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In memoriam Prof. Alberto Binia

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Prof. Alberto Binia, M.D.
(1933-2002)

Alberto Binia was born in Buenos Aires. He got his medical degree as one of the first 25 physicians graduated at the Medical Sciences School of Cuyo National University. Afterwards Dr. Binia was awarded scholarships by the Argentine National Council of Scientific and Technical Research (CONICET) and by the Rockefeller Foundation and began his career in cardiovascular research as the main collaborator of Prof. Juan Carlos Fasciolo. Besides his original research contributions, particularly in the field of arterial hypertension, Dr. Binia directed doctoral candidates and trainees who are active both in Argentina and abroad.

After Prof. Fasciolo's retirement, Dr. Binia succeeded him as full professor of Pathophysiology. He was active in virtually every conceivable academic area, from teaching to undergraduate students to research promotion. He served as Secretary of the University Research Council and as the representative for the Cuyo region before the National Secretariat of Science and Technology. Prof. Binia was appointed vicerector of Cuyo National University (1986-1988). In 1988 he was elected Dean of the Medical Sciences School and reelected for this position in 1992. After his retirement he was appointed Emeritus Professor and stayed quite active in the field of medical education. In 1992 he was granted the title *Master of Medicine* by the Argentine National Academy of Medicine.

Prof. Binia was a member of a number of national and international scientific societies. He also honoured the Cuyo Biology Society as a long-time member and enriched it with his ideas and deeds, serving as its President and Board member in several periods. His intelligence, soundness and last but not least his bright sense of humor will be sorely missed.

L.1.**IN VITRO REGULATION OF THE SERTOLI CELL***Anna Steinberger, Ph.D.**Professor Emeritus,**University of Houston Medical School**Houston, Texas (USA)*

Already in the nineteenth century, Enrico Sertoli (1842-1910) suggested that the supporting cells of seminiferous tubules which bear his name performed functions of nutrition and support of developing germ cells during spermatogenesis. Since then the nature of Sertoli cell function and its underlying mechanisms of action have been extensively investigated both *in vivo* and *in vitro*. Early electron microscopy findings showed specialized tight junctions between adjacent Sertoli cells, which establish a functional blood-testis barrier allowing selective transport of circulating substances to the luminal compartment of the seminiferous tubules. Sertoli cells were also considered as secretors of fluid into the tubular lumen. Cytochemical experiments by Mancini *et al.* (1967) suggested that Sertoli cells could be a primary target for the effects of follicle-stimulating hormone (FSH) in the testis. However, more direct evidence about Sertoli cell secretion and its regulation came from later experiments performed *in vitro*. These studies were made possible by the development of techniques for the isolation and culture of Sertoli cells (and most other testicular cells) in a chemically defined medium. Although *in vitro* results may not be representative of normal physiological conditions, culture models yielded useful systems for research in Sertoli cell secretions and its direct responses to hormonal stimulation and other factors. Studies from our and other laboratories have furnished clear evidence showing that Sertoli cells secrete a variety of proteins, including androgen binding protein (ABP), transferrin, and inhibin among others. All of these secretions are increased by addition of FSH to the medium and in many cases, also by testosterone. Evidence was obtained indicating that in the testis, receptors for FSH and FSH-induced increase in cAMP are located exclusively in Sertoli cells and are age-related. It was also found that Sertoli cells have cellular and nuclear receptors for androgens, indicating that they are under hormonal regulation both by FSH and testosterone. Moreover, since neither FSH nor androgen receptors have been detected in germ cells, it seems that the effects of these hormones on spermatogenesis must be, in a still poorly understood way, mediated by Sertoli cells. Regulation of Sertoli cell function also involve complex paracrine interactions with other testicular cells. Although a wealth of information has been gathered, a complete understanding of Sertoli cell function and its role in mammalian spermatogenesis remains elusive.

L.2.**ANDROGENS AND SPERMATOGENESIS: A historical perspective***Emil Steinberger, M.D., F.A.C.E.**Consultant**Texas Institute for Reproductive Medicine and Endocrinology. Texas, Houston (USA)*

Androgens enjoy a long and distinguished history. Already in 300 B.C., Aristotle dealt with the effects of castration in fowl and man. Somatic changes which follow castration were a subject of discussion and practical applications for centuries.

In 1885, Berthold showed atrophy of the rooster comb after castration and its regeneration with a testicular transplant. This work introduced the first androgen bioassay, which was employed well into the twentieth century. In 1903 Loisel found lipidic granules in Leydig cells and afterwards McGee showed the androgenic activity of lipidic testicular extracts. Testosterone was soon isolated and identified, and the steroid was synthesized in 1939 by Butenandt and Ruzicka, who shared the 1939 Nobel Prize on account of this feat. Notwithstanding this fast early advances, to date there is no androgenic compound suitable for oral administration.

In 1927, P.E. Smith found that hypophysectomy prevented testicular development in immature rats and that it caused atrophy of the testes and accessory sexual glands in mature animals. These changes could be avoided by administration of pituitary homogenates. Greep (1934) and others showed that follicle-stimulating hormone (FSH) promotes spermatogenesis, and that interstitial cell-stimulating hormone (ICSH = LH) stimulates androgen production in Leydig cells. In the next year, Welsh and others found that, in the rat, testosterone can support spermatogenesis when given alone. However, it was later observed that testosterone-induced spermatogenesis was quantitatively abnormal; also, attempts of inducing or keeping spermatogenesis with testosterone alone in men were unsuccessful. Indeed, high testosterone doses actually depress spermatogenesis in men (Hotchkiss, 1944). In the normal human male, plasma testosterone shows fast oscillations which are synchronous with ICSH plasma levels. Chronic administration of testosterone enantate results in normal plasma testosterone levels, suppresses gonadotropin and testicular testosterone secretion and stops spermatogenesis. It remained to be shown, however, that a high local level of testicular testosterone was able to induce spermatogenesis.

The proof came from a clinical case of precocious puberty in a 6-year old boy, whose plasma testosterone was in the normal adult male level (384 ng/dL); plasma gonadotropins were undetectable. Testosterone was markedly high in the right spermatic vein (17,100 ng/dL). After ablation of the abnormal testis, plasma testosterone fell to 4 ng/dL. A Leydig cell tumor was found, which was shown *in vitro* to have high testosterone concentration and *in vitro* synthesizing capacity. Tubules located close to the tumor showed spermatogenesis up to the latest stages of spermiogenesis, while more distant tissue featured prepuberal seminiferous tubules. This case demonstrated that a high local concentration of testosterone, even with undetectable plasma gonadotrophins, is able to initiate the process of spermatogenesis. The inability of hypogonadic human males to initiate spermatogenesis, even when given massive testosterone doses, was explained by fundamental differences in the kinetics of spermatogenesis in humans compared with rats, and by the practical impossibility of administering to humans the equivalent of doses given to rats: 1-2 mg (4-8 mg/kg) in a 250- g BW rat corresponds to 320 to 480 mg in a man! Advances in molecular biology allowed identification of the testosterone receptor, sequencing of the androgen binding protein and to single out the Sertoli cell as the target of testosterone and FSH, although the complex interactions at this level between the two hormones still remain unclear.

Conclusions: 1) Testosterone is essential for initiating and maintaining spermatogenesis in the human male. 2) hormonal requirements for initiating spermatogenesis and restarting or maintaining it in the adult are different. 3) Hormonal requirements for maintaining a complete spermatogenesis and their quantitative aspects are different. 4) In the human male both FSH and high testicular testosterone are needed for a quantitatively normal spermatogenesis. 5) Currently a high testicular testosterone concentration is therapeutically attainable with hormones with ICSH-like activity such as hCG or LH. 6) FSH and testosterone exert specific effects in different stages of spermatogenesis; clinical use has to take into account their cooperative interactions. 7) Clinical applications and availability of androgens and gonadotrophins are way ahead of our current understanding of the basic role of testosterone in spermatogenesis.

L.3.**IN VIVO TRANSFECTION OF MAMMALIAN REPRODUCTIVE TRACT EPITHELIA**

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Injections of DNA/liposome complexes in the female mouse reproductive tract produces transfection, with 14 % of epithelial cells which line the uterus and oviduct expressing the transfected gene. In the uterus, transfected cells are mainly those of the glandular epithelium, while in the oviduct they are those epithelial cells of the isthmus and ampulla. The efficiency of transfection was relatively high, since transgene expression was detected for one week in the oviduct and for two weeks in the uterus. An analysis of factors controlling transfection showed that this phenomenon is regulated mainly by the hormonal status of the animals. Specifically, transfection efficiency was highest when the procedure was carried out in females with high progesterone and low estradiol levels.

In male mice, transfection was also obtained in the vas deferens epithelium through injection of an exogenous DNA associated with several lipids or cationic liposomes. In this case transfection efficiency was quite high, with regions of the epithelium expressing the transfected gene for two months after injection. Recently we have been able to transfect the cauda epididymis with similar results. Furthermore, when a construct containing genes expressed as a secretion, a modification of the fluid composition of transfected vasa was obtained.

These results demonstrate the feasibility of *in vivo* transfection of epithelial cells lining both the male and female mammalian reproductive tracts, and even the potential to modify the secretion produced by these cells. In the near future these techniques might become an important tool for the study of reproductive physiology. The possibility of applying transfection technology to practical goals (e.g., veterinary and clinical) may also be envisaged.

L.4.**ALGAE AND FORENSIC SCIENCES****with particular reference to diatoms**

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When a person drowns, water enters the lung in increasing amounts and reaches the bloodstream through injured peripheral alveoli. Any other particulate material present in water (besides plankton) is convectively transported with it and as long as the heart continues to pump blood, will be distributed all over the body.

In many natural and artificial water sources, this particulate material includes diatoms with a characteristic profile according to the specific source. Therefore, the species found at necropsy may provide information about the place of drowning.

Increasingly, the study of diatoms for the precise diagnosis of death by drowning has led to clarification of doubtful death causes. This implies a direct relationship with criminalistic investigation and ulterior trial. In these cases, the forensic scientist assists the coroner and a joint work protocol may be established.

In practice, many deaths caused by drowning occur in sweet water environments. The first question is whether the death really was caused by drowning. If so, then the next question is where did it happen. At this point the analysis of diatoms recovered from the body, which may be compared with those from the putative place of death becomes important.

It is possible to find diatoms in organs from non-drowned corpses. Recognition of "naturally appearing" diatoms from those which suggest drowning is a challenge, and it takes an experienced diatomologist to make the distinction. Expertise is needed for a detailed study leading to identification of species and inference of the most probable ecological scenario where death actually took place.

1. POLLEN FLORA OF SAN LUIS, ARGENTINA, I: POLLEN ATLAS OF ALLERGENIC PLANTS

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Pollinosis (or hayfever) is the most important cause of allergy in Argentina. The Poaceae pollen is the main aeroallergen responsible for sensitizations among atopic inhabitants in San Luis City. Botanic and aeropalynological studies are essential for correct diagnosis and treatment of patients, including the identity of plants known and/or suspected as allergens, a blooming calendar from them; and an allergological reference palinoteca for future aerobiologic studies. During a study on the pollination syndrome of anemophilous plants that fulfil Thommen's Postulates, and/or those that were mentioned as allergens, there were established the presence of 175 species native and exotic plants related with allergies, distributed in urban and periurban areas of San Luis City. Were processed more of 130 pollen samples of 75 different species (part of the regional palinoteca of Herbarium UNSL). There were registered the blooming phenodates of 120 species since July 2002, too. These studies can carry to a more adequate knowledge of local pollinosis syndrome and states the bases for the projected local aeropalynological study.

3. PRELIMINARY MAP OF RISK OF EROSION OF THE "PAMPA DE LAS INVERNADAS" (SAN LUIS, ARGENTINA)

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Starting from the photointerpretation one can obtain information about the characteristics of the current erosion and of the involved processes. Our objective was the use of the photointerpretation applying the physiographic analysis method for the making of a preliminary map of risk of water erosion in the sector that is located at the 32° 47' of South latitude and 66° 07' of longitude West. The landscape is a high plateau that was subdivided in sublandscapes by slope degree, it forms and longitude. The area understands soils developed on the rocks mentioned with contributions of eolic loess and something of volcanic ash. Inside the opposing sublandscapes, a gradation of the processes erosive is observed, from those slightly affected to the areas gravely erode. However, he/she stands out the advance of the erosion in the sector, new excavates and gully in whose areas are exposed in many of the cases the superficial rock taking place. These degradational processes are increased by an increase of the precipitations and a bigger pressure in these lands for the pasturing to which they are subjected.

2. EVOLUTION OF C-CO₂ OF SUNFLOWER RESIDUALS IN CONVENTIONAL AND NO TILLAGE IN SAN LUIS SOIL

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The growing deterioration of the atmosphere has caused a renovated interest in evaluating the incidence of different systems on the organic matter of the soil. The quantity of this in semi-arid soils is relatively low, influencing the properties of more importance. The objective of this work was to study the evolution of carbon (C) of the CO₂ of the residuals of sunflower crop simulating two practices: conventional (CT) and no tillage (NT). The C was determined by measuring CO₂ in alkaline trap. The obtained data show that of evolved C-CO₂ of the different treatments is intense during the first days of incubation. The data of the curves adjusted significantly ($r^2 > 0,97$ in all the curves) to the pattern of square root ($y = a + b \cdot x$) with $p < 0,01$. Significant results were obtained among the two simulations in favor of the CT with regard to the NT. The curves of NT begin to fall starting from the 150 to 160 days, what would imply a smaller rate of decomposition and therefore the necessity to fertilize the sunflower cultivations since in NT its mineralización under field conditions will be smaller.

4. INTRODUCTION OF A BOOK REFERRED TO THE TEACHING OF AGRICULTURAL BOTANY

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The objectives of this work are: a) to write a book that includes the main concepts about Agricultural Botany and allows university students to handle easily didactic and consulting material. b) To achieve a high- quality and low-cost book. The content was selected according to the authors' experience of teaching and investigation. The book consists of 426 pages and 704 illustrations. 156 families of vascular plants are described and illustrated. There is an introductory section related to Botany in which the main morphological levels of organization, which are complemented with conceptual tables, are described, illustrated and discussed. On the second part of the book, the divisions Pinophyta and Magnoliophyta are enumerated, emphasizing those families with an agronomical interest. The third part describes and analyzes the phytogeographical regions of Argentina, and at the end, the appendix includes an agronomical classification of species (forestal plants, weeds).

5. NOGOLÍ RIVER BASIN DIATOMS (SAN LUIS, ARGENTINA). PRELIMINARY QUANTITATIVE DATA.

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This work shows the quantitative results of the plankton diatoms collected at sampling spots along the Nogolí River basin. 1: upstream; 2: El Molle River; 3: La Quebrada River; 4: El Molle and La Quebrada Rivers confluence, and 5: near the dam. The results corresponded to 2000 spring at all the five sampling spots and to 2001 fall for the first three spots. The most abundant species was *Cymbella affinis* Kütz, associated to *Cymbella cymbiformis* Agardh. In spring, it kept its dominance and abundance with 220 org/ml at La Quebrada and a minimum of 72 org/ml upstream. In the fall, *Cymbella affinis* Kütz showed a means of 229 org/ml, (max=265 org/ml in the La Quebrada River and min=195 org/ml upstream), associated to *Achnanthes minutissima* Kütz., means density=144 org/ml (max.=205 org/ml at La Quebrada). Except for *Synedra ulna* Ehr. (100 org/ml at El Molle) and *Epithemia sorex* Kütz (98 org/ml at the confluence), the rest of the species did not surpass 50 org/ml. La Quebrada River, thus, presents the greatest population density of these studies.

7. OCCURRENCE OF ANOMALOUS STRUCTURE IN ROOTS FROM THE HILLY AREA

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The adaptative characters of radical system from the hilly area have been investigated. High percentage of Fabaceae species have succulent root, with characteristic shapes. These modifications could be consequence of unusual growth root. The objective of this work is a) carry out an ontogenetic and comparative study of roots of *Pfaffia gnaphalioides* (L. Fil.) Mart. (Amaranthaceae), *Adesmia cordobensis* Burkart, *Coursetia hassleri* Chodat, *Rhynchosia diversifolia* Micheli var. *diversifolia*, *Rhynchosia diversifolia* Micheli var. *prostrata* Burkart, *Rhynchosia edulis* Grisebach and *Galactia marginalis* Benth (Fabaceae), and b) characterize different secondary growth. Samples of roots of seedlings and adults were fixed in FAA and included in Histowax, subsequently processed for histological studies. Histologically, the roots showing secondary structures with predominance of parenchymatous tissue. This parenchyma is related with water and starch accumulation. The analyzed unusual secondary growth were: 1- formation of extensive radials in *A. cordobensis*, 2- differential activity of cambium (*C. hassleri*, *G. marginalis*), 3- behavior of normal cambium and supernumerary cambia (*P. gnaphalioides*, *R. diversifolia* var. *diversifolia*, *R. diversifolia* var. *prostrata*, *R. edulis*). This type of growth has not been mentioned for roots of Fabaceae.

6. TRICHOPTERA'S FOOD CATEGORIES IN GRANDE RIVER (SAN LUIS, ARGENTINE)

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The Trichoptera Family has a great importance in trophic chains of rivers and streams. Food habits determination and the functional classification of aquatic insect species improve the understanding of ecological process that take place in the lotic ecosystems. The classification of invertebrates in the Functional Feeding Groups (FFG) is a classification based in the feeding mechanism. It has four categories: shredders, collectors, scrapers and predators. The main objective of this study was confirm or reassign the FFG of the Trichoptera Family present in the Grande river based in the diet analysis composition. Field sampling were produced during the high and low water period in four stations of the river. The gut content was analyzed and were identified eleven categories of feeding items and the relative importance of each one. Tree species were recategorized and five were confirmed. *Smicridea* spp. and *Chimarra* sp. acts as filterers-collectors *Marilia cinerea* behave as collector-gatherer during the first stages and in bigger specimen abundant rest of invertebrates were observed. *Metrichia* sp. and *Hydroptila* sp. was categorized as collector-gatherer and piercer-herbivore. The diet analysis of *Helicopsyche* sp. and *Protophila dubitans* confirms the descriptions founded until now *Polycentropus joergenseni* could be playing not just a predator but also a collector-gatherer.

8. ADVANCES IN THE STUDY OF QUALITY OF AIR AND DIVERSITY OF LICHENS, IN AN URBAN AREA OF THE PROVINCE OF SAN LUIS

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One of the most used biological methodologies to evaluate air quality is to measure lichens diversity. The higher air pollution, the lower lichens diversity. This study was carried out in a developing urban area: city of Juana Koslay (J.K.), (33° 21' 19'' S, 66° 14' 64'' W). In this area we have identified different sources of contaminants. The objective of these study was to compare the lichen diversity between J.K. to an ecologically equivalent area but without contamination sources: Donovan (Dv). Lineal transects, were performed. In all cases, we evaluated the trees of the tree side facing West. 290 trees were sampled. We used one grid of 0,5x0,1m, with 10 subdivisions. Parameters: lichen species, frequency, cover in percentage, wealth condition and diversity. Found species: 12. Diversity index J.K.: 0,274 +/- 0.14. Diversity index Dv: 3,27 +/- 0.87. Species by transect J.K.: 3,71 +/- 2,7; in Dv: 7,83 +/- 1.32. Richness index J.K.: 3,71 +/- 2,7; in Dv 7,83 +/- 1.32. Considering lichen diversity index, one could argue that there is air pollution in J.K. compared to Dv. Lichen diversity at Dv is ten times greater than J.K. and so it is the number of species that appear by transect.

9. MYCORRHIZAL STATUS OF THE FLORA OF SIERRA DE LAS QUIJADAS NATIONAL PARK (SAN LUIS, ARGENTINA), I

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Mycorrhizas are symbiotic associations between some fungi and roots and underground organs of Vascular plants and Bryophyta. These relationships improve host water and nutrients uptake from soil, and resistance to pathogens and herbivory. There are two main mycorrhizal types: ecto- (EM) and endomycorrhiza (arbutoid, ericoid, orchid and vesicular-arbuscular or VAM). Different biomas are associated with diverse mycorrhizal types. *Sierra de las Quijadas* Natl. Park is an arid area at NW of San Luis province. There are 206 taxa into 45 families of Vascular plants; 62 are endemic taxa from Central Argentina, San Luis or the Park. The aim of this work was to detect different types of mycorrhizas into the Park Flora. Twenty two plant taxa were collected in summer; in each sample, roots were washed, fixed in FAA, clarified, stained, placed onto slides and observed under microscope to detect the mycorrhizal type and to quantify colonization. From 22 plant taxa analyzed, 58% were associated with fungal symbionts (12 formed VAM and 1 colonized by "dark septate endophytes"). These associations are new records for the science.

11. IMMUNOLOGICAL EVALUATION OF NATIVE PLANTS FROM CENTRAL-WESTERN ARGENTINA, I

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Many native plants used in popular medicine in Cuyo region, Argentina, are not at all known regarding phytochemical, pharmacological and therapeutic properties, as well as toxicity, side effects, etc. Because the scarce immunologic information about 5 of them, the phagocytic murine macrophage activity was analyzed in aqueous extracts (24 hs, 4° C) from aerial dried and powdered parts of *Baccharis trimera* and *Cyclolepis genistoides* (Asteraceae), *Schinus areira* (Anacardiaceae), *Larrea divaricata* (Zygophyllaceae) and *Lippia turbinata* (Verbenaceae), as well as infusion and decoction obtained in agreement with Argentine Pharmacopoeia; these solutions were sterilized by filtration and dried by lyophilization. Extracts of *Schinus areira* and *Larrea divaricata* show a significant increase in their phagocytic activity as compared with the control. On the other hand, aqueous extract, infusion and decoction of *Schinus areira* have microbicidal activity against *Staphylococcus aureus*, but not the simulated digested extract. These results confirm the activity of the extracts on components of the immune system and on microbes.

10. CUARTO RIVER (CÓRDOBA, ARGENTINA) DIATOMS. ANNUAL CYCLE DISTRIBUTION

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The space-time distribution of Cuarto River diatoms monthly collected at Piedra Blanca was analyzed following a longitudinal and altitudinal gradient (from June 1988 to June 1989): in the hilly area, in the alluvial plain (Tres Acequias and Río Cuarto) and in the ponds area (La Carlota), as well. Physical-chemical and hydraulic parameters were registered. Aliquots for the collected samples were studied according to Hasle and Frixell (1970) methods. A taxonomic study and a similarity index were performed, with the species-sampling seasons association being determined. 164 taxa were determined. 111 at Piedra Blanca, 124 at Tres Acequias, 123 at Río Cuarto and 105 at La Carlota. The greatest number of species came out to belong to the genera *Nitzschia* (22), *Fragilaria* (15), *Cymbella* and *Pinnularia* (13). Many of these genera and species were representative of the spot and showed an sporadic occurrence, except for *Nitzschia gandersheimiensi*, at La Carlota, which showed to be present all the year long. The species association registered the following patterns (mean \pm SD): Piedra Blanca, 0.267 \pm 0.03; Tres Acequias: 0.433 \pm 0.07; Río Cuarto: 0.442 \pm 0.05 y 0.447 \pm 0.16 at La Carlota. The greatest similarity: between Tres Acequias and Río Cuarto (SDmax = 0.815; GGmax = 0.6793). The research showed that the species distribution was more related to the space gradient than to the temporal distribution.

12. CANINE SUBCUTANEOUS DIROFILARIASIS DUE TO DIROFILARIA SUBGENUS NOCHTIELLA IN MENDOZA, ARGENTINA

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Dirofilariasis is a disease that affects various species of animals and humans. Two subgenus exist; *Dirofilaria Dirofilaria* and *Dirofilaria Nochtiella*. In dogs and humans, *Dirofilaria* causes subcutaneous Dirofilariasis, with many cases reported in Europe and Asia, with scant reports in domestic dogs in the American continent. We present a case of a mongrel male dog, three years of age, that during surgery an helminth was visualized in the subcutaneous tissue in the tarsal region. It was sent to the laboratory where it was fixated in 10% formaline and histological sections were performed and stained with Hematoxilin and Eosin. The fragments sent to the laboratory had a total length of 7 cm. The sections were 0,4 mm in diameter with a thick cuticle (8 microns) with fine external longitudinal ridges and a circumferential muscle cell layer. According to these features, it was identified as *Dirofilaria* subgenus *Nochtiella*. Finding this subgenus in the Americas is relevant due to the implications it can have in human and animal health. Further taxonomical and epidemiological studies should be performed.

13. COCCIDIA IN CATTLE OF MENDOZA RANGELAND

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Information on the occurrence and diversity of coccidia infecting cattle in Mendoza Rangeland is lacking. The objective of this study was to determine the levels of infection of coccidia in cattle during the different seasons and to identify the species involved. Fecal samples from cross-bred (Aberdeen Angus x Criollo Argentino) and Criollo Argentino weaned calves were collected during the following seasons: dry, spring and wet season during 2001-2002 from the "El Divisadero" Cattle and Pasture Experimental Station in Mendoza, Argentina. Teuscher modified technique was used to investigate the presence of coccidia, Wisconsin technique was used to determine OPG (oocysts per gram) and species identification was done by measurement and morphological characteristics. From a total of 172 samples, the following were positive for coccidia: Dry Season 19 (33,3%), Spring 14(26,4%) and Wet Season 13%(26,7%). 169 samples has < 10 OPG, 2 had 10-20 OPG, and one had >100 OPG. The species involved were: *E. bovis*, *E. auburnensis*, *E. ellipsoidalis*, *E. illinoisensis*. Even though they can be potentially harmful, the low OPG counts would not be a relevant factor in herd health in range conditions.

15. *Sisymbrium irionis* - *Malvetum parviflorae* AND *Poo annuae-Coronopetum didymi* IN SAN LUIS

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San Luis Province is barely explored from the phytosociological point of view. There is currently no study of this nature that includes its urban vegetation. Two nitrophilous communities of hiemo-vernal phenology were detected. The first one presents as main characteristic species *Sisymbrium irio* and *Malva parviflora*, and the second *Coronopus didymus* and *Poo annuae*. In order to characterize them phytosociologically, we carried out phytosociological inventories in different points of the city following Braun-Blanquet methodology. The sampling units oscillated from 1 to 10 m². The inventories were tabulated and compared with those presented in the original description of the syntaxa in which these species are present. Both communities are frequent from the end of winter to middle spring. Both of them developed on fairly humid soils of urban habitats. *Malva parviflora* and *Sisymbrium irio* occur on no compacted soils and the other one on compacted soils of the ditches for water round trees of the pavements. We can conclude that the present communities in San Luis city belong to the described associations for Spain: *Sisymbrium irio-Malvetum parviflorae* and *Poo annuae-Coronopetum didymi*.

14. PRELIMINARY SURVEY OF SOME POACEAE FAMILY SPECIES IN "PAMPA DE LAS INVERNADAS", SAN LUIS

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In the hills, "pampas" denotes a roughly level surface, with sandy loamy soils and xerophytic herbaceous vegetation. "Pampas" are sometimes disturbed by crystal outcrops and a unique plant cover. In San Luis, they occur at three different levels: one, above the rejuvenated "penillanura" (elevated plain), from 1,750 m to 1,800 m a.s.l.; another, above the old "penillanura", from 1,350 m to 1,000 m a.s.l.; and the other, the peripheral inner "pampas", below the 1,000 m a.s.l. "Pampas de las Invernadas" is situated at the first mentioned level. As to vegetation, the "pampas" belong to the "pastizales y bosques serranos" unit, marked by savannas dominated by grass and latifoliated species, woody species are scarce. The aim of this study is to determine the grassland composition in the "Pampa de las Invernadas". We established some 200 long x 10 m wide "transectas" (sort of plots), from the La Carolina - San Luis road side towards the West. Grass species were collected, pressed, dried, identified and included in the "Herbario de la FICES" (VMA). 29 Poaceae family species were identified. 1 of them are new for San Luis: *Muhlenbergia peruviana* (P.Beuv.) Steud. 23 have a medium to high forage value, 4 a poor one and 2 an unknown value. 44,83% of the total are winter species - an invaluable resource in a forage scarce season.

16. GROWTH FORM AND ROOT ANATOMY OF *Zornia gemella* (WILLD.) VOGEL AND *Zornia trachycarpa* VOGEL

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A growth form is the complex of characteristics that define the habitat of a plant. In the hilly area of the south-west of Córdoba province, observations were done during three consecutive years. Seeds were placed in a glass container in order to observe their root development. For histological studies, the roots were fixed in FAA, added to histowax and stained with Safranin-Astrablau. Both species show phaneroepigeal seedling. If the characteristics of the aerial and subterranean root are compared during their first year of life, it is observed that the main differences are present in the aerial part, in *Z. trachycarpa*, 3 or 4 orthotropous shoots are developed, showing a clear acrotonia. In *Z. gemella*, except for the first two nodes, all of them are branched and oriented in a nearly plagiotropous way. Transversal root cuts reveal an usual secondary structure with a central cylinder in which scarce conducting elements of xylema with a great diameter are observed. Around this structure, there is an abundant reserving parenchyma corresponding to the secondary phloem and phelloderm. All the studied characteristics show that the surviving organ of these species is the root which accumulates water and starch, and favors the sprout after winter. Being the innovation area at soil level, the presence of reserving roots is common in the hilly environment, though the structural strategies to increase the parenchymatic area are very variable within the Fabaceae family.

17.

CHORRILLO RIVER BASIN PHYCOFLORA COMPARATIVE SURVEY (DEPT. CAPITAL, PROV. SAN LUIS, ARGENTINA)

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Algae communities categorization, the knowledge of the diversity and its own pattern of distribution allow to assess the ecosystems primary productivity, population dynamics and its responses to the anthropic and eventual natural disturbances. Chorrillo river phycoflora comparative survey with the aim of stablishing persistence and tolerance of the species for the same dates and places in yearly periods was made. During 2002 year fixing points in 1999 Chorrillo river samples were taken: Point 1 (Tanque de agua), Point 2 (Dique Chico), Point 3 (B° Tibiletti section). Both plankton samples by using 10 µm net and abiotic parameters were found. A number of 47 taxa belonging to *Cyanophyceae*, *Euglenophyceae*, *Dinophyceae*, *Chlorophyceae* and *Bacillariophyceae* were determined. Species associations for each points were also found. By mean of applying the Sorensen Similitude Index it was observed: Smin: 0,676 between points 1 and 3, while Smáx: 0,750 was between points 1 y 2 for the present year. On the other hand, obtaining Sorensen values for 1999 were Smin: 0.503 between points 1 and 3 and Smáx: 0.552 between 1 and 2. During the passing period average similitude from 25% more than further survey were modified. We can conclude in the end of 2000 and the beginning of 2001 river increasing streams have caused population homogenization between different points and the following increasing of similitude.

19.

IN SACCO RUMINAL DEGRADABILITY DYNAMICS OF DRY MATTER OF FERTILIZED KLEINGRASS

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Panicum coloratum L. (Kleingrass) is a perennial estival bunchgrass. The aim of the paper was to evaluate the kinetics of ruminal degradability of fertilized accumulative Kleingrass growth (T1) and regrowth dry matter (T2) and its correlations with different nutritional fractions (fiber and protein). In situ degradability technique and exponential models were applied to determine accumulated (AD) and effective degradability (ED) (Orskov and Mc Donald, 1977), analysis of mean differences (Fisher Test) ($\alpha = 0.05$) and correlation among variables ($P < 0.05$). T1 exceeded T2 in DM solubility. In T1, AD and ED decreased in winter and in T2, high percentages were kept during summer. AD and ED depend on insoluble nitrogen in neutral detergent variation and lignin of the entire plant and stem true protein (TP) in T1 and TP and neutral detergent fiber of the entire plant in T2. Conclusion Better forage quality can be obtained from T2 during summer. The advance of phenological stages results in poor quality in both treatments. The fiber fractions limit degradability and nitrogenous ones favor it.

18.

CHANGES IN THE NUTRITIONAL PROFILE OF FERTILIZED KLEINGRASS ACCORDING TO CLIMATIC FACTORS

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Panicum coloratum L. is a perennial summer grass, considered promissory for the cattle systems of semiarid regions. The aim of this paper was to evaluate the correlation between different climatic factors: precipitations (PP); minimum, maximum and average temperatures and effective sunlight (EH) with nutritional quality parameters of Kleingrass accumulated growth (T1) and regrowth (T2) dry matter. Material and Methods Correlation analysis ($P < 0,05$) between each nutritional parameter (nitrogenous, fibrous and degradability) and the different climatic factors. Results Higher correlations (r : more than $\pm 0,70$) were found between HE and/or PP before the cutting with nitrogenous, fibrous and degradability fractions in T1 and T2. Temperatures influence the quality of T2 (r : $\pm 0,88$). Conclusions Quality parameters are correlated in higher or less proportion with climatic factors. Before the cutting, whatever the treatment, PP and EH influence on the variations of different fibre and nitrogenous fractions and finally on dry matter degradability. Nitrogenous fractions have contrary behaviors to fibrous ones with regarding to environmental factors.

20.

GENOMIC DNA METHYLATION IN TWO cvs. OF *Digitaria eriantha*, CORRELATED WITH LOW TEMPERATURE

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Changes in the percentage of 5-methylcytosine (5-mC) have been determined in angiosperms during agein-maturation. The aim of this paper was to evaluate if DNA methylation state were correlated with cold tolerance in two cvs. of *Digitaria eriantha*. The percentage of genomic DNA methylation was determined in plantlets of Sudafricana cvs. (native) and Avanzada INTA (synthetic), grown at 25°C during 30 days and subjected to low temperatures (10°C) during 24, 48 and 72 hours. Quantification of relative methylation in DNA samples was calculated as follow: %5mC = $mC * 100 / (5mC + C)$. Sudafricana cv. did not show important differences in methylation % in all evaluated moments showing suppression of genes expression in response to cold in agreement with its natural adaptation to high temperatures (30-40°C). The Avanzada INTA cv., decreased the relative percentage of 5-mC to 24 and 48 hours of treatment, which can be considered a response to this cv. to cold stress possibly through transcription for resistance proteins. This is the first time that the relation DNA methylation with cold stress processes in plants is reported.

21. PARAMETERS OF PRODUCTION IN *Digitaria eriantha* UNDER COLD STRESS

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Digitaria eriantha is a species that was introduced in Argentine in 1991. The debility of this species is her low tolerance to the early frosts and the late ones. The objective of this work was evaluate the low temperatures' effects on the parameters of the production like dry weight, cool weight, leaves and root's longitude. There weren't variations in any parameter measured after 48 hours of treatment with cold neither the entire plant nor in the leaves and roots. The variations happened with 96 hours of treatment (4°C). The results suggest that the growing and the water contain, in the entire plant and the leaves are affected by the cold stress, immediately then have a slowly recovery; moreover the biomes is affected 14 days lastly stress. The effects on the roots is more important, the water contain and the biomes lowed at 7 and 14 days. The growing in roots was more affected that the foliage.

23. PRESERVATION OF *Tolypothrix tenuis* INOCULA USED AS BIOFERTILIZER IN RICE

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The production of a cyanobacterial biofertilizer for rice at industrial level implies the availability of viable inocula for the mass culture in photobioreactors. The usual technique by periodic subcultures under photoautotrophic conditions requires special environments and a high frequency of operation. In the present study two preservation methods of biomass were analyzed: calcium alginate encapsulation and freezing. The cellular survival was established through growth kinetics and several qualitative parameters over a storage period of three to fifteen months. The encapsulated biomass as either beads or dishes showed chlorosis, bacterial contamination and viability losses amounting to 82.73% after a three month-storage, while the freezing results depended on the nature of the protective agent. There was a complete cellular death with glycerol and variable results with methanol depending on the condition of the biomass produced and the storage time. For biomass raised under low light intensities and three month-storage a viability retention of 84.36% was observed.

22. RESPONSE OF NATURAL GRASSLANDS TO THE SOWING OF *Digitaria eryantha* OBSERVED IN THE OF SEED BANK

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This study was conducted in the establishment "Santa Teresa", near Granville (San Luis Province, Argentina) This estate is located in the "Area of dunes with grasses and islet of Chañar". This phytogeographic area is an undulating steppe with sandy soils and fixed as well as mobile sand dunes. Part of the estate natural grassland has been ploughed up to intersow *Digitaria eryantha*. We selected a 250 m (EW orientation) x 20 m (NS orientation) plot located in the natural grassland; the plot was burned and will be sown with *Digitaria eryantha*. We took 20 samples longitudinally, 12.5 apart, at depths of 5 cm and 10 cm. Sampling was carried out using a 62.8 cm³ capacity sample borer. We recorded the plant specimens found in the plot. The samples were divided into two groups; one half was sired for seeds and the other was cultivated in separate dishes. Seeds of *Avena fatua*, *Conyza bonariensis*, *Cynodon hirsutus*, *Digitaria californica* *Sorghum sp.* y *Stipa tenuissima* were found in samples taken at a depth of 5 cm.

24. LEAF MORPHO-ANATOMY AND GROWTH FORM OF *Acantholippia seriphioides* (A. GRAY) MOLD. IN THE CENTRAL ZONE OF SAN LUIS PROVINCE

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In the central zone of San Luis province, *Acantholippia seriphioides*, grows as a perennial aromatic subshrub. "Tomillo del campo" undergoes human action, like harvest and fire. Leaf morpho-anatomy and characterization of growth form contribute to provide basic information for use and management and to estimate potential regeneration in the natural environments of the province. Leaf transverse sections embedded in paraffin were cut with a slide microtome at 10 µm. The branching system was observed in natural environment and in cultivated plants during two years. Leaf anatomy presents xeromorph characters, like thick cuticle and compactness of palisade parenchyma. Plagiotropic growth of *in situ* plants favors the asexual reproduction. The thickness of roots in the proximal zone indicates a possible adaptation similar to that of a reserve organ, probably related to environmental conditions.

25. SOLID-STATE CULTIVATION OF *Fusarium oxisporum* ON WASTE FROM OLIVE OIL INDUSTRY

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Even if solid-state fermentations (SSF) have been studied, reports related to SSF on olive oil mill solid waste, (OO) are limited. This work study *F. oxisporum* growing on OO and its production of extracellular enzymes. Cultures were carried-out in Raimbault columns, at 30°C. Culture media used included OO, green pea residues (GPR), and apple residues (AR), humidified with a saline solution. Initial water content and pH were set at 65% and 4.8, respectively. Enzymatic activities (EAs) (amylolytic, AA; cellulolytic, CA; pectolytic, PA; and lypolytic, LA) were determined using current analytical procedures. Growth was estimated through measures of carbon dioxide evolution. Records of pH, reducing sugars and dried weight loss, were also done. Growth and extracellular enzymes activities (AA, CA, PA and LA) profiles, were made. Cultures carried out on OO only, showed little growth and no Eas. GPR or AR were added to the OO medium. Biomass estimation reached to 0.15 g biomass/ g dried culture, in OO(60%) + AR(40%). As for EAs, AA was found in both cultures; PA was found only in OO + AR; and LA was not detected. Although biomass and EAs found are smaller than those reported in another SSF on agroindustrial residues, they result encouraging, keeping in mind the kind of substrate employed.

27. MICROPROPAGATION OF *Prosopis caldenia* BURK

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During the last 35 years the wooden demand has increased hard of quality, constituting the wood of the *Prosopis caldenia* Burk, one of the potential products of the arid lands of more unitary price. The objective of the present work is to establish an appropriate protocol for its massive micropropagation. They were used explantos the segments binodales that were sowed in to half it completes of Murashige and Skoog (1962), suplement with 2% sucrose, ascorbic acid (2 ppm), gibberellic acid (AG3) 1 ppm. The regulators of growth used in different concentrations were: Acetic naftalen acid (NAA), Kinetin (K) and Indole 3 butyric acid (IBA). 20 segments were sowed by treatment, half of them between liquid with bridge of filter paper (Watman N°5) and the other half between solid (agar 0,6%). The number of roots average for segment binodal was of 4 and the percentage of rooting was 90%, with K (0,1 ppm) and NAA (10 ppm). The results confirm the necessity of light for the formation of roots. This situation can be related with the high level of auxinas production for the exposed leaves to the light, and the addition of AG3.

26. METHODOLOGICAL ASPECTS OF THE DISINFECTION AND EXPLANTOS PREPARATION FOR THE *IN VITRO* CULTURES OF *Piptochaetium napostaense*

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Piptochaetium napostaense (Speg.) Hack. it is a gramineous, perennial, very felt like by the livestock. This native species is in regression process due to disturbances caused by the man. To avoid this genetic erosion, it is necessary to apply biotechnologies that help to the germoplasma conservation. With the purpose of being able to diminish the initial contamination a series of rehearsals it was designed with different disinfectant agents. The tillers was treated with the combination of the following disinfectant agents: bleach and Tween 30; ethanol 70%; benomyl and ampicilina. In the aseptic camera continues with three laundries with sterile bidistilled water; the basal meristem was extracted that were sowed in a half basic of Murashige and Skoog. The evaluations began to carry out every 7 days of incubation at 28°C, with a fotoperíodo of 16 hs. light. When adding benomyl (2 g/L) the contamination was controlled with fungi and the contamination with endogenous bacterias decreased 60% adding ampicillin (350 mg/L), in both cases the concentration of bleach was of 10%.

28. ANGIOTENSIN II MODULATES TYR PHOSPHORYLATION IN PRIMARY CULTURED FIBROBLASTS

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Angiotensin II (Ang II) exerts its biological actions through binding to two membrane receptor subtypes, AT₁ and AT₂. AT₂ receptors are widely expressed during fetal life and a possible role of these receptors in development was proposed. In this study, we analyze both receptor expression and signal transduction pathways in primary cultured fibroblasts obtained from rat fetuses (E20). Cells were cultured until 80% confluence and arrest was induced by serum deprivation 24 hs before stimulation with Ang II. Cell proteins were separated by SDS-PAGE, transferred to PVDF membranes and developed with specific antibodies. Ang II induces PKC translocation to membrane fraction, a response mediated by AT₁ receptors. In cultured fibroblasts, Ang II stimulated tyrosine phosphorylation of several proteins, an effect inhibited by Losartan. RT-PCR of RNA from cultured fibroblasts exhibited higher expression of AT₁ receptors and lower expression of AT₂ receptors with respect to the original source. In summary, primary cultured fibroblasts from rat fetuses E20, exhibited an increase in AT₁ receptor expression, which modulate PKC activation as well as an increase in phosphorylation level, a mechanism usually related to growth.

29. EFFECT OF pH ON THE INHIBITION OF XANTHINE OXIDASE BY 4,4'-DIHYDROXY-BENZOPHENONE

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The effect of pH on the inhibition of xanthine oxidase (XO) by 4,4'-(OH)₂-Benzophenone (BP) was analyzed in order to determine the active form of the inhibitor in solution. The kinetic experiments were performed in phosphate buffer (pH 6.0 and 7.8) and carbonate buffer (pH 9.6). The substrate initial concentration was 20 μM, while the BP was modified between 15 μM and 200 μM. Control experiments (without BP) were carried out simultaneously. The initial reaction rates (V_o), the degree of inhibition and the IC₅₀ were determined. The effect of the pH on the xanthine oxidation reaction is reflected in the V_o of the control experiments, which is taken as reference to estimate the degree of oxidation and the IC₅₀. The variations observed in these parameters match changes in the *neutral molecule-ionic form* relation of BP. At pH 7.8, IC₅₀ is 59 μM, while at pH 6.0 and 9.6, these values are 179 and 120 μM, respectively. It can be inferred that the ionic forms of BP are always more active than the neutral form. In particular, of all the BP molecular forms present in solution, the monoanionic form is the one with the greatest XO inhibitory activity.

31. EFFECTS OF DIETARY PROTEIN ON PLASMATIC AND LIVER CHOLESTEROL. ITS RELATION WITH FECAL LIPID EXCRETION

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Prior studies have shown the cholesterol-lowering effect of the protein concentrate of *Amaranthus cruentus* (Ac), on livers of male Wistar rats, compared with those fed with casein as protein source. We determined the cholesterol (Ch) amount in plasma and liver. Simultaneously we measured total lipid (TL) and Ch in feces in order to determine whether Ac enhances Ch excretion. We worked with a lot of eight rats, 200 g BW. The animals were separated in two groups: one control fed with casein as protein source, a problem group with Ac as protein source. Both diets have 11.9% of protein, supplemented with 1% Ch. During the last week feces were collected. The experiment lasted 28 days. Ch, HDL, LDL were measured (Wiener kits). TL (Folch) and Ch (Zak) were determined in feces. Liver Ch was measured by Zak's method. Rats fed with Ac diet had an increase in HDL and a concomitant decrease in LDL. Liver total Ch showed a clear decrease in rats that received the experimental diet; esterified Ch was also decreased. The Ac diet promoted a higher fecal Ch and TL excretion, compared with controls. The presence of soluble fibers in Ac might diminish liver total Ch and Ch esterification and augment fecal Ch excretion.

30. EFFECT OF VITAMIN A DEFICIENCY ON KIDNEY OXIDATIVE STRESS AND INFLAMMATION

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Vitamin A participates in tissue differentiation, growth and maintenance. We have showed that vitamin A deficiency modifies kidneys lipidic profile and increases superoxide dismutase (SOD) activity. We studied the effect of this deficiency on iNOS and COX-2 expression in renal papilla and medulla. Male Wistar rats, 21-day old, were fed during three months with vitamin A-free diet (-A) and the same diet plus 8 mg of retinol palmitate/kg of diet (+A). Renal papillae and medullae were homogenized in presence of protease inhibitors and proteins were measured. 40 μg of proteins were loaded on 8% SDS-PAGE gel. iNOS and COX-2 were identified by Western blot with specific antibodies and bands were revealed using Vectastain ABC detection kit. Both tissues (papilla and medulla) showed iNOS and COX-2 expression without significant differences between -A and +A. Then even if there is some oxidative stress, it does not modify the expression of these enzymes.

32. EFFECT OF MODERATE ZINC DEFICIENCY ON THE RAT EPIDIDYMAL LIPIDS

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Previous studies of our laboratory show an elevated index of lipoperoxidation in epididymis. Our work aimed at studying the effect of zinc deficiency in rat epididymis lipids. Wistar male rats (200-230 g b.w.) were separated in two groups: I) Control, (Co), fed with a diet containing 30 mg Zn/kg of diet, and II) Zinc deficient (ZD). After 2 months, animals were killed and the determinations were carried out in head and cauda of epididymis. Total lipids (TL), total cholesterol (TC), esterified cholesterol (EC) and free cholesterol (FC), triglycerides (TG) and total phospholipids (TPL) were determined. The values were EC (mg/g tissue) in Co: 882 ± 179 and ZD: 458 ± 103 (p < 0.05) in head. FC (mg/g tissue) in Co: 2794 ± 233 and ZD: 2158 ± 157 and EC (mg/g tissue) in Co: 763 ± 128 and ZD: 1357 ± 163 in cauda, while TC and FC in head and TC in cauda did not change. TG did not modify in head and cauda of ZD in relation to Co. TPL (mmol P/g tissue) in Co: 0.84 ± 0.05 and in ZD: 1.26 ± 0.1 (p < 0.01) in cauda and they did not change in head. Phospholipid percent composition in head of ZD was higher for LPC, PC and PS+ Pi (p < 0.01) and lower for PE and PG (p < 0.05), without differences for Sph. In cauda there was higher (%) PC (p < 0.01), lower (%) Sph, PS+ Pi and PE (p < 0.05), without change in percent LPC and PG. In a moderate Zn deficiency, both head and cauda of the epididymis undergo an alteration of the lipid pattern, which is more marked in the latter. These alterations might conceivably lead to modifications in the spermatid maturation.

33. iNOS AND eNOS EXPRESSION IN VITAMIN A DEFICIENT RAT AORTA

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We have shown an increase of oxidative parameters in serum and heart due to vitamin A deficiency. Vascular endothelium participates in the control of vascular tone through the release of nitric oxide (NO) which is synthesized by endothelial NO synthase (eNOS). Inducible NO synthase (iNOS) expression in endothelial cells occurs under conditions of oxidative stress and inflammation. Wistar male 21-day old rats were fed during three months with vitamin A-free diet (-A) and the same diet plus 8 mg of retinol palmitate/kg of diet (+A). Thoracic aortas were homogenized in presence of protease inhibitors and proteins were measured by Biuret method. Proteins (40 µg) were loaded on 8% SDS-PAGE gel. iNOS and eNOS were identified by Western blot with specific antibodies and bands were revealed using Vectastain ABC detection kit. The expression of the enzymes increased in -A rats when compared to +A. Thus, vitamin A deficiency modifies the expression of both isoforms of NO synthase *in vivo*, which are implicated in the regulation of vasomotor tone.

35. EFFECT OF ORGANIC SOLVENTS ON THE PREFERENCE OF ARAUJIAIN BY SYNTHETIC SUBSTRATES

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The objective of this work was to determine the effect of the non-aqueous media on the preference of araujiain by different synthetic substrates (N- α -carbobenzyloxy-p-nitrophenyl esters of amino acids). The selected media were Tris-HCl buffer (pH 8), NN-DMF-Tris-HCl buffer (50:50, pH 8) and ethylene glycol (aw: 0.5). Measurement of esterolytic activity was performed with Z-p-nitrophenyl esters of amino acids and expressed as Ucbz/mg of proteins. An arbitrary enzyme activity unit (Ucbz) was defined as the amount of enzyme that releases 1 µmol of p-nitrophenolate per min in the assay conditions. The greater specific activities were found in buffer. The preferences of araujiain in buffer and NN-DMF (50%) were: Gln > Gly > Leu > Tyr > Phe > Pro, and in ethylene glycol (aw: 0.5) were: Phe > Gln > Pro > Leu > Gly > Tyr. The esterolytic activity of araujiain in buffer depended on hydrophobic, electronic and steric factors of the amino acids side-chains. The preferences of araujiain in media with low water activity were also affected by the capacity of desolvation of the substrate and, probably, by the conformational and dynamic changes of the protein.

34. EFFECT OF CADMIUM ON ANTIOXIDANT DEFENSE SYSTEM IN SOYBEAN (*Glycine max. L*) LEAVES

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This work aimed at studying the relationship between cadmium toxicity and the mechanisms of antioxidant defense of plant cells. Germinated and grown soybean plants, were exposed on the 6th day of germination and adaptation to hydroponics conditions in nutritive solution to intoxication with CdCl₂ (10mg/L) for 8 days. Seeds were separated in two groups: control (Co) and Cadmium (Cd). The determinations carried out were total glutathione (Gt), glutathione disulfide (Gox), non-protein thiols (NPT). Protein oxidation was measured as the total content of carbonyl groups (C), and the difference between NPT and GSH were considered representative of the phytochelatin (PCs-SH) content. Proteins and chlorophylls were also determined. An increase of Gt ($p < 0.0001$) and a decrease of Gox ($p < 0.01$) were observed in Cd-treated samples, thus increasing the GSH/GSSG ratio. NPT and PCs-SH showed an increase in the Cd group. Carbonyls were increased ($p < 0.01$) in the Cd group. The latter corroborates previous results which showed that the plant suffers oxidative stress induced by reactive oxygen species (ROS) generated by activation of endogenous systems. If the ROS increment is relatively small, the antioxidant response (judged by GSH levels) may be enough to balance the ROS rise allowing a return to the original state. Therefore, physiologic manifestations of redox regulation imply a temporal change of the redox thiol/intracellular disulfure state toward more oxidative conditions.

36. PEPTIDE ENZYMATIC SYNTHESIS IN DIFFERENT ORGANIC MEDIA

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In this work we selected different organic media for the enzymatic synthesis of Z-Phe-Phe.OMe peptide (intermediary of a viral replication inhibitor, Z-Phe-Phe-Gly), using araujiain proteases. Hexane or NN-DMF and buffer (pH 8) or ethylene glycol (aw: 0.5 in the enzyme) were used as a reaction media. Reaction media were selected because of they were the most stabilizer for the enzyme. Ethylene glycol showed the greater esterolytic activity/hydrolytic activity ratios. In hexano-buffer, the enzyme was able to produce the wished peptide. It is probable that the synthesis in NN-DMF (50%) and in ethylene glycol (aw: 0.5) has not taken place due to hydrolysis of the peptide, to viscosity of ethylene glycol (affecting the diffusion of the substrates towards the active site), to the presence of an unable nucleophile (probably one derivate more strongly activated will be required), and changes that could have undergone the enzyme (preventing the union of nucleophile to the intermediary acyl-enzyme).

37. STABILITY OF MORRENAIN IN HYDRATED MISCIBLE ORGANIC SOLVENTS

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The objective of this work was to study the stability of the crude extract of morrenain in miscible organic solvents (30%, 50%, 70%) - Tris-HCl buffer (pH 8) for its application in the flavoring peptide synthesis. Latex of the *Morrenia odorata* fruit was gathered on citrate-phosphate buffer (0,1M, pH 6.5), EDTA and Cys, centrifuged and conserved at 20°C. The supernatant is the crude extract. Stability assays were determined as residual caseinolytic activity (RCA). A caseinolytic unit (Ucas) was defined as the amount of enzyme that produces an increase of a unit of absorbance in the assay conditions. 70% miscible organic solvents led to the smaller RCA and the threshold of inactivation in methanol, acetone, tetrahydrofuran, NN-DMF and ethylene glycol was reached. The highest RCA were obtained in 30% NN-DMF and methanol. Morrenain needed more than 70% of water to express its catalytic potential, because miscible organic solvents remove the water layer, which surrounds the enzyme molecule and keeps it catalytically active.

39. VITAMIN A DEFICIENCY MODIFIES FATTY ACID COMPOSITION AND METABOLISM IN MITOCHONDRIA OF RAT HEART

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It is known that fatty acids (FA) are used as energy source by the heart. The effect of vit A deficiency on FA composition and utilization by β -oxidation pathway were studied. Male rats at 21d age were fed on a vit A deficient diet (-A) or the same diet with 8 mg retinol/kg diet (control), for 3 months. Serum, liver and heart Vit A were measured by HPLC and heart FA synthesis by incorporation of [¹⁴C]acetate. In mitochondria were measured: lipid contents after TLC separation; FA by gas liquid- chromatography and carnitine palmitoyltransferase I (CPTI) activity by using [¹⁴C] carnitine. In relation to control, mitochondrias of -A group showed an increase in phosphatidylcholine, triglycerides and fosfatidic acid; a decrease in cholesterol esters; an increase in palmitic (16:0) and a decrease in oleic (18:1), linoleic (18:2) and arachidonic (20:4) acids. Regarding phospholipids, -A increased 18:0 and 10:4 and decreased 18:2 acids. FA synthesis decreased. This suggests a low availability of malonylCoA, an inhibitor of CPT, which agrees with the high CPTI activity observed. Thus vitamin A deficiency alters cardiac FA metabolism.

38. STABILITY OF MORRENAIN IN BIPHASIC SYSTEMS

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In order to study the stability of morrenain in two-phase media, 9 organic solvents of varied hydrofobicity were mixed with (30, 50, 70%, pH 8) buffer. The more hydrofobic ones (log P: 0,7-0,85) like ethyl acetate, ethyl ether and butanol allowed to obtain the maximum stability of morrenain. The generalized belief that organic solvents with log P > 4 causes less inactivation than hydrophilic ones did not allow to explain the stability of this enzyme. The percentage of water in the mixture did not affect in the same way all the studied cases and its activity depended of the nature of the organic solvent. The smaller caseinolytic residual activities observed at 30% organic solvent could be due to the formation of "cluster" in the active site or enzymatic aggregates, with the formation of intermolecular disulphides bridges. The lower activities at 50 and 70% organic solvent can be justified by the presence of a greater interface superficial area of organic solvent and therefore, a greater probability of inactivation. The remarkable stability of morrenain in (70%) butanol and ethyl acetate could be because the enzyme could have acquired catalytic conformations more active than the native one, due to the competition of solvents by the water of media.

40. EVALUATION OF VITAMINS C AND E IN OXIDATIVE STRESS CONTROL

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Oxidative stress by radical reactions has received growing attention in biomedical sciences. An adequate cellular pro-oxidant - antioxidant balance controls the free radicals generation to avoid its reaction with lipids, proteins, RNA and DNA, altering its structure and/or function. In previous works we have demonstrated the increase of tissue lipid hydroperoxides (LOOH) as determination of early oxidative stress, induced *in vivo* in mice by different compounds. In the present work we evaluate the possibility of using antioxidants, vitamin C (100mg/L in drinking water *ad libitum* during 15 days), or vitamin C (500mg/Kg/day ip, 7 days) with vitamin E (200mg/Kg/day ip, 7 days) as alternatives of protection against oxidative stress induced by ciprofloxacin (10mg/Kg) in 8-12 weeks mice. Vitamin C in drinking water reduced the physiological hepatic levels of LOOH from 145 \pm 15 to 45 \pm 11 (p < 0.001) nmol/g, and the induced ones from 174 \pm 29 to 54 \pm 9 (p < 0.01) nmol/g. The renal levels of LOOH did not show significant differences. Vitamin C given during 15 days decreased in 70% the physiological and induced LOOH hepatic levels, a protection not observed with the simultaneous administration of both vitamins.

41. MICROBIOLOGICAL QUALITY OF COMMERCIAL AND HOME-GROWN CONDIMENTS CONSUMED IN SAN LUIS

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We understand as condiments the nutritious substances used to season, to improve or to enhance the taste of foods. The aim of this work was to establish the microbiological quality of condiments in San Luis city. Sixty samples were processed. The following determinations were carried out: count of total aerobes in agar medium count, count of fungi and yeasts in dicloran bengal rose chlortetracycline agar, coliforms count in Mc Conkey broth and EC broth (MPN), count of *Clostridium perfringens* in milk iron medium (MPN), determination of enterotoxigenic strains of *C. perfringens* by RPLA. *Coliforms* were detected in 36.80% of the commercial condiments and in 46.30% of home-grown condiments, overcoming the allowed limits in the most cases. *C. perfringens* was detected in 25 samples. Seven enterotoxigenic strains were obtained. A high degree of contamination is observed, due to the high percentages of coliforms and *C. perfringens* isolated mainly from home-grown condiments. Most of these samples exceeded the limits suggested by the Alimentary Code of Spain for *coliforms* (10/g) and only one sample was above the limits of *C. perfringens* (10³/g). The presence of enterotoxigenic strains suggests potential risks in the event of improper handling during food preparation.

43. SUPERFICIAL MYCOSIS IN SAN LUIS CITY: A MULTICENTER STUDY

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Superficial mycosis are caused by fungic parasitism in the corneous structures of the skin, hair and nails. The purpose of this work was to perform a mycological diagnosis of patients with superficial lesions in the city of San Luis, during the period January-October 2002. Samples were obtained by scraping. Direct testing was performed with 20% KOH and methylene blue in suspected cases of pityriasis. Samples were directly sowed on Sabouraud glucose, lactrimel agar and DTM. Identification was performed by macro-, micromorphological and biochemical tests. Of a total of 191 samples, 88 (46%) were positive and 103 (54%) were negative. The most frequently isolated agent was *Microsporum canis* (22%) followed by *Trichophyton mentagrophytes* (18.2%), *Malassezia* spp (17%), *Candida albicans* (11.4%), *Trichophyton rubrum* (10.22%), *Candida parapsilosis* (6.8%), *Trichophyton tonsurans* (4.5%), *Candida guilliermondii* (2,3%) and other fungi (8%). Among dermatophytes, the most frequent was *Microsporum canis*, while *Malassezia* spp was the most frequent among the dermatomycosis. Localization in nails and glabrous skin was most frequent, followed by scalp and small skin folds.

42. OBTENTION OF A SPECIFIC CONJUGATE FOR THE RAPID DETECTION OF VIBRIO PARAHAEMOLYTICUS

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Vibrio parahaemolyticus causes gastroenteritis associated with the consumption of seawater fish and shellfish. The purpose was to obtain a *V. parahaemolyticus*-specific fluorescein isothiocyanate (FITC) conjugated IgG, in order to be used for the rapid detection of this bacterium in clinical and food samples. Three rabbits were immunized with a formalised *V. parahaemolyticus* ATCC 17802. The sera were titled by ELISA and agglutination. The IgG was purified using protein A-Sepharose affinity chromatography and elution with glycine buffer pH 3. The purified antibody was labeled with 5 mg/ml FITC in DMSO (20µl/mg of antibody). After removing unbound FITC by dialysis, protein concentration, FITC/antibody ratio was calculated. The conjugate was tested by direct immunofluorescence (IFD). The serum title was 1/800 by ELISA and 1/64 by agglutination. The FITC/protein ratio was 4.12. The conjugate reacted with *V. parahaemolyticus*. The conjugate obtained is optimal for IFD and can be used in the rapid detection of this bacterium.

44. AIRBORNE Clostridium botulinum SPORES IN MENDOZA, ARGENTINA

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Botulism is caused by intestinal absorption of botulinum neurotoxin, that is produced by *Clostridium botulinum* (Cb). Cb spores are distributed to a large extent in nature. They spread from soil that serves as its natural source of infection, especially for infant botulism (IB), whose transmission is under investigation. In most arid biographical regions, hot and dry climate, with strong winds make propitious environmental conditions with persistent suspension of dust. During Spring of 1999, air biological studies were performed to detect Cb spores in Mendoza. Samples were taken through filtration capture of particules, according to WHO. Filters were seeded in cooked meat medium and incubated for 5 days at 31°C. For toxin detection, supernants were inoculated intraperitoneally in mice. Serological typing was carried out by neutralization test at 2000 DL₅₀/mouse. From 1,725,720 L of filtered air, one sample was positive to Cb toxin type A, corresponding to 40,800 L in the last week of September. In that period, Zonda wind was noticed. The high incidence of IB and high prevalence and load of Cb spores recorded in arid regions of Mendoza suggest airborne as an important way of transmission of IB.

45. INFECTION WITH *YERSINIA ENTEROCOLITICA* O:8 IN CYTOKINE KNOCKOUT MICE

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The cytokines γ -IFN, α -TNF, IL-12, and IL-18 participate in the immune response against *Yersinia*. C57BL/6 mice are relatively resistant to *Yersinia* due to a rapid and pronounced Th1 response. The purpose of this work was to analyse the affected immune mechanisms in cytokine knockout mice infected with *Yersinia*. Normal C57BL/6, TNFRp55^{-/-}, IL-12p40^{-/-} and IL-4^{-/-} mice were infected orally with 1×10^8 CFU. On 5 days after infection, the bacteria number in spleen and Peyer's patches (PP) was determined. *Yersinia* intracellular killing by peritoneal macrophages was studied. The bacteria number in spleen was significantly increased in TNFRp55^{-/-}, IL-12^{-/-} and IL-4^{-/-} ($p < 4 \times 10^{-5}$, $p < 0.0003$ and $p < 0.008$, respectively). Intracellular killing of *Yersinia* was decreased in TNFRp55^{-/-} macrophages (11-27%) compared with the normal macrophages (65-85 %) ($p < 0.05$). We concluded that IL-4 participates in the response against *Yersinia* oral infection. Deficiency in the activity of macrophages could explain the increased susceptibility of TNFRp55^{-/-} to *Yersinia*.

47. USE OF BLUE LAKE AS AN INDICATOR OF BACTERIAL PENETRATION INTO EGGS, AFTER DISINFECTION WITH CHEMICAL AGENTS

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Certain agents used in the disinfection of the eggshell surface might alter the thin shell cuticle and thus enable future recontamination of the egg. The possible alteration the shell microstructure and egg cuticle after disinfection with chemical agents was evaluated by blue lake, an indicator of bacterial penetration. Chemical agents: sucrose monolaurate (SMC) and sucrose monolaurate (SML), 500 μ g/mL and 1000 μ g/mL, alone and in combination with citric acid 0.1% (CA), propionic acid 0.1% (PA) and ethylenediaminetetracetic acid 300 μ g/ml and 600 μ g/ml (EDTA). **Blue lake**: an insoluble dye. After disinfection with chemical agents, eggs were dipped in 0.25% blue lake prepared in 0.1% Triton X-100 solution for 30 min and stored for 24 h at 25°C. Eggshell were broken into two pieces and the numbers of blue dots inside of the shell, were counted. Significant differences were considered to be $p < 0.05$. The greater number of blue dots was obtained with SMC 1000 μ g/mL in combination with EDTA 600 μ g/mL, followed by SMC 1000 μ g/mL in combination with EDTA 300 μ g/mL. Different egg-shell alterations caused for some chemical agents also affect the structure of the inside layer.

46. EFFECT OF ORGANIC ACIDS AND DIFFERENT THAWING METHODS ON THE NATURAL AND PATHOGENIC MICROFLORA OF FREEZED SAUSAGES

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Pathogenic and spoilage bacteria might be controlled in fresh sausages by cold and acidic conditions. In this study, total aerobe counts in noncontaminated "chorizos" (nCC), and total aerobe, *Escherichia coli* O157:H7 or *Yersinia enterocolitica* O:9 counts in artificially contaminated "chorizos" (CC) were performed after treatment of food with 3.4% ascorbic acid (AA) and 2% lactic acid (LA) on day 0. Nontreated group was used as control. Samples were frozen at -18°C for 7 days, and then, thawed using four methods: microwave (MW), refrigerator (R), flowing water (FW) and on bench at room temperature (RT). New bacterial counts were performed. In nCC, best reductions of total aerobes were obtained in nontreated (-2.2 log) and AA (-2.19 log) groups. In *Y. enterocolitica* O:9 CC, count of this microorganism decreased 3.27 log in LA group. All of these results were reached by thawing in R. In *E. coli* O157:H7 CC, the best total aerobe reductions were produced by AA using RT (-1.57 log) and R (-1.70 log). *E. coli* O157:H7 decreases were observed with LA treatment: -1.37 log in R, and -1.65 log in FW. The combination LA-R might be effective in the reduction of pathogens.

48. POTENTIATION OF *Leishmania* sp. MACROPHAGE PHAGOCYTOSIS IN CO-INFECTION WITH *Escherichia coli*

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A restricted number of macrophage receptors are known to mediate the internalisation of many kind of different particles. Examples are Fc γ , complement and mannose receptors. *Leishmania* sp is the responsible agent of Leishmaniases disease. On the host side, Ig G macrophage receptor is considered to play a major role during amastigote's infection, in addition to CR3 and mannose receptor. We will study the cultured macrophage activation cells with *E. coli* in regarding parasite phagocytosis. *Leishmanias* sp and *Leishmanias* sp - *E. coli* cultures were used for macrophage infection. Percentage of J774 (mouse macrophage cell line) infection and vacuoles number by phagocyte, were studied after two, four and twenty hour culture under optical microscopy. As a result we have 58% infected macrophages in the coinfection group and 21% in the other, after two-hour culture. More vacuoles were also counted, after twenty hours in the first group. In conclusion, *E. coli* coinfection increases macrophage captation of *Leishmania*. Other intracellular mechanisms are yet to be known involved with increased coinfection efficiency with *E. coli* as well as its medical perspectives.

49.

LISTERIA SPP ISOLATION FROM FOOD SAMPLES

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Several reports have described the presence of *Listeria* spp. in dairy products, meat and poultry, vegetables, mineral oil and soft cheeses. In this study 55 food samples were investigated. The enrichment procedure included a 48 h incubation at 37°C in a Palcam Broth. After the first culture step, 0.1ml of Palcam Broth were spread in Palcam Agar. After 48 h incubation, typical colonies of approximately 2 mm in diameter, grey-green in colour with a black sunken centre and a black halo were considered like *Listeria* spp. Of the 25 meat products 5 strains of *Listeria innocua* were isolated. Listeriosis outbreaks analysis occurring in several countries have shown food products as the most common vehicle of transmission of human listeriosis. The selective enrichment method used in this study allowed *Listeria* spp. isolations from food samples.

51.

POSSIBLE VEHICLES IMPLICATED IN INFANT BOTULISM TRANSMISSION

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Intestinal colonization of *Clostridium botulinum* (*Cb*) and later absorption of its toxin cause infant botulism (IB). It affects infants under one year of age. The main source of infection would be the soil, but other vehicles, like honey and medicinal herbs, must be considered. Our principal aim was to contribute with the knowledge of transmission of IB. The following samples were examined for *Cb*: 276 of soil from Mendoza, 50 of honey, and 23 of *Matriaria sp* (*Mt*) acquired in Mendoza. The most probable number (MPN) method was used to estimate the spore number in all samples of *Mt* and 76 of soil. *Cb* was detected in 117 soil (42.4%) and 6 *Mt* (63.7%) samples. The honey samples were negative. The MPN/g *Cb* spores in 6 *Mt* samples was 0,31 (0,09-1,03)*. In 30 soil samples it was comprendido between 0,14 (0,03-0,63) and 7,59 (1,67-34,37). *(95% confidence limits). The high prevalence and concentration of *Cb* in soils would be the principal factors in the air borne IB transmission. In addition, *Mt* would be a potential vehicle of *Cb* spores. Even though honey samples were negative for *Cb*, previous works indicate it should be taken into consideration.

50.

VIRULENCE OF *Yersinia enterocolitica* IN FOOD

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In humans, *Yersinia enterocolitica* is a causative agent of gastroenteritis. Just some bioserovars are associated with human infections. The pathogenesis of *Y. enterocolitica* is related with a 70-75 Kb plasmid (pYV). This work studied the virulence characteristics of *Y. enterocolitica* isolated from food. Nine strains were studied: *Y. enterocolitica* B1 O:5 (chicken)(2), *Y. enterocolitica* B1A O:9 (chicken), *Y. enterocolitica* B1 O:5 Lis Xz (fish), *Y. enterocolitica* B2 O:9 (egg), and four more strains which classification is on course. The virulence was tested by: a) calcium dependence (Laird y Cavanaugh), b) auto agglutination (Higuchi and col.), c) pyrazinamidase (Kandalo y Wauters) and d) alkaline plasmid extraction (Birnboim and Doly) in agarose gel 0,8%, at 100V during 1h. Two strains showed a 70-75 Kb plasmid and were positive for all the virulence tests. Among the others strains, 2 were calcium depended, 3 showed auto agglutination and 4 were pyrazinamidase negative. These results showed that 2 strains should be virulent *Y. enterocolitica* in raw or slightly cooked food, might be a health risk for consumers.

52.

DETECTION OF *Clostridium septicum* CELLULAR AND EXTRACELLULAR α -TOXIN

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Clostridium septicum is a pathogen which causes the classical malignant edema after injuries. Because of its strong hemolytic and cytotoxic α -toxin, infections are often lethal. The aim of this study was to identify cellular and extracellular α -toxin production of *C. septicum* ATCC 12464, cultured in anaerobic conditions. No significant differences of cellular and extracellular specific hemolytic activity was observed at 0.1, 0.5 and 1% glucose concentrations. In 5 times concentrated supernatants the 45.4 band (protoxin) and 42 band (active form) corresponding to the α -toxin was observed by SDS-PAGE, at all glucose concentrations, while in cellular sonicated cell, only the 45.4 kDa band was identified. These results were in agreement with a lower cellular hemolytic activity.

53. CONFIRMED CASES OF BRUCELLOSIS IN SAN LUIS AND INFLUENCE ZONE – A PRELIMINARY INVESTIGATION

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Brucellosis is an endemic disease in San Luis province. The investigation it is realized for to confirm the clinic diagnostic by specific and sensible immunological tests: Plate Agglutination test (PAT), Rose Bengal (RB) and Tube Agglutination test (TAT). In 2002, it was studied the serum of 120 individuals from San Luis city and 109 of rural zone (both sexes and different ages). In the first group five samples were reactive for TAP and one for RB; two of them were reactive by TAT. In rural zone, 16 were reactive for TAP and four by RB; four of them were positive by TAT (cut-off by TAT : \oplus 1:100). Since direct diagnosis by means of bacterium isolation it is not possible in all cases, antibodies detection is an alternative and fast form of diagnosis in clinically suspect patients. Thus, this study demonstrates that the reactivity in patients of rural zone is twice greater. In the other hand a predominance of male infection was found.

55. YERSINIA ENTEROCOLITICA: A COMPARATIVE STUDY OF INVASIVE CAPACITY AND PATHOGENICITY OF TWO LOCAL STRAINS

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Yersinia enterocolitica causes enteritis and enterocolitis. A 70 kb plasmid (pYV) is necessary for the virulence. The purpose of the present work was to study the pathogenicity and invasive capacity of *Yersinia* local strains. *Y. enterocolitica* O:9 and O:5 (local strains) and *Y. enterocolitica* O:8 (reference) were used. Autoagglutination and calcium dependence tests were realised. Plasmid was extracted and electrophoresed on agarose. Proteins from cultures of 2 h at 25°C and then shifted for 4 h at 37°C were analysed by SDS-PAGE. Mice were orogastrically infected with 2×10^8 *yersiniae*. Three days later, the number of bacteria in spleen (S), mesenteric lymphoid nodes (MLN) and Peyer's patches (PP) was determined. The plasmid was present in all strains. *Y. enterocolitica* O:8 invaded PP, MLN, and S. *Y. enterocolitica* O:9 invaded PP and ML. *Y. enterocolitica* O:5 invasion was limited to PP. We concluded that *Y. enterocolitica* O:9 local strain is more pathogenic and invasive than O:5.

54. COMPARATIVE STUDY OF DIAGNOSTIC CRITERIA FOR H. PYLORI IDENTIFICATION

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The methods used for *Helicobacter pylori* identification show advantages and disadvantages, so making it difficult to establish the reference one. The most accepted criterion comprise the combination of urease test with histology. The aim of this study was to compare different diagnostic criteria using two simultaneously positive tests. The diagnosis methods were: urease, Gram stain, culture, histology and PCR. Based on the combination urease-histology the percentage of infected patients was 54.3% while on Gram stain- histology was 54.8%. All the combinations studied were equally useful for *H. pylori* diagnosis except the criteria that included culture ($p \leq 0.05$), because the low sensibility of this method.

56. VARIATION OF HEMATIMETRIC VALUES WITH ANTICOAGULANT VOLUME

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Anemia constitutes one of the most frequent causes of medical consult, so its study is of particular interest. The first step towards a diagnosis of anemia is the hemogram, a test that can be performed at any routine laboratory. However, in the pre-analytical stage, the use of the inadequate type or proportion of anticoagulant may lead to errors in the results. Protocols indicate the use of 20 μ l of 10% EDTA in 2 ml of whole blood. The purpose of this work was to determine whether the different hemogram parameters vary significantly with different volumes of anticoagulant. Blood was collected by vein puncture from 45 patients and 2 ml was placed into two Kahn tubes containing 20 and 50 μ l of 10% EDTA-Na₂, respectively, as anticoagulant. A Cell-Dyn-1400 hematological counter was used. The variation of hematic parameters was analyzed by means of Student's t test, verifying the method normality with the Shapiro-Wilk test. The data were processed with software XLSTAT 4.2, using a significance level of 0.05. It was found that the number of leukocytes and red blood cells as well as the values of hemoglobin, hematocrit and VCM varied significantly with the different volumes of EDTA, indicating that the proportion of this anticoagulant affects the determination of hematic parameters, but does not influence the count of platelets.

57.

VARIATIONS OF THE CARRIERS PROTEINS OF THYROID HORMONES*Pereyra A, Sturniolo A, Ferrari C, Miotti A.**Cat Anal Clin, Fac Quím Bioquím Farm, Univ Nac San Luis, 5700 San Luis. E-mail: amiotti@unsl.edu.ar*

The principal carriers proteins of thyroids hormones are: TBG, Prealbumina and Albumina. In the pregnancy the variations of this transporters proteins can affect the availability of thyroid hormone and the valves of the same can be affected by changes in the concentration of this proteins. The objective of present work was the study the changes of the transporters proteins of the thyroid hormone in the different trimesters of pregnancy. We analyzed 150 samples of serum from euthyroid pregnant women negative for antithyroid antibodies: 35 during the first trimester, 55 during the second trimester and 60 during the third trimester. A group of 20 euthyroid non-pregnant women served as control. TBG was determined by chemoluminescence, albumina by automatic analysis (CCX – ABBOTT) and prealbumin by IDR. As pregnancy progresses, the main transporting protein TBG increases up to 100% while albumin decreases by 25% at the end of gestation and prealbumin shows no significant change. These modifications are accompanied by an increase in total thyroxine and a slight decrease in free thyroxine.

59.

CALCIUM INTAKE AND BONE MINERAL DENSITY IN MENDOZA POSTMENOPAUSAL PATIENTS*Zavaroni N, Rodríguez C, Saravi FD***Serv Nutr, Hosp Militar Mendoza; *Esc Med Nuclear y Fac Cs Méd, Univ Nac Cuyo, 5500 Mendoza. E-mail: ferniel@msn.com*

Low calcium intake is a risk factor for osteoporosis. Calcium intake and bone mineral density (BMD) were assessed in 138 postmenopausal women referred during 2001 to the Bone Densitometry Unit of the Mendoza Nuclear Medicine School. Patients were not taking any medication known to affect BMD at the time of measurement. Calcium intake was estimated from an alimentary survey and BMD by dual energy X-ray absorptiometry (DXA; Lunar DPX-L). Sample data (mean \pm SD): Age 57.3 \pm 8.8 yr; time since menopause 10.4 \pm 9.9 yr (median 9.0); height 157 \pm 6 cm; weight 65.5 \pm 10.6 kg; body mass index 26.47 \pm 4.48 kg/m². Current mean calcium intake showed a non-Gaussian distribution with a median of 400 mg/day (range 0 to 2,200), significantly below the recommended minimal intake of 1,000 mg/day ($P < 0.0001$). Less than 20% of patients had a calcium intake \geq 1,000 mg/day. Lumbar spine BMD = 1.038 \pm 0.184 g/cm² (n = 136); femoral neck BMD = 0.864 \pm 0.122 g/cm² (n = 106). There was no significant linear correlation between current mean calcium intake and BMD at either site. BMD was significantly correlated with both body mass index and body weight, but was best for the latter (lumbar spine, $r = 0.4208$; $P < 0.0001$; femoral neck, $r = 0.4034$; $P < 0.0001$). According to WHO criteria, only 37.5% of patients had BMD within the normal range, while 43.5% were osteopenic and 19% osteoporotic. A comparison of present results with those of a similarly designed British study showed low spine BMD in a higher proportion of our patients ($P < 0.0001$). These results underline the need of a local public health policy addressed to osteoporosis prevention, early diagnosis and treatment.

58.

DYSLIPIDEMIA DETECTION IN COMPLEJO SANITARIO SAN LUIS OUTPATIENTS*Pellegrini MF, Dato M*, Moyano M*, Zirulnik F, Giménez MS.**Bioquím Molec, Univ Nac San Luis; *Secc Quím Clín, Compl Sanit San Luis, 5700 San Luis. E-mail: fernypelle@hotmail.com*

The objective of this work was to detect cases of dyslipidemias in the masculine and feminine population of different ages in the period March- October of the current year in the Complejo Sanitario San Luis. The determinations were carried out in an automatic TECHNICON RA-XT equipment. The numbers of subjects of both sexes that presented values above the normal range of cholesterol, triglycerides, LDL and below the normal range for HDL were determined as percentages. *Cholesterol* in men (M) age 20-50: 37% in 85 cases and 50-80: 33% in 177 cases. Women (F) 20-50: 10% in 252 cases and 50-80: 27% in 256 cases. *Triglycerides* (M) 20-50: 39% in 89 cases, 50-80: 34% in 170 cases and (F) 20-50: 27% in 254 cases and 50-80: 30% in 236 cases. *LDL* (M) 20-50: 17% in 80 cases, 50-80: 25% in 173 cases; (F) 20-50: 36% in 251 cases, 50-80: 6% in 257 cases. *HDL* (M) 20-50: 7% in 84 cases: 50-80: 13% in 73 cases; (F) 20-50: 9% in 252 cases and 50-80: 8% in 264 cases. The results show that men suffer hypercholesterolemia more often than women, although in the female group of 50-80 years old the fraction is increased, compared with the group of 20-50 years old. Hypertriglyceridemia is also more frequent in men, showing a more elevated percentage in the group of 20-50 years old, while in the women the highest percentage corresponded to the group of 50-80 years old. Levels of HDL were lower in 50-80 years old men. LDL levels were higher in 20-50 years old women; a lesser increase was found in the age-matched male group.

60.

ENHANCEMENT OF FLAVONOIDS WITH BACTERIOSTATIC ACTION AGAINST *Escherichia coli* ATCC 25 922*Alvarez MA, Debattista NB, Pappano NB.**Proyecto 2-9101, Univ Nac San Luis, 5700 San Luis. E-mail: npappano@unsl.edu.ar*

In this work, the enhancement of flavonoids with bacteriostatic activity against *E. coli* ATCC 25 922 was investigated. Kinetic growth experiences of the microorganism in nutritive broth with increased concentrations of flavonoids were performed. The specific growth rates and minimal inhibitory concentrations (MICs) in presence of rutin (5,7,3',4'-tetrahydroxy-3-O-rutinosyl-flavone), a compound inactive, quercetin (3,5,7,3',4'-pentahydroxyflavone) MIC = 77.75 μ g/mL and morin (3,5,7,2',4'-pentahydroxyflavone) MIC = 109.5 μ g/mL, were evaluated. Using identical procedure, MICs of quercetin and rutin were determined in presence of constant concentration of inactive flavonoid rutin, resulting quercetin MIC = 44.57 μ g/mL and morin MIC = 78.5 μ g/mL. The decrease of MIC values shows that the inactive flavonoid enhanced the bacteriostatic activity of quercetin and morin. This fact might be attributed to rutine (glycoside)- induced facilitation of access to *E. coli* of the more polar molecules, quercetin and morin.

61. POTENCIAL IRRITANT ACTIVITY OF *Acacia caven* (Fabaceae): A HISTOLOGICAL STUDY

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Acacia caven (Mol.) Molina (Fabaceae), commonly known as "espinillo", "aromo", "aromito", "espino", "churque", is an aromatic plant that grows in Argentina. The objective of this work was to investigate the irritant activity of the crude extract of pollen of *Acacia caven* on rabbit skin (OECD, 2000) through a histological study. Sterile gauze patches were soaked with the crude extract and they were fixed on the dorsal part of albin rabbits of both sexes, which were previously shaved. Readings took place at 4 and 24 h. Crude extract of pollen of *Acacia caven* did not show irritant activity on the skin (Index of primary cutaneous irritation in the rabbit, OECD, 2000). Skin was processed and fixed for light microscopy study. Crude extract of pollen of *Acacia caven* lacked of irritant activity according to macroscopic study. Nevertheless, the histological study indicated few scaly alterations with lost of epithelium continuity and leukocytarie invasion. Further studies will be necessary to determinate the reversibility of these injuries.

63. DNA DAMAGE AND REPAIR INDUCED BY TAXOL

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Taxol is a clinically effective antitumor drug. The major cellular target for taxol is the tubulin / microtubule system. Taxol promotes the assembly of stable microtubules and inhibits their depolymerization and may also induce DNA damage in some cell types. We report the formation of DNA strand breaks induced by taxol in peripheral blood lymphocytes of healthy donors. We also evaluate if such damage could be repaired. Venous blood (10ml) was collected by routine venipuncture into a tube with heparin. Lymphocytes were isolated with Histopaque 1077. The cells were cultured in RPMI 1640 supplemented with 10% of fetal bovine serum, 1% penicillin /streptomycin and 0.02 ml of lectin. The alkaline Comet Assay was used to evaluate DNA damage and repair capacity. Lymphocytes were treated with 100, 500 nM, 10 and 30 mM of taxol for 4 h. A significant increase in DNA strand breaks was achieved when cells were incubated with 10 mM of taxol. Lymphocytes were left to recover in taxol-free medium) for 2, 4, 6 and 24 h. DNA damage was almost completely repaired after 24 h, demonstrating a time-dependent repair capacity. We thus demonstrated that taxol induces DNA damage and this damage can be repaired.

62. EFFECT OF DEHYDROLEUCODINE ON MOTILITY OF HUMAN SPERMATOZOA

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The spermatozoon is a peculiar cell: Haploid, highly differentiated and transcriptionally silent. It has been shown that the sesquiterpene lactone dehydroleucodine (DhL) reacts with thiol groups interfering the production of glutathione (GSH) in *Trypanosoma cruzi* and decreasing the flagellar motility. The object of the present study was to evaluate the effect of DhL on the motility and the viability of human spermatozoa. Semen samples from normal donors were utilized. After swim-up for 1 hour at 37°C-5% CO₂ in gamete preparation media (GPM), concentration was adjusted to 10 x 10⁶ cells/mL. Aliquots were subjected to one of the following treatments: DhL 10 µM, 100 µM and 1 mM and dithiothreitol (DTT) 100 µM. Motility and viability was evaluated at time 0 and 60 min. At time 0, DhL 10 µM and 100 µM did not show any deleterious effect in either motility or viability. DhL 1mM decreased both sperm motility and viability suggesting a toxic effect. Interestingly, at DhL 100 µM there were qualitative changes on the sperm motility, i.e. new patterns of abnormal movements (helical, backward, "jumping" cells and so on). Effects were time-dependent since there was a decrease in motility but not in viability after 60 min of treatment with DhL 10 µM and 100 µM. DhL could act as an stabilizing agent of -S-S-bridges on the sperm flagellum. In turn, this situation could lead to the loss of sperm motility. The situation is quite the opposite when using DTT, a reducing agent that breaks -S-S- and that increases the motility in a way which closely remembers the so-called hyperactivated motility.

64. EFFECT OF CADMIUM ION ON PHOSPHOLIPASE D ACTIVITY IN ADENOHYPOPHYSIS

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It is well known that cadmium ion administered in drinking water alters prolactin (Prl) secretion in adenohipophysis by a mechanism that requires phosphatidic acid (PA). Phosphatidylcholine (Pc) is a precursor of PA through the action of phospholipase D (PD). Thus, a decrease in the activity of this enzyme might be related to a lower Prl secretion. At the same time, we showed that animals exposed to cadmium present a highly significant decrease in the number of cells immunomarked for anti-Prl in adenohipophysis when compared to controls. Two experimental models with Wistar male rats were used. *In vivo*: rats drank 3 mg Cd/100 mL in drinking water for 7 weeks and *in vitro*: Adenohipophysis cells of control male rats were incubated in presence of 10 mM Cd. In both cases, the PD activity was measured using myristic [³H] and ethanol in the incubation medium. With both experimental models, similar results were obtained: significant decreases in PD activity. Therefore we conclude that cadmium intoxication *in vivo* as well as *in vitro* modifies adenohipophyseal Prl secretion through a mechanism involving a decrease in PD activity.

65.

ALTERNATIVE METHODS IN CYTOTOXICITY ASSAYS OF COMPOUNDS LICHENS. REFINEMENT, REDUCTION AND REPLACEMENT (3RS)

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The use of laboratory animals has been rationalized gradually for scientific community, according with the legislation of Europe and U.S.A. The three Rs (*refinement*, *reduction* and *replacement*) are the root of the development of *in vitro* alternative methods to animal experimentation. *Refinement* is the elimination of any incidence or severity of inhumane procedures applied to those animals which have to be used, *reduction* is to decrease the number of animals needed to obtain information, and *replacement* is the substitution of animals by non-sentient material. In this work, experimental models as primary culture of hepatocytes and continuous cell lines HeLa and L-929, are used considering *refinement* and *reduction* concepts. To study the cytotoxicity of usnic acid (commercial and obtained in laboratory) was evaluated using: a) tetrazolium reagent (MTT), b) neutral red (NR) and c) lactate dehydrogenase (LDH). The usnic acid (commercial) shows a higher toxic activity compared with hepatocytes and L-929 in continuous cell lines HeLa. The cytotoxic of usnic acid (obtained in laboratory) was similar in the models used. These results indicate the need to use different alternative methods for guarantee the quality and specificity of the datas.

67.

PHAGOCYTIC ACTIVITY OF PLANT EXTRACTS USED IN POPULAR MEDICINE IN ARGENTINA

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The available information on the immunostimulatory activity of plants used in popular medicine in Argentina is scarce. The aim of this work was to apply bioassays to evaluate the effect upon the phagocytic activity of murine peritoneal macrophages of aqueous extract of: jarilla (*Larrea divaricata*), palo azul (*Cyclolepis genistoides*), poleo (*Lippia turbinata*), aguaribay (*Schinus areira*) and carqueja (*Baccharis trimera*). The leaves were collected and identified by the Botany team of the UNSL. For the preparation of extract, the leaves were dried and kept at 4°C for 24 h to 5% (w/v). Rockland mice were employed. Macrophages from peritoneal fluid were resuspended in DMEM at 1×10^6 cél/ml. They were incubated for 2 h at 37°C and 5% CO₂. Adherent cells were treated with 1 ml of extract. The phagocytosis test was carried by using opsonized zimosan and nitro blue of tetrazolium. Only jarilla and aguaribay extracts showed increase of phagocytosis. The knowledge of the immunostimulatory effects of plants used in popular medicine will permit us to use them on a more rational and scientific basis.

66.

FUNGI ISOLATED OF SOIL AND PLANTS IN SEARCH OF ANTIMICROBIALS

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A high number of substances with biological effect are produced by microorganism. These can give solutions to the search of new compounds with biological activity and they could be considering standard to developed new herbicide, fungicide and bactericide. Fungus of typical plants from San Juan and of the soil associated to them were isolated. The samples were collected from Bauchazeta- Department of Iglesia-San Juan in February 2002. They were taken of different species plants like: *Tagetes mendocina*, *Baccharis grisebachii*, *Acaena magellanica*, *Baccharis incarum*, *Microcanto*, *Oxalis erythorhiza* y *Senecio volckmanni* and of the soil. The samples were cultivated in soil and liquid suspension in Czapeck, under isolation's standard protocol. Thirty one samples (20 of plants and 11 of soil) were analyzed. Eight filamentous fungus were isolated of plants and one of soil. The suspensions were analyzed macro and microscopically to determinate morphological and reproductions feature. Nine fungus isolated will be used to produced substances in liquid suspension and analyze them as potential herbicides, fungicides and bactericides.

68.

CARDIOVASCULAR EFFECTS OF ACETYLSALICYLIC ACID IN AN EXPERIMENTAL MODEL OF METABOLIC SYNDROME

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We evaluated the effects of chronic treatment with acetylsalicylic acid (ASA) on tensional and metabolic changes in fructose-fed rats (FFR), a model of syndrome X. Rats were divided in 4 groups (n = 6 in each): 1-Control; 2-C + ASA (10 mg/Kg/d) in drinking water in the last 4 weeks; 3-FFR (10%fructose in drinking water during 8 weeks) and 4-FFR (idem 3) + ASA (idem 2). Systolic blood pressure (SBP mmHg) was measured weekly and at the end of the experimental period, glucose tolerance test (GTT mmol. L⁻¹.90min) and relative heart weight (RHW mg.100⁻¹g BW) were determined. Data (mean ± SEM) were processed by ANOVA. * p < 0.001 vs Control; † p < 0.01 vs FFR.

	Control	C+AAS	FFR	FFR+AAS
PAS	101 ± 4	110 ± 2	133 ± 3 *	112 ± 3 †
GTT	881 ± 64	886 ± 128	1292 ± 31*	839 ± 51 †
RHW	225 ± 4	234 ± 4	298 ± 18*	242 ± 4 †

We confirmed that FFR develop hypertension, cardiac hypertrophy and glucose intolerance, as previously observed. Chronic treatment with ASA normalized tensional and metabolic variables in FFR, suggesting the involvement of an inflammatory component in the development of the cardiovascular alterations associated to this pathology.

69. ACTIVITY OF *Artemisia douglasiana* BESSER ON EXPERIMENTAL COLITIS

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Artemisia douglasiana Besser is used in folk medicine as a cytoprotective agent. The effects of extract of *A. douglasiana*, on two models of experimental colitis were examined. Colitis was produced in male Wistar rats by rectal instillation of 30 mg of trinitrobenzene sulfonic acid (TNBS) in 50% ethanol and 3% N-ethylmaleimide (NEM). Rats were killed on 7 days (TNBS) and 24 h (NEM), and the colon rated macroscopically. The TNBS treated rats showed extensive and widespread ulcerations of the mucosa. Macroscopic scoring showed a decrease ($p < 0.001$) in the degree of damage in the group of rats treated with extract post TNBS. Colon mass was decreased ($p < 0.001$). *A. douglasiana* has an effective healing capacity at macroscopic level. The administration of NEM caused severe diarrhea, adhesion and dilatation of the colon. Extract of *A. douglasiana* inhibited the mucosal lesion formation ($p < 0.01$) and diarrhea, but did not inhibit adhesion and dilatation. Our study suggest that *A. douglasiana* may prevent or reverse the inflammation process, in two models of experimental colitis.

71. *Prosopis flexuosa*: ANTINUTRIENTS AND ANTI-ULCEROUS ACTIVITY OF EXTRACTS

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The purpose of the present work was to study the gastric anti-ulcerous activity of *Prosopis flexuosa* leaf methanolic extract (*Pf.m.ex.*) and the presence of antinutrients such as hemagglutinins and saponins in *P. flexuosa* leaves (*Pf.l.*). Wistar rats were given p.o.: saline (normal and ulcer control groups); 25, 50, 100 or 200 mg/Kg of *Pf.m.ex.* (experimental groups). After 1 hour, absolute ethanol was administrated to experimental and ulcer control groups. The ulcer grade was evaluated after 1 hour according to Marazzi Ubeti and Turba scale and expressed as ulcer index (UI). Hemagglutinins (Do Prado, 1980) were extracted from leaves with saline, the dilutions were assayed with trypsin treated goat erythrocytes. Title was measured 24 h later. Saponins (WHO/PHARM, 1992) were extracted with 100°C water. Foam index was calculated. The UI decreased significantly from 50 mg/kg *Pf.m.ex.* The *Pf.l.* showed hemagglutinating and hemolytic activity (dilution °). The foam index was 125. Conclusions: The *Pf.m.ex.* has anti-ulcerous effect and posses antinutrients such as hemagglutinins and saponins. The anti-ulcerous effect could be attributed to the presence of active compounds with free radical scavenger effect.

70. MOLECULAR MECHANISMS INVOLVED IN THE INHIBITORY ACTION OF DEHYDROLEUCODINE ON MAST CELL DEGRANULATION

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In previous works we have shown that dehydroleucodine (DhL) is able to inhibit mast cells (MC) degranulation induced by various chemical agents. In this analysis, we compared the effect of DhL on different degranulation activators in order to trace its molecular mechanism of action. Peritoneal rat MC were isolated and incubated for 20 min at 37°C with: 1) buffer; 2) DhL (1.6 mM); 3) 48/80 (Gi protein activator; 10 µg/ml); 4) DhL+48/80; 5) A23187 (calcium ionophore; 10 µg/ml); 6) DhL+A23187; 7) Ethanol (PLC and PLD activator; 10%); 8) DhL+Ethanol. Released and remnant serotonin (5-HT) was quantified by HPLC. Compound 48/80, A23187 and ethanol increased 5-HT secretion compared to controls. DhL totally inhibited 5-HT secretion induced by 48/80 and by ethanol, and partially that induced by A23187. MC from 48/80, A23187 and ethanol groups showed evident morphological alterations and decreased granular density. DhL inhibited those alterations. Inhibition in MC degranulation appears to be related to adenilatocyclase activation, or to phospholipase C and/or D inhibition.

72. CADMIUM AFFECTS TRIGLYCERIDE METABOLISM

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Cadmium (Cd) is a toxic pollutant. Chronic intoxication with Cd modifies liver lipid metabolism in rat. Previously, we showed that Cd increases triglyceride, decreases esterified cholesterol, alters the percent concentration of fatty acids (increases palmitic acid and decreases linoleic acid) and increases Fatty Acid Synthetase (FAS) activity in liver, and serum triglycerides in Cd animals that were starved 12 h. The aim was to determine the effect of Cd in liver triglyceride production. Adult male Wistar rats, weighing 180-200 g were divided into two groups: controls (Co) and cadmium (Cd). Cd was administrated in the drinking water, as CdCl₂ in a concentration of 15 ppm during 7 weeks. VLDL production assay: Anesthetized male rats starved 12 h, were injected with 500 mg/kg of Triton WR 1339 (i.v.) using a 10% (wt/vol) solution. Plasma triglyceride levels were measured at T = 0 and 60 min after Triton injection (T1). Serum triglycerides (g/L): Co T0: 1.427 ± 0.1343, Co T1: 2.545 ± 0.2737, Cd T0: 2.240 ± 0.1218, Cd T1: 3.980 ± 0.6705, P < 0.05 for the Cd groups T0 y T1. A significative increase of serum triglyceride content in Cd animals after Triton injection is due to an increase in the synthesis and liberation of liver triglycerides.

73. GASTROINTESTINAL ACTIVITY OF *Artemisia douglasiana* Besser IN RATS AND MICE

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Artemisia douglasiana Besser (*A. d.*), popularly known as "matico", is placed in the Cuyo region. It has been used in folk medicine as a cytoprotective agent against the development of peptic ulcer. This work aimed at evaluating the gastrointestinal activity of *A. d.* in rats and mice. Twenty four hours before the experiments, Wistar rats were fasted and the duodenum was ligated (Melchiorri *et al.*, 1997). Absolute ethanol (AE) was employed as ulcerogenic agent. After ligation, rats were divided in groups with the following treatments: I: vehicle and AE.; II: *A. d.* 10% extract and AE. The results were expressed in mm². *A. d.* prevents the formation of gastroduodenal lesions induced by absolute ethanol. Intestinal transit was measured by method of Ueda. *et al.* (1969). 10% and 20% extracts decreased small intestinal transit in mice. The free radical scavenging effect of *A. d.* (DPPH decoloration assay, Joyeux *et al.*, 1995) was demonstrated. Thus, *Artemisia douglasiana* Besser reduces the intestinal transit in mice and prevents ethanol induced gastroduodenal damage in rats. This activity could be due, in part, to their capacity for scavenging free radicals which may be involved in peptic ulcer development. These facts support the use in traditional medicine of *Artemisia douglasiana* to treat digestive disorders.

75. EFFECT OF *Artemisia douglasiana* BESSER ON ACUTE LIVER INJURY INDUCED BY CCl₄ IN RATS

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Artemisia douglasiana Besser is used in folk medicine and known by the common name of "matico". The aim of the present work was to study the hepatoprotective activity of the extract of this plant by using the model of experimental liver damage induced by carbon tetrachloride (CCl₄). Male Wistar rats (90-110 g) were used. Control group: received CCl₄ (0.15 mL/kg); experimental group: received extract (1g/kg) and CCl₄; blank group: received vehicle. Serum aspartate (AST) and alanine aminotransferase (ALT) was determined. The extract of *A. douglasiana* produced marked reduction of both, AST and ALT ($p < 0.001$. ANOVA-Tukey), relative to the control group. Liver injury induced by CCl₄C, is attributed to the production of free radicals (trichloromethyl) by the liver cell microsomes during the metabolism of CCl₄. Recently, it was demonstrated that an extract of *A. douglasiana* has antioxidant capacity. This capacity may be the result of several antioxidant compounds in *A. douglasiana* (flavonoids). The present study shows that the extract prevents significantly acute liver injury induced by CCl₄, and this activity, could be due, to their capacity for scavenging free radicals.

74. ANTIBACTERIAL ACTIVITY OF DIFFERENT EXTRACTS FROM *Schinus areira* L ON *Staphylococcus aureus*

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Schinus areira L popularly used by its antiseptic properties, anti-rheumatic, among others. The aim of this study was to evaluate the antibacterial activity of different extracts and to determine the concentration responsible for this effect. Pulverized dry leaves were employed, which they were submitted to different extraction methods: infusion, decoction, aqueous extract and simulated digested extract. The extracts were lyophilized and 1:2 serial dilutions were tested from 40 g/l. The bacterial activity was tested using the hole plate diffusion method. The results were compared with the bacterial growth inhibition by concentrations of gentamicin. Results obtained showed that the infusion, decoction and aqueous extract exhibited activity against the *Staphylococcus aureus* growth. *S. areira* L possesses antibacterial activity and the different extraction methods modified the antibacterial effect. Future studies in the characterization of the product (s) with antibacterial activity will contribute to a better scientific knowledge and rational use of plants used in popular medicine.

76. ANTI-INFLAMMATORY ACTIVITY OF *LARREA DIVARICATA* CAV. INFLUENCE ON SPLEEN AND THYMUS

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Larrea divaricata Cav contains nordihydroguaiaretic acid with anti-inflammatory properties (Franchi - Micheli *et al.*, 1986). The purpose was to investigate the anti-inflammatory activity of a methanolic extract of *Larrea divaricata* Cav leaves (*L.d*) and its influence on spleen and thymus. Granulomas were induced in Wistar rats by implanting of cotton pellets into the dorsal zone; 24 h later and during 6 days the following was administered: Saline (inflammation control) (G1), dexametaxone (7mg/kg, s.c.) (reference) (G2), *L.d* (200 mg/kg) s.c. (G3) or i.p. (G4). Seven days later, granulomas, thymus and spleen were weighed. The granuloma weights were G1 = 0.338 ± 0.02, G2 = 0.246 ± 0.02 (**p < 0.0003), G3 = 0.257 ± 0.03 (* p < 0.005), G4 = 0.374 ± 0.10 (not significant). In contrast to dexamethasone, s.c. *L.d* did not change corporal, spleen and thymus weights. I.p. *L.d* decreased thymus weight. Conclusions: *L.d* has anti-inflammatory activity by s.c. administration without influence on body, spleen and thymus weights. This anti-inflammatory effect might be partly attributed to nordihydroguaiaretic acid.

77. DEHYDROLEUCODINE INHIBITS MAST CELL DEGRANULATION INDUCED BY COMPOUND 48/80

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Dehydroleucodine (DhL), a lactone isolated from *Artemisia douglasiana* Besser, prevents gastrointestinal damage elicited by necrosis-inducing agents. This work examines the effect of DhL on compound 48/80-induced degranulation from rat peritoneal mast cells, to determine whether DhL may act as a mast cells stabilizer. Rat peritoneal mast cells were purified in Percoll and incubated with: 1) PBS or 2) 10 µg/mL compound 48/80 or 3) DhL (20, 40, 80, 200 and 2000 µM) + 48/80. Serotonin release studies by high performance liquid chromatography (HPLC), evaluation of mast cell ultrastructure by transmission and scanning electron microscopy, and dose-response studies were carried out. Compound 48/80 increased serotonin release by the cells and elicited evident granule ultrastructural changes. These effects were inhibited by dehydroleucodine in a dose-dependent manner. The lactone inhibits compound 48/80 mast cell activation, probably acting as a mast cell stabilizer.

79. SALBUTAMOL DOSE-DEPENDENT BIOCHEMICAL EFFECTS ON ADULT MALE RAT LUNG SURFACTANT AND ALVEOLAR MACROPHAGES

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The effects of the β_2 -adrenergic agonist salbutamol in low and in high (therapeutic) concentrations on the composition of lung surfactant and alveolar macrophages of adult male Wistar rats were assessed. Total lipids (TL), triglycerides (TG), total cholesterol (TC), free cholesterol (FC), phospholipids (PL) and proteins (Pt) were determined after 5 min inhalation of nebulized saline (control) or salbutamol at 1.3 µmol/mL (Low) and 13 µmol/mL (High), b.i.d. for 15 days. For pulmonary surfactant: TL (mg), Control 14.2 ± 2.5; Low 28.4 ± 2.3; High 30.4 ± 0.5 mg (p < 0.001). TC (µg/mg lipid), Control 64.42 ± 1.3; Low 14.3 ± 1.1 (significant decrease); High 83.86 ± 5.5 (p < 0.01). FC (µg/mg lipid), control 51.3 ± 3.4; Low 10.7 ± 0.1 (p < 0.0001); High 62.4 ± 2.2 (NS). PL (µmol P/mg lipid), control 0.02 ± 0.006; Low 0.089 ± 0.005; High 0.116 ± 0.01 (p < 0.001). No significant differences were found for TG or Pt. For alveolar macrophages: TL (mg), Control 13.5 ± 0.7; Low 23.23 ± 4.4; High 25.5 ± 1.8 (p < 0.001). TC (µg/mg lipid), Control 27.4 ± 3.4; Low 44.4 ± 2.5; High 47.2 ± 2.4 (p < 0.001). PL (µmol P/mg lipid), control 0.036 ± 0.01; Low 0.065 ± 0.02 (p < 0.001); High 0.026 ± 0.007. No significant differences were found for TG or Pt. Thus, inhaled salbutamol modifies the lipid profile in both lung surfactant and alveolar macrophages in a dose-dependent way.

78. LUNG SURFACTANT AND ALVEOLAR MACROPHAGE PHOSPHOLIPIDS IN MALE ADULT RATS INHALING HIGH-DOSE SALBUTAMOL

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The effect of a high concentration (130 µmol/mL 5 min b.i.d. for 15 days) of inhaled salbutamol (Sal), a β_2 -adrenergic agonist, was compared to that of nebulized saline in adult male Wistar rats. Total phospholipids (TP) and phospholipid fractions separated by thin-layer chromatography were measured. Results are expressed as mean ± SEM in µmolP/mg lipid of control vs Sal. Lung surfactant: TP 0.060 ± 0.006 vs 0.293 ± 0.2 (p < 0.0001); lysophosphatidylcholine, 0.010 ± 0.001 vs 0.024 ± 0.002; sphingomyelin, 0.006 ± 0.0001 vs 0.034 ± 0.002; phosphatidylcholine, 0.026 ± 0.006 vs 0.103 ± 0.02; phosphatidylglycerol, 0.008 ± 0.0002 vs 0.013 ± 0.0007 (p < 0.0001). Alveolar macrophages: TP, 0.086 ± 0.01 vs 0.224 ± 0.02 (p < 0.0001); lysophosphatidylcholine, 0.009 ± 0.003 vs 0.026 ± 0.001; sphingomyelin, 0.013 ± 0.002 vs 0.036 ± 0.004; phosphatidylcholine, 0.036 ± 0.001 vs 0.083 ± 0.01; phosphatidylglycerol, 0.008 ± 0.0004 vs 0.034 ± 0.008 (p < 0.0001). Salbutamol inhaled at a high concentration increases phospholipid concentration in both surfactant and alveolar macrophages, an effect which might be related to its therapeutic benefit on respiratory function.

80. CATALPOL, A DNA POLYMERASE INHIBITOR FROM *Buddleja cordobensis* HIERON

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Both DNA polymerases and topoisomerases have been identified as a target for anticancer drug development. The iridoid glucoside Catalpol was isolated from the shrub *Buddleja cordobensis* and the structure was elucidated using 1D and 2D NMR techniques. Catalpol was screened in order to detect potential taq DNA polymerase inhibitory activity handling polymerase chain reaction (PCR). This molecule showed a strong inhibition against taq DNA polymerase with IC₅₀: 42µM. Chemical derivatives such as peracetylcatalpol; 3,4-dihydrocatalpol; 10,6'-O-di-terbutyl-difenylsilylcatalpol; 10,6'-O-di-oleylcatalpol, and 4',6'-O-isopropylidencatalpol were prepared in an attempt to modify estereoelectronic factors. Structure-activity relationships will be discussed from Molecular Mechanic (MMFF), conformational analysis, and semi-empirical calculations (HOMO, LUMO and MEP) carried out with Spartan Pro software.

81. TOXIC ACTIVITY OF TRITERPENOIDS AND DERIVATIVES FROM *Junellia aspera* AGAINST *Sitophilus oryzae* (COLEOPTERA)

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A large number of plant-derived substances possess physiological and behavioral effects against stored grain insect pests, and may provide new sources of natural pesticides and antifeedants. As part of an interdisciplinary work directed to detect insect-bioactive plant secondary metabolites we studied the triterpenes from *Junellia aspera* (Gillies ex Hook) and their effect on the pest insect *Sitophilus oryzae* (Coleoptera:Curculionidae). Oleanolic acid (**1**) was the main secondary metabolite and was used as starting material to prepared a series of derivatives: methyl-3 β -hidroxyoleanol-12-en-28-oate (**2**); 3 β ,28-dihidroxyolean-12-en (**3**); ac. 3-oxo-olean-12-en-28-oic (**4**); ac. 3 β -acetoxiolean-12-en-28-oic (**5**); ac. 3 β -hidroxyoleanol-11-bromine-12-en-28-oic (**6**); 3 β -acetoxiolean-12-bromine-(28 \oplus 13)-olide (**7**); 3 β -hidroxy-12-bromine-(28 \oplus 13)-oxide-oleanane (**8**). Results showed that compounds **4**, and **8** were the most active molecules as ingested toxicants. When all compounds were topically applied no significant changes were observed.

83. LOSARTAN EFFECT ON CARDIAC NITRIC OXIDE SYNTHESIS IN AN EXPERIMENTAL MODEL OF SYNDROME "X"

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We evaluated the effects of chronic treatment with an AT₁ antagonist, losartan (L), on cardiac endothelial nitric oxide synthase (eNOS) expression and activity from fructose-fed rats (FFR), a model of syndrome X. Rats were divided in 4 groups: Control (n = 6); FFR (10% fructose in drinking water, n = 8) and FFR+L (20mg.Kg⁻¹.d⁻¹, n = 8). After 8 weeks systolic blood pressure (SBP mmHg), glucose tolerance test (GTT mmol.L⁻¹.90min), relative heart weight (RHW:mg.100⁻¹g body weight), eNOS expression by semiquantitative RT-PCR (eNOS/GADPH ratio) and eNOS activity by conversion of [³H]-arginine in [³H]-citrullin (dpm.mg⁻¹prot.min⁻¹) in cardiac homogenates were determined. Data (mean \pm SEM) were processed by ANOVA. Compared to Control, FFR increased PAS (114 \pm 1 vs 13 0 \pm 1; p<0.001), GTT (880 \pm 64 vs 1293 \pm 31; p < 0.001) and RHW (225 \pm 4 vs 239 \pm 5; p < 0.001) and decreased eNOS expression (1.05 \pm 0.1 vs 0.63 \pm 0.23; p < 0.05) and activity (27.1 \pm 2.6 vs 12.3 \pm 1.1; p < 0.05). Losartan reduced SBP (105 \pm 2; p < 0.001), GTT (1102 \pm 44; p < 0.05), RHW (202 \pm 0.7; p < 0.05) and partially restored cardiac eNOS expression (0.86 \pm 0.14; p < 0.05) and activity (22.5 \pm 0.7; p < 0.05). Data suggest that changes in eNOS expression and activity could be involved in the pathology mechanisms as well as in the therapeutic action of AT₁ receptor antagonists.

82. DIFFERENT ACTIVITY OF FLAVONOIDS IN ACUTE INFLAMMATION

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Flavonoids are natural compounds with different effects on biological systems. Acute inflammation can be manifested through models, which have different cellular mechanisms. The aim was to evaluate five flavonoids on two acute inflammatory models. *Paw edema (PE)*: 35 mice, divided into groups, received SF i.p.(control), quercetin (Q), rutin (R), hesperedin (Hd), hesperetin (Ht) and morin (M) (75 mg/kg) or phenylbutazone (standard, 80 mg/kg). After 1 h, 0.05 mL carrageenan (3.5%) was injected in the left paw. Edema was measured at 1, 3, 5 and 7 h by plethysmography. *Ear edema (EE)*: 30 mice, divided into groups, received via i.p. SF (control), Q, R, Hd, Ht and M (0.25 M/kg). After 1 h 0.03 mL xylene was applied on the right ear. Thirty minutes later, both pinnae were removed and weighed. *PE*: At 3 h, the inflammation was inhibited by Ht (flavone 46%) and Q (flavonol 51%). Flavonol M inhibited at 3 h (43%) and 5 h (54%). R and Hd did not show effect. *EE*, flavonols Q, R and M did not have antiinflammatory effect, while flavanones Ht and Hd inhibited the inflammation in 45 and 44%. Only flavanones showed antiinflammatory effect on *EE*. In *PE*, Q, Ht and M inhibited the inflammation, while glycoside flavonoids did not.

84. *Brucella abortus* TRAFFIC IN MACROPHAGE ENDOCYTIC AND AUTOPHAGIC PATHWAYS

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Maturation of phagosomes containing *Brucella abortus* (virulent strain 2308 and attenuated virulence strain S19) was studied by monitoring of intracellular transport through the endocytic and autophagic pathways of J-774 murine macrophages with fluorescence microscopy. Acidification of compartments containing bacteria was assessed with the fluorophore LysoSensor (5 μ M), a weak base. Monodansylcadaverine and DiOC6 (3,3'-dihexyloxycarbocyanine) were employed to assess, respectively, whether *Brucella* was located in autophagic vesicles and was related through this pathway with the endoplasmic reticulum. Co-localization of virulent bacteria within acidic compartments, while lower than that of attenuated virulence bacteria for long incubating periods (46 vs 66%), anyway indicates that virulent *Brucella* is able to resist acidification. Still more important is that the fraction of both strains in autophagic and endoplasmic reticulum compartments is quite low (< 6 %) even after 24 h follow up. This is at variance with the behavior of *Brucella* in HeLa cells as reported by other authors. Present results suggest that *Brucella abortus* delays the fusion of phagosomes with lysosomes. This delay does not prevent acidification, which *Brucella* can endure. A third type of compartment, which does not interact with endosomal or lysosomal markers, might also not interact with the autophagic pathway, thus keeping the bacterium in the endocytic pathway. Further study on *Brucella* interference with fusion proteins (Rabs) expression is needed.

85. RELATIONSHIP BETWEEN RAB 1 AND AUTOPHAGIC PATHWAY

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Autophagy is a process in which cytoplasmic proteins and organelles are sequestered into a membranous compartment, the autophagosome, to be finally degraded by the lysosomes. This process contributes to the cellular homeostasis under nutrient deprivation conditions, cellular differentiation, aging. Like apoptosis, it is a kind of programmed cell death. Rab GTPases comprises a large family of proteins, localized in distinct subcellular compartments where they coordinate consecutive stages of membrane transport. Rab 1 is a member of this family which is essential for ER to Golgi transport. Recently, the yeast homologue of Rab 1, Ypt 1, has been shown to be involved with the autophagic pathway. To study the role of Rab1 in autophagy, we transfected CHO cells with the GFP-Rab 1 wt and mutants. Under control conditions Rab1wt and mutants colocalized with the Cis-Golgi markers. Surprisingly, after induction of autophagy by amino acid deprivation, Rab 1 moved to vacuoles that do not colocalize with Golgi markers. These vacuoles were labeled with monodansylcadaverine, a specific marker for autophagosomes. Thus, these data suggest that Rab 1 is related to the autophagic pathway.

87. THE SNARE PROTEIN SYNTAXIN 1B IS PRESENT IN RAT BRAIN BUT NOT IN TESTIS

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The SNARE proteins participate in the molecular mechanism of exocytosis, a cellular process, under a strict regulation involving the fusion of membrane of secretory vesicles with plasma membrane. One of the mentioned SNARE proteins is syntaxin, which is thought to be present in the plasma membrane (PM) of neurons and forms an exocytosis fusion complex with other proteins (e.g., NSF, SNAPs, SNAP-25 and VAMP). In order to determine whether this protein participates in exocytosis of other cells, we have studied expression and structural localization of syntaxin 1B in rat testis by western blotting and colloidal gold immunoelectron microscopy. Brain tissue served as a positive control. A high expression of syntaxin 1B in whole brain homogenates, as a single band of 36 kDa, was found. This protein was localized in synaptic vesicles and PM of cortical axonal buttons. However, in the testis we found no syntaxin 1B. The presence in the testis of other isoforms of syntaxin with specific roles in exocytosis regulation has not been ruled out.

86. MEMBRANE DOMAINS IN MOUSE SPERM (RAFT) DURING SPERMATOGENESIS, MATURATION, AND CAPACITATION

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Sperm maturation or capacitation process are defined by sphingolipids re arrangement or cholesterol lost, respectively; but most of them are poorly defined. Our lab is characterizing the lipids domains using filipin (fluorescent cholesterol marker), Alexa conjugate with cholera toxin – fracción β (CT β , ganglioside marker) and antibodies to caveolin 1 (cav1)- raft protein. Immature, mature and capacitated sperm were incubated 15 min alone or in combination with filipin (0.1mg /5 x10⁵ sperm/mL), CT β (100 μ g / 5 x10⁵ sperm/mL) and anti cav1 (1:20; 2nd antibody IgG-FITC) and processed for fluorescent microscopy. Immune recognition was performed in permeabilized cell with SLO (16UI/mL) and paraffin sections of mouse testis using a 2nd antibody with Px by routine techniques. Cav1, filipin ant CT β were colocalized from round spermatid to acrosomal sperm, except in capacitated sperm that lost cholesterol and move CT β to post acrosomal region. These changes suggest that raft is present at the sperm but rearranged after capacitation.

88. CHARACTERISTICS OF THICK FILAMENT ASSEMBLY FROM MYOID CELL MYOSIN

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Peritubular myoid cells contain abundant actin filaments and they are contractile. Previously, we have show that rat myoid cells contain an isotype of smooth muscle myosin which is not assembled in filaments. In order to determine whether this myosin isotype is able to assemble in vitro, myosin of rat myoid cells was purified and it's salt-dependent solubility was assessed in a buffer containing different concentrations of NaCl. 200 μ g/ml of myosin in phosphate-buffered 350 mM NaCl were dialyzed for 12 h again 100-350 mM NaCl. The samples were centrifuged, the pellets and supernatants analyzed by 7.5% SDS-PAGE, and the fraction of assembled myosin quantified by comparison of staining intensity of the bands. The assay showed that 95% of the myosin in 100 and 150 mM NaCl was assembled in filaments, 50% in 200 mM NaCl, and 10% in 350 mM NaCl. These results indicates that myoid cell myosin is able to assemble in vitro in a similar manner to other smooth muscle myosin II isotypes.

89. ANGIOTENSIN II TYPE 1 RECEPTOR A¹¹⁶⁶C GENE POLYMORPHISM AND ITS ASSOCIATION WITH ESSENTIAL HYPERTENSION

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An adenine/cytosine (A/C) base substitution at position 1166 in the angiotensin II type 1 receptor (AT₁R) gene was associated with essential hypertension in humans. We examined 25 individuals with primary hypertension and 10 normotensive controls. DNA was extracted from whole blood and mismatch PCR combined with RFLP was used for analysis of AT₁R polymorphism. PCR products were incubated overnight with the restriction endonuclease DdeI at 37°C, which cuts the product into two pieces, 600 and 250 bp long. An additional DdeI recognition site is created in the C-type variant, at nucleotide 1166, which is found in the 250 bp fragment. Thus, the homozygote CC produces three bands (600, 140, and 110 bp long), the homozygote AA produces two bands (600, 250 bp long) and the heterozygote produces four bands. Although the polymorphism is within a non translated region, detection of this polymorphism will allow us to carry out preventive studies on the patient's family.

91. PLASMIDS DESIGN TO PRODUCE MEMBRANE PERMEANT RECOMBINANT PROTEINS

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Several arginine-rich peptides are transported into the cells and can carry covalently bound proteins. Introducing exogenous proteins into the cytosol is a useful tool to study protein function in cells that cannot be transfected. To produce membrane permeant proteins two plasmids have been generated, pGEX-2T-R and pGEX-2T-R-EGFP, both of them are pGEX-2T derived. The proteins GST-R (glutathione-S-transferase C-terminally bound to the peptide RRRQRRKRRRQ) and GST-R-EGFP (GST-R C-terminally bound to green fluorescent protein) were produced by inducing with IPTG bacteria transformed with these plasmids. Permeability assays were performed incubating CHO cells and human spermatozoa with the proteins for three hours at 4°C. To visualize GST-R, indirect immunofluorescence was performed, whereas GST-R-EGFP was directly observed by fluorescence microscopy. These results suggest that GST-R was internalized while GST-R-EGFP was not. It is possible that the arginine rich peptide is not accessible in GST-R-EGFP to interact with membranes. This work shows that these plasmids can be used to study protein function in several cell types.

90. RECONSTITUTION OF PHAGOSOME TRAFFICKING IN STREPTOLYSIN O PERMEABILIZED MACROPHAGES

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Phagocytosis is a process through which macrophages internalize large extracellular particles. By fusion with endosomes and lysosomes, phagosomes mature and acquire degradative ability. During phagocytosis a large amount of plasma membrane is internalized, implying an active mechanism for the retrieval of membranes to the cell surface. The objective of this work was to reconstitute phagosome transport along the phagocytic pathway in cells permeabilized with Streptolysin O (SLO). In this system, trafficking could be manipulated by the addition of proteins or reagents that usually do not cross the plasma membrane. Phagocytosis was assessed by the uptake of *St. aureus* coated with ¹²⁵I-antibodies and recycling or degradation were measured by the efflux to the medium of precipitable or soluble radioactivity. In permeabilized cells, the presence of cytosol and an ATP regenerating system facilitates the recycling from phagosomes and the degradation of the particles internalized. These effects are in agreement with the requirement of cytosolic proteins and energy for phagosome maturation. In conclusion, SLO-permeabilized macrophages could be an useful tool to study the molecular machinery involved in trafficking along the phagocytic via.

92. DEHYDROLEUCODINE SLOWS THE GROWTH OF WILD-TYPE YEAST BUT DOES NOT AFFECT A STRAIN DEFICIENT IN THE DNA CHECKPOINT

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The sesquiterpene lactone, dehydroleucodine (DhL), selectively induces a transient arrest in G2 in meristematic cells. In order to elucidate the molecular mechanism of DhL action on the cell cycle, we assayed the growth kinetics of two strains of *Saccharomyces cerevisiae*: strain 9154 (wild-type) and strain 9155 (chk1 deficient). Growth kinetic assay: cells at 0.5-1 x 10⁶ cells/mL (grown in YPD to early stationary phase) were incubated in fresh YPD medium with 0 (control) or 0.5 mM DhL (treated) at 30°C with continuous aeration and agitation. Aliquots were taken every 2 h and the number of cells per ml were determined in a hemocytometer. The number of cells (x 10⁶) under each condition at 0, 2, 4, 8, 10 and 24 h of incubation was the following: strain 9154, control: 3, 4, 9, 18, 27 and 41 respectively, and treated 4, 4, 4, 5, 7, and 10 respectively. Strain 9155, control: 4, 5, 9, 17, 30 and 45 respectively, and treated 4, 5, 10, 20, 26 and 39 respectively. These results show that DhL reduced the growth rate in the wild-type strain, without affecting the strain inactive in chk1. Thus, it is possible that DhL acts at the level of the DNA damage checkpoint.

93.

ACROSOMAL REACTION IN MOUSE SPERM: SIGNAL TRANSDUCTION THAT RESPONDS TO PROGESTERONE

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Mouse sperm acrosomal reaction (AR) is a highly regulated process triggered by specific signals of zona pellucida proteins through a non well characterized signal transduction pathway. Progesterone (P) can initiate the cascade leading to the AR. Our previous work suggested that the sequential activation of G proteins (G) → phospholipase A₂ (PLA₂) → increase in intracellular Ca²⁺ (Ca²⁺) promotes AR. It is possible that this cascade is activated by P in mouse sperm; other phospholipase could also be involved. Using specific inhibitors, GDPβ (for G), ONO-RS 082 (for PLA₂) and neomycin (for PLC) or mimetics, GTPγ (G), PMA or imipramine (phorbol ester for PLC) and arachidonic acid (PLA₂) alone or in combination, their participation in AR was tested both in the presence and in the absence of P. Percentages of reacted sperm were obtained by the chlortetracycline stain technique. FURA-AM – an Ca²⁺ fluorescence marker – was determined by spectrofluorometry. P (10 μM) was used to trigger AR. Sperm was capacitated in HMB medium for 60 min. Mimetics of both phospholipases were involved in promoting AR, as well as GTPγs. Conversely, phospholipase or G inhibitors blocked AR. But, both are independent. An Ca²⁺ increase was detected follow activation of PLC or PLA₂. These results suggest that P activates G, and simultaneously and independently G activates PLC or PLA₂. Both promote an Ca²⁺ increase that finally leads to AR.

95.

ANDROSTENONE AFFECTS PERCEPTION OF PEOPLE PICTURES IN WOMEN

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Objective: to study the effects in women of androstenone pheromone on the perception of people pictures. Twenty one women (ages 18-28), evaluated 7 woman and 7 man pictures, after inhaling vehicle or androstenone. The 21 women fulfilled a questionnaire with 13 attributes that describe how they perceived the photographed people, scale 1 to 10. Student's paired *t* test was used. Inhaling androstenone, these effects were found: *Women pictures*: N° 1: more attractive ($p = 0.0052$) and sexier ($p = 0.0675$), n° 3: warmer ($p = 0.0177$) and less dominant ($p = 0.0028$); n° 6: less reliable ($p = 0.0160$), less sensitive ($p = 0.0145$), less good ($p = 0.0167$) and less pretty ($p = 0.0570$); n° 7: warmer ($p = 0.0357$) and less feminine ($p = 0.0592$). *Men pictures*: n° 9: less virile ($p = 0.0724$), n° 11: less sexy ($p = 0.0212$) and less attractive ($p = 0.0609$). In young women, androstenone might affect people's perception of both sexes in a variable way. This pheromone may increase the sexual attraction to women who were evaluated as less attractive in control condition, without changes in women pictures who were seen as very attractive in control condition. About the photographed men, androstenone would decrease the sexual attraction to those photographed men who were evaluated as more virile and attractive in control condition.

94.

BRAINSTEM EVOKED RESPONSE AUDIOMETRY IN OTOSCLEROSIS WITH CONDUCTIVE HEARING LOSS

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Otosclerosis is a primary illness of the otic capsule and the auditive bones. The Brainstem Evoked Response Audiometry (BERA) is an objective method for to study the ear. *Objective*: to investigate the contribution of BERA in the differential diagnosis of Otosclerosis. Sample OCHL: subjects with otosclerosis with conductive hearing loss (15 affected ears). Controls: 10 subjects with normal hearing (20 ears). Controls latencies to 80 dB: Wave I: 1.28 ± 0.28 ms (median \pm SD); Wave III: 3.40 ± 0.30 ms; Wave V: 5.27 ± 0.39 ms; interval I-V: 3.91 ± 0.31 ms. OCHL latencies: Wave I: 2.04 ± 0.47 ms; Wave III: 3.92 ± 0.55 ms; Wave V: 5.80 ± 0.56 ms; interval I-V: 3.80 ± 0.26 ms. It was observed lengthened OCHL latencies: Wave I, $p=0.000$; Wave III, $p=0.000$; Wave V, $p=0.004$. Conservation of central transmission time (interval I-V) ($p=0.059$); high auditive threshold ($70 \text{ dB} \pm 12.56 \text{ dB}$) in correlation with tonal audiometry ($p=0.090$); bony-air audition separation: 30 dB. The delayed latencies of I, III and V waves is due to the impedance of the transmission component. The interval I-V is normal because the internal ear and the auditive pathway work with normality. The BERA objectively allows to determine the auditory threshold, type of hearing loss and topographic localization of the probable lesion.

96.

VARIATIONS IN SHORT TERM MEMORY DURING THE MENSTRUAL CYCLE

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Memory amplitude (MA) (Weschler) is the number of non related stimuli that are remember forward (>) or back (<). The Exploration in Memory (EM) of Sternberg study the human system of information, especially the short term memory (STM). Objective: To investigate the variations in STM during the menstrual cycle (MC). 28 women (18-30 years) with regular MC, were studied in the half (HC: day 13 \pm 1) and when concluding (EC: day 28 \pm 1) their MC. Automated tests were used. In EM were registered Answers (A): Correct (+ and -), Incorrect, no A, reaction times (RT), S.D. and Variation Coefficient (CV). Student's *t* test was used. In MA there were not differences between HC ($>5.2 \pm 1.0$; $<4.5 \pm 1.4$) and EC ($>5.5 \pm 1.2$; $<4.6 \pm 1.0$). EM: The correct + A are slightly larger in EC than in HC ($P=0.077$). The incorrect totals A and the incorrect + A are smaller in EC than in HC ($P = 0.012$ and 0.020 respectively). The RT of total A is smaller in EC than in HC ($P = 0.023$). The S.D. of RT of the total A and of the - A is smaller in EC than in HC ($P = 0.020$ and 0.007 respectively). The CV of RT of the - A is lightly smaller in EC than in HC ($P = 0.051$). These results suggest that women have better STM and a bigger answer speed in EC. Also, the variability in the answer speed would be smaller in EC.

97.

HAMSTER OOCYTE ELECTRICAL RESPONSE TO FECUNDATION WITH HOMOLOGOUS SPERMATOZOA*Ituarte LME, Viera TB, Ibañez JE, Saldeña TA, de Rosas JC*, Gutiérrez G***Area Fis Biol; *Area Histol Embriol, Fac Cs Med, Univ Nac Cuyo, 5500 Mendoza. E-mail: patchuncuyo@yahoo.com*

Recent studies in *Xenopus laevis* oocytes have shown an electrical response to fertilization with homologous spermatozoa. The occurrence of a comparable response in mammalian (hamster) oocytes was assessed through single-channel patch clamp recording of the response to homologous spermatozoa. Seal resistance (R_s) between the membrane and the microelectrode tip was measured. Currents were recorded during a voltage clamp ramp protocol (20 runs per record, from -100 mV to $+100$ mV, 2.5 s). With this protocol, a graph was obtained for control recordings with curve fitting by the least mean squares method. Control resistance was 30 ± 7 G Ω ($n = 18$) with a slope of $0.11 \pm 0.01\%$. In the presence of homologous spermatozoa the slope was $0.16 \pm 0.01\%$ ($n = 20$; $p < 0.01$). Both the number of channels and single channel conductance was decreased in the presence of homologous spermatozoa, while the voltage value for zero current did not change. Thus hamster oocytes display an electrical response to fertilization similar to that reported by others for *Xenopus laevis* oocytes.

99.

RENAL INTERSTITIAL FIBROSIS. ANGIOTENSIN II AT₁ RECEPTOR-INDEPENDENT FUNCTION OF LOSARTAN*Manucha W, Rüttler ME, Oliveros L*, Carrizo L, Vallés P.**Área Fisiopatol, Fac Cs Med, Univ Nac Cuyo, 5500 Mendoza; *Quím Biol, Fac Quím Bioquím Farm, Univ Nac San Luis, 5700 San Luis.*

Obstructive nephropathy results in tubulointerstitial fibrosis. Vasoactive peptides, antioxidant enzymes and reactive oxygen species, are included in this model. We examined the effect of Losartan, on the development of renal fibrosis, by studying the involvement of NO, reactive oxygen species and eicosanoids. Losartan (10mg/kg/day) was administrated to experimental and control Wistar female rats for 15 days, unilateral ureteral obstruction was performed for 24hs. Results: No change in blood pressure was observed after Losartan administration. Increased expression of iNOS (0.407 ± 0.03 vs 0.441 ± 0.04) and nNOS (0.847 ± 0.034 vs 0.72 ± 0.22) were observed in obstructed cortex. In obstructed medulla, increased expression of iNOS and COX₂ were observed related to control (46% and 49%). After Losartan administration decrease expression of iNOS in medulla and nNOS in cortex of obstructed kidneys were observed. A significant decrease on the SOD activity in obstruction was observed in cortex (81 ± 5 vs 18 ± 4) and in medulla (39 ± 4 vs 18 ± 3). Simultaneously, in obstructed cortex and medulla increased total cholesterol 8.39 ± 0.2 vs 6.27 ± 0.17 and 8.17 ± 0.4 vs 6.72 ± 0.52 was shown, without changes in total phospholipids (15.64 ± 0.7 vs 12.84 ± 0.48) in cortex. Parameters of renal fibrosis (interstitial volumen and tubular atrophía) were decreased after losartan treatment. Our results showed Losartan modulation of renal fibrosis in a non hypotensive dose with decreased expression of iNOS, nNOS, COX₂, and decreased activity of ROS, suggesting the interaction of endogenous nitric oxide, reactive oxygen species and prostaglandins in slowing down the rate of renal injury in obstruction.

98.

EFFECT OF NEONATAL SECTION OF THE SUPERIOR OVARIAN NERVE ON THE *IN VITRO* RELEASE OF PROLACTIN FROM RAT HYPOPHYSIS*Lafarque M, Forneris ML, Aguado LI.**Lab Biol Reprod, Univ Nac de San Luis, 5700 San Luis.*

We have shown that superior ovarian nerve (SON) section in 4-day-old rats sacrificed at 41 days (41-SON) causes delay of the puberty and decreases the circulating levels of FSH, prolactin (PRL) and GH, with no change in LH levels, when compared with 35-day controls (C). No correlation was observed between the serum levels of hypophyseal hormones and the release of ovarian steroids. Now, we studied the behavior of the hypothalamus-hypophysis-ovary (H-H-O) axis through PRL release in a scheme that magnifies the neural effects caused by early denervation. We performed superfusion of dispersed adenohypophysis cells (AH) of 41-SON and C rats stimulated with incubation liquids of: **1)** hypothalamus (HIL)-median eminence (ME) of C rats; **2)** HIL-ME of C rats+CLK; **3)** HIL-ME of C rats+ Adrenaline (A); **4)** HIL-ME of C rats + Noradrenaline (NA) **5)** HIL-ME of SON rats + CLK; **6)** HIL-ME of SON rats + A; **7)** HIL-ME of SON rats + NA. In the collected fractions, the PRL levels were determined by RIA. The PRL release in 41-SON rats vs C: increased with 1, 2, 3 and 5; decreased with 4 and 7; and did not change with 6. These results suggest that the H-H-O axis of SON rats is affected by early denervation, modifying the sensitivity of the AH cells to different stimuli.

100.

TEMPERATURE EFFECT ON PATCH-CLAMP ELECTROPHYSIOLOGIC VARIABLES IN RAT CARDIOMYOCYTES*Pocognoni JD*, Saldeña TA, Ituarte LME, Viera TB, Ibañez JE.**Area Fis Biol, Fac Cs Med, Univ Nac Cuyo, 5500 Mendoza; *Becario. E-mail: patchuncuyo@yahoo.com*

The effect of bath temperature on patch-clamp electrophysiological recordings was assessed in adult male rat cardiomyocytes. Under ether anesthesia, the heart was excised, mounted in a Lagendorff-type set up at 37°C and perfused with oxygenated solutions with decreasing calcium concentration and proteolytic enzymes. After mechanical disaggregation, cells were exposed to BK solution, rinsed with solutions of increasing calcium concentration and seeded on a Petri dish. Temperature was monitored with an electronic sensor placed on the Petri dish. Cells were studied at 25°C and 37°C in single channel, inside out configuration. After the seal between the microelectrode and the membrane was established, seal resistances > 1 M Ω were obtained. Stimulating pulse protocol included 20 ramps with holding potentials from -60 to $+60$ mV in 50 ms with filtering set at 1.25 kHz. Data were analyzed with a Digidata 1200 A device. I-V curves crossed the voltage axis at $V = -20.4 \pm 0.9$ mV and $V = 1.3 \pm 0.4$ mV at 25°C and 37°C, respectively (mean \pm SEM; $n = 6$, Student's *t* test for paired data, $p < 0.01$). It is concluded that the current-voltage relationship of isolated rat cardiomyocytes is significantly modified at room temperature as compared with normal body temperature.

101. SEROTONIN-STIMULATED CHLORIDE SECRETION INCREASES OXYGEN CONSUMPTION IN RAT DISTAL COLON

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Under basal conditions rat distal colonic mucosa oxygen consumption (QO_2) is linearly correlated with short-circuit current (Isc, representing mostly Cl^- secretion) with a ratio of 5 monovalent ions transported per O_2 molecule consumed. To assess whether this relationship holds true during stimulated Cl^- secretion, QO_2 and Isc were simultaneously measured in isolated epithelium mounted as a flat sheet in a modified Ussing chamber under basal condition for 30 min and after addition of serotonin (5-HT, 0.1 mmol/L at the serosal side) for another 30 min. 5-HT increased Isc ($\mu A \cdot cm^{-2}$) and QO_2 ($\mu mol \cdot h^{-1} \cdot cm^{-2}$) respectively by 50.9 ± 7.5 and 0.92 ± 0.08 (mean \pm SEM; $n = 14$; $P < 0.0001$ for both), with a linear relationship between them (slope 0.181 ± 0.060 nmol/nEq; $r = 0.654$; $P = 0.011$). Thus 5-HT increased Isc by 59% and QO_2 by 33%, with a monovalent ion: oxygen ratio of 5.5. Cl^- secretion blockade with bumetanide and diphenylamine-2-carboxylate ($n = 6$) decreased Isc and QO_2 and blunted proportionally the effect of 5-HT on both: Baseline, Isc 85.9 ± 6.0 , QO_2 2.90 ± 0.13 ; blockade, Isc 27.1 ± 3.9 ; QO_2 2.32 ± 0.11 (both $P < 0.001$ vs baseline); plus 5-HT, Isc 43.7 ± 4.1 ; QO_2 2.54 ± 0.13 (both $P < 0.001$ vs baseline; Isc $P < 0.05$, QO_2 $P < 0.01$ vs. blockade). Thus the apparent 5:1 stoichiometry holds during 5-HT-stimulated Cl^- secretion. The effect of blockers on both Isc and QO_2 suggests that the 5-HT effect on QO_2 is mostly due to stimulation of Cl^- secretion.

103. APOPTOSIS AND BLOOD TESTIS BARRIER (BTB) ASSEMBLY IN PUBESCENT RATS

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The aim of this work was to determine the sequence with which the following components appear in pubescent rats: the tubular lumen, the first spermatocytes with synaptonemal complexes (zygotene and pachytene, ZP), the competent BTB onset, and its relation with the presence of apoptotic cells. We studied male rats from 14 to 36 postnatal days by light and electron microscopy using lanthanum as an electron-opaque tracer of intercellular spaces which stops on reaching the BTB. We observed that: 1) BTB assemblage occurs before the solid cords change into tubules, and not simultaneously as previously described; 2) the first spermatogenic waves are unfruitful if ZPs have appeared when BTB is still disassembled, and 3) apoptotic process begins in ZP spermatocytes when BTB is incompetent. This is shown by synaptonemal complexes interspersed among chromatin clusters. The present results, in agreement with earlier publications, confirm that communication and synchronisation that take place between the germinal and Sertolian populations are a condition to ensure cell survival and spermatogenesis progression.

102. EFFECT OF CHRONIC INTOXICATION WITH CADMIUM ON RAT PROSTATE HISTOARCHITECTURE

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Cadmium (Cd) is a toxic heavy metal. We have shown that Cd intoxication produces oxidative stress and modifications on rat prostate lipidic profile. We studied prostate histoarchitecture under different concentrations of Cd. Wistar 21-day old male rats received 15 ppm (Cd-1) or 100 ppm (Cd-2) of Cd in water while Controls (Co) did not receive Cd. After 3 months, prostates were obtained and fixed on Bouin Co; normal pseudostratified epithelium, with deep invaginations and active glandular cells. Acini have abundant and homogeneous secretion. Interstice has some fibroblasts and undifferentiated mesenchymatous cells. Cd1: epithelium height decreases. Lumen diameter enlargement with a non homogeneous, particulated material suggesting absence of functionality. Cd2: cubic cell monolayer epithelium. Lumen diameter enlargement with loss of the typical invaginations. Colloidal, non homogeneous and amorphous material. Interstice loses its structural integrity, an effect that may be due to alterations on glycoproteins and proteoglycans. Thus, Cd produces a dose-dependent toxic effect on rat prostate histophysiology.

104. IMMUNOCYTOCHEMICAL STUDY OF THE PITUITARY PARS TUBERALIS OF THE VISCACHA (*Lagostomus maximus maximus*)

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The mammalian pituitary pars tuberalis (PT) is a well developed area and structurally different from the pars distalis (PD). The PT of viscacha consists of the granular, agranular and folliculo-stellate (F-S) cells. The purpose of this work was to investigate immunocytochemically the expression of different hormones in PT. Adult male viscachas were captured in their habitat, the pituitary gland was rapidly dissected and processed for light microscopy. Anti- β LH, anti- β FSH, anti-prolactin, anti-ACTH and anti-S-100 protein antibodies were used. The present study revealed immunoreactivity for β -LH in the caudal part and for ACTH in the zona tuberalis, but not in the PT. After incubation with the anti- β FSH and anti-prolactin antibodies, no cells in the PT could be stained. S-100 protein was detected in some PT cells which showed characteristics typical of PD F-S cells. These results suggest a probable cellular migration from PD to PT caudal part. This hypothesis is supported by our results and those of other authors.

105.
FOLLICULO - STELLATE CELLS OF THE PITUITARY GLAND OF VISCACHA: IMMUNOHISTOCHEMICAL AND ULTRASTRUCTURAL STUDY

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The morphological and ultrastructural features has been investigated in folliculo-stellate (F-S) cells; however, their function is still a matter of debate. The purpose of this work was to study the F-S cells in pituitary pars distalis from adult male viscachas. This study showed that immunoreactive cells with anti- S 100 protein serum were small in size and stellate in shape. Their cytoplasmatic processes were extended around colloidal accumulations and neighbouring endocrine cells. Ultrastructural study showed cells lacked secretory granules. Nucleus were oval, elongated, or irregular, and relatively euchromatic. Cytoplasm contained moderate numbers of mitochondria and scanty amounts of rough endoplasmic reticulum. The lateral membranes of the apical region were joined by prominent junctional complexes and desmosomas. F-S cells are important and interesting type cellular. They are forming the colloidal structures and are well-connected secretory glandular cells by means of junctional complexes. This cells are a model for future studies considering the hormonal and structural seasonal variations in this rodent.

107.
IMMUNOCYTOCHEMICAL STUDY OF PROLACTIN SECRETING CELLS OF ADENOHYPOPHYSIS OF RATS TREATED WITH CADMIUM

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It is well known that cadmium ion (Cd) is a toxic element that affects prolactin (Prl) secretion in adenohypophysis. It was studied whether the administration of Cd 3 mg/100 mL in drinking water for 7 weeks modifies the content of that hormone. Pituitary glands of control rats treated with Cd were fixed in Bouin liquid, dehydrated and enclosed in paraffin. Histological cuts about 5 μ m thick were performed. Immunocytochemical technique: an anti-Prl monoclonal antibody was used with a Biotin-streptavidin (B-SA) amplifier system and it was developed by a D.A.B. (3,3'diaminebenzidine) chromogene. Contrast with hematoxylin was carried out. Samples of animals exposed to Cd showed a highly significant decrease of the number of cells immunomarked for anti-Prl in pars distalis adenohypophysis pituitary gland when compared to control samples. Then, Cd probably interferes in prolactin synthesis. Angelina Bernardi is gratefully acknowledged for her technical cooperation.

106.
VARIATION IN LANGERHANS CELLS BETWEEN TWO REGIONS OF THE HUMAN ESOPHAGEAL EPITHELIUM

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Langerhans cells (LCs) are dendritic components of stratified epithelia, presenting antigens to other cells of the immune system. The paucity of information about their significance in the esophageal mucosa was addressed by studying their distribution and morphology in this particular location. LCs were identified by immunohistochemical detection of CD1a, a cell-specific marker, as well as by electron microscopic identification of characteristic Birbeck granules. Cell counts carried out at 25 and 35 cm distal to the dental arch demonstrated significant differences in number and size between the two locations. The upper region contained 10.4 ± 0.8 cells (mean \pm SEM) vs. 18.4 ± 1.4 cells in the lower region. Also, cells in the lower region were larger and appeared to have longer dendritic processes. Several recent studies indicated that these cells participate in antitumor immunity. It may be significant that squamous cell carcinoma of the esophagus is more frequent in the middle and upper portions where LCs, according to the present findings, are comparatively scarce. This points to a role for LCs in anti-tumor defense, adding new data to the scarce reports available to date. Supported by CONICET and CIUNC. Argentina.

108.
AFTER TREATMENT ANTIMICROBIAL RESIDUES IN CHICKENS INFECTED WITH MYCOPLASMA SYNOVIAE

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Enrofloxacin (EFX) and ciprofloxacin (CFX) are antimicrobials of intensive use in medicine. EFX is employed in poultry and pig production. CFX, used in humans is an alternative for farm animals. Both antimicrobials were detected in muscle of chickens infected with *M synoviae* and administered with EFX or CFX. Groups of 18 chickens were treated with 5mg/Kg/12h of EFX or CFX during 5 days. Three animals per group were sacrificed 24, 48, 72, 96 and 120 hours after treatment. Muscle samples (200 mg) were homogenized, and extracted twice with dichloromethane. EFX and CFX were detected by high performance liquid chromatography with fluorescence detection at 295-500 nm. CFX residues were maximum at 72 h after treatment, with 0.021 μ g/ml. From 24 h to 120 h CFX levels were close to 0.014 μ g/ml. The maximum, concentration of EFX, 24 h after treatment, was 0.075 μ g/ml, which gradually declined to 0.0004 μ g/ml at 120h. The Codex Alimentarius (1999) does not determine maximum residue levels for EFX or CFX. Consumers and authorities must be concern about the control of veterinary products residues in farm animals.

109.
CARRIAGE OF STAPHYLOCOCCUS SPP. IN HEALTH CARE WORKERS OF A NEONATAL INTENSIVE CARE UNIT

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The detection of a cluster of infections in a neonatal intensive care unit (NICU) caused by strains of *Staphylococcus epidermidis* showing similar characteristics and antibiotype, led us to the investigation of a possible common source. This was carried out by swabbing nares and hands of 14 health care workers of the NICU. The specimens were cultured in blood agar and salt manitol agar at 37°C during 48 hs. Twenty-five *Staphylococcus* spp isolates were randomly chosen and studied. Tube coagulase test and disk diffusion susceptibility test (NCCLS 2002) were performed. All the workers were determined to be carriers but the strain investigated was not found. There were 3 strains of *S. aureus*, all of them susceptible to oxacillin but resistant to penicillin. The remaining 22 were CoNS. Four of them (18%) were resistant to oxacillin but only one had associated resistance. All the strains studied were susceptible to teicoplanine and vancomycin by this method. As the reservoir was not found, a follow up to this work with the remaining strains as well as the inanimate environment is needed. Given the importance of oxacillin resistance as a risk factor, it was worthwhile its determination though the low rate detected.

110.
ELECTRICAL RESPONSE OF HAMSTER OOCYTES TO HIGH EXTERNAL SODIUM CONCENTRATION

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The electrical response to a high external sodium concentration of hamster oocytes devoid of zona pellucida was studied by the patch-clamp method. Oocytes were placed in HECM-3 medium on Petri dishes with their bottoms filled with Sylgard 184 to minimize noise. The single-channel configuration was used to monitor the time course of current under voltage clamp conditions. Currents were recorded with a protocol of ramp potential of 20 events per stimulus within a range from -100 mV to +100 mV in 2.5 s. Seal resistance was $30 \pm 7 \text{ G}\Omega$ (mean \pm SEM; n = 18). Baseline recordings were compared with those obtained after increasing external Na^+ concentration from 130 mmol/L to 163 mmol/L. Results were analyzed graphically using the least mean squares method and by comparison of mean I-V slopes with Student's *t* test for paired data. The control slope was $0.11 \pm 0.01\%$ while in high Na^+ it was $0.51 \pm 0.01\%$ ($p < 0.01$). Thus, high external Na^+ caused a decrease in the number of open membrane channel and their conductance. It also led to a reduction in the voltage value at which current is zero, indicating that high external Na^+ has a depolarizing effect under these conditions.

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