologous growth factors in what is termed platelet-rich plasma (PRP) or growth factor-rich plasma (GFRP) and different techniques have been employed to achieve this. The aim of the present work was thus to compare the different bone regeneration techniques using lyophilised bone (2 cases), autologous bone (2 cases) plasma rich in GFRP or a combination thereof (20 cases). To accomplish this, we performed radiological and clinical studies in selected patients undergoing regenerative surgery, followed at different times by a biopsy and histological study to assess bone maturation. Although all the

cases studied developed bone that was suitable for implantation, the histopathological trend varied, since in the cases in which lyophilised bone was used fragments of this persisted at the time of biopsy, showing signs of decalcification with low cellular activity around them.

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P-53 REACTION OF BONE TISSUE AFTER LESION. A HISTOLOGICAL AND MORPHOMETRIC STUDY OF THE ALBINO RAT HINDLIMB.

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Osteoporosis related to age, menopause or disuse is one of the major problems in clinical medicine. Here we used a model of neurectomy, or compression of the sciatic nerve, in rats divided into four groups and sacrificed at 2,4,6,8,12,16 and 24 weeks, dissecting out the femur and tibia, whose diaphyses were studied after embedding in plastic, transversally sectioned, and stained using the Goldner and von Kossa techniques, then performing the corresponding histological and morphometric studies. From the histological point of view, there was a striking periosteal reaction associated with scant osteoclastic activity. The cortical initially displayed a large increase in intracortical porosity but, later on, this regressed and there was also an initially strong endomedullar osteoblastic activity, which also decreased with time. Morphometrically, the most relevant observation was a decrease in the femoral cortex area of the neurectomized part in comparison with the contralateral part.

Funded by MAT 2004- 016 54.

P-54 MESENCHYMAL STEM CELLS FROM SYNOVIAL MEMBRANE REPAIR IN VITRO ARTICULAR CARTILAGE OF OSTEOARTHRITIC PATIENTS. PRELIMINARY RESULTS.

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Mesenchymal stem cells (MSC) are defined as pluripotent progenitor cells with the ability to repair damaged tissues.

The purpose of this study is to determine if human MSC from synovial membrane repair cartilage of osteoarthritis patients.

Cells extracted from synovial membrane of osteoarthritic patients were positives for characteristics markers of

MSC (CD29, CD44, CD73, CD90, CD117, CD166, PAX-7 and STRO-1) and negatives for hematopoietic markers (CD34 and CD45). Cartilage discs of osteoarthritic patients were obtained at autopsy from femoral heads of 3 donors (mean 65 years). The discs were divided in two groups. In Group I, only culture media was deposited over the cartilage surface, while in Group II 200.000 MSC suspended in 10 µl of culture media were deposited over the surface of osteoarthritic cartilage discs treated with fibronectin (45 minutes at room temperature). Discs were incubated for 4 and 8 weeks at 37°C in a humidified atmosphere of 5 percent CO₂/95 percent air. Culture media consisted of chondrogenic medium with 33,3 ng/ml transforming growth factor (TGF)-beta3 and 10 ng/ml basic fibroblast growth factor (FGFb). Frozen sections were processed for standard histologic staining (hematoxylin-eosin, safranin O, Masson trichrome) and immunostaining with monoclonal antibodies against type I and II collagen, integrin subunit beta1, chondroitin-4-sulfate and keratan sulfate.

Repair was only observed in the cell-treated cartilages. After 4 culture weeks, the cartilage surface did not show consistent signs of repair. After 8 weeks culture the cartilage surface was covered by a neo-synthesized tissue that consisted of several layers of rounded cells. This tissue showed a high cellularity, a moderately regular surface, and zones of excellent integration with the native cartilage. Furthermore, the neo-synthesized tissue showed no staining for safranin O (indicating low content in proteoglycans). Immunostaining for type I collagen and characteristic markers of articular cartilage (type II collagen, chondroitin-4-sulfate, keratan sulfate and integrin subunit beta1) were positive.

These dates suggest that the MSC from synovial membrane of osteoarthritic patients can have the ability to repair osteoarthritic cartilage.

P-55 CORRELATIVE RADIOLOGICAL, HISTOLOGICAL AND MORPHOMETRIC STUDY OF HUMAN FEMUR HEADS.

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Currently, the two diseases with the greatest social and economic repercussions within the field of traumatology are arthrosis and osteoporosis. These pathologies mainly affect the proximal end of the femur. This load-bearing joint must adapt to the stress it must support through a complex system of trabecular bone.

The aim of the present study was to gain further insight into these two diseases, both histomorphometrically and radiologically, and compare the results of both analyses.

During 2000-2003, we collected 100 femur heads from patients receiving a hip replacement. From these samples, we obtained a central slice that included the hard core and the zone of the round ligament, embedding them in methacrylate. We then performed Goldner and von Kossa trichrome stainings and analysed -histomorphometrically- the osteoid area and the trabecular volume of the

histological sections. From the complete samples of femur head we obtained X-ray images for assessment of the Singh Index (degree of osteoporosis).

Significant differences ($p < 0.05$) were obtained regarding sex and pathology in the peripheral area of the osteoid, the trabecular bone volume, both central and peripheral, and the radiological Singh Index. Differences in the central trabecular volume and the Singh index were only found as regards the age variable.

Women, subjects who had broken a hip and older individuals showed a lower percentage of bone, less osteoid substance and a pathological Singh Index.

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P-56 ANTHROPOLOGICAL IDENTIFICATION OF THREE CASTILIAN PRINCES.

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The contents of the sarcophagus of the Castilian prince Don Alfonso was given to the Museum of Anatomy of the University of Valladolid in order to carry out an anthropological study.

The following results were obtained after proceeding to the separation of the different individuals, the making of an inventory of the remainings, the determining of the ages, the estimation of the sexes, the radiological studies, the histological study of the soft parts and the DNA studies:

INDIVIDUAL 1: According to the stages of dental eruption and the measurements of the long bones the age would be about 3 years old. All the bones show morphology absolutely compatible with normality. No alterations on the skin or subcutaneous tissue were observed. No proper material for a DNA study could be extracted.

INDIVIDUAL 2: Thanks to the good conservation of the orbital and nasal regions, and even counting on the retraction due to dehydration, a certain grade of hypertelorism was obvious. According to the stages of dental eruption and the measurements of the long bones the age would be about 2 years old. The ends of the long bones showed a certain grade of widening, being especially obvious on the distal ends of femurs and on the proximal ends of tibiae. In these same places the bone cortical was thinned. The scanner showed mummified ocular structures in the right socket. No alterations were found on the processed skin remainings. Only a tooth sample was suitable for DNA studies, and it confirmed the presence of the "Y" chromosome. The allele DSY389-II*27 revealed a slightly higher value in 3 consecutive experiments. The obtained haplotype had a low enough frequency to be used as a potential marker all throughout the descendant generations from king Sancho IV.

INDIVIDUAL 3: According to the stages of dental eruption and the measurements of the long bones the age would be about 10 months old. All the bones show morphology absolutely compatible with normality. No alterations on the skin were observed. No proper material for a DNA study could be extracted.

P-57 BONE COLLECTIONS OF THE MUSEUM OF ANATOMY OF THE UNIVERSITY OF VALLADOLID.

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The bone collection of the Museum of Anatomy of the University of Valladolid dates from 1856 when the studies in Medicine were re-established in Valladolid. In just a few years it already had hundreds of skulls and complete skeletons cleaned in the modern facilities and use almost exclusively for teaching purposes. Since the sixties two main factors influenced on the decreasing of the bone material: On the one hand the diminishing on corpses, and on the other hand the students overcrowding, which made the material to faster deteriorate.

The contents of these collections are the skeletons or parts of them. This way every vertebrate group is represented.

The unbuilt skeletons are mainly used as scientific collections, for studies in anatomy (human and animal), compared anatomy, anthropology, paleobiology, archeology and forensic osteology. In these kinds of collections a good dating of the specimens is very important and also the existence of type specimens. The skeletons built in the anatomical position are used in teaching collections or for exhibits. The osteological material constitutes a valuable source of information, for, apart from the morphostructure, it can provide genetic material, so important for modern studies on taxonomy, population ecology, molecular biology and forensic techniques. The optimum use of the human and animal material requires facilities and staff, and also the establishment of a biological material compilation net, signing agreements with cemeteries, zoos and fauna recovery centres.

Since 1987 a job of increasing the human osteology collection is being carried out. It has to main objectives, on the one hand to get quality material for the pregrade practical classes and on the other hand the establishment of a reference collection for anthropological research. There are at the moment 238 human complete skeletons with age, sex and origin known, 460 crania and also post-cranial bones.

Of recent creation is the collection for compared osteology, used mainly for research purposes, although it is also used for display and teaching purposes. There are, so far, 3,266 skeletons, including fish, amphibians, reptiles, birds and mammals. We would like to underline the order Primate, including 87 different species within the collection so far.

A good cataloguing, computerization and storage are fundamental in order to keep the specimens readily accessible for their examination.

Scientific examination on morphology is more and more demanded and an interinstitutional collaboration is needed in order to make a proper use of the specimens and to optimize the generated biological material, creating, apart from the bone collections, others of hides, eggs, organs, histological material and genetic material.

P-58 BIOMETRIC AND ORAL HEALTH DATA COMPARISON BETWEEN ARCHAEOLOGICAL AND MODERN IBERIAN POPULATIONS: THE "ENCANTADES DE MARTÍS" CAVE.

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The aim of this study is to describe the biometric data and the oral pathology signs present in the teeth and the osseous fragments found in the "Encantades de Martís" cave, compared with the data obtained from the present time population of the Iberian Peninsula. The cave was used as a burial site during 2500 years, from the Neolithic until the Roman Empire. The archaeological materials found in this cave were not, unfortunately, properly classified, making its study quite difficult. 4274 teeth have been identified from the 4325 teeth found, from which 3877 were definitive and 397 deciduous. Moreover, 774 osseous fragments, 170 maxillae and 604 mandibles, were studied.

75 permanent teeth were selected, following two criteria: dentary type and absence of deforming lesions and of abrasion. The teeth were measured for all dimensions (crown length, root length, total length, mesio-distal diameter, vestibulo-lingual diameter) and their crown morphological index, crown strength index and crown module, as well as the Bolton index were calculated. These data were compared with those previously obtained in modern populations of the Iberian peninsula.

The results obtained show that the archaeological population teeth were smaller than the teeth from the modern population. According with our previous results, some dental measurements are statistically correlated with the height of the individual. A comparative study of the length of the femoral bones stated that the individuals buried were significantly shorter than our present population. This could be the reason of the differences both in dental size and in dental volume indexes observed between the two populations.

The osseous fragments studied do not differ significantly in shape from those from the modern population. Size measurements have been limited by the fragmentation of the bone structures. Only a 1,64% of the teeth were affected by caries, situated in areas that suggest the concomitant presence of periodontal disease. Abrasion is a constant finding in the teeth, both the isolated and those still anchored in bone structures, either permanent or deciduous. These data indicate that the ancient inhabitants of the area were healthy, and that their diet was rich in fruits, vegetables and fiber, allowing a good self-cleaning of the teeth.

P-59 EXPRESSION OF MAJOR HISTOCOMPATIBILITY CLASS I ANTIGENS IS MODULATED BY P-GLYCOPROTEIN TRANSPORTER: IMPLICATIONS IN MUSCULAR DIFFERENTIATION.

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Muscle differentiation may be influenced by modulation of resistance genes expression (*mdr1*) which are able to induce changes of myosin and to generate the apparition of myofilamentous material which is considered a clear evidence of myogenic differentiation. On the other hand, the activity of this gene may be involved in the control of the MHC class I expression. This relationship could be explained by the homology found between the product of the *mdr1* gene, termed P-glycoprotein (P-gp), and the putative peptide transporters TAP1 and TAP2. Because of the amount of the MHC class I on the cell surface is related to the quantity and quality of peptides in the endoplasmic reticulum, transport of protein fragments from the cytosol to the endoplasmic reticulum by P-gp may modulate HLA expression. Interestingly, modulation of HLA class I antigens expression has also been related to the degree of differentiation in some cell types. Using embryonal rhabdomyosarcoma (RMS) samples and cell lines as an experimental model to study myogenic differentiation, we have examined the resistance mediated by *mdr1* and its correlation with the HLA class I expression. Analysis by PCR indicated that positive RMS to *mdr1* show higher HLA class I expression than those which were negative to *mdr1* PCR. To demonstrate if the *mdr1* gene expression in these cells modifies the MHC molecules expression, we generated two resistant RMS cell line (A-204-1 and 2) using actinomycin D. FACS analysis of both cell lines showed an increase of *mdr1* and HLA class I expression. Moreover, the blockade of P-gp in RMS cells using a non-toxic treatment with verapamil, caused a significant decrease in the HLA class I expression. Our results confirming that the product of *mdr1* modulates the expression of HLA class I antigens in RMS cells. However, further investigations will be necessary to determine the mechanism by which the development of MDR mediated by *mdr1* can modulate HLA class I expression. Our findings may be relevant to understand the myogenic differentiation process and may have some application in RMS immunotherapy against tumor-associated antigens presented by HLA class I molecules.

P-60 MUSCULAR DIFFERENTIATION PROCESS: ANALYSIS IN RHABDOMYOSARCOMA CELLS.

Prados, J.¹, Melguizo, C.², Carrillo, E.¹, Vélez, C.², Caba, O.¹, Marchal, J.A.³, Boulaiz, H.³, Rodríguez, F.³, Martínez, A.³, Fernández, J.E.¹, Suárez, I.¹, Rama, A.R.¹, Álvarez, L.¹, Aránega, A.¹

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Understanding the molecular mechanisms that control muscle gene expression is an important requisite to develop therapeutic strategies that correct muscle dysfunction. Rhabdomyosarcoma (RMS) which derives from striated muscle, is an in vitro model to analyse the myogenic differentiation. Our analysis of these cells demonstrate that myogenic differentiation program involves to separate phases; a biochemical differentiation with the expression of nonmuscle and muscle specific gene and a morphological differentiation in which the mononucleated myoblasts fuse to form multinucleated myotubes. Treatment of RMS with DMSO results in an increase of desmin and a decrease of vimentin expression with a rapid accumulation of actin, tropomyosin and alpha-actinin. This structuration of the cytoskeleton is involved in modulating signal

transduction and in the morphological changes during cellular differentiation. At the same time, a biochemical modulation of the muscle isoenzyme of creatin kinase (CK-MM, MB and BB) expression happened. In this process, transcriptional factors that are expressed exclusively in skeletal muscle are decisives. So, our results show that a decrease in Id, a negative regulator of MyoD, induce the activation of muscle CK expression. However, MyoD family may not represent the only mechanism for activation of the myogenic program. These factors present an amino acid segment which shares significant homology with the members of the c-myc family. In RMS, we have observed modulation of c-myc expression during the process of myogenic differentiation whereas levels of *N-myc* showed no modulation. The exact mechanism through which these factors activate their target genes is unknown. On the other hand, using low concentrations of actinomycin D, we have obtained an ultrastructural myogenic differentiation in RMS cells that showed cytoplasmic projections, contact between cell membranes and formation of myotubes. By electron microscopic, RMS cells contained increased numbers of cytoplasmic organelles, lipid vesicles, intermediate filament and, the main indicator of complete myogenic differentiation, the presence of numerous myofilaments which formed well-defined bundles (Z-bands). In conclusion, myogenesis follows a succession of developmental stages, each one characterized by distinct morphological events and patterns of gene expression. RMS cell lines are an in vitro model to analyse the process of myogenesis.

P-61 DETECTION OF MUSCLE DAMAGE BY THE DETERMINATION OF SERUM ALPHA-ACTIN LEVELS.

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Intense physical exercise is associated with the development of muscle injuries. Their severity and evolution are variable, depending on the intensity, duration, and type of exercise carried out. To date, enzymes (CK, LDH, MDA) and proteins (troponin and myoglobin) in serum have been used as markers of skeletal muscle damage. However, all currently available parameters present limitations in their sensitivity, specificity, and/or release-time, and they do not clearly reflect structural muscle damage. The skeletal muscle protein α -actinin was investigated in the serum of subjects with severe skeletal muscle damage in order to assess its utility as a reliable predictive marker of muscle contractile dysfunctions.

METHODS: Serum samples were obtained from 33 healthy controls and 33 patients with serious skeletal muscle damage, defined by a total CK value of > 500 IU/L (Rosalki method). Troponin I, T and myoglobin concentrations were determined by immunoassay and α -actinin concentrations by Western blot and densitometry.

RESULTS: The mean serum concentration of α -actinin in controls and patients with skeletal muscle damage was 600.9 and 1968.51 ng/mL, respectively, a statistically significant difference. The sera of the patients with muscle

damage showed higher concentrations of α -actin than of troponin or myoglobin. No significant difference in troponin I levels was observed between the groups.

CONCLUSIONS: According to these results, α -actin was the most significant skeletal muscle damage marker analyzed and may be an ideal candidate for the early detection of skeletal muscle damage in sportspeople.

P-62 MONOLAYER CELL CULTURE AND GENERATION OF AUTO-INDUCED TISSUE FROM HUMAN HYALINE AND FIBROUS CARTILAGE SAMPLES

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In synovial joints, confronted articular surfaces of bones are coated by hyaline cartilage. Hyaline articular cartilage is avascular, alymphatic, and aneural, and its nutrition mainly depends on the synovial fluid. The only cells present in hyaline cartilage are chondrocytes, which are responsible for the synthesis and maintenance of the extracellular matrix.

Joint cartilage differs from other tissues in its response to injury. Due to its avascularity, the natural healing capability is poor. Although there have been many attempts to treat cartilage injuries, no treatment has produced long-lasting hyaline cartilage.

Autologous chondrocyte implantation (ACI) is a form of tissue engineering that is being increasingly used to treat damaged articular cartilage. The success of ACI depends to a large extent in the proper tissue differentiation, the architecture of the final tissue, the mechanical properties and its integration in the recipient tissue.

In the present work, fragments of human meniscus and joint cartilage were obtained by means arthroscopic methodology as result of the treatment of diverse knee pathologies. Tissues were disgregated by collagenase and pronase and then cells were cultured in monolayer in order to expand cell population. Because three-dimensional structure is key in the development of the morphofunctional cellular pattern, we centrifuged cultured cells allowing the formation of a fine cellular lamina in the flat bottom of a tube constituting, in this way, an artificial three-dimensional tissue.

After different growing periods, either monolayer cultures or laminas were fixed in 4% paraformaldehyde and stained with Masson's trichrome and safranin O techniques in order to analyze the cell characteristics and the extracellular matrix production. The presence of hyaline cartilage markers Col-2 and Sox-9 was analyzed by means immunohistochemistry. Different cell morphologies and grades of intracellular secretion were observed in monolayer cultures depending on the cells source: meniscus or joint cartilage. Laminas displayed a compact disposition, adequate cell viability and diverse cellular patterns from fibroblastic to more chondrocytic.

Laminas constitute a first step in the generation of an implantable tissue that can potentially restore damaged hyaline cartilage; next steps include the use of biopolymers and the addition of specific growth factors in the culture medium.

Cancer, Proliferation, Apoptosis

P-63 PROGNOSTIC VALUE OF RT-PCR TYROSINASE DETECTION IN PERIPHERAL BLOOD OF MELANOMA PATIENTS.

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The prognosis for malignant melanoma (MM) has been related to different factors such as tumor thickness, clinical stage. The risk of metastasis has been related to the presence of tumoral cells in peripheral blood.

For detecting tumour cells in melanoma patients reverse transcriptase-polymerase chain reaction (RT-PCR) has been used to identify tyrosinase, a key enzyme in melanin biosynthesis in the circulation of patients with disseminated disease.

We report results of the detection of tyrosinase by RT-PCR in peripheral blood samples from 58 MM patients with all clinical stages (I, II, III and IV). The aim of this study was to determine the clinical relevance of this procedure, exploring the relationship between presence of tyrosinase in peripheral blood of MM patients and their clinical stage and prognosis.

Blood samples were obtained from 58 consecutively selected MM patients at time of primary tumour diagnosis or at relapse. All stage I-IV patients were visited every 4 months during the first 2 years after the diagnosis and every 6 months thereafter.

We used RT-PCR assay to detect tyrosinase mRNA in the patients with MM. Atwo-tailed Fischer's exact test was used to assess the association between RT-PCR results and clinical stage. Relationships among RT-PCR results, clinical stage and overall survival were evaluated in all patients.

The results of the RT-PCR assay for tyrosinase were related to two prognostic markers used to evaluate these tumors: clinical stage and thickness. Positive PCR results were more frequent in primary tumors measuring > 4mm (83%) than in thinner tumors (1.1-4.0mm, 74%; ≤ 1.0 mm, 23%) (p= 0.005).

After a median follow-up of 24 months (range, 3-48 months), statistically significant correlation between RT-PCR results and recurrence (p<0.05) and clinical stage III (p<0.05) was found. Analysis of stage III tumours alone to determine the prognostic value of melanoma cell presence in peripheral blood found 24-month overall survival to be 70% in the RT-PCR negative group versus 10% in the positive group (p<0.02). The prognostic value of the detection of circulating melanoma cells may be especially relevant in stage III patients, whom the RT-PCR positivity seems to define a group of patients with a high risk of recurrence.

P-64 DETECTION OF CIRCULATING TUMOR CELLS IN PATIENTS WITH MELANOMA BY RT-PCR: CORRELATION WITH CLINICAL STAGE, TUMOR THICKNESS AND HISTOLOGICALTYPE.

Carrillo, E.¹, Prados, J.¹, Marchal, J.A.², Boulaiz, H.², Vélez, C.³, Melguizo, C.³, Rodríguez-Serrano, F.², Martínez, A.², Caba, O.¹, Fernández, J.E.¹, Suárez, I.¹, Alvarez, L.¹, Aránega, A.¹

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For detecting tumour cells in melanoma patients reverse transcriptase-polymerase chain reaction (RT-PCR) has been used to identify tyrosinase, a key enzyme in melanin biosynthesis, in the circulation of patients with disseminated disease. The tyrosinase gene is actively expressed only in melanocytes cannot be detected in the circulation, the detection of tyrosinase RNA indicates the presence of melanoma cells.

The prognosis for malignant melanoma (MM) has been related to different factors such as tumor thickness, invasion level and histological tumor type although these features are of limited value.

We studied the detection of melanoma cells using RT-PCR in the peripheral blood of 58 patients with MM. Healthy subjects or non melanoma patients were used as negative controls. As a positive control, we used a lymph node metastasis from a patient with MM.

Blood sample were collected in tubes containing edetic acid (EDTA), was centrifuged and the plasma was discarded of the serum. Our primers ME-1 (5' TTT 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. α -actin primers spanning an intron were devised from the gene and used to check the integrity of the RNA samples for RT-PCR.

The primers were 5' ATC ATG TTT GAG ACC TTC AA3' and 5' CAT CTC TTG CTC GAAGTC CA3', produce a 248 pb fragment of α -actin. We used RT-PCR assay to detect tyrosinase mRNA in the patients affected of melanoma. For statistical evaluation the Pearson χ^2 test and Mann-Whitney test was used to correlate the PCR results with clinical stage, tumor thickness and histological type.

We tested peripheral blood samples from 58 patients with MM in different stage for the presence of tyrosinase transcripts by clinical stage the distribution was the primary tumor was classified as nodular melanoma (NM, n=19), superficial spreading melanoma (SSM, n=30), acrolentiginous melanoma (ALM, n=7) or lentigo maligna melanoma (LMM, n=2). No tyrosinase mRNA was detected in any of the healthy control patients and with other malignancies.

The results of the RT-PCR assay for tyrosinase were related to two prognostic markers typically used to evaluate these tumors: clinical stage and thickness. Positive PCR results were more frequent in primary tumors measuring > 4mm (83%) than in thinner tumors (1.1-4.0mm, 74%; < 1.0 mm, 23%) (p=0.005). No statistical correlation was found between the PCR results and histological appearance of the primary tumor.

P-65 MORPHOLOGICAL CHANGES, MODULATION OF CELL CYCLE, AND APOPTOSIS IN HUMAN BREAST CANCER INDUCED BY A NOVEL HIGHLY LIPOPHILIC 5-FLUOROURACIL DERIVATIVE.

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Breast cancer is among the most common tumours affecting women. The morbidity and mortality rates for this type of cancer have prompted a search for novel therapeutic strategies, such as the use of novel antitumour drugs that are less toxic to normal tissues and more specific to malignant cells. The fluoropyrimidines, especially 5-fluorouracil (5-FU), are antimetabolite inhibitors of de novo purine and pyrimidine syntheses. They have played an important role in standard chemotherapy protocols for a range of solid tumours, including breast and colorectal cancers. The antitumour activity of 5-FU is dependent on the ability of the drug to bind and inactivate the enzyme thymidylate synthase (TS). However, the intravenous administration of 5-FU in patients with metastatic breast cancer is highly toxic to normal cells and present major toxicities that include mucositis and hand-foot syndrome. One approach to overcome this toxicity of 5-FU is to construct prodrugs with lower hydrophobicity and cytotoxicity, preferentially activated in cancer cells.

The research reported investigates antitumour activity, the cell cycle arrest and apoptotic properties of novel lipophilic benzene-fused seven-membered 5-fluorouracil (5-FU) analogs in comparison to 5-FU on MCF-7 human breast cancer cells.

In the present investigation we demonstrate the high antitumour properties of three novel lipophilic benzene-fused seven-membered 5-FU analogs (ESB-786B, ESB-252A and ESB-928B) by apoptosis induction and cell cycle arrest in MCF-7 breast cancer cells.

Our results indicate a dose-dependent activity against MCF-7 and IC₅₀ values in the low micromolar range. The novel derivatives slow down the cell cycle progression, as indicated by the decrease in the %S and the increase in the %G₀/G₁. Moreover, because a larger proportion of cells (> 60%) is recruited into early apoptosis and DNA strand breaks are produced in the MCF-7 cells, a cell line where the induction of DNA fragmentation is very difficult, we suggest that novel 5-FU derivatives can be considered as specific apoptotic inducers. These experimental findings provide evidence of specific antitumour activity of these new substances having the 5-FU moiety and warrant further evaluation in *in vivo* models of breast cancer for future clinical applications.

P-66 BONE ANATOMICAL LOCALIZATIONS OF THE METASTASES IN BREAST CANCER.

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The bone invasion by a malignant tumour is due, with a lot more frequency, to the metastasis than to the primary tumours, being the breast cancer one of the tumours that with more frequency metastasize in the bone.

The metastases dissemination of the breast cancer can occur in several organs, but the bone is one of the most frequent localizations, which occurs in a 73% of patients.

The importance of the metastases localization in the bone is basically the pain and the pathological fractures, in addition to other metabolic complications as the increase of calcium.

The morphologic standard of these injuries can be lytic, blastic or mixture.

The diagnosis of the bone metastasis is done by x-ray being complementary the explorations with simple Rx, TAC and RM, also the bone scan with technetium 99 can detect more precociously injuries and direct towards the injured areas the selective x-ray studies. When these signals are unique they are not always metastasis and therefore force a diagnosis with other techniques and even a biopsy. However if the isotopic receptions is multiple is highly suspicious of metastases.

Within the skeleton regions more affected are the spinal column (88%), the pelvis (64%), the skull (43%), the ribs (46%), and the inferior (39%) and superior (17%) limbs.

In the limbs, the outstanding localizations are the long proximal bones (femur and humerus), being the distal regions very sporadically affected.

In different series the frequency of metastases in the bones of the carpal or of the foot is very low and some times are confused the symptoms with other pathologies.

We present the skeleton regions more frequently affected in metastatic breast cancer and two unusual metastasis cases in the astragalus, calcaneus and metatarsal. Both patients had other areas of metastases localizations in spinal column and pelvis. Both received palliative antialgic treatment with radiotherapy with very good local response.

When painful distal exists in patients affected by breast cancer, we can not exclude the presence of metastases; therefore we will have to do radiological studies to exclude this localization.

P-67 APOPTOSIS AS TARGET IN HUMAN GENE THERAPY: MORPHOLOGICAL CHANGES AND MODULATION OF GROWTH MARKER EXPRESSION INDUCED BY GEF GENE IN BREAST CANCER CELLLINE.

Boulaiz, H.¹, Prados, J.², Marchal, J.A.¹, Melguizo, C.³, Carrillo, E.², Martínez, A.¹, Rodríguez, F.¹, Caba, O.², Vélez, C.³, Álvarez, L.², Fernández, J.M.², Aránega, A.²

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Nowadays numerous research fronts exist in the fight against cancer that attempt to overcome the main obstacles of standard therapeutic approaches. New therapeutic strategies are required to overcome the limitations of conventional breast cancer treatment. Suicide gene therapy offers a potential approach to this type of tumour, since systems based on the use of prodrugs may present some drawbacks related to toxicity, drug release and bioavailability. This lead to the development of strategies based on

the use of toxic genes that do not need a prodrug to be effective in tumor cells. The *gef* gene has cell-killing functions in *Escherichia coli* and does not depend on the use of a prodrug for its action, making it an attractive target for suicide gene therapy.

We created a *gef*-overexpressing human breast cancer cell line (MCF-7TG) by transfecting the *gef* gene under the control of a pMAMneo promotor. Dexamethasone-induction of *gef* gene expression in MCF-7TG cells produced a significant decrease in Ki-67 expression, which is a known proliferation marker. In addition, Annexin-V-FITC and propidium iodide assays showed the presence of apoptotic cell death, which was confirmed by scanning electron microscopy. The most significant finding was the presence of "craters" in the cell membrane, as previously described in other apoptotic breast cancer cells.

In conclusion, the present data demonstrate that the induction of *gef* gene expression in breast cancer MCF-7 cells induces a decrease in the expression of Ki-67, an antigen related with the proliferation rate of these tumour cells, and produces a process of cell apoptosis characterized by major modifications in the cell morphology. Although the mechanism has yet to be established and further research is required, these results suggest that the *gef* gene is a potential candidate for novel gene therapy strategies against breast cancer cells. This approach would be simpler than other suicide gene therapy model systems because no prodrug is required. Furthermore, the use of selective transcriptional control sequences of *gef* may offer the gene therapist additional tools of great potential.

P-68 DIFFERENTIATION OF THE INTESTINAL EPITHELIUM MEDIATED BY EXOGENOUS NUCLEOSIDES.

Rodríguez-Serrano, F.¹, Marchal, J.A.¹, Boulaiz, H.¹, Caba, O.², Martínez, A.¹, Prados, J.², Carrillo, E.², Suárez, I.², Melguizo, C.³, Vélez, C.³, Fernández, J.E.², Ríos, A.², Aránega, A.²

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In the Lieberkühn intestinal crypts, it's carried out a cellular dynamic process that allows the epithelial renovation. The progenitors cells, derived from stem cells located in the base of the crypts, migrate towards the intestinal villus. During this course takes place the differentiation, morphologic and functionally, that it reaches his maximum degree in the apex of the villus, where the cells are finally flaked.

We present a study where we evaluate the effects that produce the exogenous nucleosides on the enterocytic differentiation. For it, intestinal epithelial cell line IEC-6 has been chosen, that is derived from rat jejunal crypts. The behavior of IEC-6 line in cell culture is resembled to which experience the enterocytes *in vivo*. These cells present initially undifferentiated phenotypic characteristic, but when they get cellular confluence begin to differentiate themselves, and finally they are flaked in the culture medium. The nucleosides were administered in two mixtures, constituted by thymidine (NTmixture) or uridine (NU mixture), together with guanosine, cytidine and inosine, at a final concentration of 100µM of each nucleoside.

The confluent cultures treated with the nucleosides mixtures, had a similar microscopic appearance, where

we could find structures characteristic of differentiated IEC-6 cells, whereas the control group, treated with a culture medium without nucleosides, lacked them. On the other hand, the villin expression, a structural marker of enterocytic differentiation, was bigger in both groups, NT ($7.17 \pm 0.65\%$, $p < 0.01$) and NU ($6.76 \pm 0.59\%$, $p < 0.01$), regarding the control group ($2.76 \pm 0.27\%$). These results of the marker expression were corroborated by Western Blot. Finally, the quantification of the total protein amount of the different cultures, gave a bigger amount in the groups NT ($128 \pm 4\%$, $p < 0.01$) and NU ($136 \pm 9\%$, $p < 0.01$), respecting to the control group ($100 \pm 5\%$).

As a conclusion, the exogenous nucleosides stimulate the differentiation of intestinal epithelial IEC-6 cells. In addition, the behavior of the used mixtures, NT and NU, is similar in this process.

P-69 ACTION OF EXOGENOUS NUCLEOSIDES ON INTESTINAL EPITHELIAL PROLIFERATION.

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Several studies point out to the exogenous nucleosides as important elements for the normal development and gastrointestinal maintenance. Thus, the absence in the diet of these compounds has been related with a height reduction of the intestinal villus, a smaller content of RNA, DNA and smaller rates of protein synthesis.

The objective of our study is focused in evaluating the effect of two nucleosides mixtures, constituted by thymidine (NT mixture) or uridine (NU mixture), together with guanosine, cytidine and inosine, on the proliferation of an established intestinal epithelial cell line denominated IEC-6, coming from rat jejunal crypt.

Paradoxically, our results show deep differences between both mixtures, on the proliferation of IEC-6 cells, at a final concentration of $100 \mu\text{M}$ of each nucleoside. The NT mixture, increased the cell content of the cultures over a 51.89% with respect to the control without nucleosides ($p < 0.01$), whereas NU mixture reduced the proliferative capacity over a 25.39% ($p < 0.01$). The effects produced by the NT mixture could be associated to a reduction of the percentage of cells located in the synthesis phase and to an increase in phase G2-M of the cell cycle. On the other hand, the antiproliferative action of uridine in NU mixture, didn't associate neither to morphologic changes, nor to variations in the cell cycle phases, nor to a modification of the viability levels, necrosis or apoptosis, nor to the acquisition of a bigger degree of cellular differentiation.

These data indicate that intestinal epithelium is sensitive to the presence of exogenous nucleosides, being able to produce an increase of the cellular proliferation. However, the consideration of the uridine at $100 \mu\text{M}$ as a situation of physiological range seems an error, reason why in ulterior assays it must be considered the administration of a lesser amount of the nucleoside.

P-70 THE PERITONEAL MESOTHELIUM COVERING THE INFUNDIBULUM IN THE SOW: ASCANNING ELECTRON MICROSCOPY STUDY.

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This study was undertaken to describe the surface features of the peritoneal mesothelium covering the infundibulum and the transition between oviductal and mesothelial epithelium in the sow, and to compare them with those previously described in the cow (Yániz et al, 2000). The external infundibulum of 12 cyclic sows was examined by scanning electron microscopy. Tissue specimens 0.5 to 1 cm² were taken from the infundibulum, at a point adjacent to the free margin and another 1-2 cm distal. The pieces were fixed in 2.5% glutaraldehyde, postfixed in 1% osmium tetroxide, dehydrated and subjected to critical-point drying using liquid CO₂ substitution. The dried specimens were coated with gold and examined and photographed in a Zeiss DSM 940 scanning electron microscope at 15 kV. As in the cow, the oviductal mucosa exceeded the free margin in the external side of the infundibulum, forming a continuous band measuring 0.25 to 1 cm in width. This oviductal epithelium showed cyclical variations with a predominance of ciliated cells during the follicular phase. Differences with the cow include the transition between the mucosa and serosa, which was abrupt in the cow and gradual in the sow, with oviductal epithelium penetrating between mesothelial cells, both individually or in groups forming islets. Mesothelial cells showed also morphological peculiarities in the sow: they were flat or slightly prominent and were covered either by short microvilli with low density, allowing observation of cell plasma membrane, or by a dense mat of medium/long microvilli, making it difficult to appreciate the limits between cells. Additionally, the presence of solitary cilia in mesothelial cells was frequently observed in transitional areas. In the mesothelial side numerous bulbous processes on the exposed surface of mesothelial cells were frequently detected, suggesting a secretory-like activity. This work describes the peritoneal mesothelium covering the infundibulum in the sow, showing numerous peculiarities when compared with observations previously performed in the cow.

Embryology

P-71 PROTEOMIC ANALYSIS OF THE GALLUS GALLUS EMBRYO AT STAGES 13^{HH}, 21^{HH}, 29^{HH} AND 40^{HH} OF DEVELOPMENT.

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The chicken embryo is one of the most widely used experimental model organisms and has been for many years the most advanced model organism suitable for experimental embryology. Moreover, the chicken represents the model system which, permitting experimental intervention *in ovo*, most resembles other higher vertebrates. As such, it represents an important complement to mouse model systems (Gregory, C. *et al*, 1998).

Measuring gene expression at the protein level is potentially more informative than mRNA analysis. In contrast with the genome, which is essentially static, a proteome is highly dynamic. Processes such as differentiation, cell activation, disease or invasive infections can all significantly change the relative protein repertory.

In order to understand the molecular mechanisms underlying the normal and abnormal development of the chicken, we used two-dimensional electrophoresis (2-DE) to construct a proteome reference map of different stages of development (Hamburger, V. and Hamilton H. L. 1992) (Kawakami, Y. *et al*, 2003).

Proteins were separated by isoelectric focusing on immobilized pH gradient (IPG) strips, and by 11% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gels. Protein identification was done by peptide mass fingerprinting with matrix assisted laser desorption/ionization-time of flight-mass spectrometry (MALDI-TOF-MS) (Agudo *et al*, 2004).

In all, 4 stages of embryonic development were compared (stages 13, 21, 29 and 40), and were detected about 300 spots that appears in different stages, or in different amounts in each studied stage.

These maps will be update continuously and will serve as a reference database for several investigators, studying changes at the protein level under different physiological conditions. These results suggest that the proteomic approach is valuable for the study of the embryonic development.

P-72 BMF, A PROAPOPTOTIC "BH3-ONLY" PROTEIN IDENTIFIED DURING CHICKEN EMBRYOGENESIS.

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Apoptosis, a process critical for sculpting organs during development and ensuring homeostasis throughout life, has been conserved during evolution. The "BH3-only" proteins are proapoptotic members of the Bcl2-family that share with their relatives only the short BH3 domain (9-12 amino acids), which allows their interaction with prosurvival Bcl-2 family members to trigger apoptosis. Genetics experiments have demonstrated that they are essential for initiation of programmed cell death and stress-induced apoptosis and are highly conserved in species as distantly related as *C. elegans* and *H. sapiens*. However outside the mammals, just a few reports about "BH3-only" have been published. Recently, a draft genome sequence of *Gallus gallus* has been published, providing a new perspective on vertebrate genome evolution and consolidating the chicken as an important model organism that bridges the evolutionary gap between mammals and others vertebrates. In this context, we report here the identification and characterisation of BMF from *Gallus gallus*. The amino acid sequence of chicken BMF

show 61%, 43% and 60 % identity to human, mouse and rat BMF, respectively. The very high interspecies conservation of BH3 domain (100% identity) and DCL2-binding motif (100% identity) is consistent with an involvement of these regions in the functional activity. Exogenous expression of BMF caused apoptotic cell death in two different cell lines and mRNA^{BMF} expression was detected along all whole embryo and tissues at different stages of development. In addition, a novel isoform has been detected (sBMF: short BMF). The sBMF amino acid sequence was identical to the BMF except for a deletion of 75 aa (from 31 to 106 aa), including the DCL2-binding motif. The developmental expression pattern and the ability to induce apoptosis of this isoforms were also examined.

P-73 PROAPOPTOTIC BH3 ONLY BCL-2 FAMILY MEMBERS IN GALLUS GALLUS, A MODEL ORGANISM FOR INVESTIGATION OF EMBRYONIC DEVELOPMENT.

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Apoptosis is an evolutionary conserved process involved in proper embryonic development, tissue homeostasis and also in the pathogenesis of a variety of diseases. The Bcl-2 family is an important regulator of apoptosis that can be grouped into subfamilies of pro-survival and pro-apoptotic molecules. Inducers of apoptosis members include BH3-only proteins. These proteins have been suggested to play an important role in initiating mitochondria-mediated apoptosis. At least eight "BH3-only" have been identified in mammals, whereas in *C. elegans*, a single "BH3-only" protein, EGL-1, is required for the initiation of all developmentally programmed deaths. From the complexity of the mammals to the simplicity of the invertebrates, other organisms exist in which apoptosis mechanisms are clearly conserved. From an evolutionary standpoint, the chicken is well positioned model organism to provide an intermediate perspective to understand a process as complicated as apoptosis. The recent publication of a draft genome sequence of *Gallus gallus*, together with the accessibility of chick embryos, consolidating this organism as an important model mainly for development experimentation.

To identify chicken orthologs of mammalian BH3-only genes, we search at the TIGR Chicken genome database for sequences that encode conserved BH3 domain. The search revealed three different ORFs with a putative BH3 only region (70% homology with mammals BH3 domains). Two of them correspond to Bim and Bid human orthologs. The other one, named Bcr, correspond to a new BH3-only with no mammals orthologs. The proapoptotic activity of chicken BH3-only proteins was demonstrated by rapid induction of apoptosis after transiently transfection in HeLa and MCF-7 cell lines. The mRNA expression of chicken BH3-only were detected along different embryo stages and tissues examined suggesting a role for these proteins in the regulation of cell life and death in various types of cells during the developmentall process.

These results indicated the functionality of BH3-only mammalian orthologs in chicken and the expression of

these genes during the embryonic development providing a valuable genetic tool and preparing the way for the studies of their roles in the development and even in disease.

P-74 FETAL DEVELOPMENT OF HORNER'S MUSCLE.

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In the study of 18 human fetuses ranging from 38-137 mm CR development we have tried to analyse and systematise the development of the Horner's muscle.

At the beginning of the fetal period, 9th week of development, we observed myoblasts at palpebral level which formed the primordium of the orbicular muscle of the eyelids. At the 10th week, coinciding with the appearance of lumen in the lacrimal system, muscular fibres around the lacrimal canaliculi were observed. Alax mesenchyme was interposed between the muscular fibres and the canaliculi.

The presence of the lacrimal portion of the orbicular muscle dorsal to the lacrimal sac was observed from the 12th week onward. The fibres of Horner's muscle ran dorsal to the lacrimal sac, surrounded the canaliculi and were continuous with the fibres of the orbicularis oculi muscle. At the 13th week, the mesenchyme which surrounded the canaliculi and lacrimal sac condensed and the direct tendon of the medial palpebral ligament appeared on the anterior lacrimal crest. The reflex and direct tendon of the medial palpebral ligament was observed at the 15th week of development.

We concur with Fernández Valencia and Gómez Pellico (1990) in that the fibres of Horner's muscle are not fixed in the lacrimal sac but run dorsal to it, while the pre-tarsal fibres of the orbicular of the eyelids are fixed in the mesenchyme which surrounds the the lacrimal sac.

Shinolara and col. (2001) point out that the fibres of Horner's muscle reach the external palpebral ligament. However, we believe that Horner's muscle is a clearly differentiated part of the orbicular muscle of the eyelids and although its deep portion surrounds the canaliculi, its superficial fibres are continuous with the orbicular muscle of the eyelids.

P-75 EFFECTS OF PULSED MAGNETIC FIELDS ON EYE DEVELOPMENT.

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Little is known about the harmful action of magnetic fields on eye development. Fertilized eggs of Gallus domesticus were exposed during incubation to a harmonic signal of 10mT intensity and 50 or 100 Hz frequency, acting for 1 s with a pause of 0.5s. Embryos were divided into two groups. One was exposed to pulsed magnetic field for only the first 15 days of incubation and the other throughout incubation (21 days). Embryos were extracted at 15 and 21 days of development. The following parameters of the eye were studied by ultrasound: anteroposterior diameter of cornea, anterior chamber of eye and anteroposterior diameter of lens and eye.

At 15 days of incubation, the anteroposterior diameter of the lens alone was significantly ($p < 0.01$) larger in embryos exposed to a 10 μ T 50 Hz field than in controls. In embryos exposed to a 10 μ T 100 Hz field, the anterior chamber of eye was significantly smaller ($p < 0.05$).

At 21 days of incubation, the group exposed to a 10 μ T 50 Hz field for 21 days had significantly ($p < 0.05$) larger anterior chamber of eye and anteroposterior diameter of cornea versus controls. In the group exposed to 10 μ T 100 Hz field, the anterior chamber of eye and the anteroposterior diameters of cornea and eye were significantly smaller than in controls ($p < 0.05$; $p < 0.01$ and $p < 0.05$, respectively).

In the group exposed for only the first 15 days of incubation, only the anteroposterior diameter of the eye showed a significant difference versus controls ($p < 0.05$).

These findings indicate that pulsed magnetic fields of 100 Hz affect the development of the eye.

P-76 PULSED MAGNETIC FIELDS AND EMBRYONIC DEVELOPMENT.

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The possible effects of magnetic fields on embryonic development have been well studied. However, whereas some authors found no alterations in embryos exposed to low-frequency pulsed magnetic field, others reported that this exposure produced embryonic malformations.

We exposed fertilised Gallus domesticus eggs during their incubation to pulsed magnetic fields of 10 μ T intensity and 50 or 100 Hz frequency, acting for 1 s with a pause of 0.5 s.

Embryos were extracted from both groups at 45 h of incubation and somite count and development staging were performed.

At 15 days of incubation, further extractions were made to assess the somatic weight and development stage (third-finger measurement). Also at 15 days of incubation, half of the exposed eggs were moved to another incubator, removing them from the action of the magnetic fields.

In exposed embryos extracted at 45 h of incubation, there were significantly fewer somite pairs versus controls ($p < 0.05$); somatic weight and development stage of embryos extracted after 15 days of exposure to 50 Hz field were significantly higher versus controls ($p < 0.05$).

At 21 days of incubation, the group exposed to a 50 Hz field throughout incubation had a lower somatic weight versus controls ($p < 0.05$), while those subjected to a 100 Hz field significantly differed from controls both in somatic weight and development stage ($p < 0.01$ and $p < 0.05$, respectively).

In the group removed from exposure at 15 days, there were no statistical differences with controls in somatic weight or development stage.

Our findings support the hypothesis that magnetic fields act on embryonic development.

P-77 TGF- β 1 AND CELLDEATH IN THE PALATAL MIDLINE EPITHELIAL SEAM AND DENTAL KNOT.

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During embryonic development, cell death is a crucial process that allows the correct morphogenesis of several organs, such as the palate and the tooth. TGF- β_1 is a growth factor highly involved in the appearance of cell death, but also of cell survival, differentiation and the production of extracellular matrix molecules. All these events take place during the palatal midline epithelial seam (MES) and dental knot formation and disappearance. In order to gain insight on the role that TGF- β_1 can play during the development of these structures, we aimed to compare in them the presence of TGF- β_1 and cell death. We labeled embryonic day 14.5 mouse gelatin sections with an antibody against TGF- β_1 , or with TUNEL (terminal deoxynucleotidyl transferase-mediated d-UTP nick-end labeling) or with both, and observed the sections using both an optic and confocal microscope. Our results show temporospatial differences in the expression of TGF- β_1 in the MES, and that its highest expression is, as for the dental knot, strikingly related to the presence of cell death. We therefore suggest that TGF- β_1 is likely having a role as cell death inductor in these structures, although experimental *in vitro* analysis are needed to confirm our suggestion.

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P-78 ROLE OF THE MEDIAL EDGE EPITHELIUM EXTRACELLULAR MATRIX IN THE ORIGIN OF THE CLEFT PALATE PRESENTED BY TGF- β_3 NULL MUTANT MICE.

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TGF- β_3 null mutant mice show all the possible kind of cleft palate, from complete to submucosa, depending on the strain of mice where the gene is targeted, thus implying different degrees of adhesion/fusion of palatal shelves. Our aim has been to analyse the presence/distribution of several extracellular matrix and adhesion molecules in two strains of TGF- β_3 null mutant mice to investigate their importance in the process of palatal shelf adhesion and fusion. We have performed immunohistochemistry with antibodies against fibronectin, laminin, colagens IV and IX, α_5 and β_1 integrins and ICAM-1 on heads of embryonic day (E) 14.5 Albino Swiss and C57 wild type and TGF- β_3 null mice. In addition, we have performed E13.5 palate cultures, treated or not with TGF- β_3 or treated with blocking antibodies against fibronectin or the integrins α_5 and β_1 . Our results show differences in the presence of the molecules analysed between the wild type and null mutant palates, more severe in the C57 (the one having complete cleft palate) than in the Albino-Swiss strain (where the cleft palate is incomplete). The addition of TGF- β_3 rescues the normal presence of these molecules. The use of blocking antibodies against fibronectin and the α_5 integrin

results in a statistically significant decrease of the adhesion and fusion of the palatal shelves. We therefore conclude that the presence of some extracellular matrix and adhesion molecules is very important to palatal shelf adhesion/fusion and that is part of the pathogenesis of the cleft palate presented by the TGF- β_3 null mice.

This work has been granted by the Comunidad Autónoma de Madrid (Ref. GR/SAL/0539/2004) and Fondo de Investigación Sanitaria (PI030185).

P-79 EXCESS OF TGF- β_1 AND GENESIS OF THE CLEFT PALATE PRODUCED IN THE ABSENCE OF TGF- β_3 .

Murillo, J., Barrio, C., Del Río, A., Trinidad, E., Izquierdo, C., Amorós, M., Garcillán, B., López, M., Resel, E., González, I., Martínez-Sanz, E., Martínez-Álvarez, C.

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TGF- β_1 is a growth factor highly involved in both cell proliferation and death. In the mouse, TGF- β_1 is observed in the epithelium covering the tips of palatal shelves (medial edge epithelium -MEE-) and in the midline epithelial seam (MES) that forms when they contact in the midline, but is weakly seen in the underlying mesenchyme. However, in the TGF- β_3 null mutant mouse, that bear cleft palate, TGF- β_1 is strikingly increased, both in MEE and mesenchyme. There is a great decrease in the presence of dead cells in the MEE of these mice with respect to the wild type, and cell death is crucial to the MES disappearance and the final fusion of palatal shelves. The aim of this work was to determine whether excessive TGF- β_1 is able to decrease MEE cell death during *in vitro* palatal fusion, what would give it a role in the persistence of the MES observed in those TGF- β_3 null mice that have incomplete cleft palate. We have performed embryonic day 13.5 paired palatal shelf cultures, adding or not a supraphysiological doses (-400 ng/ml- physiological doses: 10 ng/ml) of TGF- β_1 to the culture medium. After 18 hours, cultures were fixed and embedded in paraffin. Sections were labeled with TUNEL (TdT mediated dUTP Nick End Labeling) to detect dead cells in the MES. Using a Zeiss Axioplan 2 Microscope, the MES area of each culture was measured and TUNEL positive cells present per area were counted. Acquisition of the images and statistical treatment were performed by using the MetaMorph 5.07 and SPSS 12.0 programs respectively. Our results show a significant ($p < 0.01$) decrease in the number of dead cells in the MEE of TGF- β_1 treated cultures related to controls. We have also found significant decrease of the MES formed, what suggests that the excess of TGF- β_1 may also reduce palatal shelf adhesion.

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P-80 TOWARDS A NON SURGICAL TREATMENT FOR CLEFT PALATE?

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dad, E.¹, Izquierdo, C.¹, Amorós, M.¹, López, M.¹, Garcillán, B.¹, **Martínez-Álvarez, C.¹**

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Cleft palate is a congenital malformation whose current treatment includes several surgical procedures throughout the patient's life. We are trying to find an alternative method to surgery. Our hypothesis is that once early orthopedics has helped the approach of the cleft palate edges, the *in situ* synthesis of extrabone could allow their total contact in the midline. Immediately, the chemical removal of the epithelium covering these edges (medial edge epithelium –MEE–) would permit the contact of the underlying connective tissues and the adherence of the cleft palate tips, thus resembling a normal palate.

OBJECTIVES: 1) To explore the possibility of inducing *in vitro* with BMP new osteogenic cells from the fibroblasts located at the edges of the cleft palate. 2) To remove the MEE and to achieve the subsequent adhesion of the underlying connective tissue.

METHODS: We used as experimental models the cleft palate presented by *TGF-β₃* null mouse and small fragments of tissue obtained during surgery from the edges of human cleft palates. First we placed heparin beads soaked with BMP2 below the MEE of embryonic day 18 (E18) *TGF-β₃* null mouse cleft palates and cultured these specimens. The presence of induced osteogenic cells was identified by immunolabelling with an anti-osteocalcin antibody. We then removed the MEE from both E18 *TGF-β₃* null mouse cleft palates and fragments from human cleft palates with a trypsin solution, followed by apposition of the opposing underlying tissues.

RESULTS: 1) The fibroblasts located at the edges of the cleft palate can be induced to form osteogenic cells. 2) It is possible to remove the MEE covering these edges and to achieve subsequent adhesion of the underlying tissues.

CONCLUSION: These *in vitro* results allow to hypothesize that a future non surgical treatment for the cleft palate is possible.

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P-81 EFFECTS OF PROINSULIN ADMINISTRATION ON CARDIOGENESIS IN DEVELOPING CHICK.

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Proinsulin gene expression regulation and function during early embryonic development are remarkably different from those found in postnatal organisms. During development, apoptotic cell death is temporally and spatially regulated during proliferative stages of neurogenesis, and embryonic proinsulin is presumably an endogenous protective factor. Here we characterize a new proinsulin mRNA variant generated by retention of intron 1 in the 5' untranslated region. Inclusion of intron 1 did not affect nuclear retention or cytoplasmic decay but it

inhibits proinsulin translation almost completely. The novel proinsulin mRNA isoform expression was developmentally regulated and tissue-specific. The proportion of intron retention increased from gastrulation to organogenesis, and was highest in the heart tube and presomitic region, whereas it could not be detected in pancreas. We implanted proinsulin-coated beads into the lateral limit of the precardiac region of chick embryos. Proinsulin led to formation of ectopic tissue encircling the bead. *In situ* hybridization showed that this tissue expressed *VMHCl*, a marker of differentiating cardiac myocytes, later restricted to ventricular myocytes. These effects were observed regardless of the distance of the bead to the host heart, relatively distant or near. Moreover, the ectopic tissue contained cells positive by immunohistochemistry for MF20, a myosin heavy chain normally expressed in muscle cells in the myocardium of the major chambers. Proinsulin beads did not induce expression of other mesodermal markers such as *Shh*, specific to the axial mesoderm, or *paraxis*, specific to lateral mesoderm. These results indicate that proinsulin induced ectopic expression of cardiac lineage markers and that it could thus play a crucial role in cardiogenesis. Proinsulin did not act as an initial inductor of non-specified mesoderm, which later could differentiate into cardiac mesoderm. These results are in accordance with previous studies in which insulin induces terminal cardiac differentiation. We propose that regulated unproductive splicing and translation is a mechanism for regulating proinsulin expression, according to specific requirements in developing vertebrates.

P-81b DEVELOPMENT OF CHICK CARDIOMYOCYTES: MODULATION OF INTERMEDIATE FILAMENTS BY ALL-TRANS AND 13-CIS RETINOIC ACID.

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Retinoids, the active metabolites of vitamin A, regulate complex gene networks involved in vertebrate morphogenesis, growth, cellular differentiation, apoptosis and homeostasis. Early heart development is known to be sensitive to retinoid. Retinoic acid (RA) provides a critical signal to allow progression of heart development to adult phenotype. The molecular mechanism by which RA exerts its biological function 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 and gene expression during development is largely uncharacterized. In order to test the effect of all-trans RA (ATRA) and 13-cis RA on cardiomyocyte differentiation, we studied the level and the subcellular compartmentalization of intermediate filaments desmin and vimentin 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 a humid atmosphere. Hearts were ablated when the embryos had reached HH22, HH32 and HH40. Chick

cardiomyocyte were isolated and treated during 24 and 48 h with ATRA and 13-cis RA to a final concentration of 10⁻⁶ M in the growth medium. Sodium dodecyl sulfate-gel electrophoresis, immunoblotting and fluorescence activated cell sorter analysis (FACSscan) were used to quantify the effects of retinoids on cultures of chick cardiomyocytes during development. In general, the retinoids increased the concentration of desmin and reduced of vimentin in cardiomyocytes cultured at all three stages of development. The effects of RA were time-dependent. Our results shown a marked decrease in vimentin levels at HH22 cells cultured for 24 and 48h in both, cytoplasmic (CP) and cytoskeletal (CS) compartments. The greatest increases in desmin concentration occurred in more mature stages (HH40) cells exposed to retinoids. We found that after 48h of RA treatment the increases in desmin levels were larger in the CS than in the CP fraction. These findings are further evidence that RA plays a modulating role in the reorganization of cytoskeletal proteins during the process of cardiomyocyte maturation.

P-82 ANALYSIS OF THE EMBRYOLOGICAL DEVELOPMENT OF THE CHICKEN LIVER USING TWO-DIMENSIONAL REFERENCE MAPS.

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Liver development needs to be analysed in depth for medical and scientific reasons because it is a very interesting embryological process. In order to begin to understand the molecular mechanisms underlying the normal and abnormal development of the chicken, we used two-dimensional electrophoresis (2-DE) to construct a proteomic reference map. Proteins were separated by isoelectric focusing on immobilized pH gradient (IPG) strips, and by 11% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gels. 2-DE reference maps are very useful for these studies because most of the main proteins can be identified.

The chicken (*Gallus gallus*) embryo is one of the most widely used experimental model organisms and has been for many years the most advanced model organism suitable for experimental embryology. Moreover, the chicken represents the model system which, permitting experimental intervention in ovo, most resembles other higher vertebrates. As such, it represents an important complement to mouse model systems.

The liver 2-DE reference map compared with the complete embryo 2-DE reference map, let us identified protein not present in the complete embryo map. This analysis allowed us to identified 1800 new spots in the chicken embryo proteomic map.

When we compare 2-DE liver reference map of the stages 30 and 38 we observed differences in the proteins present at these two stages possibly owing to liver developmental processes. The differences observed between liver and complete embryo chicken maps could be due to liver specific proteins. This possible result could be very useful in order to study metabolic paths involved in the liver embryological development.

P-83 THE URORECTAL SEPTATION COMPLEX OF THE HUMAN EMBRYO.

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In order to analyze the patterns of cellular proliferation both in the mesenchyme of the urorectal septum (URS) and in the adjacent territories (posterior urogenital mesenchyme, anterior intestinal mesenchyme and cloacal folds mesenchyme), as well as their contribution to the process of cloacal division, a computer-assisted method was used to obtain the nuclear area of 3874 mesenchymal cells from camera lucida drawings of nuclear contours of selected sections of human embryos (Carnegie stages (CSs) 13-18). Based on changes in the size of the nucleus during the cellular cycle, we considered proliferating cells in each territory to be those with a nuclear area over the 75th percentile and from them we determined by embryo and territory the rate of proliferating cells that has been used as comparative parameter. The URS showed increasing cell proliferation, with proliferation patterns that coincided closely with cloacal folds mesenchyme, and with less overall proliferation than urogenital and intestinal mesenchymes. Furthermore, at CS 18, we observed the beginning of the rupture in the cloacal membrane; however, no fusion has been demonstrated either between the URS and the cloacal membrane or between the cloacal folds. The results suggest that cloacal division depends on a morphogenetic complex where the URS adjacent territories could determine septal displacement at the time that their mesenchymes could be partially incorporated within the proliferating URS.

P-84 DIFFERENTIATION AND EMBRYONIC DEVELOPMENT IN THE DIFFERENTIATION-BASED CANCER THERAPY.

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Differentiation is a complex multistep process of cell specialization that begins with the installation of a genetic programme, named determination, specific for a cell lineage. Terminal differentiation is the end stage of this process where the cells irreversibly lose their proliferative capacity. In embryonic development, differentiation begins with early determination processes on the cleavage divisions in the egg reaching intermediary levels of differentiation. Finally, after amplification divisions, each cell lineage mature to specialized cell types and tissues. The knowledge of the mechanisms involved in differentiation and malignant transformation has allowed the search of alternative routes for antitumoural therapy that

does not imply cellular death. In this study we tackle different aspects of differentiation from a view point of embryonic development, the relationship with the neoplastic transformation and the application of this knowledge to the anticancer differentiation therapy, using agents that re-enter the tumoural cells into the normal pathway of development.

We present a series of natural and pharmacological agents, enhancers of differentiation used in clinical trials or recently proved in experimental assays. Our results indicate that the use of low doses of antineoplastic drugs can induce therapeutic differentiation in some human tumours and the synthesis of new differentiative drugs from well-known chemical structures allowed the lessening of the adverse cytotoxicity. Analogues of purines and pyrimidines inhibit the growth of tumour cells, interfere in the synthesis and metabolism of nucleic acids acting on key enzymes for the synthesis of DNA and induce cell differentiation. We used specific markers of normal muscular differentiation to decide if these compounds led to the re-entry of the tumoural cells into the normal pathway of development. These 5-FU derivatives induced myogenic differentiation in the rhabdomyosarcoma human cells not inducing the multidrug resistance phenomenon, indicating that this type of tumour may be amenable to treatment by differentiation therapy.

We conclude that the better understanding of the molecular mechanisms responsible for the multistage process of differentiation, embryonic development and neoplastic transformation, will be allowed a more rational clinical application of differentiation cancer therapy.

Nervous System

P-85 TGF-BETA FAMILY DURING NEURULATION AND DEVELOPING SPINALCORD.

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Development of the nervous system involves a serie of morphogenetic steps leading to the formation of highly organized structures such as the brain and spinal cord. The transforming growth factor (TGF- β s) is involved in the regulation of crucial processes through the regulation of proliferation, cell movements along the radial glia and neuronal differentiation. It has been reported that TGF- β 2 and - β 3 are present in glial cells and along developing tracts, being considered as the "neural" TGF- β s isoforms, while TGF- β 1 is normally restricted to the meninges and coroid plexus. However, some functions attributed to TGF- β 1 like cell-cycle control, neuron survival, extracellular matrix formation and differentiation, support the hypothesis that this factor might have important roles during the formation of the central nervous system (CNS). We have studied the expression of the three isoforms during neurulation and early stages of spinal cord development (E7.5- E13.5 d.p.c.). Our results indicate that the expression of TGF-betas shows a temporal and spatial dynamic pattern. During neurulation, TGF- β 1, - β 2 and - β 3 proteins were present in the neuroepithelium, although TGF- β 3 showed the weakest immunoreactivity (IR). In the spinal cord, the three isoforms were detected on different spatial localization. As expected, TGF- β 2 and TGF- β 3 showed

wed overlapping areas of expression at the basal plate region of the spinal cord, the ventral commissure, dorsal funiculus, ventral root fibers and fibers at the marginal layer (E13.5). The expression pattern of TGF- β 1 is detected quite homogeneously within the spinal cord at E11.5, while at E13.5 the periventricular region, and the half dorsal zone of the spinal cord showed more intense IR. The TGF- β 1 protein is observed at the region where proliferation and migration of neuroblasts occur as detected by BrdU staining. However, the pattern of TGF- β 2 and - β 3 is completely different, related to axons and with the fiber component of the spinal cord mantle. Together with the known biological activities of TGF- β s, we propose that these factors are important mediators of cell-cell interaction during development of the CNS and that the presence of these isoforms might be related to migration of neural cells on a glial substrate.

P-86 BRAIN DEVELOPMENT AND TGF-BETA FAMILY.

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The transforming growth factor isoforms (TGF- β 1, - β 2 and - β 3) are the most abundant cytokines in the central and peripheral nervous system. A high degree of overlapping has been described in the expression pattern of TGF- β 2 and - β 3 in the telencephalic cortex, mesencephalon and cerebellar anlage, detected along peripheral nerves, in radial glial cells and along central nervous system (CNS) axon tracts. In contrast, TGF- β 1 expression seems to be confined to the meninges and coroid plexus in the unlesioned nervous system. According to their expression pattern, it has been proclaimed that a possible role in the control of cell division is excluded since no one of the isoforms seems to be detectable in the periventricular zones throughout the CNS. However, studies *in vitro* suggest that TGF- β s play a role in proliferation although positive or negative effects depend on culture conditions. We propose to study the pattern of TGF- β 1, TGF- β 2 and TGF- β 3 protein expression detected by immunostaining on free-floating gelatin sections, a system that provides mainly two advantages: it avoids epitope retrieval necessary in paraffin sections, and detect weak immunoreactivity due to the use of 50 μ m thick vibratome slices. We have studied mouse brains of embryonic stages ranging from E11.5 to E14.5. In our experimental system, TGF- β 1 immunoreactivity (IR) was observed in the ventricular zone in the prosencephalon, mesencephalon and rombencephalon. The IR seems to be similar to the proliferation pattern observed by BrdU staining. The TGF- β 2 isoform is expressed in a homogenous and intense pattern in the CNS, with peak of expression in the eye lens, the pallium/subpallium of the telecephalic cortex and some encephalic regions where differentiation is more advanced (intermediate and marginal zones). TGF- β 3 is clearly visible at the fibers of the cephalic marginal zone. In summary, all the three isoforms can be detected in the embryonic nervous system, with a differential and dynamic expression pattern. In addition, we have found a striking, not yet reported, complementary pattern of expression between these two isoforms. In this study, we analyse the possible functions of TGF- β s in the CNS during mouse embryogenesis.

P-87 ADJUSTMENT OF THE NUMBER OF CELLS DURING VERTEBRATE RETINALNEUROGENESIS.

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During the development of the retina a high number of cells need to be adjusted to get a normal and functional structure in the adult nervous system. Apoptosis seems to be one of the major mechanism involved in this specific selection of functional neurons for the mature nervous system to work. The prevalence of programmed cell death affecting connecting neurons during late neural development is well established. On the other hand, the incidence of apoptosis during neurogenesis on proliferating neuroepithelial cells and recently differentiated neuroblasts has only recently been recognized.

Using the terminal transferase deoxyuridine-triphosphate nick end labelling (TUNEL) as a technique and the embryonic chick retina as a model system we have designed several *in vivo* experimental interferences with naturally occurring cell death. *In ovo* treatment with caspase inhibitors resulted in decreased apoptosis and increased number of retinal ganglion cells.

We then approached the cell death naturally affecting ganglion cell generation and found that more than 50% of recently born ganglion cells die within 6-9 hours after their last S-phase. While these results demonstrate an early phase of neurogenic cell death of similar magnitude than the later occurring neurotrophic cell death, the critical developmental and cellular processes that underlie this early phase of cell death during neurogenesis are still poorly understood.

P-88 THE CHICKEN CIRCUMVENTRICULAR LATEROSEPTAL AND PREOPTIC ORGANS ARE FORMED ALONG THE STRIATOPALLIDAL MOLECULAR BOUNDARY.

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The lateroseptal organ (LSO) is a circumventricular specialization present in the avian and reptilian telencephalon (Kuenzel y van Tienhoven, 1982). It has a population of liquor-contacting neurons which are VIP immunoreactive. Another neighboring circumventricular organ is that of the preoptic recesso. Recent data suggest that the LSO is associated to extraretinal photoreception, insofar as its neurons express molecules of the opsin family and their activity changes with exposure to light. Although according to its name the LSO would seem to lie in the septum, actually only a small portion of it has this localization, whereas the largest part extends below the lateral ventricle and lies in its lateral wall, associated to the ependymal region of striatum or pallidum. Its position within the subpallium has not been investigated precisely, although this represents a prerequisite for any causal embryological interpretation of the LSO.

To this end, we studied the topography of the chicken LSO relative to the striatopallidal complex, delineated molecularly. It is known that during development the subpallium generally expresses *Dlx* family genes (in both striatal and pallidal portions) and *Nkx2.1* signal (only in the pallidum and preoptic area), as a substrate for production of various GABAergic neurons. Another subpallial histogenetic zone, the anterior entopeduncular area (AEP), shows, in addition to *Dlx* and *Nkx2.1*, signal of the gene sonic hedgehog (*Shh*), a pattern apparently associated to production of the cholinergic neurons of the basal forebrain.

Using immunohistochemical and *in situ* hybridization techniques, we mapped calbindin (which identifies early on the LSO), the genes *Nkx2.1*, *Nkx2.2*, *Shh*, *Dlx5* and the protein BEN. The results jointly define with precision the topography of the chicken LSO in the subpallial telencephalon, show for the first time a group of distal neurons associated to the LSO, and provide embryological evidence that this organ actually forms throughout its length along a striatal band parallel and in contact with the molecular striatopallidal boundary, as defined by the limit of *Nkx2.1* expression. This band is continuous with an extra-telencephalic counterpart along which the preoptic circumventricular organ develops. Accordingly, there seems to exist a common molecular background for the formation of these two ventricular organs.

P-89 NEURONAL ORIGIN OF PALLIAL AND SUBPALLIAL AMYGDALAR SUBDIVISIONS.

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The amygdala is a very complex structure, located in the ventrolateral telencephalon and involved in a large variety of functions, such as control of homeostasis and emotional behavior. The anatomical and functional complexity of the amygdala is partially explained by its multiple developmental origins in several histogenetic compartments (Puelles et al., 2000). Thus, the nuclei/areas of the amygdala derive from different sectors of pallium or subpallium, each one expressing a different combination of developmental regulatory genes. The complexity of the amygdala is also related to the existence of tangential cell migrations in the telencephalon, mainly from the subpallium to the pallium. The aim of this study has been to investigate in mouse the neuronal origin of pallial and subpallial amygdalar subdivisions, and analyze the contribution of tangential migrations to their complexity. For that, we prepared organotypic cultures of mouse embryonic telencephalon (embryonic day 14.5), and labeled cell clones in different telencephalic ventricular areas, using the fluorescent cell trackers CMTMR or CMFDA. The results on cell migrations were compared to the different molecular domains of the telencephalon, based on expression patterns of the genes *Tbr1*, *Dlx5*, *Nkx2.1*, *Lhx6*, *Lhx7*, *Gbx1* and *Shh*. Our results offer further support for the multicompartmental organization of the amygdala, and provide new data on the specific subpallial origins of amygdalar nuclei. Thus, the central amygdala originates in the caudal ganglionic eminence, whereas the posterodorsal part of the medial amygdala and a related part of the extended amygdala originate in the medial ganglionic

eminence plus the anterior entopeduncular area of the subpallium. In contrast, the basolateral complex of the amygdala originates in the ventrolateral pallium. Further, our results provide evidence for the existence of tangential cell migrations from the subpallium to the pallial amygdala (including the basolateral amygdalar complex). Immigrant neurons of the pallial amygdala appear to integrate as different subtypes of GABAergic interneurons, modulating the activity of principal neurons. Finally, we present novel evidence indicating the existence of dorsoventral tangential migrations in the amygdala, further contributing to the high complexity of this structure.

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P-90 STUDIES ON THE DEVELOPMENT OF THE ECTOMAMILLARIS NUCLEUS IN THE CHICKEN.

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The Ectomamillaris nucleus (EM) or the nucleus of the basal optic root (nBOR), is a diencephalic caudoverstral nucleus that is located under the Tecto-thalamic tract in reptils (Martinez de la Torre, 1985) in the prosomere 1 (Puelles, 1995), but in chicks the EM seems exceed the Sinencephalon- parencephalic limit.

This nucleus is very important in the analysis of optic flow and the generation of the optokinetic response, reason why we studied its embryologic origin in the chicken.

In the present study we've used chick embryos from 8 (HH34) to 19 (HH45) days of incubation. Brains were sectionated in the horizontal plane at 100 µm and then we have compared parallel series dyed with a mixture of Thionine-Neutral Red with Acetylcholinesterase (AChE), the acetylcholine degradation enzyme.

Expression of AChE starts around embryonic day 6 in some structures and follows mainly increasing time-courses in the chick brain.

P-91 MOLECULAR PROFILING INDICATES AVIAN BRANCHIOMOTOR NUCLEI INVADE THE HINDBRAIN ALAR PLATE.

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It is generally believed that the spinal cord and hindbrain consist of a motor basal plate and a sensory alar plate. We now have molecular markers for these territories. The relationship of migrating branchiomotor neurons to molecularly defined alar and basal domains was examined in the chicken embryo by mapping the expression of cadherin-7 and cadherin-6B, in comparison to genetic markers for ventrodorsal patterning (Otp, Pax6, Pax7,

Nkx2.2, and Shh) and motoneuron subpopulations (Phox2b and Isl1). We show cadherin-7 is expressed in a complete radial domain occupying a lateral region of the hindbrain basal plate. The cadherin-7 domain abuts the medial border of Pax7 expression; this common limit defines, or at least approximates, the basal/alar boundary. The hindbrain branchiomotor neurons originate in the medial part of the basal plate, close to the floor plate. Their cadherin-7-positive axons grow into the alar plate and exit the hindbrain close to the corresponding afferent nerve root. The cadherin-7-positive neuronal cell bodies later translocate laterally, following this axonal trajectory, thereby passing through the cadherin-7-positive basal plate domain. Finally, the cell bodies traverse the molecularly defined basal/alar boundary and move into positions within the alar plate. After the migration has ended, the branchiomotor neurons switch expression from cadherin-7 to cadherin-6B. These findings demonstrate that a specific subset of primary motor neurons, the branchiomotor neurons, migrate into the alar plate of the chicken embryo. Consequently, the century-old concept that all primary motor neurons come to reside in the basal plate should be revised.

P-92 MRI IMAGES OF THE MEDIAL TEMPORAL LOBE IN POSTMORTEM FIXED HUMAN BRAINS: NEUROANATOMICAL CORRELATION.

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The anatomical detail provided by modern neuroimaging techniques open new perspectives on the correlation of anatomical structures that, because of their saliency, is relatively easy to identify with a certainty close to the neuroanatomical histological detail. We, and others, have reported different strategies to segment structures within the medial temporal lobe in order to assess volume variations in patients with various pathologies, mainly Alzheimer's disease and other dementias, and temporal lobe epilepsy.

We report the findings obtained in the post-mortem fixed human brains that were encased in 1.5% Agar, and explored with a Siemens 1.5T and with a Philips Intera, also of 1.5T. The sequence used was FLAIR 3D, TR:6000, TE:353, TI:2200, 1mm thickness and FOV 260. After completion of the MRI examination, the brains were freed from the Agar encasing and sectioned in the coronal plane (orthogonal to the AC-PC line) into 1 cm. thick slabs. The medial temporal lobe was dissected by a section that encompassed from the endorhinal sulcus to the inferior limiting sulcus in the insula, in such a way that the whole temporal lobe was separated for sectioning. Every slab of the temporal lobe was cryoprotected with 10 and 20% glycerol solutions containing

2% DMSO and sectioned at 50 μm in a sliding microtome provided with a freezing unit. A one-in ten series was immediately mounted onto subbed slides. The nine remaining sections in the series were stored in a cryoprotectant solution and stored at 4°C. The series was stained with thionin for cytoarchitectonic evaluation of the medial temporal lobe structures.

MRI images were of enough good quality as to offer a good contrast between grey and white matter in T2. All of the major landmarks in the medial temporal lobe were recognizable, and additional details are offered as to the distances and border definition between neighbouring cytoarchitectonic fields. In conclusion, this proves a very powerful and useful technique for evaluation of MRI-Neuroanatomical correlation in the medial temporal lobe, thereby simplifying the interpretation of pathological images obtained in patients affected with Alzheimer's disease, medial temporal lobe epilepsy and other diseases.

P-93 THE CONNECTIONS OF THE NUCLEUS INCERTUS IN THE RAT AND ITS RELATION IN THE HIPPOCAMPAL THETA RHYTHM.

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The hippocampal theta rhythm is generated by the pacemaker activity of the medial septum-diagonal band of Broca neurons (MS/BD_v). These nuclei are influenced by brainstem structures that modulate theta rhythm. The nucleus incertus in the dorsal tegmental area, has been described as the origin of an extensive projection to prosencephalic structures, MS/BD among others. The aim of the present work is to determine whether the nucleus incertus contributes anatomically and physiologically to the activation of the hippocampal theta rhythm.

In the present study, anterograde tracer injections in the nucleus incertus showed terminal-like labeling in a widespread pattern of subcortical structures. These results served like target for retrograde tracer injections in order to extend and confirm the efferent connections of the nucleus incertus. These data led us to conclude that there is a widespread system of ascending projections arising from the nucleus incertus to the median raphe, mammillary complex, hypothalamus, habenula, amygdala, entorhinal cortex, medial septum, and hippocampus. Many of the targets of the nucleus incertus were involved in arousal mechanisms including the hippocampal theta rhythmicity.

The anatomical evidences of the possible relation between nucleus incertus and theta oscillations were confirmed through physiological experiments. The hippocampal field activity was recorded in urethane anesthetized rats. Electrical stimulation of the nucleus incertus evoked theta rhythm in the hippocampus, and decreased the amplitude of the delta waves. No high frequency activity (>10 Hz) was spontaneously observed during the electrical stimulation of the nucleus incertus, suggesting that the nucleus incertus selectively induced hippocampal theta rhythm without altering the general arousal state. Conse-

quently, the nucleus incertus may be a relay station between brainstem structures and the MS-DBB in the control of the hippocampal theta rhythm.

P-94 QUANTITATIVE CHANGES OF SEROTONERGIC FIBERS IN THE RAT CINGULUM BUNDLE AFTER MDMA ADMINISTRATION.

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MDMA (Ecstasy) is a common drug of abuse, particularly among young adults, that is neurotoxic to the serotonergic system. In order to assess this phenomenon, the change in the number of serotonergic fibers of the cingulum bundle in MDMA treated Wistar rats was quantified. We used 17 rats in three experimental series: controls (n=7), and two MDMA-treated groups (15 mg of MDMA in saline per kg) and either one week (n=5) or four weeks (n=5) survival. The quantitative study was performed at two levels of the cingulum bundle (anterior and posterior portions) with the CAST-Grid system for estimation of the number of profiles (5-HT immunolabeled fibers) per area QA. Data were pooled for rostral and caudal levels and for right and left hemispheres. 5HT-positive fibers showed a tortuous course, they were fine in diameter and contained small varicosities. The fiber density per area of cingulum bundle showed a statistically non-significant 19% decrease one week after MDMA administration compared to controls. Four weeks survival group showed a decrease of 55% in the occurrence of 5HT positive fibers (p<0.001). In Wistar rats the 5HT positive fiber decrease (possibly due to toxic damage) was protracted in time, and it might happen, not only at the terminal site, but somewhere along the axonal pathway, excluding cell bodies of the raphe nuclei, according to other studies. In summary, the susceptibility of the serotonergic pathway in Wistar rats after MDMA administration was highly significant at four weeks survival.

P-95 OREXINERGIC CONNECTIONS TO THE ORAL PONTINE RETICULAR NUCLEUS IN THE RAT. IMPLICATION IN SLEEP-WAKE STATES.

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Orexine (hypocretine) is a neuropeptide located in neurons of the perifornical region of the posterior hypothalamus that has been implicated in promoting arousal in cats and rats. Degeneration of orexine-containing hypothalamic neurons in humans induces narcolepsy. On the other hand, it has been described that the cholinergic sti-

mulation of the oral pontine reticular nucleus (RPO) in the rat induces paradoxical sleep (PS). The aim of present communication is to investigate the possible influence of the orexinergic hypothalamic neurons on the pontine PS-induction neurons, in the rat. Under general anaesthesia, five animals received stereotaxic injections of the retrograde tracer cholera-toxin B subunit (CT) in the RPO PS-induction region by means of a 500 nl Hamilton syringe. After seven days survival time, animals were perfused and their brains frozen sectioned at 40µm in consecutive series to be processed for immunostaining with 1:2000 goat anti-orexin antiserum (Santa Cruz Biotechnology) and for revealing CT using a silver enhancement kit (Amersham) procedure. One parallel series was devoted to Nissl staining to delimit the different nuclei and anatomical structures. The study of histological material revealed the presence of orexine immuno-positive neurons in the lateral hypothalamic area, dorsomedial hypothalamic nucleus and perifornical nucleus according with data previously described. A large number of neurons retrogradely labelled with CT was detected mainly ipsilateral but also contralateral in the ventral, dorsolateral and medial hypothalamic areas extending also to zona incerta. An important number of neurons displaying both CT labelling and orexine immunostaining were located ipsi and contralaterally in dorsolateral hypothalamic areas and around the fornix, being less abundant in posterior hypothalamic area. Our results demonstrate the existence of projections to the pontine PS-induction region from several hypothalamic areas and a consistent orexinergic connection arising in neurons mainly located in dorsolateral hypothalamic and perifornical nuclei. These results suggest that degeneration or hypofunction of this orexinergic projection in narcoleptic subjects could underlie pathological alternation of the different phases of the sleep-waking cycle.

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P-96 VENTRAL PALLIDAL INNERVATION OF THE RAT MESOPONTINE TEGMENTUM AND ITS RELATIONSHIP TO CHOLINERGIC NEURONS.

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The downstream projection of the ventral pallidum (VP) to the mesopontine tegmentum is part of the cortico-striato-pallido-pontine circuits, which are altered in some neurodegenerative diseases such as Parkinson's disease or Huntington's chorea, as well as in neuropsychiatric disorders such as schizophrenia.

Pharmacological, physiological and lesion studies in rats have reported an effect of the VP on the activity of the pedunclopontine tegmental nucleus (PPTg), thus providing indirect evidence of putative VP-PPTg projections. Anatomical studies carried out so far, however, have not clearly shown ventral pallidal terminal fibers in the PPTg to support the physiological and pharmacological findings. In order to analyse in detail the ventral pallidal innervation of the rat mesopontine tegmentum, small injections of the sensitive anterograde tracer biotinylated dextran amine were performed in different rostrocaudal and mediolateral portions of the ventral VP by iontopho-

resis. The precise localization of the injections within the VP was neurochemically assessed by means of double labeling with Leu-enkephalin, a specific marker of the VP. The subsequent anterograde labeling in the mesopontine tegmentum was analyzed throughout the rostrocaudal extension of PPTg. In addition, the relationship between the terminal fibers and the cholinergic neurons in PPTg was studied by means of double labeling using the vesicular acetylcholine transporter (VACHT) as a cholinergic marker. Significant anterograde labeling was found in the mesopontine tegmentum, although its density varied in relation to the size of the injection. Most of the fibers were terminal fibers displaying putative synaptic boutons. Though at low magnification the terminal fields were dorsomedially located relative to PPTg cholinergic neurons, at higher magnification, several varicose fibers were intermingled among the cholinergic neurons. Our results clearly demonstrate the VP-PPTg projection in the rat, thus providing the anatomical substrate for the functional modulation of PPTg neurons from the basal forebrain.

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P-97 TOPOGRAPHIC LOCATION AND CIRCUANNUALEVOLUTION OF THE SYNAPTIC BODIES IN THE PINEAL GLAND OF THE ALBINO RAT.

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The synaptic bodies of mammalian pineal gland are poorly understood ultrastructural organelles into the cytoplasm of the pinealocytes. Previous studies in the rabbit have shown that these organelles are rather heterogeneous in shape, decrease in number during the light phase and increase in number at dark phase. It has also found pronounced season-related differences as well as differences related to pineal regions.

No studies are currently available on seasonal changes in other species and it is unknown whether the biological rhythms are identical in the proximal, intermediate and distal parts of the elongated pineal.

Our study was carried out on 120 male albino rats kept under natural lighting conditions in order to examine numerical variations of the different types of synaptic bodies in the proximal, intermediate and distal regions of pineal glands procured at different time points of 24-hour cycle and in the four annual seasons.

In the present study rod-like, sphere-like and triangular synaptic bodies were distinguished, being the first two types the most abundant. Results also displayed circadian changes in the number of synaptic bodies with the lower values during light phase and higher ones at dark phase. We have also found pronounced season related differences as well as differences related to pineal regions.

The circadian and seasonal results shown that day and night length are structurally coded in the pineal gland by means of the number of synaptic bodies. The regional differences are probably related to the different origin of the innervation: the sympathetic fibres enter to the gland distally while the proximal fibres come from the central nervous system.

P-98 VASSOPRESIN, TYROSINE-HYDROXYLASE AND DOPAMINE β -HYDROXYLASE DISTRIBUTION IN SUPRAOPTIC AND ARCUATE NUCLEUS. A COMPARATIVE STUDY BETWEEN FARMER AND COST GOAT.

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The hormone vasopressin (A-V) is well known for its pressor and antidiuretic effects, is primarily synthesized in the magnocellular neurons of the SPO and other hypothalamic structures. The SPO participate in the salt-water balance thorough of A-V secretion, also the vasopressin appears to modulate the cardiovascular reflex control of the sympathetic nervous system. The (TH) and (DBH) are present in the catecholaminergic neurons located in the hypothalamus and brain areas related with the vascular regulation. The secretion of A-V by the SPO is controlled, at least in part, by angiotensinergic (AGII) inputs that come from the subfornical organ (SFO). The purpose of the present work is to study angiotensin-vasopressin system in the hypothalamus of the "majorera" goat (*Capra hircus* variation), comparing the goat development in farmer with goat that live in extreme condition of drinking intake of the arid climate in Fuerteventura island cost.

We have used a total of six adult male and female goats divided in two groups: a farmer group (well feeding) and a cost groups (feeding in natural way in the arid climate of the cost). We have found the vasopressin-ir and TH-ir are decreased in the different hypothalamic nucleus of the cost goat, contrarily DBH immunoreactive is augmented in this goat. We could conclude that the decrease of vasopressin-ir and TH-ir and the increase of DBH-ir in the hypothalamus of the cost animal, could be an expression of the water retention, since in this kind of goat the daily drinking water is scarce.

P-99 DISTRIBUTION OF IL-6 AND nNOS IN VASOPRESSINERGIC AND CRF-ERGIC NEURONS OF THE RAT HYPOTHALAMUS.

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Corticotropin-releasing factor (CRF) and arginin-vasopressin (AVP) are the two major regulatory peptides in the hypothalamus-pituitary-adrenal (HPA) axis. Parvocellular neurons of the paraventricular hypothalamic nucleus (PVN) are the main source of CRF in the hypophysial portal vascular system, after being released in the median eminence (Sawchenko and Swanson, 1990).

AVP, however, is synthesized in both magnocellular and parvocellular regions of the PVN, and in the supraoptic (SON) and suprachiasmatic (SCN) nuclei. Furthermore, CRF and AVP colocalize in parvocellular PVN neurons (Grino and Zamora, 1998) and secretory granules at the nerve endings in the ME (Gillies et al., 1982). Numerous studies indicate that interleukin-6 (IL-6) and nitric oxide (NO) play regulatory roles on the HPA axis response to different kinds of homeostatic threats. IL-6 stimulates synthesis and secretion of CRF and AVP at hypothalamic levels, and is robustly expressed in the PVN and SPO nuclei, largely co-localized in these centres with AVP (Ghorbel et al., 2003). Nitric oxide synthase (nNOS), NO synthesis enzyme, has been detected at different levels of the HPA axis. However, the involvement of NO in the axis modulation is still controversial. It is known the existence of a biological master clock localized in the hypothalamic SCN, and circadian variations in plasma IL-6 levels have been recently described (Abreu et al., 2004).

The aim of the present study is investigate co-localization of IL-6 and nNOS in CRF and AVP neurons of different hypothalamic nuclei of the rat using immunohistochemistry and double staining immunofluorescence in sham and colchicine intracerebroventricular injected animals in order to a best visualization of the markers.

Our results show the different distribution of these peptides in the PVN, SPO and SCN hypothalamic nuclei, and the existence of two different populations of AVPergic cells, one containing CRF, but no IL-6, that project to the median eminence, and another one that expresses IL-6, but no CRF, and project to the neural lobe of the hypophysis. This last one also coexpresses nNOS in most of its neurons, suggesting that IL-6 and nNOS are involved in AVP release from posterior lobe of the pituitary. In the SCN, we have detected a cell subpopulation expressing AVP and IL-6, and another expressing CRF.

P-100 COMPARATIVE STUDY OF nNOS IN THE SUPRAOPTIC NUCLEI OF THE HYPOTHALAMUS OF HYPOPHYSECTOMISED RATS AS COMPARED WITH RATS SUBJECTED TO STRESS.

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Nitric oxide synthase is the enzyme responsible for the synthesis of nitric oxide and its neuronal isoform (nNOS) can be detected in hypothalamic nuclei by immunocytochemistry. Accordingly, here we performed a study of nNOS in the supraoptic nuclei of animals with opposing functional states and different from normal, such as survival at 40 days post-hypophysectomy and stress induced by 4 hours of immobilisation, with a view to determining the neuronal behaviour of nitric oxide under such conditions. Adult male Wistar rats were used: untreated (n=5); hypophysectomised (n=5) and animals subjected to stress (n=5). All brains were fixed in 4% paraformaldehyde, embedded in paraffin and sliced in serial sections of 6 mm. nNOS was detected in the hypothalamic neurons by single-label immunocytochemistry, streptavidin-biotin peroxidase, using anti-nNOS polyclonal antiserum raised

in sheep and diluted 1:15000. Together with the morphological study we performed another morphometric analysis, calculating the cellular and nuclear areas of the immunoreactive neurons. In the untreated animals, nNOS-immunoreactive neurons appeared, especially in the dorsal region of the prechiasmatic subdivision of the supraoptic nucleus, with a double immunostaining pattern: either as an intense immunoreaction covering both the perikaryon and the prolongations or as a weak reaction limited to the perikaryon. Hypophysectomy led to a decrease not only in the number but also in the intensity of reaction of the neurons to nNOS. This appeared almost exclusively in the prechiasmatic subdivision, although it was also detected both in the perikaryon and in the prolongations. Finally, stress induced a qualitative and quantitative increase in the nNOS reaction in both subdivisions (pre- and retrochiasmatic), with an intense reaction in the perikaryon and neuronal prolongations. The morphometric values of this group of animals were significantly greater than any others analysed.

P-101 DOPAMINE β -HYDROXYLASE AND P73 EXPRESSION IN THE ORGANUM VASCULOSUM OF THE LAMINA TERMINALIS AND SUBFORNICAL ORGAN. A STUDY OF SHR AND WKY RATS.

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The organum vasculosum of the lamina terminalis (OVLT) is a circumventricular organ located in the anteroventral region of the third ventricle. The OVLT is rich in neuropeptides such as angiotensin II and catecholamines. The subfornical organ (SFO) and OVLT are characterized by the absence of a blood-brain barrier. The SFO has connections with the brain regions implicated in the central regulation of drinking, blood pressure and cardiovascular function, among them the anteroventral region of the third ventricle and OVLT. The protein p73 is a member of a family of transcription factors, which also includes p53 and p63. The p73 is present in developing neurons as a truncated isoform whose levels are dramatically decreased when sympathetic neurons apoptosis after nerve growth factor (NGF) withdrawal. Thereafter p73, is necessary for survival and long-term maintenance of CNS neurons, including postnatal cortical neurons. The purpose of present work is study the expression of the dopamine β -hydroxylase (DBH) and p73 in the OVLT and the SFO and their variations in the ventricular dilatation and arterial hypertension.

Brains and cerebrospinal fluid (CSF) from control Wistar-Kyoto rats (WKY) and spontaneously hypertensive rats (SHR) were used. The paraffin section containing the OVLT and SFO were immunohistochemically processed with anti-DBH and anti-p73. DBH and p73 band were identified in SFO extract by western blot.

We found that DBH was increased in the hypertensive animals only in the SFO while the increase of the p73 expression was found in both SFO and OVLT of the hypertensive rats. Also the SFO extract band was marked with anti-DBH and anti-p73 and the intensity of the reaction was higher in SHR than in the WKY rats. The present results and the fact that the deltaNp73 is essential for survival of sympathetic neurons, would indicate that p73 in an essential survival protein in CNS catecholaminergic neurons, therefore there would be a correlation between p73, DBH and these cerebral centres implicated in cardiovascular regulation.

P-102 SUBFORNICAL ORGAN EXPRESSION OF CERTAIN PROTEINS FOUND IN CEREBROSPINAL FLUID OF THE SPONTANEOUSLY HYPERTENSIVE RATS.

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The subfornical organ (SFO), like other circumventricular organs, is characterized by the absence of blood-brain barrier. The SFO has connections with the brain regions implicated in the central regulation of drinking, blood pressure and cardiovascular function, among them the anteroventral region of the third ventricle. Alterations in the Angiotensin II receptor have been described in the SFO of the SHR, moreover underdevelopment of the SFO and variations of the angiotensin II Receptor content of the subfornical organ have also been found in hydrocephalic animals. The aim of this work was study the possible role in the concomitance among hydrocephalus, arterial hypertension and the variation in subfornical organs. Brains and cerebrospinal fluid (CSF) from control Wistar-Kyoto rats (WKY) and spontaneously hypertensive rats (SHR) sacrificed with chloral hydrate were used. CSF and extract were processed by western blot. Antiserum against proteins band of 141, 117 and 48 kDa (anti-B1, anti-B2 and anti-B3) were used in the immunohistochemical and Immunoblotting study of the subfornical organ and adjacent structures. The SFO, third ventricle ependyma and choroideus plexus (CP) showed immunoreactive material (IRM) for antibody against 141kDa, 117 and 48 kDa proteins band. The larger amount of the IRM was found in the SFO of the SHR. Several protein bands were marked mainly with anti-b3 and an increase in the IRM was found in the SFO SHR extracts. Our results and the alterations observed by other authors in the SFO in hydrocephalic and hypertensive rats, support the possibility that these circumventricular organs, some proteins of the CSF and ventricular dilatation could be connected with the physiopathology of this type of the hypertension.

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P-103 EFFECTS OF THE HYPERTENSION AND ITS CAPTOPRIL TREATMENT ON THE ADRENAL MEDULLA. A CATECHOLAMINERGIC AND CHROMOGRANIN STUDY.

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We investigated the alteration of tyrosine hydroxylase (TH), dopamine-beta-hydroxylase (DBH), phenylethanolamine-N-methyltransferase (PNMT) and chromogranin A (ChA) expression in the adrenal medulla of spontaneously hypertensive rats (SHR) and its variation with the captopril treatment.

We have used a total of 20 male rat divided in four groups: a control group formed by five WKY rats, a control treated group formed by five WKY rats treated with captopril, a hypertensive group formed by five SHR rats and a hypertensive treated group formed by five SHR rats treated with captopril.

In the adult adrenal gland, chromogranin A and TH were increased in the SHR, while dopamine beta-hydroxylase activity was diminished. The captopril treatment produce a decrease in IRM in the hypertensive group. The PNMT was also diminished in the hypertensive groups. These results are partially in concordance with another works on the Chromogranin A mRNA expression where it was also elevated in the SHR adrenal medulla, contrarily, in another rodent model of hypertension, the hypertensive mouse (BPH/2), adrenal chromogranin A and noradrenaline were diminished. We conclude that over-expression of chromogranin A and the alteration found in the different enzymes of catecholamines are a variable feature of mammalian genetic hypertension.

P-104 INFUNDIBULAR CATECHOLAMINES VARIATIONS IN ARTERIAL HYPERTENSION.

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The infundibular area include the arcuate nucleus (AN) and median eminence (ME), that are brain structures closely related with hypothalamic-hypophyseal axis and play an important roll in the cardiovascular regulation throughout of its catecholaminergic innervations which act on the angiotensin II and vasopressin release. The axons origina-

ting in these neurons pass through the internal zone of the median eminence and reach the posterior lobe of the hypophysis. The secretion of this hormone is controlled, at least in part, by angiotensinergic inputs from the subfornical organ (SFO) and reaches the hypothalamus.

We have used a total of 20 male rat of 10 and 15 week old divided in two groups: a control group formed by WKY rats, a hypertensive group formed by SHR. We have found TH and DBH immunoreactive material (IRM) in neurons and fibres of the AN, periventricular nucleus (PEV) and in fibres of the ME. At ten weeks old the hypertensive group shows TH-IRM slighter augmented than the control groups, moreover the hypertensive group has a greater number of fibres marked than the control group, this fibres go into the PEV. Contrarily, at 15 weeks old the control group shows the amount of TH-IRM elevated and a greater number of marked cells than the hypertensive group. The DBH-IRM at ten week old was more intense in the control group. While the AN of the SHR groups at 15 weeks old showed the intensity of DBH immunoreaction augmented in a 20%. The amount of TH and DBH identifying by Western blot in the hypertensive group was greater than in the control group. These findings indicate that catecholaminergic projections on the AN could be implicated in the changes of angiotensin and vasopressin secretion described in the SHR rats.

P-105 IMPLICATION OF AROMATASE ON PITUITARY FUNCTION, EVIDENCED BY WESTERN BLOT, IMMUNOHISTOCHEMISTRY, IN SITU HYBRIDIZATION AND AROMATASE-ANTAGONISTS.

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In previous studies we demonstrate the immunocytochemical expression of aromatase in pituitary cells. The aim of this study is demonstrate the expression of mRNA for the enzyme in pituitary cells by in situ hybridization and correlate with immunocytochemical expression of aromatase in the pituitary of male adult rats. In situ hybridization demonstrates that aromatase-mRNA is located in the cytoplasm of 41% of pituitary cells and it correlates well with immunocytochemical staining.

In order to determine the physiological relevance of pituitary aromatase in the control of prolactin pituitary cells an immunocytochemical and morphometric study of prolactin-positive pituitary cells was carried out on pituitary of adult male rats treated with fadrozole. After treatment, the cellular and nuclear areas of prolactin cells, as well as the percentage of prolactin-positive cells and percentage of proliferating-prolactin cells was significantly decreased ($p < 0.01$, in relation to untreated and control animals). Moreover, fadrozole decreases serum prolactin levels. Our results suggest that, in physiological condi-

tions, aromatase P450 exert a relevant control on prolactin-cells, probably transforming testosterone to estradiol in the pituitary gland.

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P-106 GONADAL STEROIDS REGULATE AROMATASE P450 EXPRESSION IN THE RAT PITUITARY.

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Rat aromatase immunohistochemical expression is different in male than in female adult rats. In order to analyze in these differences are related to presence of gonadal steroids, an study was carried out on pituitaries of adult castrated and castrated and treated with gonadal steroids rats using immunohistochemistry, western blotting and in situ hybridization for Rat Aromatase P450. Rat aromatase P450 mRNA was detected in the pituitary of male and female rats. Sex-related variations of mRNA evidence were observed, the mRNA signal was more abundant in males than in females; moreover the male pituitaries showed more immunohistochemical positive cells than females and by western blotting the enzyme was most abundant in males than in females. With the three methods assayed, ovariectomy elicited a considerable increase in the reaction to aromatase in females; in male rats, castration reduced the number of reactive cells, although the reaction persisted. Treatment with gonadal steroids after castration modified aromatase expression in the sense that in testosterone-treated castrated males the expression of aromatase was increased while in castrated females treated with estradiol decreased. Our results demonstrate the synthesis of aromatase in the pituitary and its immunohistochemical expression in the gland 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.

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P-107 COEXPRESSION OF AROMATASE, ESTROGEN RECEPTOR α AND DOPAMINERGIC RECEPTOR D2L IN HUMAN PROLACTINOMAS.

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In previous studies we demonstrate the immunocytochemical expression of aromatase in pituitary cells and prolactinomas in the rat, suggesting that aromatase could be involved in the genesis of prolactinoma in rodents. The aim of this study is demonstrate the expression of the enzyme in the human prolactinoma cells by immunocytochemistry and in situ hybridization and the relation among immunocytochemical expression of aromatase and dopamine receptor D2L and/or estrogen receptor α in human prolactinomas. Immunocytochemistry and in situ hybridization demonstrate that aromatase is expressed in 100% of human prolactinomas, which express moreover estrogen receptor α . Dopamine receptor D2L was expressed in 98% of human prolactinomas. These findings demonstrate the ability of human prolactinomas to bind dopamine through to dopamine receptor D2L and the synthesis of the aromatase in human prolactinomas and its functional ability to develop the estrogenic effects mediated by estrogen receptor α , suggesting that these effects could be involved in the growth of human prolactinoma.

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P-108 CORRELATION OF ESTROGEN RECEPTOR COACTIVATOR AIB1, ESTROGEN RECEPTOR α , AROMATASE WITH CELLULAR PROLIFERATION AND APOPTOSIS IN HUMAN PROLACTINOMA.

Sierra, E.¹, Blanco, E.¹, Rubio, M.¹, Carretero, M.², Pérez, E.³, Herrero, J.J.³, Font de Mora, J.⁴, Burks, D.J.⁴, Carretero, J.¹

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Although differential responses of hormone-dependent tumors to sex steroids could be strongly influenced by the relative amounts of co-activator protein AIB1, AIB1-expression and its correlation with aromatase, enzyme responsible of the local aromatization of testosterone to estradiol, and estrogen receptor α in these tumors are not yet established. The aim of the present study was to investigate the protein expression of the co-activator AIB1 in human prolactinomas and compare this expression with the expression of aromatase and estrogen receptor α and with the cellular proliferation in these tumors. Of a series of 87 adenomas studied, 56% showed high population of prolactin-positive cells and were classified as prolactinomas because they show hyperprolactinemia. Moreover, 80% of the adenomas were aromatase-positive

tumors. Interestingly, 100% of the prolactinomas were aromatase-positive tumors. Western blotting with anti-aromatase antibodies revealed a twice increase in expression of aromatase in pituitary tumors as compared to non tumoral human pituitary gland. All prolactin- and aromatase-positive tumors were positive for estrogen receptor α and AIB1. Eighty one percent of prolactinomas were PCNA-positive tumors. Nuclear expression of AIB1 was predominant expression in PCNA-positive prolactinomas. Seven percent of prolactinomas non PCNA-positive showed cytoplasmic reaction for AIB1 and were active caspase 3-positive. Our results demonstrate for the first time the pituitary expression of AIB1 in prolactinomas and their correlation with the cellular proliferation of the tumor, suggesting the possibility that AIB1 could be involved in the growth of the tumor and an abnormally high conversion of testosterone into estradiol mediated by aromatase in pituitary cells.

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P-109 INVOLVEMENT OF THE ARCUATE NUCLEUS DURING COMBINED GROWTH HORMONE (GH) SECRETAGOGUE STIMULATION IN THE STUDY OF THE PITUITARY GH RESERVE.

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Combined use of growth hormone-releasing hormone (GHRH) and GH secretagogues has been proposed for studying the pituitary growth hormone (GH) reserve. However, very little is known of the response of hypothalamic arcuate nucleus neurons to these stimuli. The present study sought to identify that response through analysis of neuron activation using c-fos protein (neuron "trigger") activation and GH release prompted by combined GHRH and GH-secretagogue stimulation, with and without somatostatin (SRIH) pretreatment.

A total of 120 female Wistar rats aged 28-30 days were used. Half received SRIH and the other half saline, at -90 minutes (SRIH-pretreated and saline-pretreated groups, respectively). At 0 minutes, both groups were given one of the following stimuli: saline, GHRH, GHRP-6, GHRELIN, GHRH + GHRP-6, GHRH + GHRELIN (1 μ g/Kg dose in all cases). Rats were slaughtered at -90, 0 and +90 minutes, and blood samples were taken for RIA determination of GH; brains were fixed in paraformaldehyde, frozen and sectioned for immunocytochemical determination of c-fos protein.

Combined GHRH and GH-secretagogue stimulation enhanced GH release, both with and without SRIH pretreatment. However, in isolated cases stimulus was enhanced by SRIH pretreatment.

In the arcuate nucleus, all GH-releasing stimuli (except GHRP 6) prompted neuron activation. SRIH pretreatment also prompted neuron activation in the arcuate nucleus; subsequent stimuli were unable to enhance this activity, with the exception of the GHRH + GHRP6 combination.

These data suggest that the maximum somatotropic response to combined GHRH and GH-secretagogue stimulation may be limited by the activation of somatostatic-type neurons in the arcuate nucleus, which exercise ultrashort control over neuron GHRH activity.

P-110 INFLUENCE OF DIETARY FOLIC ACID SUPPLEMENTATION ON CEREBELLUM FISSURATION IN YOUNG RATS.

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It is well known that low levels of folic acid (FA) cause hiperhomocysteinemia, which modifies methylation process and development of neuronal network pattern secondary, as well as potentially vasculogenesis, resulting in cognitive dysfunction in the elderly. In addition, during prenatal development, maternal availability of choline FA has been suggested to influence on cerebral neuroblasts and angioblasts in mice at the end of development in mice.

The aim of this work is to evaluate the influence of different levels of FA supplemented diets on several brain structures (e.g. cerebellum). Four groups of 10 each old rats were fed with an pure amino acid diet: Group 0 (0 mg FA/kg diet), Group 2 (2 mg FA/kg diet, control), Group 8 (8 mg FA/kg supplemented diet), Group 40 (40 mg FA/kg supplemented diet), for four weeks.

Brains were fixed in 4% neutral tampon phormalin and processed for histological study. Serial sagittal sections of 8 mm thickness were performed. Sections were stained with Klüber-Barrera, Nissl, Hematoxiline-Eosine, Bodian and metanamine silver techniques. Immunohistochemistry for glyal fibrillar acid protein in order to visualize Bergman's gly arrangement, and calbindin for Purkinje's cells were done.

Macroscopical study of the cerebellums and distribution and number of folia was normal in all groups. However, Group 0 cerebellums presented significant larger size when compared with Group 40.

Microscopic analysis showed alterations on Groups 0 and 40, with partial fission of opposite slopes of prima and secunda fissures, appearance of early fissures in development, going with pia mater missing, ectopic islands of granular cells appearance in molecular layer and abnormal pattern of Bergman's gly prolongations. The observed pattern closely remains to the one that occurs when rats are chronically fed with alcohol.

In conclusion, either no folic acid or highly supplemented diets cause corticogenesis failure in cerebellum. A later functional evaluation is needed to confirm if these cerebellum cortical anomalies may have some influence on several cognitive and behavioural marks under different dietary treatments.

P-111 DISTRIBUTION OF CATECHOLAMINE BIOSYNTHETIC ENZYMES IN THE RAT DORSAL VAGALCOMPLEX.

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The dorsal vagal complex (DVC) is formed by the nucleus tractus solitarius (NTS), the dorsal motor nucleus of the vagus nerve (DMV) and the area postrema (AP). The AP function has been connected with the cardiovascular regulations. The nucleus tractus solitarius (NTS) and dorsal motor nucleus of the vagus (DMV) constitutes sensory and motor nuclei of the dorsal vagal complex, respectively. The (DMV) is a relay centre for integrating central and peripheral signals related to cardiovascular regulation. The NTS is the primary site for cardiovascular afferent fibres termination and it is innervated by catecholaminergic neurons. The present work quest more evidences on the implication of the DVC contains catecholaminergic neurons in the arterial hypertension.

We have used 30 male rats, fed with a standard diet, sacrificed at 2, 4 and 12 postnatal months, divided in two groups: Group 1 control Wistar-Kyoto rats (WKY) and Group 2 spontaneously hypertensive rats (SHR). Paraffin serial coronal sections and DVC extract were performed. Antibodies to the catecholamines synthetic enzymes tyrosine hydroxylase (TH) and dopamine-beta-hydroxylase (DBH) were used in the immunohistochemical and western blot analysis.

The TH and DBH material immunoreactive were located throughout the rostrocaudal and dorsoventral extent of the area postrema, as well as in neurons within the NTS. The distribution and the number of TH neurons were the similar as those DBH, except in the dorsal motor nucleus of the vagus. In the area postrema the number of neurons immunocytochemical labelled for TH was higher than those labelled for DBH. Thereafter, we conclude the increase of the catecholamines in neurons of the DVC in the hypertension, are influenced by the increase the AGII level in the DVC caused by augment of gene expression of the AT1 receptor in SHR rats.

P-112 MORPHOLOGY AND MORPHOMETRY OF THE INFERIOR LARYNGEAL NERVES OF THE RAT.

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The inferior laryngeal nerves (ILN) of the left and the right sides, due to their dissimilar course around the aortic and subclavian arches (recurrent nerves), are of substantial different length. This variation in extent, in plain terms of conduction velocity, could involve a significant gap in the time of action of the musculature controlled by each nerve. Thus, several explanations have been addressed to elucidate the closest of the glottis at the same time despite the different length of the nerves in both sides.

Therefore, we undertake this study with the aim of analyze and compare the length and fibre composition of the ILN, as a background for further studies in the function of the laryngeal nerves.

Adult male Sprague-Dawley rats were used in the study. Previously to any experimental procedure, the function of the larynx was confirmed in each animal by means of laryngoscopy, in order to discard any animal with abnormalities in the laryngeal function. In one group, the ILN were dissected from its origin to the entrance in the larynx. Then, the nerves were measured in length. In other group, the ILN was excised distally to its origin - below the caudal pole of the thyroid gland-, and the sample was processed in order to obtain plastic semithin sections, later stained with toluidin. Morphometry of the myelinated fibres present in the sections were developed and analyzed using "Visilog" software.

The averaged length of the ILN was 2.79 cm in the right side and 3.63 cm in the left side, i.e., 30% longer in the left side.

The mean number of myelinated fibres was 182, with no significant differences by side. The calibre distribution pattern of the myelinated fibres was unimodal, with a maximum distribution range between 2.5 and 7.0 micrometers. No differences by side were observed in the mean calibre of the fibres.

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P-113 PROJECTIONS OF THE RAT LARYNGEAL NERVES. A NOVEL STUDY WITH DEXTRANES.

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The knowledge of the precise connections of the laryngeal nerves is of capital relevance for the larynx function comprehension. Since the beginning of the forties decade, several works have approached to the topic by means of distinct techniques including lesions in the brainstem, sections of the nerves, and, more recently, tracing studies with a variety of neuronal tracers. From the preceding studies, some discrepancies arise concerning to the organization of the central motor nuclei of the laryngeal intrinsic muscles and, in addition, less attention is paid on the central distribution of the afferents forming the laryngeal nerves.

We have used, for the first time in the literature for this model, dextrans as tracers for the superior (SLN) and recurrent (RLN) laryngeal nerves. Dextrans, when used in certain experimental conditions, are anterogradely and retrogradely transported, allowing, in the same specimen, the study of the central and peripheral projections of a given nerve.

Dextrans of several molecular weights (3.000, 10.000) were applied on the SLN and RLN of healthy Sprague-Dawley adult male rats. After a survival period, the larynx and brainstem were processed, in order to analyze the central and peripheral distribution of the components of the nerves. The SLN appears to project exclusively on the cricoid muscle, and the central projections are distributed within the nucleus ambiguus, the

dorsal motor nucleus of vagus, and the nucleus of the solitary tract. The RLN provides innervation to the intrinsic laryngeal muscles, except the cricotiroid, and, centrally, it appears to project exclusively on the ambiguus nucleus. This results open the possibility that, at least in the rat, the RLN does not convey afferent fibres of the infraglottic territories or that these afferent fibres could be send to the SLN by means of a connecting branch/es.

Our results, compared when the scarce literature on this subject, open new insights on the neural systems controlling the laryngeal function.

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P-114 KIT POSITIVE CELLS IN THE HUMAN URINARY BLADDER.

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Interstitial cells of Cajal (ICCs), which are found throughout the gut, are now known to be the origin of pacemaker signals having a major role in the transmission of signals from enteric neurons to smooth muscle cells. The discovery that ICCs in the gastrointestinal tract could be labelled with antibodies to the proto-oncogen c-kit, which codes for a tyrosine kinase c-kit receptor, led to the successful characterization of ICCs in the gut. In fact Cajal (1904) had already deduced that these cells could be present in all those organs with autonomous innervation (blood vessels, glands and smooth muscle fibres). The aim of the present work has been to investigate the distribution and morphology of c-kit positive ICCs in the normal urinary bladder. We have used urinary bladders samples from human necropsies (Hospital Clínico Universitario Lozano Blesa, Zaragoza). The samples were processed for immunohistochemical (IC) methods (En Vision, Dako®), using antibodies against glio fibrilar acidic protein (GFAP) (specific of glia cells), enolase (specific of neurons (NSE)) and CD 117 which reveals the c-kit receptor on the ICCs membrane. Our results show nervous ganglia in the submucous layer next to the smooth muscle bundles. Neurons somas have been observed in the nervous trunks placed at the submucous layer. GFAP positive reaction has been observed in the nervous trunks located in the submucous layer and inside the muscle fascicles. In the lamina propria it has been observed isolated big nuclei piriform cells. The identification of urinary bladder ICCs and their interrelationships with smooth muscle fibres innervation will lead to the interpretation of their function in this organ.

P-115 WILLIAM HUNTER'S SPECIMENS OF THE CENTRAL NERVOUS SYSTEM.

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William Hunter, the famous 18th Century London anatomist, was born near Glasgow in Scotland and left his anatomical collection to Glasgow University. This

demonstration shows 9 of the remaining 76 of Hunter's specimens on the central nervous system. Hunter carried out research into the placenta, lymphatics and bone but the specimens illustrating the nervous system seem to be a teaching collection.

The specimens illustrated are:

- 21.06 - The vertebral canal of a child.
- 21.13 - The cranial dura mater.
- 21.14 - Dried specimen showing the dura mater of a child.
- 21.17 - The external surface of the dura with the superior sagittal sinus and its lacunae laterales opened.
- 21.24 - Specimen of pia and arachnoid mater showing the vascularity of the pia.
- 21.31 - Dissection of the brainstem showing the floor of the fourth ventricle.
- 21.45 - Cerebellum of a man who had been mad for many years before his death.
- 21.50 - Sagittal section of the brain of a young woman.
- 21.54 - The olfactory nerves running from the olfactory epithelium to the cribriform plate.
- 21.61 - The cauda equina dissected.

The numbers and descriptions are quoted from the Museum Catalogue.

In a lecture (c1770), Hunter said it was highly probably that we would always remain ignorant of how the nervous system worked. In his lectures, Hunter described the meninges but gave little detail of the internal features of the brain. He did, however, cover the corpus callosum, corpus striatum, fornix, septum lucidum, thalamus and choroid plexus. In one set of notes he is recorded as saying, "I have shown you the parts of the brain in a general way so that you are able to describe the sites of disease, and understand the anatomical language for the various parts of the brain: as to the use of these parts neither I nor anyone else knows it. One of the sets of notes (c1770) contains a remarkable insight – that there might be a link between the nervous system and electricity that had recently been discovered.

P-116 CONTRIBUTION OF GEOMEDIA PROFESSIONAL TECHNOLOGY TO THE COMPILATION OF DOCUMENTAL BRAIN MAPS.

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We describe an Informatic Application (IT) development that has allowed us to capture, edit and manage spatial data by means of the interrelationship among different standard industrial databases, including Microsoft Access and Oracle SDO/SC.

As reference images we used axial sections from the Visible Human Project (*National Library Medicine, Bethesda, Maryland, USA*). The sections were obtained at a resolution of 2048 x 1216 pixels by 24 bits of colour per pixel and a separation between slices of 1mm. However, each section was stored in a single file compressed in Unix form and, once decompressed, affords a native

image of 512 x 512 pixels in size and with 16 bits/pixel. The collection of sagittal and coronal sections was accomplished by means of the so-called re-slicing technique.

Vectoring and segmentation of each brain structure allows the collection of three-dimensional reconstructions of these, after which they are assigned textures, semi-transparencies, etc.

Guided by a defined system of stereotaxic coordinates, based on the atlases of Tailarach and Tournoux (1988), the information was introduced into the databases. This offers a true georeferenced information system (GIS) of the human brain.

Geomedia Professional is a last-generation ITtool for the compilation of maps, allowing a smooth link-up and integration with other standard programs such as Visual basic and Visual C++, Power Builder, Delphi, Excel, or Foxpro among others. Also, this tool supports simultaneous access to different GIS databases, and with different formats, such as MGE, FRAME, ArcView, MGE Segment manager, MGE GeoData manager, and MAPINFO, among others.

The convergence of data joined by the association of different articulated databases is of great interest in the field of neuroanatomy since it allows the simultaneous management of abundant interrelated information.

TAILARACH J and TOURNOUX P (1988). Coplanar Stereotactic Atlas of the Human Brain Thieme. Stuttgart.

P-117 IMPLEMENTING A NOVEL TOOL FOR ULTRADISECTION OF TISSUES.

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Introduction: Antibody arrays are being proposed as a useful tool for identification and simultaneous quantification of proteins. Samples from topographically selected areas can identify specific regional proteic profiles.

MATERIAL AND METHODS: Extracting of proteins from the selected tissue area was performed using special lysis buffer and after several incubation times with the antibody array, we studied the protein profile of 35 samples of different brain tissues; 5 from healthy individuals (CTRL), 15 belonging to low grade astrocitoma (LGA) and 15 from glioblastoma (GBM), using a 19 antibody array (Bcl-2, Caspase-1, Caspase10, E2F1, E-Cadherin, EGFR, HSP-70, Integrin alfa 5, Integrin beta 3, MGMT, MMP-9, P21waf1/cip1, p53, PTEN, Rb (p110), VEGFR1, VEGFR2, ZO-1 and Actin; Hypromatrix). Quantification of immunopositive spot areas had been done by Image J computer software.

RESULTS: We found differential protein expression between CTRL and GBM groups, and between CTRL and LGA; those differences increased between the two studied tumoral stages for almost all the proteins included in the assay and analysed results by a univariate varianza SPSS software analysis; obtaining significant differences $p < 0.05$, for the expression of Caspase-1, Caspase10, E-Cadherin, p53, VEGFR2 and $p < 0.005$ in the case of Bcl-2, EGFR, MMP-9, PTEN, Rb (p110) expression.

CONCLUSIONS: Identification of differential protein expression gives a morpho-functional view of tissue composition and physiological processes (metabolism, signalling etc...) taking place in it.

P-118 DISRUPTION OF PRECISION GRIP PATTERN IN ON/OFF PARKINSONIAN PATIENTS USING AN ANTHROPO-MORPHIC DEVICE.

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Several parameters of grip and load force have been studied in Parkinson's Disease (PD) by means of precision grasping tasks. The aim of this work was to carry out an accurate study of reach-load-grip-hold-place-release tasks in 27 patients (17 women, 10 men, 62.70 ± 10.4 years), in ON and OFF state, and 27 aged matched control subjects (16 women, 11 men, 61.26 ± 20.74) using a sensorized anthropomorphic device (5DT data Globe); to measure the grip force (GF) it was used a piezoresistive sensor located over the data glove in the position of the index fingertip and an accelerometer was placed on the wrist and over the data glove in order to measure the accelerations of the hand. This apparatus can measure precisely several parameters related to abnormalities in the movements performed by PD patients. The experimental task consisted of gripping four experimental objects of different textures and weights, one by one, using index and thumb to carry out the reaching, loading, holding, placing and releasing tasks.

RESULTS: PD patients (ON and OFF states) needed significantly more time than the control group to complete the task; load velocity, acceleration, grip force and velocity in the application of the grip force during execution of the tasks were lower in PD patients (independent of their state) than in the controls. Coordination between the two forces involved was impaired and down movement made by PD patients was delayed with respect to the control group, and to a greater extent in OFF than in ON patients.

CONCLUSION: these delays could be a kind of freezing phenomenon occurring at the beginning of the different subtasks of the movement. This phenomenon would have its origin in the basal ganglia, which has lost the capacity to generate discrete control actions at specific time landmarks during the execution of the task.

P-119 PROTECTIVE EFFECTS OF MEMANTINE AGAINST NEURODEGENERATION OF THE BASALOCORTICAL CHOLINERGIC SYSTEM.

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Memantine (1-amino-3,5-dimethyladamantane) is an uncompetitive N-methyl-D-aspartate (NMDA) glutamate receptor antagonist. We aimed to investigate the therapeutic efficacy of memantine in different behavioural outcomes in a rat model of unilateral and bilateral cortical devascularization (which induces a loss of the cortical cholinergic terminal network and a retrograde degeneration of the cholinergic projections that originate in the nucleus basalis de Meynert). A total of 28 Sprague-Daw-

ley rats were randomly divided in 7 groups: control, three groups of animals with unilateral lesion (without treatment/saline, treated from 3 days before the lesion, and treated after the lesion), and three groups of animals with bilateral lesion (without treatment/saline, treated from 3 days before the lesion, and treated after the lesion). The dose of memantine was 20 mg/kg/day was administered via Alzet minipumps implanted subcutaneously in the posterior thoracic region. Control and treated rats were trained in an elevated T-maze and in the water maze before starting the experiment and the behavioural effects were assessed following a postsurgical delay of one week, and then repeated once a week after the lesion, until the 28th day. All memantine-treated rats: i) recover spatial abilities lost after cortical devascularization (both unilateral and bilateral, and with treatment and ii) were significantly able to make more efficient use of their capacities to master the T- and the water maze tasks. These results suggest a neuroprotector action of systemically administered memantine, both starting before and after cortical devascularization, and mitigates cholinergic degeneration *in vivo*.

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P-120 TIME COURSE OF THE MORPHOLOGICAL EXPRESSION C-FOS AND HSP-90 IN THE CNS AFTER ACUTE EXPOSURE TO 900 MHz GSM RADIATION OF PICROTOXIN MODEL OF EPILEPTIC RATS.

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Electromagnetic fields (EMFs), with no thermal stress, are suspected to have several biological effects and induction of genes. In previous studies we have found neuronal activation markers (c-fos) in the brain of rats pre-treated with subconvulsive doses of picrotoxin, after 2hs exposure to a 900 MHz GSM (Global System for Mobile communications) radiation, at intensities similar to those emitted by mobile phones. In this study we analyzed –by means of immunochemical testing of relevant anatomical areas– the relationship between the induction of c-fos and heat shock proteins HSP90 markers in acute experimentally models of epilepsy (induced by picrotoxin), to establish the effects on the CNS (Central Nervous System) of 50 adult male Sprague-Dawley rats after 60 minutes, 24 hours and 3 days of exposure to EMF radiation. After exposure, the animals were sacrificed and their brains were cut into sections, which were processed for c-Fos and HSP90 immunodetections. C-Fos positive cells were counted in cortical and hippocampal areas, using a microscope connected to a morphometric software, having expressed counts per field as means \pm s.e.m. The significance of between–group differences was estimated through two–way analysis of variance Tukey or Kruskal–Wallis tests. 60 minutes after the radiation (AR), the c-Fos positive cells were double in all cortical areas, except in entorhinal cortex, where there were smaller differences; 24 hours AR there were differences in paleocortex and none in neocortex; after 3 days the counts were similar in the same areas of the exposed–and–treated and non–exposed–and–treated animals. In hippocampal areas

the c-Fos expression showed differences after 60 minutes and 24 hours in CA₁ and CA₃ areas, but did not in Dentate Gyrus. After 3 days, none of them showed differences. The HSP90 expression showed differences in Dentate Gyrus, CA₁ and CA₆₀ minutes AR, while did not show after 24 hours in all areas; after 3 days in there were differences only in CA₁.

The time course of the effects after the GSM radiation on the CNS showed to be recovered after 3 days.

P-121 C-FOS DISTRIBUTION-PATTERN IN BRAIN OF MICE CHRONICALLY EXPOSED TO POWER-FREQUENCY ELECTROMAGNETIC FIELDS AT ENVIRONMENTAL POLLUTION DENSITIES.

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Last years some epidemiological studies related several pollution levels of power-frequency electromagnetic fields (50 Hz) with different pathologies, particularly children's leukaemia. These people were chronically exposed to high levels (upper 0.2 microTesla) of these physico-polluted environmental as those living near power-lines. However these results have been refuted by other studies and today these health-effects are still confused. In order to explore effects into laboratory, we have developed an experimental system that produces electromagnetic fields similar to the above mentioned. Inside a large tube-solenoid ten mice were introduced in individual dielectric-boxes where animals were chronically exposed at very homogeneous electromagnetic fields (10.0 microTesla and 50.0 Hz) during a very long period (9 month) and a sham-system with ten mice were used as a control. Immediate early genes were used as a model to explore different distribution-patterns in cortical and sub-cortical brain areas compared to exposed and sham groups. We have found some differences in both exposed/sham brains and a statistical significant reduction of c-Fos expression at both habenular and paraventricular thalamic nucleus and some evidences to a global reduction of immuno-reactive expression in the brains exposed.

P-122 MORPHOMETRIC EVALUATION FOR THE USE OF MAGNETIC RESONANCE IMAGING TO CHARACTERIZE NEURODEGENERATION IN MOUSE MODELS.

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Common anatomical manifestations of neurodegenerative diseases such as Alzheimer include loss of neurons, formation of plaques and tangles, and inflammation. However, detection and analysis of these pathological changes by histological methods can only be performed postmortem, thus complicating an accurate diagnosis in humans or limiting aging studies and therapeutic trials in the case of animal models. Therefore, approaches allowing the direct, *in vivo* visualization of brain pathophysiology would greatly facilitate assessment of disease status in humans and would enable researchers to fully exploit mouse models of neurodegenerative disorders. Here we present the use of magnetic resonance imaging (MRI) as an analytical tool in the characterization of neurodegeneration the IRS-2 knockout model.

Insulin and IGF-1 exert their various physiological effects by phosphorylating and activating insulin receptor substrate (IRS) proteins. Deletion of IRS-2 in mice dramatically reduces brain size due to impaired neuronal proliferation during development. The absence of IRS-2 in adult brain produces an additional pathology: hyperphosphorylation and accumulation of the microtubule-associated protein tau. Young IRS-2 knockouts display defects in learning and memory as assessed by tests such as Morris water maze. We reasoned that IRS-2 deficiency might represent a reasonable mouse model of neurodegeneration. Therefore, we allowed these animals to age and subjected them to MRI at 18 months of age (Bruker Pharmascan system, Instituto Biomedicas, Madrid). This analysis revealed a very striking pathological difference in cerebral ventricles of aged KOs as compared to WT controls. We used these images to measure area, diameters and perimeter of lateral, medial and fourth ventricles and thus, have detected significant increases of these structures in the brains of IRS-2 KOs. Interestingly, enlarged ventricles have been reported for several neuronal disorders including schizophrenia and Alzheimer's. Subsequent histological analysis of these same brains revealed other abnormalities including neuronal loss and amyloid deposits joint to decreases of the thick of cortical areas and basal ganglions. Thus, our initial observations suggest that MRI provides a valuable tool for *in vivo* detection of pathological changes in neurodegenerated brains of mouse models.

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P-123 MORPHOLOGICAL DATA SUPPORT INDUCED SYNCHRONIZING ACTIVITY UNDER APPLIED MAGNETIC FIELDS IN ELEMENTAL TWO-NEURON NETWORKS OF MOLLUSCS NERVOUS SYSTEM.

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Synchronizing activity in pairs of pacemaker neurons from the central nervous system (CNS) of molluscs (*Helix aspersa*) has been observed to be induced under applied sinusoidal magnetic fields (AMF) of extremely low frequency (50 Hz) and low intensity (1-15 mT) (1, 2). The aim of this work has been to find out a morphological support for the synchronizing activity. It has been widely accepted that in mammals the synchronization of neurons bioelectric activity is promoted through gap junctions, being astrocytes the main cells responsible for the transmission of excitability between neuron distant networks. We have found by electron microscopy (EM) and immunocytochemistry (IC) methods morphological data which support the described synchronization in molluscan neurons induced by applied AMF. EM shows neuron-neuron, neuron-glia and glia-glia gap-like junctions. We have made the characterization of *Helix aspersa* nervous cells with connexin 26 (Chemicon), connexin 43 (Chemicon), enolase (Chemicon) and glio fibrillary acidic protein (GFAP, DAKO) antisera. Positive and negative controls have been made on Wistar rat brain samples. We have concluded that in molluscs CNS: i) there are two kind of glia cells distributing respectively among neurons soma and neuron fibres in the neuropile; ii) neurons and glia cells express connexin 26; iii) neither enolase nor connexin 43 and GFAP are good markers for molluscan CNS cells. Such a reaction differences could be the consequence of either non-specificity for mammals antisera or phylogenetic differences in the expression of the proteins tested in molluscs with respect to mammals nervous cells. We have shown that applied 50 Hz MF mimic the effect of glutamate on neurons (3). MF stimulation is mediated by calcium ions which are free liberated due to the interaction of MF with neuron membrane accordingly with our proposed model (2). We have come to the conclusion that applied 50 Hz-MF induces simultaneous stimulation of neuron-glutamergic and glutamate-glia-dependent pathways, as a result neurons synchronization is facilitated by applied sinusoidal MF.

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P-124 CORTICAL AXONAL PROJECTIONS TO SOMATO-SENSORY AREAS IN RABBIT CORTEX.

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The axonal connections presented here had been scarcely studied in rabbits to date. In doing this, we have additionally estimated the occurrence of inverted pyramidal (IP) cells out of the examined projection cells. Young adult rabbits received 1% CTb (0,2-0,4 µl) injections either in primary somatosensory cortex (SI), the rim between somatosensory and visual areas (S-V), or in precentral areas, both medial (PrCM) and lateral (PrCL).

• Injections in PrCM caused retrograde labelling that was important in PrCL, SI, SII and anterior limbic cortex

and scarce in the posterior limbic cortex and prefrontal cortex.

- *Injections in PrCL* yielded important retrograde labelling in SI. They also labelled SII, PrCM, prefrontal areas and anterior limbic areas.

- *Injections in posterior SI* produced retrograde labelling in SII, PrCL and S-V. In turn, *injections in medial SI* did so in PrCM and several polymodal associative areas such as area 25 (which is prefrontal), anterior cingulate areas (especially the rostralmost one) and perirhinal cortex.

- *Injections in posterior S-V* revealed major retrograde labelling in SI and VI, minor in SII and scarce in VII, PrCL y PrCM.

In the insular claustrum, there was either minor retrograde cell labelling following the injections in SI, S-V, or major following those in PrCM. The latter injections also labelled projection cells in anterior endopiriform claustrum and basolateral amygdala.

There was usually many retrogradely labelled IP cells among labelled infragranular neurons. Out of the latter cells, IP-cell percentage was higher for projections from associative areas (S-V, VII, SII) to SI, PrCL to S-V and VI-VII to PrCM, in said order.

The results of the present study show that the motor precentral cortex can be further subdivided in the rabbit cortex. Because of its axon connections, in particular with the amygdala, PrCM would correspond with the motor supplementary area of other mammals.

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P-125 ANALGESIC-INDUCED ANTINOCICEPTION MAY BE MEDIATED BY THE A7 NORADRENERGIC NEURONS IN THE RAT.

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The endogenous antinociceptive circuitry, originally described by Basbaum and Fields, involves the mesencephalic periaqueductal gray (PAG) and the rostral ventromedial medulla (RVM). It has been clearly established that spinally-projecting neurons located in the RVM, which includes the nucleus raphe magnus and the nucleus reticularis gigantocellularis pars a, constitute an important component of this descending system. However, modulation of nociceptive transmission induced by activation of RVM neurons is mediated in part by noradrenergic neurons that project to the spinal cord. Anatomical tract tracing studies have demonstrated that a significant population of neurons in the RVM project to the pontine A7 catecholamine cell group that provide the major noradrenergic innervation of the spinal dorsal horn. The aim of

this work was to provide more evidence that activation of neurons in the RVM produces antinociception by activating noradrenergic neurons in the A7 cell group.

We studied the expression of Fos-like immunoreactivity into the tyrosine hydroxylase-positive A7 neurons of the rat after intraperitoneal injection of two analgesics: morphine and metamizol.

Fos-like immunoreactive neurons were localized near the A7 cell group both in the morphine treated and in the metamizol-treated animals. However, only a few Fos-positive cells were also tyrosine-hydroxylase-immunoreactive A7 neurons.

Our results are consistent with the possibility that some of the analgesic effects of morphine and metamizol may be attributable to activation of the A7 noradrenergic neurons.

P-126 ANALGESIC-INDUCED FOS-LIKE IMMUNOREACTIVITY IN THE RAT ROSTRAL VENTROMEDIALMEDULLA.

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The rostral ventromedial medulla (RVM) is a critical relay from the mesencephalic periaqueductal gray (PAG) to the spinal cord dorsal horn. The RVM includes the nucleus raphe magnus and the nucleus reticularis gigantocellularis pars a. All these structures were originally described by several authors as a part of an endogenous antinociceptive circuitry. The goal of the experiment was to determine if the intraperitoneal injection of a mild analgesic such as the metamizol or an opioid analgesic such as the morphine were able to induce expression of c-fos proto-oncogene into the nuclei of the RVM.

Experiment was carried out in adult male Sprague-Dawley rats. We used Fos immunocytochemistry to demonstrate the activated neurons. A systemically administration of metamizol was chosen as antinociceptive non-opiate stimulus while morphine was the narcotic analgesic. Rats were subdivided into three groups: a metamizol-treated group (500 mg/kg, i.p.), a morphine-treated group (10 mg/kg, i.p.) and a control group (saline, i.p.). Animals were sacrificed 2 hours and half an hour after the analgesic (or saline) stimulation.

The results showed Fos-like immunoreactivity in the nucleus raphe magnus and nucleus gigantocellularis pars a of both metamizol-treated and morphine-treated rats in contrast with the control rats where Fos-like immunoreactive elements were absent.

These results support that the RVM is an important component of endogenous antinociceptive circuitry. The results are also consistent with a role of descending projections from RVM in both opiate and non-opiate antinociception.