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**IV ARGENTINE MICROSCOPY SOCIETY**  
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*In memoriam Prof. Dr. Francisco Bertini*

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**CUYO BIOLOGY SOCIETY  
XXIV Annual Scientific Meeting**

**IV ARGENTINE MICROSCOPY SOCIETY**

**SYMPOSIUM ON EXPERIMENTAL REGULATION OF CELL CYCLE**

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**S.1.**

**ROLE OF ORGANOSULPHURIC COMPOUNDS FROM GARLIC ON THE REGULATION OF CELL CYCLE**

*Castro C, Cacciamani V, Galmarini C, Risler N, Miatello R, Cruzado MC.*

**S.2.**

**ANTIPROLIFERATIVE EFFECT OF DEHYDROLEUCODINE ON RAT AORTIC SMOOTH MUSCLE CELLS IN CULTURE**

*Cruzado MC, Castro C, Giordano OS, Lopez LA.*

**S.3.**

**EFFECT OF DEHYDROLEUCODINE AND 11,13-DEHYDRO-DEHYDROLEUCODINE ON AMPHIBIAN OOCYTE G2-M TRANSITION**

*Sánchez Toranzo G, Giordano OS, López LA, Bühler MI.*

**S.4.**

**ANTIPROLIFERATIVE EFFECT OF NATURAL COMPOUNDS ON TRYPANOSOMATIDAE**

*Sosa MA, Jiménez-Ortiz V, Barrera P, Lozano E.*



**1. DESIGN OF ORAL PHARMACEUTICAL FORMS FROM *Paullinia cupana* MART. SEEDS**

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Guaraná, *Paullinia cupana* Mart. is a native plant from Brasil with widely used seeds because of their medicinal properties. Its seeds possess a high concentration of caffeine, theophylline and theobromine in low quantities, terpenes, flavonoides and tanines as well. In this work the pharmacognostic study of a commercial powder from Guarana seeds is presented; the design of 4 pharmaceutical oral solid dosage forms; a caffeine dissolution assay and 2 determination methods. The commercial powder was analyzed microscopically. The hydroalcoholic solid residue was adsorbed over an inert excipient (fumed silica) forming a solid extract (SE). Different formulations were proposed by using various excipients. The biopharmaceutical behavior of the obtained tablets was studied by performing a dissolution test. The caffeine determination was achieved with 2 different methods: spectrophotometry UV and capillary electrophoresis (CE). The powder authenticity was confirmed by microscopic analysis and identification reactions. The SE exhibited suitable rheological, physical and mechanical properties, making possible the design and tablet manufacture using direct compression technology. The developed CE methodology was adequate for the evaluation of the designed tablets allowing the study of the pharmaceutical dosage forms performance.

**2. DIAGNOSIS OF BRUCELLOSIS IN CANINE BREEDING GROUNDS**

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*Brucella canis* it is a rough strain whose specific host is the dog. However, dogs may also become infected with other *Brucella* species. In the pregnant female, *Brucella* is found in the placenta and fetal intestine, stomach, and lung. It vigorously proliferates in the epithelium of chorionic villi. We isolated a *Brucella canis* strain from a 2 year-old pregnant bitch with a history of late-term miscarriage. The animal belonged to a commercial breeding ground. Antibodies were demonstrated in blood by an immune test with antigen from *B. ovis*. Blood culture for bacteriological diagnosis was performed. Isolated bacteria were further cultivated in *Brucella* agar and tryptose agar at 37°C for 72 h, with CO<sub>2</sub> and without it, with antibiotics and without them. Colonies with morphologic, staining and growth characteristics compatible with *Brucella* were submitted to the following tests: catalase, oxidase, citrate, methyl red, urease, SH<sub>2</sub> production, fuscine/tonine, and acriflavine. Afterwards, they were challenged with an antibody against *B. canis* and agglutination was observed. *Brucella abortus* strains 2308, S19, RB51 (rough) were used as negative controls.

From the economic viewpoint, stillborn puppies and those dead at embryonic stages, and the consequent loss of service expenses, should be taken into account. The use of infected animals for breeding should be avoided to prevent the spread of the infection to other animals.

**3. PRELIMINARY STUDY OF GENETIC DIVERSITY OF *Rosa rubiginosa* BY RAPDs MARKERS**

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*Rosa rubiginosa* is an exotic species introduced in Argentina which posses economic value because of the content of poliinsaturated fatty acid, antioxidants and trans retinoic acid in seeds. Leaves and fruits have a vitamine C content higher than citric fruits. Diversity conservation, exploitation and management of the genetic resources of species of ecological, cultural and economic interest requires the knowledge in detail of the quantity and distribution of genetic diversity. The aim of the present study was to study the genetic diversity of rose mosqueta in populations of San Luis province by means of molecular markers RAPDs. Samples were collected in a transect between Potrero de los Funes and El Volcán. The yields and quality of genomic DNA are considerably affected when the common protocol for DNA isolation is applied to *Rosa rubiginosa*. Thus, different protocols were assayed to obtain genomic DNA from leaves to find the appropriate one. RAPDs molecular markers were obtained by using primers Kit A of Operon technologies (OPA). Polymorphic level of the populations studied was analyzed by using the Jaccard's coefficient and similarity between individuals was analyzed by constructing the dendrogram by means of the UPGMA algorithm.

**4. SECHIAM EDULE: A NUTRICIONAL PROPOSAL**

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*Sechium edule* is an edible plant coming from Mexico and widely cultivated in the nor east region of Argentina. In our country it is known as chayote being the less known member of the pumpkin family (curcubitáceas). It can be described as a climbing vegetable which is cultivated because the fruit. The main aim of the present work is to evaluate the protein quality of the seed coming from the cultivated areas of the province of San Luis, Argentina. The seed extracted from the fruit were conditioned, dried, grinded and sieved. The determination of protein (N x 6,25) was done by Kjeldhal, while the aminoacidic profile by autoanalyzer. The quality of the protein was measured by means of the biological indexes: net protein utilization (NPU), biological value (Bv) and true digestibility (tD). The protein analysis presented a relevant value in the context of this family: 21,98 g %. Once obtained the aminoacidic profile, the corresponding value of the chemical score (CS) resulting lysine as a first limitant (CS: 81). The biological quality reveals a good NPU (54%) and an excellent tD (80). Then, it is inferred that *Sechium edule* is a vegetable which can be used for different propose (leave, fruit, seed) due to the capacity of adapting to the climatological conditions of San Luis. Their production and marketing should generate good quality resources due to their nutritional properties constituting an interesting economic and agroindustrial alternative for the region.

**5. ANTIBACTERIAL AND ANTIOXIDANT ACTIVITY OF EXTRACTS FROM AERIAL PARTS OF *Colliguaja integerrima* AND *Euphorbia schickendantzii* (EUPHORBIACEAE)**

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*Colliguaja integerrima* and *Euphorbia schickendantzii* (Euphorbiaceae) are used in Argentinean folk medicine in wart treatments and other skin diseases. Other species of the *Euphorbia* and *Colliguaja* genii have been reported for their antibacterial property. This work was focused to determine the potential antibacterial and antioxidant activities showed by acetone, methanol and aqueous extracts. Antibacterial activity was measured by determination of minimum inhibitory concentration (MIC) using the broth microdilution method. The antioxidant activity was determined by bleaching of DPPH (1,1-diphenyl-2-picrylhydrazyl) in methanol solution at 517 nm. Results showed that methanol extract of *C. integerrima* was the most active against *B. subtilis* with MICs lower than 31.2 µg/mL, but *E. schickendantzii* showed a weakly activity. The antioxidant activity was incredible strong in the all extracts with the same value for rutin, flavonoid used as standard with 100% of antioxidant activity.

**6. AMARANTHUS A FOOD OF THE FUTURE**

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Amarantos has been widely studied in the literature. Such studies include cultivations in different climatological and edafological conditions and they inquire about the bromatological features of the whole plant in order to be used in the human and animal diet. The main aim of the present contribution is to perform a chemical and nutritional analysis of the seed of many species of Amarantho: *A. dubius* (Ad), *A. quitensis* (Aq), *A. standleyanus* (As), *A. mantegazzianus* (Am) y *A. cruentus* (Ac). Thus, the objective is to recommend their consumption as a new feeding alternative. The seeds of Ad, Aq, As, Am y Ac (family Amaranthaceae, subfamily Amaranthoideae) were dried in a forced air oven at 50°C during 48 hours, grinded and sieved. The flour is hermetically conserved. The chemical study consist in the determination of moisture, ether extract and ash by AOAC, protein (N x 6,25) by Kjeldhal, total carbohydrates by differences. Total dietary fiber (FDT), insoluble dietary fiber and (FDI) and soluble dietary fiber (FDS) were determined by using the multienzimatic method due to Prosky for Ad. The quality of the protein was measured by means of the biological indexes: net protein utilization (NPU), biological value (Bv) and true digestibility (TD). The fiber content for Ad (15,33 g/100g) (FDI). It is also important to emphasize the Bv of Ad: 82, being acceptable the values for the remaining species. The results confirm that amaranto raise the desirable nutritional attributes.

**7. IN VITRO ACTIVITY OF ESSENTIAL OIL OF *Origanum x applii* AGAINST *Ascosphaera apis***

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Chalkbrood is a highly contagious disease of the honeybee *Apis mellifera*, caused by the heterothallic fungus *Ascosphaera apis*. Most usual methods of control refer to prophylaxis technique and the use of agrochemical products with their resultant problems of residuals in apiculture products, which produce commercial problems in foreign trade. Natural fungicide based on essential oils may represent an alternative, in particular to those pest relate to the food provision. The oil of *Origanum x applii* (oregano criollo) was obtained from air-dried leaves by steam distillation for 4 h. The major components of the oil were: γ-terpinene (27.1%), p-cymene (15.1%) and carvacrol (11.9%). Strain of Chalkbrood fungus was obtained from mummies of naturally infected hives in Villa Mercedes, San Luis, Argentina. Isolation of the fungus was made in MY20 medium. In each Petri dish of 9 cm diameter, three disks of agar of 5 mm diameter from the border of colonies of *A. apis* of 6 days of growth were sowed symmetrically. The oil was mixed with DMSO (1:1). The concentrations assayed were at 1,000, 500 and 250 ppm (µL/L), and were added to the culture medium before inoculating it with the fungus, incubating at 30°C measuring growth after 14 days. Three repetitions and a control were tested. The Minimal Fungicidal Concentrations against *A. apis* was 500 ppm.

**8. (-)-AMBROX HYDROXYLATION BY A FILAMENTOUS FUNGUS**

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Terpenoids are one of the most important family of compounds in Organic Chemistry and are found in all parts of higher plants besides in mosses, algae, etc. and in the animal kingdom. (-)-Ambrox is a terpene like compound isolated from ambergris a prized scent which is a secretion of sperm whale. It is structurally related to a wide variety of compounds, in particular to the cytotoxic sclareol. The aim of our work was to intend to transform (-)-Ambrox (1) by biocatalysis using the enzymes excreted by the filamentous fungus *Alternaria alternata*. Biotransformation was carried out according to a two standard protocol. In stage 1 a liquid Czapek medium was inoculated with a refrigerated agar culture of the microorganism and grown during 72 hours. Stage 2 was then started by inoculating the same amount of medium containing the organic compound, with biomass from stage 1 and grown aerobically at 25°C on a reciprocal shaker at 110 rpm. After that only one compound could be obtained which was isolated, purified and analyzed by means of H<sup>1</sup> NMR. This compound was identified as 3β-hydroxyambroxide when compared with bibliographical data. We can see that *A. alternata* is capable of stereo and regioselective hydroxylation of compound 1 in the C-3 position. Thus using this methodology we proved *A. alternata* enzymes are capable of catalyze oxidation of (-)-Ambroxide in a liquid medium.

**9. PRELIMINARY STUDIES ON THE EFFECT OF SALAZINIC ACID (ALICHEN SECONDARY METABOLITE) ON ALLIUM CEPA CELLS**

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Salazinic acid is a secondary metabolite extracted from the antarctic lichen *Parmelia saxatilis* (L.) Ach. At non-citotoxic concentrations it is able to induce apoptosis in continuous HeLa cell lines and rat hepatocytes in primary culture. We used *Allium cepa* meristems because they are very sensitive to genetic damage produced by chemical substances, allowing us to determine chromosomal aberrations.

The aim of this study was to test the effect of salazinic acid on meristematic cells of *Allium cepa* L. (onion). The basal ends of the onion bulbs were submerged in distilled water at  $25 \pm 0.5^\circ\text{C}$ . Roots (2 to 3cm in length) were placed on aqueous solutions containing 10 and 50  $\mu\text{g/ml}$  salazinic acid, during 8 and 24 hours. Control treatments had distilled water and DMSO (dimethylsulfoxide). In order to perform cytologic studies, root samples were fixed, stained and squashed, following routine techniques. An average of 3000 cells were analyzed on each meristem. Chromosomal aberrations, like fragments, abnormal chromosome arrangements of anaphase, lagging chromosomes and C-mitosis were observed in roots treated with 50  $\mu\text{g/ml}$  salazinic acid during 24 hours. From these preliminary data we conclude that salazinic acid causes chromosomal aberrations in the cells studied and damage is directly related to concentration and time of exposure.

**10. SEARCH FOR ANTIFUNGAL COMPOUNDS IN ENDOPHYTIC AND SOIL FUNGI EXTRACTS**

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During an ongoing collaborative research for antifungal active compounds, endophytic and soil fungi extracts were assayed *in vitro* by dilution method agar against a panel of pathogenic yeast, including *Candida* sp, filamentous fungi, and dermatophytes. Ethyl acetate extracts (EAE) from *Penicillium citrinum* P melinii, P verrucosum, P. roqueforti, P. jensenii, *Fusarium solani*, *Trichoderma koningii*, *Sporormiella minima* and *Alternaria alternata* showed antifungal activity against *Microsporum gypseum*, *Trichophyton mentagrophytes* and *T. rubrum* with MIC &#8804;1000  $\mu\text{g/ml}$ . The highest activity was presented by EA extract from P melinii with a MIC = 31.2  $\mu\text{g/ml}$ . Also, EAE from P. citrinum displayed an important activity against all *Candidas* assayed as *C. albicans*, *C. tropicalis*, *C. glabrata*, *C. parapsilopsis*, *C. lusitaniae*, *C. colliculosa*, *C. krusei*, *C. kefyr* with MICs &#8804;125  $\mu\text{g/ml}$ . The strongest activity was observed toward *C. albicans* and *C. tropicalis* with a MIC = 8  $\mu\text{g/ml}$ . From twelve EAE assayed against filamentous fungi *Aspergillus flavus*, *A. fumigatus*, *A. niger*, just four, P. canescens, P verrucosum, P. citrinum and T. koningii displayed moderated activity with MICs &#8804;1000  $\mu\text{g/ml}$ .

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**11. PHARMACOBOTANICAL CHARACTERIZATION OF THE "ESPINA DE PESCADO" DRUG (*Tetraglochin alatum* var. *alatum*, ROSACEAE)**

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*Tetraglochin alatum* (Gill. ex Hook. & Arn.) Kuntze var. *alatum* is a 50 cm subshrub that habits the Andean Precordillera of Argentina, and neighboring Chile. Its common names in Cuyo region are "espina de pescado" and "horizonte", the first of which is referred to the spiniform aspect of their stipules and foliar rachis. Their infusion are used in popular medicine by their noteworthy diuretic properties. The present work was carried out because the scarcity of pharmacobotanical, phytochemical and bioactivity studies about this drug, which is characterized and described to contribute to the quality control. The samples were collected in the Uspallata Valley in Mendoza province, fixed and conserved in formol:acetic acid:alcohol (1:1:1), included in paraffin, cutted and mounted in DPX. The parameters value are the following: stomata number and stomata index of the upper surface = 0; stomata number of the lower surface =  $15,5 \pm 0,67 \text{ .mm}^{-2}$ ; stomata index of the lower surface =  $17,41 \pm 0,54$ ; palisade ratio =  $13,64 \pm 0,67$ ; islots number =  $17 \pm 1$  and terminal nervation number =  $27,75 \pm 1$ . Phytochemical and pharmacological studies on this species are carrying out.

**12. PHYTOMEDICINES IN CENTRAL-WESTERN ARGENTINA: MAIN FACTORS THAT AGREE THE RATIONAL USE**

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The main difficulty in the prescription and use of phytomedicines are difficult to obtain adequate stabilization and standarization of products for obtain an adequate dosification. Numerous factors affect their quality: the insufficient information sumministrated to the consumers (botanical identity, dosification, therapeutic correct use, warning, precautions, contraindications, secondary effects, technical responsible, packing and/or vanquishment dates). On the other hand, some of the products not perform with the legal standing dispositions or present impurity on high proportion, fungi attacks, a incorrectly degree of milling, decolorations, and others factors that affect seriously their quality. In the central-western argentinian a important economical activity occur vinculated with production and primary indicting of medicinal plants and with raw material of high quality. The mancomunated effort of every actors which take part in the production, quality control, comercialization and dispensation in pharmacies and herbalist shops, beside to one adequate information to the

### 13. STUDY ABOUT THE USE OF PESTICIDES IN SAN LUIS CITY, ARGENTINA

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Pesticides, chemical substances destined to prevent, to destroy and to control plagues, show important utilities in homes due to the availability of a great number of formulations. The aim of the present work is to obtain a general diagnostic about the use of pesticides in San Luis city homes.

A representative sample was calculated according to San Luis province census data from year 2001. A survey was carried out in homes from low, medium and high socioeconomic level. The most used active principles were pyrethroid (tetramethrin and deltamethrin) and organophosphorus (chlorpyrifos and fenitrothion) in the aerosol presentation. It is highlighted the use of strychnine, forbidden in Argentina, to fight against rats. The criteria of purchasing is "the most efficient against the plague", few people chose the "least dangerous for human health". The storage place is the kitchen closet and the protection measures used at the time of applying these substances are scarce. The 8.1% of those questioned mentioned intoxication antecedents. It can be concluded that all the questioned use products for plague control, especially against gnats, flies and ants. The storage in the kitchen and the few protection measures used by the questioned could promote future accidental intoxications; the diffusion of the right behaviour with the utilization of these products could be an essential contribution for the health care of our community.

*Acknowledgements: The authors thank to the Ministry of Health and Environment of the Nation the received support.*

### 14. MICROGRAPHIC ANALYSIS OF *Porophyllum obscurum* (ASTERAC.), "YERBA DEL VENADO", PHYTOMEDICINE OF CENTRAL-WESTERN ARGENTINA

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*Porophyllum obscurum* (Spreng.) DC. (Asteraceae) is a subshrub native from Central and northern Argentina, known in Cuyo region as "yerba del venado". It is marketed by their antispasmodic and diaphoretic properties. This work was carried out with the aim of perform the pharmacobotanical quality control of the drug. Fresh and/or preserved in formaldehyde: acetic acid:ethilic alcohol (FAA) samples were collected for leaf epidermis and quantitative micrography studies, applying diaphanization by the Dizeo technique. Some material were included in paraffin, cutted, stained and mounted in DPX for the micromorphologic studies. The diacritical parameters founded were: stomata number of the upper surface =  $6.66 \pm 0.88$  .  $\text{mm}^{-2}$ ; stomata index of the upper surface =  $16.09 \pm 1.6$ ; stomata number of the lower surface =  $8.92 \pm 0.9$  .  $\text{mm}^{-2}$ ; stomata index of the low surface =  $20.96 \pm 1.67$ ; palisade ratio =  $5.79 \pm 1$ ; islots number =  $19 \pm 2$  and terminal nerviation number =  $20 \pm 1.8$ . All commercial samples exactly correspond with the genuine drug *P. obscurum*. This work contributed to an effective drug quality control, mainly when it is finely crushed or powdered.

### 15. DETERMINATION OF LACTONES BY IR SPECTROSCOPY ON THREE CARDIOTONIC NATIVES SPECIES

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*Hypericum connatum* Lam., *Ligaria cuneifolia* (R. & P.) Van Tiegh. and *Tripodanthus flagellaris* (Cham. & Schltdl.) Tiegh. are used in popular medicine as cardiotoxic. The mentioned popular use was validated by bioactivity assays. From the chemical point of view the cardenolides heterosides show the characteristic nucleus C-17 substituted by the  $\alpha\beta$  insaturated 5 members lactones. In order to isolate their heterosides was carried out a specific extractive technique for lactones. These lactones were analyzed lately by IR spectroscopy; 100 g of drug were extracted with  $\text{CH}_3\text{Cl}$  for three times during 72 h; the extract were carried to dryness, rediluted with ethyl alcohol to 40 °C in double boiler; later it is added a 1 % solution of  $\text{Ac}_2\text{Pb}$ . The floated were newly extracted with  $\text{CH}_3\text{Cl}$ . The chloroformic extracts were analyzed by thin layer chromatography using *Oleum* as revealing showing an evident greenish-blue fluorescence. On the other hand the IR spectroscopy shows characteristics stretching in the fingerprint zone ( $2000\text{-}600\text{ cm}^{-1}$ ) for each samples and typical signal from  $\alpha\beta$  insaturated lactones.

### 16. GENOTOXICITY IN PREPUBERTAL GILTS INDUCED BY AFLATOXIN B1 INGESTION

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Contamination of feedstuffs by fungi and the mycotoxins they produce have a serious health risk for humans and reduce livestock production. Among mycotoxins, aflatoxin are very harmful for human health since they are a cause of liver cancer, mutations, and teratogenic impairments. Swine are the domestic animals most sensitive to aflatoxins. Signs of aflatoxicosis are feed refusal and lower growth rates. Aflatoxin B1 (AFB1) produced by *Aspergillus flavus* is particularly toxic. Previous observations made on the feed used in pig farms of the Río Cuarto region of Córdoba showed that it was naturally contaminated with AFB1. Concentrations were over 20 ppb (the maximum level allowed by FAO). Based on this fact we evaluated its genotoxic potential. Therefore, two groups of 10 sows each (G1 and G2) were fed with contaminated provisions containing 20 ppb (G1) and 40 ppb (G2) of AFB1. A third group (G3) was used as control. Cells with chromosomal aberrations were evaluated using lymphocytes cultures. Blood samples were taken at 0, 5, 10, and 20 days after the beginning of the assay. G1 and G2 groups showed a significant ( $p < 0.01$ ) increase on the frequency of cell with chromosomal aberrations contrasting with G3. Main types of alterations were breaks, rings, and end-to-end chromosome associations. The mutagenic effect were strongly dependent on AFB1 concentration but were not different for the four periods evaluated. In conclusion, these preliminary results provide evidence that AFB1 is highly genotoxic to prepubertal gilts.

**17. MICROBIOLOGICAL BAEYER-VILLIGER OXIDATION OF 1-INDANONE WITH CULTURES OF *Aspergillus terreus***  
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Biological Baeyer-Villiger oxidations of ketones provide simple routes to obtain chiral lactones. These compounds are of great interest for the synthesis of natural products, pharmaceuticals and agrochemicals. This process constitutes a green chemistry alternative allowing enhanced safety and minimum environmental impact. In this work the microbiological Baeyer-Villiger oxidation using cultures of *A. terreus* (from ANLIS Dr. Malbran) was investigated. Biotransformation of 1-indanone was carried out in two step procedure. Resting cells were harvested from 48 h-old cultures and suspended in pH 7 phosphate buffer. After 7 days of incubation the culture media was extracted with ethyl acetate. Blank assays were carried out. Each experience was performed three times with two replicates each. Biotransformation progress was analysed by GC. The oxidation product obtained with good yield was characterized by spectroscopic methods (<sup>1</sup>H and <sup>13</sup>C NMR).

**18. MEGASPOROGENESIS AND MEGAGAMETOGENESIS IN ADESMIA MACROSTACHYA**  
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Several *Adesmia* species show forage potential due to their high growth rate and to the fact that they are eaten by cattle. Therefore, the knowledge of the reproduction mode is essential to carry out breeding programs. Domestication studies started two years ago, one of the species that is being analyzed is *Adesmia macrostachya*. This species belongs to the tribe Adesmieae (Leguminosae). The objective of this work is to study the ovule development, megasporogenesis and megagametogenesis in *Adesmia macrostachya*. Flower buds in different developmental stages were collected in southern Córdoba, fixed in FAA (ethanol-acetic acid-formaldehyde, 90:5:5), dehydrated in an ethyl alcohol-xylool series, and embedded in Histowax. Longitudinal and cross sections were cut at 8-10 µm, mounted serially, and stained with safranin-fast green. These sections were photographed with an Axiophot Zeiss microscope with Axiocam HRC Zeiss camera. The ovule is campilotropous, crassinucellate, and bitegmic. The archesporial cell undergoes meiosis forming a linear tetrad of megaspores. Only a single megaspore is functional in the chalazal position, and this one develops a Polygonum type female gametophyte. The gametophyte clearly shows the egg apparatus and the opposite polarity in the egg cell and synergids. The antipodals are ephemeral and arranged in a row. *Adesmia macrostachya* is compared to other species that belong to the same genus.

**19. ANTIBACTERIAL AND ANTIOXIDANT ACTIVITY OF EXTRACTS FROM AERIAL PARTS OF *Verbena seriphioides* (VERBENACEAE)**  
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*Verbena seriphioides* (Verbenaceae) is currently used in South American traditional folk medicine against diarrhea, fever and gastrointestinal disorders. Other species of the genus *Verbena* has been reported for their antibacterial and antioxidant properties. This work was focused to determine the potential antibacterial and antioxidant activities showed by *n*-hexane, chloroform and aqueous extracts of *V. seriphioides*. Antibacterial activity was measured by determination of minimum inhibitory concentration (MIC) using the broth microdilution method. The antioxidant activity was determined by bleaching of DPPH (1,1-diphenyl-2-picrylhydrazyl) in methanol solution at 517 nm. Results showed that *n*-hexane and aqueous extracts were the most active against *Bacillus subtilis* (Gram positive bacterium) with MICs lower than 31.2 µg/mL. The antioxidant activity was incredible strong in the three extracts with the same value for rutin, flavonoid used as standard with 100% of antioxidant activity.

**20. BIOASSAY-GUIDED ISOLATION OF ANTIFUNGAL AND ANTIBACTERIAL COMPOUNDS OF PROPOLIS FROM SAN JUAN PROVINCE, ARGENTINA**  
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A bioassay-guided study by agar dilution method was undertaken to isolate and identify antifungal and antibacterial metabolites from Propolis of San Juan. Methanol propolis extract (32 g) was applied to a Sephadex LH-20 column. Eight fractions were obtained. The fraction VI (260 mg) was successively percolated by Sephadex LH-20 column to afford crysin, galangin and pinocembrin. These compounds were identified by nuclear magnetic resonance (NMR) spectra using a Bruker spectrometer, operating at 400 MHz for <sup>1</sup>H and 100 MHz for <sup>13</sup>C. *Microsporum gypseum*, *Trichophyton rubrum* and *T. mentagrophytes* were strongly inhibited by pinocembrin (MIC= 31.2 µg/ml). Also, pinocembrin were active (MICs between 62.5-250 µg/ml) against *Candida albicans* C. tropicalis, *Cryptococcus neoformans* and *Saccharomyces cerevisiae*. On the other hand, crysin, galangin and pinocembrin were active toward *Staphylococcus aureus* methicillin-sensitive ATCC 29213, *S. aureus* methicillin-resistant ATCC 43300, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 with MICs ranging from 500 to 1000 µg/ml.  
 We are grateful to ANPCyT PICT Redes 260 and CYTED Proj. X.7. We also thank CICITCA-UNSJ for financial support & grant to B Lima.

**21. BIOACTIVE COMPONENTS PRESENTS IN SECHIUM EDULE (JACQ) SW , AMARANTHUS CRUENTUS AND SUILLUS GRANULATUS**

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Diverse epidemiological studies confirm significant relationship between the high dietary intake of phenols, especially flavonoids and antocyanins and the reduction of cardiovascular and carcinogenic risk. For this reason, the objective of this work is the evaluation of these bioactive components in *Sechium edule* (seed (Ses), complete fruit (Secf), flesh (Sef), *Amaranthus cruentus* (seed) (Ac) and *Suillus granulatus* (Sg). In this investigation we work with flour of the different samples, defatted in a Soxhlet extractor. Each sample was extracted with HCl in 50% methanol/ water and heated at 90°C for 3 hours. The supernatants obtained from these extraction were used for determination of polyphenols (Folin Ciocalteu), flavonoids (aluminum chloride colorimetric assay), anthocyanins were estimated by a pH differential method. The results were for Ses, Secf, Sef, Ac, Sg: polyphenols (&#61549;g/g ) 96.76; 863.90; 1242.85; 713.56; 2694.64 respectively, flavonoids (mg/100g) 95.26; 62.33; 85.85; 45.27; 248.92 respectively, anthocyanins (mg/100g) 137.13; 93.85; 119.30; 23.31; 483.47 respectively.

In conclusion, this study show that these species have contents important of antioxidant compounds.

**22. ANTIBACTERIAL ACTIVITY OF *Acacia aroma* AGAINST METHICILLIN-RESISTANT *Staphylococcus aureus***

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The problem of microbial resistance is growing and the outlook for the use of antimicrobial drugs in the future is still uncertain. The use of plant extracts with antimicrobial properties could be of great significance in therapeutic treatments. The aim of the present study was to demonstrate the possible antibacterial activity of ethanolic extract, infusion, decoction, and ashes from leaves of *Acacia aroma* known popularly as "tusca", specie abundant in the north region of San Luis, Argentina. The antibacterial activity was tested against six local isolates of methicillin-resistant *Staphylococcus aureus* (MRSA) and two reference strains (*S. aureus* ATCC 43300 and *S. aureus* ATCC 29213 methicillin-resistant and sensitive respectively). The agar well diffusion method was applied and the Minimal Inhibitory Concentration (MIC) was determined. The infusion, decoction and ashes were inactive. The ethanolic extract showed inhibitory effect on both *S. aureus* methicillin-sensitive and methicillin-resistant strains, the last one being very difficult to eradicate. Our results suggest that *A. aroma* could be applied as alternative therapy against MRSA. Further studies would be needed to determine the responsible compounds for antibacterial activity of this plant and also to discard possible toxic effects.

**23. APOPTOSIS FOLLOWS ACTIVATION OF MACROPHAGES BY AQUEOUS EXTRACTS OF JARILLA (*Larrea divaricata* Cav)**

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It has been suggested that the apoptosis of macrophages (MØ) could be due to the activation. The induction of apoptosis correlates with NO and TNF- $\alpha$  production. Jarilla is used in Argentine in folk medicine. It is known that jarilla have a variety of therapeutic uses including anti-inflammatory and anti-rheumatic actions. Elsewhere we have showed that decoction (D) and infusion (I) of jarilla induced apoptosis on murine MØ. The aim of this work was to assess that the apoptosis-induced by *L. divaricata* is due to the MØ activation. From the dried leaves and tender branches infusion I and D were prepared. The adherent cells of mice were treated with 1 and 4 mg/ml of D or I during 1.5 and 48 h. TNF- $\alpha$  and NO were quantified by ELISA and Griess reactive respectively. Respiratory burst, alkaline phosphatase activity, LPS receptors and MØ markers were determined. The phagocytosis of zymosan (p<0.004), the lysosomal enzyme activity (p<0.03), NO production (p<0.03) and TNF- $\alpha$  (p<0.0001) release are increased when D (4 mg/ml) was used. D (1 and 4 mg/ml) also increased the binding of LPS to MØ. D at 4 mg/ml showed an up-regulation of CD14, TLR4 and CR3. I showed none of the effects of D. We confirm too that the apoptosis triggered by D on MØ is due to the cell activation. The activation and apoptosis D-induced could be used for to treat infections when MØ are chronically infected with intracellular bacteria

**24. PLANT COMMUNITIES OF THE "LAGUNAS DE GUANACACHE", SAN LUIS PROVINCE**

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The "Lagunas de Guanacache" (San Juan and Mendoza) was accepted as a RAMSAR Site in 1999. In order to include the Northwestern angle of San Luis province to this RAMSAR Site, we carried out this study during February-September 2006. Plant communities of the wetland were studied using the Braun-Blanquet method, through floristic relevés. The obtained data were analyzed with Twinspan and resumed in a synthetic table. Communities dominated by *Heterostachys*, *Allenrolfea*, *Distichlys*. and *Atriplex* were determined on saline soils; by *Baccharis* and *Tessaria* in streamflows; by *Juncus*, *Cyperus*, *Thypha* and *Phragmites* on swamp soils; and by charophytes into the lagoons. Important tree species in the vegetation of flooded woodlands were *Prosopis flexuosa*, *Geoffroea decorticans* and *Bulnesia retama*. The northeastern part of the site is influenced by the Chaco, and therefore has drier vegetation types. The major communities were included in the synclases: *Phragmito-Magnocaricetea*, *Charetea fragilis*, *Sarcocornietea perennis*, *Suaedetea divaricatae*, *Polygono-Poetea annuae* and *Stellarietea mediae*. Large variation in physical conditions in the area accounts for great diversity in vegetation communities and their important associated fauna.

**25. VARIATION IN ROOT WOOD ANATOMY IN AESCHYNOMENEAE (FABACEAE)**

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Many studies were carried out in shoot xylematic anatomy of Fabaceae, but root system investigations are scarce. The objectives of this work are: a) to investigate the xylematic structure of *Aeschynomene histrix*, *Aeschynomene rudis*, *Poiretia tetraphylla*, *Stylosanthes hippocampoides*, *Stylosanthes montevidensis*, *Zornia gemella* and *Zornia trachycarpa*, b) to relate these anatomical characteristics to the habitat. Samples were sectioned with a rotary microtome at 10 µm and stained with safranin-fast green using standard techniques. Macerations were prepared using Jeffrey's solution. From the comparative analysis of the studied species, it is deduced that *Poiretia tetraphylla* is more efficient in water use because it shows the largest diameter (79,88 µm) and the highest density (23,1 vessel member.mm<sup>-2</sup>) of vessel members. *Aeschynomene* and *Stylosanthes* species have the largest xylematic area and their histological differences show a clear adaptation to the environment. The results show that there are correlations between the anatomy of the root system and the habitat.

**26. THE EFFECT OF CADMIUM ON NON-ENZYMATIC ANTIOXIDANT DEFENSE SYSTEM IN SOYBEAN (GLYCINE MAX.L)**

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The relationship between cadmium toxicity, growth decrease and the production of oxidative stress in plant cells was studied. The level of cadmium toxicity on DM 4800 RR soybean (*Glycine max.L*) was evaluated determining the non-enzymatic antioxidant parameters in soybean roots. Grown plants were exposed on the 10th day of adaptation to hydroponic conditions in Hoagland's solution to intoxication with 40 µmol/L cadmium, during 0, 4, 6, 24, 72 hours and 6 days. Reduced glutathione (GSH), non protein thiols (NPT), phytochelatins (PCs-SH) and growth indicators were determined in the roots. GSH showed a significant increase at long time (24, 72h and 6 days) of treatment. While NPT showed a different behavior, they decrease at long time of treatment, similar results we observed with the levels of PCs-SH.

Anatomical parameters showed a slight decrease in fresh and dry weight along the time curve, as well as with the long of roots, hypocotyls and epicotyls. We can suggest that cadmium alters the non-enzymatic antioxidant system and growth indicators in soybean roots in a short and long time curve, so demonstrating a possible defense role in plant responses.

**27. EFFECT OF ARSENIC ON NADPH GENERATING ENZYMES IN SOYBEAN (GLYCINE MAX. L)**

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Species of inorganic arsenic produce oxidative stress, generating free radicals and reactive oxygen species. These species react with lipids, proteins, pigments and nucleic acids, producing lipoperoxidation, enzymatic inactivation, so affecting the cellular viability. The role of NADPH generating enzymes as NADP-isocitrate dehydrogenase (ICD) in the antioxidative response of soybean leaves and roots to arsenic contamination was studied. Grown plants were exposed on the 10th day of adaptation to hydroponic conditions in Hoagland's solution to intoxication with arsenic (6 and 16 µM) during 24 and 72 hours. The enzymatic activity of ICD was determined in soybean leaves and roots. The results showed a significant increase at short time (24h) with treatment I (6µM) and at long time (72h) with treatment II (16µM), in leaves ( $p \leq 0.001$ ). Roots showed an increase of the specific activity at 24h with treatment I ( $p \leq 0.05$ ). We can suggest that arsenic alters the NADPH production, demonstrating an essential role of this cofactor to maintain the reduction equivalents on the GSH-GSSG system, as part of the antioxidative defense responses, depending on the arsenic concentration and the time of treatment.

**28. DORMANCY BREAKOUT IN ATRIPLEX CRENATIFOLIA SEED, UNDER LABORATORY CONDITION, USING CHEMICAL TREATAMEN**

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*Atriplex crenatifolia* is native species of San Luis province. And which all native species the seeds present a noticeable state of dormance, which constitutes a disadvantage. Is important study the way of esscarifications the seeds to increase the her germinations. The objective of this work was to study dormancy breakout in *A. crenatifolia* seeds. The dispersal units of *A. crenatifolia* was collected from plants native populations situated en Las Salinas del Bebedero, Province of San Luis. The dormancy breakout trials was soaking in H<sub>2</sub>SO<sub>4</sub> at different times: 0 min (control), 2:30 min, 5 min, 7 min and 10 min and next to wash under running water during 10 min. The treatments was carried out in glass Petri with double filtre paper, moistened with 5 ml of water. Five replicates of 25 seeds each were used for each treatment. At 30°C under conditions of darkness. Percentage of germination was recorded daily during 10 days. The results indicate that there was a significative effect in the germiative capacity with esscarification treatment. The germinacions increase a 25 and 20% with treatment with H<sub>2</sub>SO<sub>4</sub> during 2:30 and 5 min in relation to control. The seeds of *A. crenatifolia* request preliminary treatment to obtain a best germination.

**29. TOXICITY AND REPELLENCY OF ESSENTIAL OILS ISOLATED FROM FOUR *Eupatorium* SPECIES AGAINST *Tribolium castaneum* HERBST**

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Insect pest often cause extensive damage to stored grains and their products. There is considerable interest in developing natural products as alternative to control insect pests. Essential oils from plants are in particular under investigation for their broad-spectrum pest control properties. Extracts of *Eupatorium* species, pure compounds and essential oils have shown biological activities. The genus *Eupatorium*, small herbs and shrubs is one of the largest and most widely distributed and grow in North, Central and South America, Eastern Asia, Taiwan and the Philippines. Among them, *E. arnotii*, *E. vicidium*, *E. bunifolium* and *E. inulaefolium* growing naturally in large areas of Argentina. As part of a program aimed to study the effects of essential oils against pest insects, we have investigated the composition and biological effects of these *Eupatorium* species essential oils against *T. castaneum* a world wide pest of stored grains. The oils composition were analysed by gas chromatography-mass spectrum (GC-MS) GC-MS analysis. The major constituents of *Eupatorium* spp were caryophyllene, germacrene D, limonene,  $\alpha$ -pinene, patchoulene and spathulenol. The essential oils of four plant species were tested in assays for toxicity and repellency in different doses. The mortality increased with increase in the dose of all essential oils. The repellency was significant at 1,69 mg/cm<sup>2</sup> dose. The results suggest that *Eupatorium* spp. essential oils could be considered a potential alternative to control pest insects of stored products.

**30. INSECT GROWTH REGULATORY EFFECTS OF LINEAR DITERPENES FROM *Baccharis thymipholia* H. & A**

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The use of fumigants and conventional organic pesticides for insect pest has caused problems of residual toxicity and developing resistance strains of insects. These problems have enhanced the need of developing more effective and safer insecticides. Insect growth regulators (IGRs) can be degraded rapidly in the environmental and generally have low mammalian toxicity to non-target species. IGRs include compounds that may affect molting and metamorphosis by mimicking juvenile hormones (JH Juvenile hormones agonist) or by interfering with the cuticle deposition. The aim of this work was to compare the bioactivity of the linear diterpenes for its similarity with juvenile hormones against insect larvae. The natural products were isolated from aerial parts of *B. thymipholia* and some derivatives were prepared. Recently ecdysed fifth instar larvae of *Tenebrio molitor* L. were selected and test solutions were topically applied to the ventral thoracic segments with a micro syringe (2  $\mu$ l/larvae). The duration (days) of pupal stage was recorded and the inhibition of the imaginal mouth. In the all tests normal adults emerged to from control larvae. Some natural diterpenes, methyl derivatives and some obtained by Sharpless asymmetric epoxidation were the most active. Morphological abnormalities were observed. These compounds affected the pupal stage duration. The results demonstrated that these compounds affect the development of *T. molitor*. The data presented are very useful for development of such IGRs, which are regarded as potential candidates for the control of insect pest.

**31. PHYTOLITHS AS PALAEOENVIRONMENTAL INDICATORS**

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The study of the sedimentologic characteristics and the description of quality and quantity of the silicophytoliths allowed the discrimination of the different pedologic horizons of the outcropping sediments in the south end of Sierra de San Luis. Records carried out in the area and based on the textural analysis, geobiochemicals (Ni, Cu, Pb and Zn) and content of phytoliths allow to identify four levels, two of them characterized by developing in edaphic-climatic conditions of ustic humidity régime similar to present-day ones and linked to the Holocene; and two levels of aridic régime related to the Late Pleistocene. The phytolith study indicate tenors that fluctuate in the 2% for the Aeolian sedimentary levels, where Prismatic, Flabelolite and Aculeolite predominate inside the so-called macrosilicophytoliths. In the fraction of thin to medium silt the estrobilolite and doliolite predominate followed by halteriolite in a smaller proportion. In the palaeo-edaphic levels the quantity of silicophytoliths exceeds the 5% in relation to the total mineralogy. The macrosilicophytoliths present morpho-kinds similar in type and quantity to those in sedimentary levels although with a greater diversity. Articulated forms are common as a conspicuous evidence of the environmental stability that accompanied the development of these palaeosoils. Within the microsilicophytoliths there is an increment of halteriolite in similar proportions to the Estrobilolite and the Doliolite scarce. The silicophytoliths constitute themselves into a good indicator of the environmental conditions of the Late Pleistocene - Holocene.

**32. DIURETIC EFFECT OF ARISTOLOCHIA ARGENTINA IN RATS**

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*Aristolochia argentina* (family Aristolochiaceae) is popularly known as "charrúa". The roots of this plant are used in folk medicine. Their infusions and tinctures are reputed to have diuretic, antidiarrheic, astringent and antihemorrhoidal properties. The aim of this study was to assess the diuretic effects in rats. Infusions of the aerial parts and roots of the plant at 10% were prepared according to Pharmacopea Argentina VI ed. Phytochemical assays were performed. Wistar rats of either sex (200-250 g) were employed. Lipschitz et al. method was used. Control (saline solution) and furosemide (reference drug) groups were established. Urinary volume was measured at 15-min intervals for 3 h to determine urinary volumetric excretion (UVE). Urinary density and pH are measured. Phytochemical screening indicated the presence of flavonoids, alkaloids, saponins, tannins among other compounds. Rats treated with root infusion showed a significant diuretic effect (UVE: 74.22  $\pm$  4.35) respect the control (UVE: 58  $\pm$  1.72), p < 0.05 (ANOVA); while aerial parts infusion was without effect (UVE: 58.70  $\pm$  3.86). Urinary density and pH were similar to controls. This diuretic activity could be due, in part, to the presence of flavonoids in this plant. Flavonoids are responsible for diuretic effect in other vegetable species. Further investigations are necessary prior to their recommendation for use as diuretic.

**33. MUTAGENIC EFFECT INDUCED BY CLASSIC SWINE FEVER LIVE VIRUS VACCINE REVEALED BY LIGHT AND ELECTRON MICROCOPY OBSERVATIONS**

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Live virus vaccine against Classic Swine Fever (CSF) was generally used to control the disease. At the cytological level we previously proved that it can induce several types of chromosomal aberrations. In this assay we evaluate (1) if chromosomal breakpoints are distributed at random or not, (2) if there is correlation between types and frequencies of chromosomal aberrations and micronuclei (Mn) frequency, (3) virus localization inside lymphocytes. *In vivo* and *in vitro* tests were made using blood samples of 20 pigs from the Río Cuarto University Experimental Farm. Lymphocyte cultures and break sites maps were made by conventional dyes and GTG banding techniques. Genotoxicity was evaluated with the Mn test *in vitro*. Virus particles were observed by electron microscopy. GTG banding showed that breakpoints were localized mainly in chromosome 13 (36% of cells) and 1 (20%). From 3 to 6% were localized in other smaller chromosomes. Mn frequency increased in proportion to virus concentrations. Electron microscopy showed that virus particles were inside the lymphocyte cytoplasm. Its morphology was similar to that found by negative stain of supernatant of CSF virus infected cultures. Therefore, live CSF virus vaccine induces non random clastogenic effects and chromosomal breakpoints coincide with fragile sites described for the pig karyotype.

**34. ENVIRONMENTAL FEATURES OF THE HABITAT OF TWO Orchidaceae SPECIES IN THE PROVINCE OF SAN LUIS**

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To date, the conducted studies show that five species of the *Orchidaceae* family, have been recorded for San Luis: *Sacoila lanceolata* (Aubl.) Garay, *Habenaria gourleiana* Gillies ex Lindl., *Habenaria hexaptera* Lindl., *Aa achalensis* Schltr. y *Aa hieronymi* (Cogn.) Schltr. The aim of this study is to contribute to identify certain important environmental factors that influence the habitat of the endangered *Aa* species of San Luis Hills. The factors studied show that these species grow under low average temperatures (less than 15°C), cold subhumid climate as the Thornthwaite index indicates. The geomorphology of the studied areas, at least where *Aa achalensis* was collected are: eroded slopes of hills, composed of granite, andesite as well as phyllite and other metamorphic rocks. They grow in colluvium- alluvial valleys, at "pampas" located at not more than 1.500 msnm., in medium to high stony soils (> 5%), and/ or lithic soils, with > 6% organic matter content, high capacity of cationic exchange and strong to weak acid pH. Is important to go on with the research of the threatened environment of the endemic *Aa* species because of their vulnerability.

**35. COMPARATIVE STUDY OF THE *Saintpaulia ionantha* AND *Sinningia speciosa* MICROPROPAGATION**

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*Saintpaulia ionantha* H. Wendl and *Sinningia speciosa* Lodd. Are species of the Gesneriaceae family, and characterized by high *in vitro* regeneration capacity, of ornamental value and high cost. The present work was done to analyze the production of adventitious shoots and roots *in vitro*, starting from leaves, using standard culture medium with hormonal combination. This response was quantified in the different micropropagation stages. The following parameters were evaluated: number of buds and roots formed *in vitro* and formation of callus. The appropriate sterilization method was determined and the highest multiplication rate, with significant differences, was achieved in Murashighe-Skoog medium with 6-benzylamino-purine (1 mg.l<sup>-1</sup>) in *Sinningia speciosa* Lodd. and is sensitive at high salts concentrations, while than *Saintpaulia ionantha* H. Wendl with  $\alpha$ -naphthaleneacetic acid (0,1 mg.l<sup>-1</sup>) and 6-benzylamino-purine (1 mg.l<sup>-1</sup>) and with major nutrients concentration. Determining the multiplication rate *in vitro* allows to specify the feasibility of its production in commercial scale.

**36. PHYSICAL AND CHEMICAL STIMULI IN THE GERMINATION OF *Hysterionica jasionoides***

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The cultivation of native ornamental plants with commercial purpose generates an alternative of economic diversification and regional development because the flower market world demands continually new species. When one wants to introduce or to diffuse a new species or variety, the main obstacle is to achieve an important number of plants, in relative short time. Therefore, it is convenient to optimize propagation methods that can be used for commercial scale. With the purpose of beginning the domestication of *Hysterionica jasionoides*, different physical and chemical stimuli on seeds germination were analyzed. The germination rehearsals on absorbent paper in plastic boxes, with the following treatments: control, gibberellic acid (500 mg.l<sup>-1</sup>), gibberellic acid (1000 mg.l<sup>-1</sup>), cool without humidity (4°C, during 7 days) and cool with humidity (4°C, during 7 days). The highest germination rates were obtained in the control and gibberellic acid (500 mg.l<sup>-1</sup>). A high pathogenic contamination was observed with pathogenic, suggesting a pretreatment for its elimination.

**37. MICROPROPAGATION OF *Oenothera biennis* AND *Hysterionica jasionoides***

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The floral genetic resources of the Argentina have wakened up interest in the last years, for the growing demand of new species or ornamental varieties. In the native flora, there is ornamental and medicinal species of great potential value. Among them stands out *Oenothera biennis* and *Hysterionica jasionoides*. With the purpose of increasing the range of available variability, to facilitate the conservation and use of the genetic resources, a *in vitro* culture was started of this species. The seeds were cultivated *in vitro* for the seedlings establishment in axenic conditions. The sterilization of seeds, its *in vitro* germination and the development of the seedlings was evaluated in Murashige and Skoog medium, with diverse combinations of plant growth regulators. The rehearsals allowed to standardize the establishment protocol and multiplication *in vitro* achieving to reliable, routine production and in the appropriate scale to produces uniforms plants and of quality for agronomic purposes.

**38. IN VITRO CULTURE OF NATIVE POÁCEAS**

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The plant tissue cultures has been applied and used with success in numerous plant species; however, few antecedents exist with regard to the *in vitro* culture of native grasses. With the purpose of being optimized the *in vitro* culture conditions of species of forage and ornamental value, protocols began with tissue culture of *Schizachyrium plumigerum*, *Trichloris crinita*, *Sorghastrum pellitum*, *Piptochaetium napostense*, *Poa ligularis*, and *Stipa tenuis*, as complementary methodology in futures domestication programs and plant breeding. The nutritional requirements achieved settled down for each species, being: embryogenesis, organogenesis and callus cultures. In all the rehearsals the recalcitrant aspect of the Poaceas, and the difficulties to execute with the introduction stages and multiplication of their cultivation *in vitro* for the high degree of contamination of the explants was proved.

**39. In vitro SELECTION OF *Medicago sativa* L. CULTIVARS FOR SALT TOLERANCE**

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The effects of the salinity in the growth of the plants have been object of intense investigations in the last years, due to the limitation that saline stress imposes to crops plants productive in arid and semi-arid areas. The alfalfa (*Medicago sativa* L.) it is one of those crops. The selection through the cultivation of tissue culture offers an alternative in the processes of crops improvement, when diminishing the space requirements and time in the selection stages and propagation. Presently work was screening salt tolerant cultivars: Mayacó, Trinidad 87, AR8 and Sirosal. The *in vitro* selection was based on the use of different concentration of CINA in tissue culture. The parameters evaluated were: the rate of growth shoots length, fresh and dry matter weigh, and percentage of survival. The Sirosal and AR8 cultivars stood out significantly in the evaluated parameters. The obtained results allow to estimate the behavior in field of these cultivars under saline stress.

**40. MICROPROPAGATION OF *Gomphrena pulchella* Mart.**

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*Gomphrena pulchella* Mart. is an native species with ornamental value for the attractive color and morphology of their inflorescences, used as dry flower in floral arrangements. This genetic resource, the same as other native species, allow the design of gardens with low maintenance requirements and with adaptation to extreme climatic conditions. The present work has as objective to determine the appropriate nutritious combination for the *in vitro* culture of this species. Different concentrations of basal Murashige-Skoog medium was evaluated (1X, 0,5X and 0,25X), without plant growth regulators. 70% of viable explantos were achieved, without oxidation problems and free of contaminants. Seedling deformation was observed in the complete medium, and don't appear in the culture medium with smaller saline concentration. This contributes to progress in the nutritious requirements for the multiplication of shoots and aspects related with the type explants.

**41. ORGANOGENESIS IN VITRO OF *Noticastrum gnaphalioides***  
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In the west semi-arid Argentina an important number of attractive native species exists for its flowers and foliage that present adaptative advantages when designing gardens and sustainable green spaces without doubts. *Noticastrum gnaphalioides* is a species that is characterized by its attractive purple inflorescence in the autumn. With the purpose of achieving their micropropagation, different disinfection methodologies and culture media were evaluated. The multiplication rate was observed by the formation of adventitious buds in seedlings cultivated in Murashige and Skoog medium with the added of  $\alpha$ -naphthalenetic acid (0,1 mg.l<sup>-1</sup>) and gibberellic acid (1 mg.l<sup>-1</sup>). The propagation *in vitro* allows to have select germoplasm and the possibility of beginning its genetic improvement by means of diverse biotechnicals, *ace in vitro* mutagenesis.

**42. MICROPROPAGATION OF *Phalaenopsis* ORCHID**  
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It is widely recognized that potted *Phalaenopsis* and its hybrids production has increased tremendously in last few years. Nature orchid seeds germinate only following infection with mycorrhizal fungus. However, seeds *in vitro* cultivated, successfully germinated in the absence of a symbiotic fungus. The present paper informs on the germination of seeds in Knudson C medium. After two months the seeds germinated and the protocorms appeared. The seedlings was cultivated in half strength Murashige Skoog medium, added sucrosa (3%) without plant growth regulators. The protocorms dark green and some were white color. After five months of cultivation *in vitro* seedlings development of shoots and roots was observed. This methodology allowed to obtain to high germination rate and multiplication, feasibility at commercial level production.

**43. PREFORMULATION STUDY IN COMMERCIAL POWDER OF *Valeriana officinalis* L. ROOT**  
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*Valeriana officinalis* L. "Valeriana" a native plant from Europe and Asia is used for its sedative activity. In this work the pharmacognostic study of a commercial powder from "Valeriana" roots is presented (microscopic assay and identification reactions). In addition, the compactation properties (compressibility and compactibility) of the powder were studied with the aim of achieving a suitable solid dosage form. The commercial powder was analyzed microscopically. The hydroalcoholic solid residue was adsorbed over an inert excipient (fumed silica) forming a solid extract (SE). Different tests were carried out to evaluate the powder rheological and physical characteristics. Finally, different tablets formulations were designed and manufactured and the pharmacotechnical tests were performed. The identity of the commercial powder was established by performing microscopic analysis and identification reactions. The SE controls comprised: solid residue assay, density and compressibility assays, angle of repose. The obtained results were appropriate. The powder ability to form coherent compacts was studied by performing the tablet compactation. The rheological properties, hardness, friability, weight uniformity and disintegration were evaluated in the obtained tablets.

**44. AMARANTHUS DUBIUS ROOT: A COMPARATIVE STUDY AND NUTRITIONAL EFFECTS**  
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The interest in the study of amaranto and maca appears due to their potential to enhance the diversity of commercial cultivation with an important contribution to the world alimentary reserve. In this work, we present a comparative study among *Amaranthus dubius*, *Martius ex Thellung* (Ad) cultivated in our research group (San Luis, Argentina) and *Lipidium meyenii* Walp (maca) cultivated in Perú. The main aim of the contribution is to evaluate the nutritive potential of Ad as an alternative cultivation. Such a study has included moisture in both fresh material and residual; dried and ether extract, ash using the AOAC methods. Protein (N x 6,25) by Kjeldhal. Total dietary fiber (FDT) soluble (FDS) and insoluble (FDI) was obtained according to Prosky. Fatty acids were determined by gas chromatography previous derivation. A uniform value of protein (10%) was determined for both studied roots. The content of fatty acids in both roots is acceptable for the human feeding (56,5% of unsaturated fatty acids for Ad and 52,7% for maca). It is important to emphasize the presence of araquidonic acid in the samples of Ad in conjunction with a good agronomic performance in 40 days with low environmental requirements and interesting bromatological indexes. It is suggested that the cultivation of Ad could mitigate the continuous and progressive damage of the physical and social conditions in the undulate pampa due to the introduction of soya and the increasing of the agricultural production.

**45. Fe AND Al CONTENT IN MARSHY SEDIMENTS OF SAN LUIS MOUNTAINS (ARGENTINA)**

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Four soil profiles were studied located among the 32° 30' and 33° 20' S and among the 65° 54' and 66° 12' O, at 1300 msnm. Under cold subhumid - dry climate conditions. Our aim was to determine the Fe and Al content of the dark sediments and hypothesize on its origin. An external and internal characterization of the soils was carried out. It was observed that these profiles are polycyclic and polygenic. This kind of sediments presents a sequence of A and E buried horizons, dark gray and gray colors respectively; with loam texture- silt loam and silt. The pH values vary from extremely to weakly acid, with good contents of organic matter whose values diminish in depth. The capacity of cationic exchange is high, while the insaturation values increase from surface, what demonstrates that the leaching processes acted intensively in depth. The contents of Fe and Al are high, generally increasing in depth. The average values of Fe are almost similar in all the studied profiles, while the Al content in these profiles show more variation. There is no relationship between Fe and Al contents in relation with pH value. We conclude that the dark sediments Fe and Al contents indicate that they developed under marshy conditions where the dominant process is that of neof ormation with strong influence of leaching processes.

**46. MORPHO-ANATOMY OF ROOTS AND NODULES IN ADESMIA BICOLOR (POIR.) DC (FABACEAE)**

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In the arid and semiarid region of central Argentina, there is a lack of forage species that can adapt to prevailing conditions, like low soil N content, presence of a dry season, and low temperatures in winter. Preliminary domestication studies started two years ago. *Adesmia bicolor* (Poir.) DC shows potential to be domesticated because of its high growth rate and tolerance to low phosphorus level in the soil. It is important to know the root system characteristics and determine its ability to fix N in symbiosis with *Rhizobium* for a species that grows in a poor soil. The objective is to analyze morphological and anatomical characteristics of the root system and nodules of *Adesmia bicolor*. In the greenhouse, this species was seeded in pots and, after germination, plants in different maturity stages were harvested. Samples were processed according to conventional techniques to obtain histological slides. The root system and nodules developed on main and lateral roots were characterized. The seedling of *A. bicolor* shows a profuse root system development. The main root is approximately 10 cm long and originates the first branching degree few days after germination, whereas the second one is produced after 15 days. The root primary structure is diarch and lateral meristems start their activity during the first stages of ontogeny. In front of protoxylem poles, two parenchymatic rays are originated. Nodules are associated or not to lateral roots and they increase their quantity as the plant grows. In cross sections, they show two well-defined areas: cortex and bacterial zone.

**47. MICROBIOLOGICAL QUALITY OF THE WATER OF CONSUMPTION IN LAVALLE, MENDOZA**

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In the desert of Lavalle the lack of superficial water leads the inhabitants to consume water from a well located in the San José place.

Physicochemical studies of the water from this well found high arsenic content. Due to the high incidence of gastrointestinal syndromes in the population of a boarding school, we decided to perform an assessment of the water microbiological quality from the source to the school. Sixty samples were obtained, cultivated using the method of membrane filtraton, in media for mesophyl bacteria, enterobacteria, *Pseudomonas*, and enterococci. The presence of fungi and parasites was also evaluated. It was found that the water is not chlorinated at any point. The results showed mesophyl bacteria close to  $5 \cdot 10^2$  cfu/100mL. *Escherichia coli* was isolated in 10% of samples and *Pseudomonas aeruginosa* was present in all samples with an average of  $3 \cdot 10^2$  cfu/100mL. In 84 % of the samples fungi were found, but no parasites were visualized. According to the current regulations demanding absence of *E. coli*, *P. aeruginosa* in water for human consumption, the presence of these bacteria, in addition to coliform ones and fungi indicates that the microbiological quality of this water requires an urgent control from regulatory authorities. The health of the Lavalle population is seriously jeopardized by the ingestion of water with these characteristics.

**48. ANTIOXIDANT AND GASTROPROTECTOR EFFECTS OF THE DIFFERENT EXTRACTS OF PLANTAGO MAJOR**

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This study compares the antioxidant and gastric cytoprotector actions of the different extracts obtained from the *Plantago major* (Pm) in experimentation animals. Material and methods: the methanolic, ethanolic and n-hexanolic extracts were tested in Wistar rats under standardized conditions and a strict protocol. The substance used to produce injury was absolute alcohol. Malonyldialdehyde (MDA) was determined as lipoperoxidation rate, and total antioxidants (TAS) and dismutase superoxide (SOD) as defense mechanisms. The removed stomachs were used for the microscopic study. The photochemical analysis obtained by gassy chromatography - mass spectrometry - revealed the presence of possible substances responsible for the antioxidant and gastric cytoprotector action. Results and conclusions: The fractions dissolved with n-hexane of Pm. indicated the presence of sterols as estigmasterol and sitosterol. From the metabolic extract it was evidenced an iridoid C-9: aucubin, a C-10: 10-hidroximajoroside and 10-acetoximajoroside, a trisacárid: plateose and cafeic acid and the flavonoids: hispiduline and napetine. The biochemical parameters showed the increase of MDA evidenced by free radicals action, while SOD and TAS decreased in the group of rats treated with 96° alcohol. A very important blood vessels and cells structure disorganization was found by electronic microscopy in the experimental injury group. The different extracts evidenced a gastric mucose unaffected in structure. The biochemical parameters and the electronic microscopy studies show a correlation regarding the antioxidant and gastric cytoprotector action of *Plantago major* against the 96° alcohol injury.

**49. ENERGY DRINKS STOP STEM CELLS PROLIFERATION AND GROWTH OF *Allium cepa* L. ROOTS**

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The recipe of energy drinks (ED) include caffeine, taurine, vitamin B(6) and glucuronolactone. Caffeine is an alkaloid that exerts significant effects in a great variety of physiologic, cellular and molecular systems. Taurine is the second amino acid more abundant in the brain that cause pharmacologic effects when is supply to the body. Vitamin B(6) cause in excess neurosis. Glucuronolactone is a metabolite relate to vitamin C. We are studying the toxicity of this cocktail of drugs in *in vivo* model, culture of onion (*Allium cepa* L.). Onion roots permit to access to stem cell proliferation that is directed related to root growth. The effect of 0 to 100% ED for 24h on growing roots and the recovery in water the next 24h were analyzed. Results are expressed as Grow Index (GI) and Mitotic Index (MI). Onion roots culture with 1 and 5% ED produce a growth reduction in GI to 17 and 83%, respectively. Moreover, MI obtained from the same model decrease to 30% just with 1% BE. In this sense, concentration near to 5% -or more- cause arrest of cell proliferation – at the interphase of the cell cycle – and consequently the MI was equal to zero. Recovery of proliferation and growth was only detected with 5% or less BE. Components of this recipe, like caffeine, interfere with cell cycle, but the BE recipe interfere more aggressively. Some negative combinations are suspected.

**50. EVALUATION OF NUTRITIONAL PARAMETERS IN *Poa ligularis*: EFFECT OF THE NITROGEN FERTILIZATION**

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*Poa ligularis* Nees. is a grasslands' kind of the semi-arid region that responds positively to the N fertilization and this is evidenced in the increase of the production of dry matter (DM). The objective of this work was to evaluate the answer of different nutritional parameters as Crude Protein (CP), Neutral Detergent Fiber (NDF) and Acid Detergent Fiber (ADF). An experiment was carried out in Nueva Escocia (San Luis, Argentina). Grassland was fertilized at three levels of N fertilizer (urea), 0, 60 and 115 kg ha<sup>-1</sup> and harvested monthly. The results indicate, according to the statistical analysis ANOVA, that the only modified parameter is the PB, observing differs significant between the unfertilized and the fertilized treatments. ADF and NDF didn't show significant differences. The conclusion of this work is that the N fertilization is a technological tool that should be evaluated especially with more detail in grasslands for its answer in species key as *Poa ligularis* since there are modifications in quality and quantity of the DM that can be important for the grassland management.

**51. A CYANOBACTERIUM AKIN TO THE FREE-LIVING GENUS *Microcystis* LIVING WITHIN CELLS OF THE APPLE SNAIL *Pomacea canaliculata***

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In previous studies we have explored the possible cyanobacterial and endocytobiotic nature of pigmented corpuscles (named C and K bodies) found in the midgut gland of *Pomacea canaliculata*. Morphological studies showed that C corpuscles are round bodies, 14 µm in diameter, of a brownish-greenish color, which are delimited by outer membrane and an electron-dense wall. On their part, K corpuscles are multilamellar, club-shaped bodies of a dark brown color (approx. 35 µm long and 14 µm wide). Numerous granules of varying sizes, as well as some irregular membranes are observed in C corpuscles. On their part, K corpuscles are formed by a center of coarse granules surrounded by numerous electron-dense lamellae. DNA was found in both C and K corpuscles, while DNA extracted from C corpuscles was purified and used as template for the PCR amplification of the bacterial gene encoding for the 16S rRNA. In the present work, this gene was cloned and sequenced, and was found akin to that of the free-living genus *Microcystis* (Cyanobacteria, Chroococcales). Also, *in situ* hybridization with probes recognizing conserved sequences of this gene (for both Bacteria and Cyanobacteria) allowed us to localize ribosomal RNA on both corpuscular types within midgut gland's epithelial cells. It seems, therefore, that pigmented corpuscles contained within these cells of *P. canaliculata* are indeed morphs of a chroococcalean cyanobacterium. And it should be stressed that this association seems to be an obligate one for the snail, since we have found such corpuscles in all populations of *P. canaliculata* that we have sampled so far.

**52. A NOVEL XANTHANOLIDE SESQUITERPENE WITH ANTIULCER PROPERTIES INHIBITS CALCIUM IONOPHORE-INDUCED MAST CELL DEGRANULATION**

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In previous work we have demonstrated that xanthatin (Xt), a novel xanthanolide sesquiterpene with antiulcer properties isolated from *Xanthium cavanillesii* Schouw, inhibits mast cell exocytosis induced by the G protein stimulant compound 48/80. The present work examines the effect of Xt on mast cell degranulation induced by the calcium ionophore A23187, to determine whether Xt acts upstream or downstream of cytosolic calcium increase. Rat peritoneal mast cells were purified in Percoll and incubated with: 1) Tyrode solution or 2) A23187 or 3) Xt+A23187. Serotonin release studies by high performance liquid chromatography (HPLC), evaluation of mast cell morphology by light microscopy, dose-response and time-response studies, cell viability evaluation by the tripan blue dye exclusion, comparative studies with ketotifen (Ket), and drug stability evaluation by thin layer chromatography (TLC) were carried out. Calcium ionophore increased serotonin release from mast cells and elicited evident morphological changes. These effects were inhibited by Xt in a dose- and time-dependent manner. The inhibitory effect exhibited by Xt was stronger than that of ketotifen, a classical mast cell stabilizer. In conclusion, the present study demonstrates that Xt inhibits A23187-induced mast cell activation, acting downstream of cytosolic calcium increase.

**53. LATERALITY IN MONKEYS: EVALUATION OF HAND SKILLNESS BY A MODIFIED EDINBURGH INVENTORY**

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Mechanisms by which man use preferentially one hand over the other have been intensely studied in the past years. Recently, it has been found that manual laterality is not an exclusively function of human beings. Evidence has supported the existence of laterality in other less evolutionary living systems, including the non human primates which share with man the use of hands. In spite that many workers have found laterality in animals, experimental tests used have been limited to just a few mechanical abilities of animals. In the present work, acknowledging the potency of the Edinburgh Inventory for evaluating hand skill in humans, a modified version adapted to monkeys was used in order to discern manual skill in *Cebus apella* species. Results confirmed that laterality for hand use is present in monkeys. Nevertheless, at population level, statistically similar proportions were found for right hand users and non-right hand users (63.6% versus 36.4%), which contrasted with the 90% right hand users and 10% non-right hand users in a sample of school children, tested with the Edinburgh Inventory. Present results give support to the Modified Inventory for monkeys and it is proposed that it could be used to test other non-human primates where manual laterality is under issue.

**54. PITUITARY PARS INTERMEDIA OF *Lagostomus maximus maximus*: IMMUNOHISTOCHEMICAL STUDY**

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The viscacha (*Lagostomus maximus maximus*) is a rodent of nocturnal habits and seasonal reproduction that lives in the semiarid region of our country. The pituitary pars intermedia (PI) of *Lagostomus* shows a developed parenchyma with typical follicular structures and scanty blood vessels. There are granulated and non-granulated cells in the parenchyma of PI. Most of non-granulated cells are the folliculo-stellate (FS) cells. The aim of this work was to study the PI of this rodent by immunohistochemical technique. The antibodies anti-ACTH, anti- $\alpha$ -MSH, anti-S-100 and anti-GFAP were used. The FS cells show cytoplasmic and nuclear immunolabeling for S-100 and cytoplasmic immunolabeling for GFAP. There are immunoreactive cells limiting with the Rathke's pouch. Sometimes the colloid lumen shows immunolabeling for S-100 and GFAP proteins. The FS cells present cytoplasmic processes. The granular cells near the follicular structures show immunolabeling for ACTH and  $\alpha$ -MSH. Our results in PI of viscacha demonstrate that there are melanotropes, corticotropes and FS cells in closely association. The intercellular network communication originated by FS cells is probably an alternative way for the hormonal distribution. Besides, the expression of S-100 and GFAP proteins might indicate a neuroectodermal origin of the FS cells.

**55. HYDROLYTIC CAPACITY OF INTESTINAL ENZYMES IN *Bufo arenarum***

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The plasticity of the digestive tract and its ecological consequences are important because any constraints on digestive performance may influence life history of organisms. This has received considerable attention among birds and mammals but little is known about intestinal enzymes of amphibians. In this study, we explored if the intestinal enzyme activities scaled to the small intestine size. In thirty toad small intestines (14 males and 16 females; body weight range: 63.13-158.35g) of Río Cuarto (Córdoba) and La Florida (San Luis) we assayed trehalase, maltase, sucrase and aminopeptidase-N activities. Total trehalase, maltase, sucrase and aminopeptidase-N activities scaled to small intestine mass ( $P < 0.001$ ). The pattern along the small intestine in trehalase and maltase was: proximal>medial>distal ( $P < 0.05$ ); while in sucrase and aminopeptidase-N was: proximal=medial>distal ( $P < 0.05$ ); similar profiles were also found for other amphibians. Thus, during growing the digestive capacity of intestinal enzymes increase as a result of the enlargement of the small intestine, and tissue-specific enzymatic activity remains constant. These results suggest that the energy requirements during the growing of toads are provided by an increase of the gut instead of an increment of the specific enzyme activity. Supported by FONCYT 255661 and UNSL CyT 22Q451 to EC-V.

**56. INTESTINAL ABSORPTION OF CARBOHYDRATES IN *Mus domesticus***

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Intestinal absorption of hydrosolubles nutrients in mammals occurs via transcellular and paracellular pathways. The contribution of both mechanisms had been measured observing that the magnitude of the transcellular absorption is larger than for the paracellular absorption. However, a controversy subsists, since the methodologies employed were not uniform and do not evaluate correctly the paracellular absorption. To test the contribution of both pathways simultaneously, we used an *in vivo* model, with intact animals. Mice ( $n=5$ ; BW  $33.37 \pm 1.15$ g), provided with glucose solution as the source of calories, were administered orally and intraperitoneally (in different experiments) with isosmotic solutions containing non-metabolizable sugars: 3-O-methyl-D-glucose (3OMG, MW192) as paracellular and transcellular marker, L-rhamnose (MW182) as paracellular marker and cellobiose (MW342) as paracellular marker too. Sugars concentrations were measured in urine collected during 72hs after administration by HPLC/fluorescence. The fraction absorbed (FA) was  $0.863 \pm 0.0011$ ,  $0.123 \pm 0.002$  and  $0.06 \pm 0.0006$  (mean  $\pm$  1 SEM) for 3OMG, L-rhamnose and cellobiose respectively. The contribution of the paracellular pathway to total intestinal absorption is smaller than for the transcellular pathway and not different from other mammals tested. The paracellular pathway exhibited the expected solute size selectivity. Supported by FONCYT 255661-UNSL-CyT-22Q451 to EC-V.

**57. TSH CELLS IN PITUITARY PARS DISTALIS OF MALE VISCACHA. SEASONAL STUDY**

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Different species show seasonal variations in the activity, number and ultrastructure of the thyrotroph or TSH cells. These variations can be directly related to the thyroid gland morphology and physiological activities of the animal. The aim of the present work was to study the TSH cells in pituitary pars distalis of male viscacha throughout the year by immunohistochemistry and image analysis. These cells were localized in the cephalic-ventral region. They were found along the surface of blood vessels, in clusters and sometimes in close proximity to follicular structures. They were oval, pyramidal or round in shape with an eccentric nucleus. During June-July, the TSH cells showed the smallest percentage immunopositive area ( $1.43 \pm 0.15\%$ ) and in August-September this parameter was the largest of the year ( $3.13 \pm 0.27\%$ ). No variations were observed in the cellular and nuclear diameters. Our results suggest that during the early winter (June-July) might exist a reduction of the cellular activity and it increase in the later winter-early spring (August-September). Environmental June-July conditions such as short photoperiod, low temperature, reduce hydric and food availability, and the reproductive state (gonadal regression period) probably provoke a decrease of the pituitary-thyroid axis activity of the viscacha (*Lagostomus maximus maximus*).

**58. MEMBRANE MICRODOMAINS (RAFTS) AND FERTILIZATION**

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Membrane microdomains (MD) are structures associated to specific functions and they are enriched in sphingolipids, cholesterol and proteins. These MD previously have been studied in diverse cellular models. The objectives of our work were the characterization of the MD components of the oocytes and their roll in the fertilization. In order to study the components of the MD, oocytes were incubated in HECM3. Later were fixed and marked: cholesterol by filipin III (FIII) and sphingolipids (GM1) by fraction beta cholera toxin (BCT). FIII is self-fluorescent and BCT is conjugated with Alexa Fluor(R). Samples were observed in a fluorescence microscope. In fecundation *in vitro* assay, we blocked lipids raft preincubating sperm with BCT (GM1) 15'. Then, mouse sperm were coincubated with hamster oocyte, and the sperm bounded were counted. Later, we blocked lipids of the MD in the oocytes and they were coincubated with spermatozoa non-blocking. First observations showed a binding decrement in sperm blocked with BCT (GM1) comparing with the control (not blocked). These results suggest that GMI, a classic raft component, and its redistribution after capacitation are involved in sperm - oocyte binding. However, in the blocked oocytes faced the sperm, they do not present a significant decrement.

**59. POSSIBLE EFFECT OF 5-EPI-ICETEXANE ON EARLY G2-PHASE OF THE *Trypanosoma cruzi* CELL CYCLE**

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*Trypanosoma cruzi* is the etiological agent of chagas disease. In cultures, these parasites cycle between the flagellate epimastigote form, and scarcely differentiate to the infective trypomastigote. Quinones derivatives have shown to be effective against the parasite, although their use is still restricted because a cytotoxic effect on the host cells. A novel hydroxylated quinone, 5-epi-icetexane (ICTX), purified from *Salvia gilliesii*, exhibited an antiproliferative effect on cultured *T.cruzi* epimastigotes, even at very low concentrations. The present work was addressed to evaluate if the drug affects selectively certain phase of the parasite cell cycle. The growth of the parasites (Dm28c strain) was synchronized at G1 phase by treatment with hydroxyurea (HU) for 24 hr, and cytostatic concentrations of ICTX were added at different time points after removal of HU. We observed that ICTX is able to arrest selectively the cell cycle between 12 and 14 hours after removal of the HU. No major effect was observed at earlier time point, corresponding to the S phase of the cell cycle. We concluded that ICTX affects early G2 phase of the cell cycle, probably by knocking down expression of important regulating proteins. Ultrastructural studies will confirm if ICTX affects somehow the chromatin condensation, impeding cell division.

**60. INTERSTITIAL CELLS OF CAJAL IN FETAL CALF ESOPHAGUS DETECTED BY IMMUNOHISTOCHEMISTRY**

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C-kit is a transmembrane tyrosine kinase growth factor receptor expressed by a variety of normal mammalian cell types, including germ cells, immature myeloid cells, mast cells and interstitial cell of Cajal (ICC). ICCs are important regulatory cells in the muscular tunica of the digestive tract. We have studied the distribution of ICC within the intra-abdominal portion of the esophagus using antibodies against c-Kit /CD117. Samples were obtained from six 5-month old fetuses. Immunohistochemistry was performed on paraffin-embedded sections, according to the LSAB® method of DAKO and examined under light microscopy. Strong immunoreactivity was observed in ICC-like cells and mast cells. C-kit positive branched ICC-like cells were detected in the esophageal striate musculature. They were distinguishable from c-kit negative unbranched smooth muscle cells and the c-kit positive unbranched mast cells. The density of ICC-like cells was greater at the circular and longitudinal muscle layers. ICC-like cells appeared as bipolar and stellate cells with long prolongations closely associated. ICCs were scarce around the ganglionic plexus where they are commonly localized. C-Kit immunohistochemistry provides an opportunity for characterizing distribution, density, organization, and relationship between ICC and other cell types.

**61. LECTINHISTOCHEMICAL CHARACTERIZATION OF INTERSTITIAL CELLS OF CAJAL IN CAT INTESTINE**

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The interstitial cells of Cajal (ICC) are pacemakers and conductors of electrical activity of the gastrointestinal muscles. Using lectin histochemical techniques, we have characterized the carbohydrate expression of the feline intestine ICC. For this purpose 7 lectins were incubated with paraffin embedded samples of ileum and colon of 3 months old cats. The following lectins were used: Con A (*Concanavalia ensiformes*), WGA (*Triticum vulgare*), DBA (*Dolichus biflorus*), SBA (*Glycine max*), PNA (*Arachis hypogaea*), RCA-I (*Ricinus communis*) and UEA-I (*Ulex europaeus*). The diaminobenzidine was used as chromogen. Furthermore, ICCs were localized using polyclonal anti c-kit antibodies in immunohistochemical assays. Con A, WGA and RCA-I lectins stained the ICC localized within the region of the myenteric plexus, closely associated with ganglia in the small and large intestine.

Con A detected oligosaccharides present in the entire cytoplasm while WGA and RCA-I bound exclusively to the plasma membrane. In the colon, Con A also bound to ICC distributed in muscle layers. UEA-I did not bound to ICC. Moreover, DBA was negative in the ileum while PNA and SBA were negative in the colon. We concluded that lectin histochemical assays using Con A, WGA and RCA-I as lectins can be a complementary study for characterizing the ICC present in the feline intestine.

**62. STUDY OF THE PHYSICAL CHEMICAL PARAMETERS IN LIQUIDS OF CONSERVES**

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Blanching is one of the food conservation process which is applied to fruits and vegetables. It consists in supplying heat at temperatures between 75°C to 95°C during one or two minutes in order to avoid the vegetables deterioration before the application of posterior processes. In the case of entire products the govern liquid, water with salt is added, just to maintain the freshness of the food during the commercialisation stage. In this work the superficial tension coefficient, viscosity and density of the govern liquid of peaches, green peas, pineapple and golden corn conserves were determined. From the differences obtained in the physical parameters related to water in the analysed marks of the peaches and pineapples syrup we can conclude that they can be due to the high content of sucrose. Beside, the concentration of sucrose is higher in peaches than in pineapples because of the necessity to conserve the swelling of the vegetables tissues avoiding sensorial changes due to osmosis or inverse osmosis. Some marks of green peas presented a considerable amount of particles of this vegetable, possibly due to an excessive treatment of blanching. For golden corn liquid conserves the differences obtained are simply due to the presence of NaCl in the batted conserve.

**63. K+ AND Cl- CHANNELS ARE INVOLVED IN ACROSOME SWELLING DURING THE ACROSOME REACTION**

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Acrosome reaction (AR) is an exocytic event that requires the attachment of the acrosome to the plasma membrane and the opening of fusion pores. Before the attachment the acrosome must swell to undergo the AR. This swelling implies the movement of ion and water molecules through acrosomal membranes. At molecular level, the AR is highly regulated by specific proteins, Ca<sup>++</sup>, ionic channels, but the molecular mechanisms of acrosomal swelling (AS) remains unclear. Our objective is to determine the ionic channels involved in the AS and AR using functional assays and transmission electron microscopy (TEM). To assess this, capacitated and SLO-permeabilized human sperm were incubated with different K<sup>+</sup> and Cl<sup>-</sup> channels inhibitors and the AR challenged with Ca<sup>++</sup>. Acrosomal status was evaluated using Pissum sativum staining. AR was inhibited when sperm were incubated with 4-AP and Apamin (K<sup>+</sup> channels inhibitors) but not with glibenclamide (KATP channels inhibitor). The Cl<sup>-</sup> channels inhibitors, DIDS and NPPB also blocked the AR in SLO-permeabilized sperm. The images obtained by TEM showed that 4-AP blocked the AS, but this swollen morphology could be recovered using K<sup>+</sup> ionophores. These results suggest the active participation of K<sup>+</sup> and Cl<sup>-</sup> channels in AS and AR.

**64. EFFECT OF MELATONIN ADMINISTRATION ON SPERMATOZOA OF VISCACHA (*Lagostomus maximus maximus*)**

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Different environmental and organic factors influence the species reproductive behaviour. The natural light is probably the most important primary signal involved in the synchronization of the sexual behaviour and the reproductive activity. The viscacha (*Lagostomus maximus maximus*), is a seasonal and photoperiod-dependent rodent. The aim of this work was to study the effects of the chronic administration of melatonin on the motility, vitality and number of the viscacha spermatozoa. Eight male viscachas captured during the maximum gonadal activity period (summer-autumn) and separated into two groups were used. The experimental groups subcutaneously injected with melatonin (Sigma) twice a day (100µg/Kg) during 7 weeks. The control group was only injected with the oily vehicle. The viscacha sperm number decreased significantly after melatonin administration: 299 ± 94 x 10<sup>6</sup>/ml; control group: 940 ± 149 x 10<sup>6</sup>/ml, p<0.01. The sperm vitality decreased significantly in the experimental group: 53 ± 1.3%, in relation to the control group 63 ± 1.79%, p<0.01. The spermatozoa motility did not show significant variations. The results showed that in the *Lagostomus* the administration of melatonin reproduces the environmental conditions of the winter season, when the levels of endogenous melatonin are high. It was also demonstrated that in these conditions there is an alteration of the analyzed parameters.

**65. RHEUMATOID FACTOR, SERUM IMMUNOGLOBULINS AND COMPLEMENT COMPONENTS IN HEPATITIS B AND C**

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Hepatitis B virus (HBV) or hepatitis C virus (HCV) infection have been associated with a variety of immunological abnormalities. Our objective was to determine the prevalence of rheumatoid factor (RF) and evaluate the serum immunoglobulins (Ig) level and components of the complement in patients with chronic HBV or HCV infection (HBV or HCV patients). In this cross-sectional study, were evaluated 17 HBV patients, 18 HCV patients and 20 healthy controls. RF was detected by latex agglutination and the levels of IgG, IgA, IgM, C3 and C4 were determined by radial immunodiffusion. RF was detected in the sera of 44% of HCV patients, but in 12% and 5% of HBV patients and healthy controls, respectively ( $p < 0.05$  vs HCV). In HCV infection, the occurrence of RF was associated to female sex ( $p < 0.05$ ). RF titers ranged from 1/40 to 1/320 in both patients groups. However, HCV patients manifested higher titers respect to positive healthy control whereas the titers in HBV patients were similar to positive healthy control. Compared with control group, significantly higher serum IgG and IgA levels were only detected in HCV patients and positive RF ( $p < 0.05$ ). There were no significant differences in the C3 and C4 serum levels between patients with or without RF and healthy controls. In conclusion, our data indicate that presence of RF and enhance of serum Igs are principally associated with HCV infection. These data would be associated to the susceptibility to autoimmunity in this chronic infection.

**66. PROGESTERONE IN COELIAC GANGLION ACTS AS A LUTEAL SURVIVAL FACTOR AT THE END OF RAT PREGNANCY**

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In rat, the corpus luteum suffers regression at the end of pregnancy. Progesterone (Pg) is a luteal survival factor, and LH at the end of gestation is luteolytic. Our purpose was to investigate the participation of coeliac ganglion in the Pg luteotrophic action and LH influence. Materials and Methods: the ex vivo coeliac ganglion-superior ovarian nerve-ovary system (CG-SON-O) of 21 days of pregnancy was used. The system was incubated in 2 ml Krebs-Ringer buffer bicarbonate pH 7.4 (glucose and albumin 0.1 mg/ml) at 37°C for 240 min, with a 30-min preincubation. The groups were: 1- control, 2- PgG (addition of Pg  $10^{-5}$ M in CG), 3- LHo (ovine LH 50 ng/ml in ovary), 4- PgG-LHo (Pg  $10^{-5}$ M in CG and ovine LH 50 ng/ml in ovary). The hormones were added at time 0 (after preincubation). Pg by RIA (30, 60, 120, 180 and 240 min) was determined in the ovarian incubation liquid, and DNA fragmentation was analyzed by ladder in the corpora lutea at 240 min. Statistics: ANOVA-Duncan test. Results: a- Pg liberation in ovarian incubation liquid: PgG group vs control group: Pg significantly increased depending on time ( $*p < 0.01$ ). LHo group vs control group: no significant differences were observed. PgG-LHo group vs LH group: Pg significantly increased after 60 min ( $*p < 0.01$ ) and at 240 min, it reached the PgG group values ( $*p < 0.01$ ). b- DNA laddering decreased in those groups in which PgG was added in the ganglionic compartment. Conclusion: Pg from CG via SON would rescue the corpus luteum from regression without significant LH influence.

**67. SEASONAL MORPHOLOGICAL CHANGES IN PROSTATE OF VISCACHA (*Lagostomus maximus maximus*)**

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The prostate is an accessory gland of the male reproductive system that morphological and functionally depends on the levels of circulating testosterone. This gland presents two lateral lobes of diffuse aspect and a central body that surrounds to the urethra. Our experimental model, the viscacha, is a South American rodent of wildlife, that low its conditions of habitat exhibits a seasonal reproductive cycle with maximum gonadal activity in summer and low activity in winter. The objective of this work is to study by light microscopy the structure of the viscacha prostate and to describe its probable morphological variations along the reproductive cycle. Prostate samples were processed by means of conventional techniques for optical microscopy. Histological courts obtained during the minimum gonadal activity shows adenomeros formed by a simple cubic epithelium, diminished projections toward the glandular lumen, surrounded by a thin layer of smooth muscular fibers and lax connective tissue. The samples obtained in the active period present alveolar adenomeros with a high cylindrical epithelium, with basal nuclei and numerous projections toward the glandular lumen. Moreover, is observed a greater development of the muscular layer and a superior number of blood vessels that in the period of minimum activity. These results show the presence of seasonal morphological changes in the prostate, coinciding these variations with the levels of circulating testosterone, determined in this rodent, through the reproductive cycle.

**68. TWO TYPES OF FOLLICULAR ATRESIA IN THE OVARY OF VISCACHA (*Lagostomus maximus maximus*)**

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The fate of the majority of ovarian follicles is the atresia. The atresia is a process in which the oocyte and the follicular cells undergo degeneration. The atresia has been widely studied, however, the process by which the follicles undergo atresia is not clearly understood. The present research was carried out to study the morphological changes in the ovarian follicles of viscacha during the atresia. Adult females were caught, anaesthetized and sacrificed. The ovaries were quickly removed and processed for light microscopy. The routine stains and the Shorr's technique were used. The results showed two types of atresia according to occur in secondary or tertiary follicles. In secondary follicles, the Shorr's technique revealed that the oocyte and zona pellucida regress and the granulosa cells become in interstitial tissue. Conversely, in the tertiary follicles, the oocyte and zona pellucida remains and the granulosa cells undergo drastic changes. The nuclei of the most granulosa cells were picnotic with apoptotic figures. Further, these cells stored a high amount of lipid droplets and the invasion of leukocytes was evident. The follicular development is a gradual process in which the follicles acquire gonadotropin receptors. The different responses by the secondary and tertiary follicles suggest that two processes take a place during the atresia. The amount of gonadotropin receptors and the complex interplay of hormones in the ovary may be factors to explain this difference.

**69. EFFECT OF MELATONIN ADMINISTRATION ON CHROMAFFIN CELLS IN THE *Lagostomus maximus maximus*. MORPHOMETRIC STUDY**

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The seasonal variations of environmental factors are transduced by the pineal gland in the synthesis and rhythmic liberation of melatonin. In mammals, the duration of the melatonin peak reaches its maximum in the short photoperiod or winter. In this work, the results of a morphometric study of chromaffin cells of adrenal medulla in a wildlife rodent are presented. Adult male viscachas were captured throughout the year, taken to the animal laboratory and administered with melatonin (Sigma) in two daily doses of 100 µg/kg (SC). The control group was only injected with the oily vehicle. The adrenal gland was immediately extracted and processed for morphometric studies (Image Pro plus®). Results. Nuclear diameter. Melatonin:  $6.66 \pm 0.091$  µm. Control:  $7.54 \pm 0.08$  µm. These results are in agreement with those obtained in previous seasonal studies. Nuclear diameter. Winter:  $6.46 \pm 0.069$  µm. Summer:  $7.03 \pm 0.056$  µm. During winter, the short photoperiod induces melatonin synthesis. These results demonstrate that melatonin actively participates in the stress process that might be a component of a general mechanism of adaptation to extreme survival conditions.

**70. INVOLVEMENT OF THE PERIPHERAL NERVOUS SYSTEM ON TESTOSTERONE RELEASE FROM THE TESTIS**

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The function of the autonomic nervous system on the testis has not been yet elucidated. The male gonads receive nerves from the autonomic ganglia that converge to the testis along two pathways, the superior spermatic nerves (SSN) and the inferior spermatic nerves (ISN). The SSN run from the superior mesenteric ganglion (SMG) alongside the testicular artery, whereas the ISN originate themselves in the pelvic and inferior mesenteric plexus (IMP). The present objective was to elucidate if the addition of the neurotransmitter acetylcholine (ACh) in the SMG or IMP modifies testosterone (T<sub>2</sub>) release from the rat testis. Two ex vivo systems (IMP-ISN-Testis and SMG-SSN-Testis) were applied. The incubation systems functioned with (experimental group) or without (control group) ACh (10-6M) in the ganglionic compartment. Krebs-Ringer buffer, pH 7.4 at 37°C was used as the incubation medium. The T<sub>2</sub> liberation in the testis compartment, was measured by RIA. The data were compared by Student's t test. Results: (Mean (ngmg testis/ml) SEM). When ACh was added in the IMP, the T<sub>2</sub> release underwent a progressive increase ( $p < 0.001$ ) while in the SMG we observed and opposite response, diminishing its liberation ( $p < 0.001$ ). These results show that the neural stimulation is significant in the T<sub>2</sub> release, which depends on physiologic status and the ganglion involved making evident that the neural function via the peripheral nervous system is directly involved in the testis physiology.

**71. INFLUENCE OF THE PERIPHERAL NERVOUS SYSTEM ON THE ENZYMATIC ACTIVITIES OF 3 β- HSD AND 20 α- HSD IN THE OVARY IN DIFFERENT STATE REPRODUCTIVES OF THE RAT**

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Most of the fibers that constitutes the innervation of the ovary, make synapses in the coeliac ganglion (CG) and superior mesenteric ganglion (SMG). Objective: to elucidate the role of the peripheral nervous system in enzymatic activity of the synthesis 3 β-HSD and degradation 20- α ; -HSD enzymes of the ovarian progesterone in different physiological conditions in the rat. Previous was standardized the integrated system CG-superior ovarian nerve-Ovary and SMG-ovarian nervous plexus-Ovary in rats. Student's t-test was applied with a significance of  $p \leq 0.05$ . 1st Androstenedione (10-6M), was added in CG in pregnancy rats at 21 days. 2nd Acetylcholine (10-6M) was added in GMS in dioestrus days during oestrous cycle. 1st. The activity of 3 β- HSD increase while that 20 α- HSD decrease, both significantly ( $p \leq 0.05$ ) respects to the control groups. 2nd. Dioestrus 1: 3 β- HSD diminished significantly versus the control group ( $p \leq 0.01$ ) while that 20 α- HSD increased with respects the control group ( $p \leq 0.05$ ). In dioestrus 2 day the activity of 3 β- HSD and 20 α- HSD do not showed significant changes with respects the group control. The results would indicate that the physiologic functioning of the corpora lutea is regulated do not only by the ovarian steroids only also by the influence of the neurotransmitters from to peripheral nervous system.

**72. SEROPREVALENCE OF CHAGAS DISEASE IN PREOCCUPATIONAL TESTS IN SAN LUIS CITY**

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American trypanosomiasis or Chagas disease is usually asymptomatic; for this reason, its diagnosis is mainly based on laboratory tests. During the indeterminate and chronic clinical periods, detection of immunoglobulin (Ig) G against *Trypanosoma cruzi* by different serologic tests is the standard for diagnosis. In the present work we communicate the seroprevalence of *Trypanosoma cruzi* antibodies in preoccupational tests in workers of San Luis city. A total of 500 blood samples were analyzed. All samples were tested with indirect hemagglutination test and IFI assay. Samples reactive for two assays were considered positive. Serological evidence of human *T. cruzi* infection was demonstrated in 36 (7.2%) out of 500 individuals. In the 18-39 years age group the percentage of negativity was 77.2% and in the 40-69 years age group 15.6%. No differences were finding when compared gender or place of birth. a) General infection prevalence in this community was similar to communicated in national average estimated rate (5.7%). b) Prevalence in the 40-69 years age group was 5 times higher in relation to that found in the 18-39 years age group ( $p \leq 0.05$ ). c) The prevalence shown no differences when compared male vs. female or place of birth. These findings show a similar prevalence compared with the one communicated in national epidemiological bulletins.

**73. CADMIUM MODIFIED THE PROLACTINE SECRETION BY DIFFERENT WAY**

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We have observed that exposition of adult male rats to 15 ppm of cadmium through the drinking water for two weeks, modified the prolactine (PI) amount in pituitary by immunohistochemistry determination and also decrease in serum. In order to determine the mechanism of cadmium action, we measured: the expression of mRNA of PI by RT-PCR, the expression iNOS, TGF beta 1 and COX 2 by Western Blot using specific antibody. We observed that mRNA of PI, and the amount of protein iNOS increase, while the amount of protein TGF beta 1 decrease and protein COX2 did not change. It is known that NO directly inhibits prolactin secretion and prolactine inhibit the expression of the inducible nitric oxide synthase (iNOS). On the other hand TGF-beta 1 suppressed PRL mRNA in a dose- and time-dependent manner, negatively controls the expression of iNOS and increase the expression of COX2. In consequence we could say that cadmium decrease the prolactine synthesis by different way: to postrancricional level, and trough NO production and down regulation of TGF beta 1.

**74. DETERMINATION OF PPAR- $\gamma$ 2 Pro12Ala VARIANT IN OBESE WOMEN**

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Several genetic variants of peroxisome proliferator-activated receptor- $\gamma$ 2 (PPAR- $\gamma$ 2), a molecule known to be involved in transcription of target genes in adipose tissue, have been identified. In this work, we study the possible relation between Pro12Ala, a missense mutation in exon 2 of PPAR- $\gamma$ 2 gene in circulating white cells and the levels of lipids and oxidative stress parameters in the serum of fertile age women. Fifteen obese and seven non-obese women were study. Fasting glucose, lipid profile (total cholesterol, HDLc, LDLc, and triglycerides), Paraoxonase-1 (PON-1) arylesterase activity, tiobarbituric acid-reactive substances (TBARS) and nitric oxide (by Griess reaction), were determined in serum. Analysis of PPAR- $\gamma$ 2 gene polymorphism was performed by Taqman Allelic Discrimination using a Standard 7500 Termocycler (Applied Biosystems). Lipid profile and oxidative parameters did not change with the body weight. The Pro12Ala polymorphism was present in 25% of obese and 14% of non-obese women, in relation to the Pro12Pro and Ala12Ala genetic variants, the most and the least prevalent PPAR- $\gamma$ 2 genotypes among Caucasian populations. The Pro12Ala variant was associated to a lower serum glucose, independently of the body weight.

**75. PRENATAL INHIBITION OF ANGIOTENSIN-CONVERTING ENZYME NO AFFECTS ITS EXPRESSION IN THE RAT LUNG DEVELOPMENT**

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Lung is the central organ in extrinsic respiration and has several important non-respiratory functions. Renin-angiotensin system in lung could mediate changes in vascular tone and permeability, fibroblast activity, and epithelial cell survival. Angiotensin II can act as a modulator of growth in a variety of cells and tissues. Different components of the RAS, including angiotensin converting enzyme (ACE) and both angiotensin type 1 and type 2 receptors, are expressed in lungs endothelial and epithelial cells. The aim of the present work was to investigate if prenatal ACE inhibition influences the expression of this enzyme in postnatal lung tissue development. Pregnant Wistar rats were treated with ACE inhibitors. Mini-osmotic pumps with Captopril, Enalapril (2,85 mg kg<sup>-1</sup> per day) and saline solution were implanted subcutaneously. The expression of ACE in lung pups at different postnatal stages: PND1, PND8, PND15, PND30 was evaluated and semi-quantified by multiplex RT-PCR. mRNA was obtained from lung tissue and we set up a procedure for co-amplification of both ACE and GAPDH sequences. Two sets of primers were used during the RT step. No significant differences in expression level at early stages of development were observed (One-way ANOVA, n.s.). However, the lung sACE exhibit time-dependent differences and very low changes were shown at PND30. We conclude that sACE expression in rat lung is not affected by treatment with inhibitors, probably the doses used was very low to interfere with functional integrity of the RAS.

**76. VITAMIN A DEFICIENCY ALTERS SERUM PARAOXONASE ACTIVITY AND INCREASES TBARS LEVELS IN SERUM AND RENAL CORTEX IN RAT**

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Vitamin A deficiency is an important public health problem in many developing nations. We have previously observed decreased serum HDLc levels and increased oxidative stress in liver from vitamin A-deficient rats. The present *in vivo* study was undertaken to investigate the effect of vitamin A deprivation on serum Paraoxonase-1 (PON-1) activity, HDL-associated enzyme that confers antioxidant activity on HDL, and lipoperoxidation in serum and renal cortex. Wistar female 21 days old rats were fed during three months with free vitamin A diet (-A) and the same diet plus 8 mg of retinol palmitate/kg of diet (+A). A group of -A rats received control diet 15 days before sacrifice (-A refed). Vitamin A deficiency was confirmed in plasma and liver by HPLC. PON-1 activity toward phenyl acetate (arylesterase activity) was determined by an enzymatic assay, and nitric oxide (NO) by Griess reaction. Lipoperoxidation was determined by quantifying the tiobarbituric acid-reactive substances (TBARS). The -A group showed decreased PON-1 activity (p< 0.001) and increased NO (p< 0.01) and TBARS levels in serum (p< 0.01) and renal cortex (p< 0.05), compared with +A rats. The oxidative stress induced by vitamin A deficiency was reversed after vitamin A refeeding.

**77. INDIVIDUAL AND FAMILIAR CHRONOTYPES: STUDY OF CORRELATION**

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The categories of chronotypes (morning-, intermediate- and evening-types) are based in the physiological and psychological circadian rhythms and refer the preferences by morning or evening activities. In this study, we investigated correlations between individual and familiar chronotypes. The sample (N=301) included young and adult women and men (mean age=32.84 years, SD=13.04). The participation was voluntary. The subjects (proband) categorized them-self and their family members (father, mother, 1 to 3 brothers and partner) by a modified version of the Munich Questionnaire (MQ). The MQ asks the chronotypes by a scale ranging from "very morning-type" to "very evening-type". The data were analysed by Spearman correlation coefficient. The correlations proband - father and proband - brothers chronotypes were highly significant ( $p < 0.001$ ) and significant with the mother ( $p < 0.05$ ). No correlation was observed with the partner. The circadian rhythms are endogenous and synchronized by a Zeitgeber or synchronizer. In the majority of the mammals, the photoperiod is the most important Zeitgeber; but, in the human being, is not possible determinate an only synchronizer. Thus, the social relationships may play an important role on the circadian rhythms. Our results show significant correlations among the family members. We suggest that the familiar relationships may participate in the modulation of the development and maintenance of the individual chronotypes.

**78. FALSE POSITIVES IN SYPHILIS**

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Treponemal and non-treponemal assays must be performed simultaneously for the serological diagnosis of syphilis. The dissociation of results between them may be due to false positives.

In order to investigate the percentage of false positives a long-term study was carried out (1990-1997; 2002-2004).

41.623 sera from patients of both sexes aged 1 to 87 were analyzed by means of VDRL test; the reactive samples were verified by TPHA and FTAabs.

947 out of the total were reactive for VDRL, while 79 out of 947 showed the following results: 37 (46,8%) with titer=1 dils; 31 (39,8%)=2 dils; 14 (17,7%)=4 dils and 3 (3,7%) between 8 and 32. The latter were non reactive for TPHA and FTAabs.

Usually, the percentage of false positives diminishes as the dilution increase.

In our laboratory the maximum limit considered as a possible false positive is 4 dils; due to this fact the samples found to have higher titers are considered an exception. They were processed through different methods at different times without showing any changes during the considered periods.

This study confirms the importance of performing both kinds of tests, since they allow to rule out or to confirm the disease as well to warn of exceptional cases.

**79. PHARMACOKINETIC OF BALOFLOXACIN IN EQUINES**  
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Balofloxacin is a fluoroquinolone with broad antibacterial spectrum against aerobic bacteria and mycoplasma, without photosensibilization effects in animals. The aim of the present study was to determine the plasma pharmacokinetics of balofloxacin following intravenous single dose (5mg/kg) administration to healthy equines (n=6). Balofloxacin concentrations in plasma was determined using a validated microbiological method of diffusion in agar, with *Bacillus subtilis* BGA. A non-compartmental pharmacokinetic data analysis program (PK-Solution 2.0) was used. Principal pharmacokinetic parameters were:  $t_{1/2} = 3,56 \pm 1,15$  h; MRT=4,7  $\pm 1,6$ ; Clearance total= 5,0  $\pm 1,0$  mL/min/kg;  $Vd_{area} = 1,46 \pm 0,19$  L/kg;  $AUC_{\infty} = 1026 \pm 204$   $\mu\text{g/mL} \times \text{min}$ . These parameters did not differ significantly from those obtained in goats and calves with the exception of AUC and clearance. The data, they can also be interpreted by a two compartments open model, since their bi-exponential curves show high correlation coefficients ( $> 0.97$ ). The value of  $Vd > 1$  L/kg, indicates good capacity of tisular diffusion, like to other fluoroquinolones and that would allow the treatment of bacterial pathologies with different localization. In turn, the values of AUC obtained with the used dose, guarantee the achievement of success theoretically in the treatment of bacterial pathologies in the equine, since the value of the index of the predictor of clinical effectiveness  $AUC/MIC_{90}$  is greater than to the conventional value of 125.

**80. PREMENSTRUAL SYNDROME IN UNIVERSITY STUDENTS**

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The severity and occurrence of premenstrual symptoms had been reported to increase with the age of the women. Moreover it was also been reported that contraceptive pill use would reduce symptomatology. This study aimed to find out the differences in frequency and severity of premenstrual symptoms with the age in university students (n=125 age 18-39). A printed structured self-reported questionnaire was utilized. The most frequent premenstrual symptom was: menstrual pain 73%; more susceptibility 51% and weakness 49.1%. Older women reported more symptoms than younger (3.53 $\pm$ 0.368; 2.46 $\pm$  0.176) (mean $\pm$ SE) respectively. The use of contraceptive pills and the regularity of menstrual cycle were not associated with premenstrual symptomatology. ( $p = 0.1348$ ;  $p = 0.1079$  respectively) The 28.8% fulfill with PMDD (premenstrual dysphoric disorder) diagnosis criteria, according to DSM IV TR; The 62.8% experiment some of the symptoms, therefore should have present premenstrual syndrome (PMS). Just the 8.8% admit do not perceive any premenstrual symptom. This preliminary study describes the pattern of incidence of symptoms. Most of the students suffer isolated symptoms from PMS and a minor proportion should suffer PMDD. Frequency results are agreed with the DSM IV data (14%-45%) No differences were found with the use of contraceptive pill.

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**81. PRELIMINARY ANATOMICAL TESTS OF COLD STRESS ON *Digitaria eriantha***

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*Digitaria eriantha* Steudel subspecies *eriantha* is a species of Southafrican origin, summer perennial, introduced from 1991 onwards and adapted to the Argentine semiarid mild central area. Currently, it constitutes an excellent forage resource but is affected by low temperatures and early and late frosts. Our goal is to assess the effect of cold on the anatomical structure of the cv. Mejorada INTA. *Digitaria eriantha* *culvitaris* in order to subsequently correlate it with physiological data of the species.

*Digitaria eriantha* cv. Mejorada INTA seeds were sown in terrines with soil-vermiculite substratum 1:1 and were grown in greenhouses. When plants had three leaves, they were exposed to 4°C temperatures during 6, 24, 72 hrs and 5 days. Stem and leaves samples were taken and fixed in FAA (formaldehyde, acetic acid, alcohol), were dehydrated and included in paraffin. Cuts were carried out in rotating microtome and were colored in Hematoxiline-Safranine- Fast Green. Out of the analysis, it was revealed that as cold hours increased, a reddish coloration rose from the caulinar apex through to the stem rest, due to the presence of necrotic cells. In addition, it was discovered that, after 5 days of low temperatures, leaves displayed a visible disorganization of chloroplasts.

**82. DENTAL PALEOPATHOLOGY PROFILE AND VESTIBULAR MICROWEAR IN AN AMERINDIAN POPULATION OF ARGENTINA**

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Oral paleopathology studies and its paleodemographic analysis are important to the reconstruction of past life. This study was made from Pampa Grande Collection (Argentina) in 15 cranium and 19 calvarium by sex and age. The pathologies that were registered: caries, alveolar reabsorption (slight, moderate and grave) occlusal wears and hipoplasias incidence. It was also selected a subregion from a digitalized photo of a molar vestibular surface impression and vertical (V), horizontal (H) and obliques striations were counted. From 298 teeth studied, 169 female, 129 male, 45 were ante-mortem lost and 37 non erupted. Forty three caries were found (27 female and 16 male); 6 slight, 7 moderate and 7 grave female alveolar reabsorption and 7 slight, 4 moderate and 4 grave male were observed. The occlusal wears were studied using the Mode: previous sector female was 2 and posterior 4 and 16; male Mode previous sector 5/6 and posterior 4/14. Moderate hipoplasias showed 3 women and 3 men cranial using Jacobi and Collins index. There were high values of vertical striations (1274), 1070 obliques MO-Dc, 873 horizontals and 769 obliques DO-MC. A low incidence of caries, moderate horizontal alveolar reabsorption, slight occlusal wears and vertical striations predominance founded, are according to with diet habits of this population like mix economy.

**83. HYPERTENSION AND LIPID PROFILE**

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Epidemiologic studies have demonstrated that hypertensive patients have unfavorable lipid profiles more frequently than does the general population. The aim of the present work was compare lipid profile of patients with or without hypertension (HTA), and to determine the distribution of the patients by sex, age, and associated pathologies. During 6 months (2006) a descriptive, retrospective and cross sectional study was realized. The diagnostic criteria ATP III (Adult Treatment Panel III) were used. The following variables were collected and analyzed: age, sex, pathologies, cholesterol total (CT), HDLc, LDLc and triglycerides (TG) of cardiologic patients both sexes (132) from private cardiologic institute. The Distribution (%) about the following parameters indicated: sex: F 67.4, M 30.3, HTA patients 67.4 and without HTA 32.6, HTA with associated pathologies: dyslipidaemia 37, hyperuricemia 12.4, hypotiroidism 10.1, hipertiroidism 5.6. Age in patients with HTA: (40-50) 4.5, (50-60) 19.1, (60-70) 23.6, and (70-80) 12.4. Lipid profile (mg/dl): With HTA: CT ( $\geq 200$ ) 30.4, HDLc (F<50, M<40) 42.2, LDLc ( $\geq 100$ ) 53, TG ( $\geq 150$ ) 28.3. Without HTA: CT ( $\geq 200$ ) 13, HDLc (F<50, M<40) 23.4, LDLc ( $\geq 100$ ) 25.8, TG ( $\geq 150$ ) 9.1. Our study reveals that the female population was the most important group. The prevalent age was between 50-70 years old. The lipid profile showed no significant differences between HTA and without HTA patients. For an unequivocal result, it would be necessary to carry out new studies.

**84. BIOASSAYS OF ACUTE TOXICITY ON AN INDUSTRIAL EFFLUENT**

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The principal objective of this work was to evaluate the possible ambient impact of an industrial effluent, through acute toxicity bioassays, using as experimental models: fish (*Poecilia reticulata* and *Cheirodon* sp.) and amphibians (*Xenopus laevis*). The design and development of the bioassays are based on a test used by U.S. Fish and Wildlife Service (Columbia National Fisheries Research Laboratory) modified by our group. This experimental technique was designed to evaluate acute toxicity of diverse chemical compounds. Our results indicate that for acute toxicity 100% mortality in a concentration until 1ppm. in fish ( $CL_{50}$  is 0.6963 ppm.) was observed. In amphibians the mortality was 20% at a concentration of 0.2 ppm. Teratogenic effects were not observed in a period of 45 days.

Based on these results and in the qualitative-quantitative chemical analysis of the effluent we proposed the followings treatments: neutralization and fractionated distillation. Thus, a decrease of its toxicity and therefore a better condition of ambient effect was obtained.

**85. SEXUAL BEHAVIOUR IN UNIVERSITY FEMALE STUDENTS**

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A descriptive, transversal, exploratory study was made in 130 women, aged 18 to 39 (mean:  $23.3 \pm 4.07$ ). A self report questionnaire was applied with the aim to know some parameters related to sexual behaviour in University female students.

Results show a menstruation beginning at a mean age of  $12.4 \pm 1.54$ , and the intercourse beginning at a mean age of  $17.7 \pm 2.52$ . The more usual sexual practices are: caresses (90.77%), vaginal (86.15%), and oral sex (67.69%). The most satisfactory are: caresses (73.08%), vaginal (70%), and oral sex (46.15%). Practices more often wanted are: sexual attachments (13.85%), and sex with 2 men at once (11.54%). Out of this sample of women, 71.42% said to have always or sometimes an orgasm and 15.58% said a few times or never had one. In the sample, 56.97% have a masturbatory activity.

Sexual practices and satisfaction in the sample would be, mostly, the commonly reported, while among the wanted practices there are some less common ones. The homosexuality percentage found (2.3%) agrees with previous reports (2%-5%). There is an important percentage in women hipo or non-orgasmic. Young women look for self satisfaction through masturbation.

*This work is part of a degree thesis made by the first author and is supported by Proy. N°: 4-0105 to R.Doña. FCH-UNSL.*

**86. DETERMINATION OF THE ADSORPTION ISOTHERM PARAMETERS FOR BOVINE SEROALBUMIN ON AN AFFINITY ADSORBENT**

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A study on the adsorption behavior under batch equilibrium binding conditions of bovine seroalbumin (BSA) with Cibacron blue 3GA ligand immobilized on yeast cells and used as affinity adsorbent, was performed. Cibacron blue molecules were covalently attached to the cells. It supposed that these linkages were formed between the reactive triazine ring of the dye and the hydroxyl groups of the glucans on the wall cell. The adsorption of BSA to the pigment Cibacron blue is attributed to the structural similarity of bilirrubine with the pigment, thus the ligand occupies the site that the protein (BSA) has to binding and transport of bilirrubine. The Langmuir model has frequently been used to evaluate the adsorption behavior of proteins with ion exchange and biospecific affinity adsorbents. The experiments were carried out at 25°C. Data of BSA equilibrium concentration in the liquid phase ( $c^*$ ) were obtained by spectrophotometric determination and the equilibrium concentration in the solid phase ( $q^*$ ) were calculated by mass balance. Values of the equilibrium adsorption parameters as the apparent dissociation constant ( $K_d = 0,16$  mg/ml) and the maximum capacity of adsorbent ( $q_m = 36$  mg BSA/g Adsorb.) were calculated by linear transformation of the Langmuir equation. From these results an application model of the adsorbent to BSA purification can be designed.

**87. PARAOXONASE AS MARKER OF ANTIOXIDANT DEFENSE IN RAT SERUM TREATED WITH CADMIUM**

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Cadmium poisoning has been known to result in a wide variety of cellular responses, including oxidative stress.

In our laboratory we observed that Cadmium (15ppm) in drinking water administrated during two months, provoke changes in the content of lipid in serum, without modification in HDL amount. On the other hand it is known that paraoxonase is an enzyme binding to HDL. In this condition our objective was measure the activity of paraoxonase enzyme (PON-1) that participate in the antioxidant defense mechanism. Simultaneously we measured the oxidative stress provoke by cadmium as sustance reactive to thiobarbituric acid. (TBARS). PON-1 activity was measured toward phenyl acetate as sustrate (arylesterase activity) by an enzymatic assay.

We observed that the TBARS were not modified. PON-1 activity increased significantly, in our experimental condition, showing that cadmium could increased the amount of enzyme binding to HDL or could increased the activity of enzyme by other mechanism in order to maintain a redox balance in serum.

**88. SERIC PARAMETER OF OXIDATIVE STRESS AND ANTIOXIDANT DEFEND IN HIPOTHIROID RATS**

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It is known that the hyperthyroidism cause oxidative damage, but the results obtained in hipothyroidism are contradictory. Our goal was to determine the thiobarbituric acid reactive substances (TBARS), paraoxonase activity (PON-1) as antioxidants and nitric oxide (NO) in serum. Ten rats were separated in two lots, one control (CG) and the other, hipothyroid group (HG) that received in the drink water 100mg/l of 6-propil-2-tiouracil during one month. (PTH). The initial and final body weight, the freeT4 and TSH were measured to confirm the experimental model. NO was measured by Griess reaction, PON-1 activity using phenyl acetate as sustrate. We observed that PON-1 was significantly increase in HG compared with the CG. No changes were observed in the amount of TBARS and NO. The hipothyroidism provoke increment of PON-1 activity in order to maintain an equilibrated oxidative stress.

**89.**  
**IS OBESITY A PREDICTIVE FACTOR OF TYPE 2 DIABETES MELLITUS IN FIRST DEGREE RELATIVES?**

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Type 2 Diabetes mellitus (DM2) is an evolutionary and silent disease, it is thought that more than half of the cases could be avoided if overweight is prevented. According to WHO (2002) approximately a 58% of world DM2 is consequence of a Index Body Mass (IBM) higher than 27.

To describe IBM, weist circumference (wc) and lipidic profile found in first degree relatives of DM2 patients in Santa Rosa, San Luis. 70 families from Santa Rosa, San Luis, with one DM2 parent and 125 non diabetics people were studied, both gender (M/F 72/53), (age 33,6 ± 1,1). IBM was calculated, and wc, blood glucose and lipids were determined.

IBM 29,13% with overweigh and 27,55% with obesity (56,68% IBM > 27); wc 65,3%; altered basal blood glucose 19,6%; triglycerides > 160 mg/dl 54,7%; total cholesterol > 200 mg/dl 52,1%; abnormal HDLc 45,6%.

The magnitud of the degrees of obesity and the other parameters suggest that the familiar relationship could be associated to a higher risk of developing DM2.

**90.**  
**PREVALENCE OF METABOLIC SYNDROME IN FIRST DEGREE RELATIVES OF TYPE 2 DIABETIC PATIENTS FROM A TOWN OF SAN LUIS PROVINCE**

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A big risk for type 2 Diabetes Mellitus development (DM2) is associated to a group of metabolic anomalies known as Metabolic Syndrome (MS) with an important inherited component.

To analyze the prevalence of MS in first degree relatives of DM2 patients using the International Diabetes Federation (IDF) criterion (2005) in Concarán.

30 families presenting one member with DM2 have been studied, 78 people were not diabetic, both gender (M/F 45/33) and age ranging 30,3 ± 2,3 years old. IBM was measured as well as weist circumference (wc), blood pressure (BP), blood insuline, blood glucose and lipidic profile. MS was defined according to IDF criteria. IBM: 26,41 ± 5,34; wc 89,15 ± 13,4 cm; blood glucose in fasting state 0,92 ± 0,1g/l; blood insuline in fasting state 8,69 ± 10,0 µUI/ml; total cholesterol 188,5 ± 46,1 mg/dl; LDLc 108,2 ± 44 mg/dl; HDLc 43,8 ± 14,3 mg/dl; triglycerides 158,2 ± 72 mg/dl; blood pressure 19,2% (15/78). Prevalence of MS according to IDF 38,46% (30/78).

Metabolic Syndrome in first degree relatives of DM2 patients is higher than in the general population (15 a 25%). This shows a similar genetic base for both gender.

**91.**  
**ANXIETY: SYMPTOMS AND DESEASES**

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This work's aim is to know the most frequent symptoms related to anxiety and to evidence the presence of any relationship between symptoms and diseases in university female students. A descriptive, transversal, exploratory study was made in 121 women, aged 18 to 39 (mean: 23.3 ± 4.07). Ss were given a self-report questionnaire: the Integral Clinical Evaluation Chart. Results indicate that, out of the 29 considered symptoms, the most frequent are (in this sample): back ache (41.3%) anguish (38%), attention problems (36.4%), fatigue (36.4%), hand perspiration (34.7%), bruxism (32.2%), head ache (31.4%), and irritability (30.6%). Persons were grouped accordingly to symptoms amount. Most frequencies are in groups of 4 (n=13), 5 (n=12), and 6 symptoms (n=12), up to a maximum of 21 symptoms, and a minimum of 0 symptoms. Out of the sample, 57.9% has more than five symptoms at a time. Out of 18 diseases considered, allergy (37.19%) was the one with more sample frequency. Ss with allergy were grouped accordingly to the anxiety symptoms amount. Greater frequency is distributed in the groups that have 4 and 5 symptoms. Data suggest a high frequency of anxiety symptoms. Besides, the person amount of symptoms, and the evidence that 57.9% of Ss with five or more symptoms are indicative of somatic anxiety manifestations, with no diagnostic category as a disease. There would be a concordance between anxiety symptoms and allergic status.

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**92.**  
**BIOTRANSFORMATION OF 3,γ,γ-DIMETHYLALIL-p-COUMMARIC ACID BY *Aspergillus terreus***

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3-γ,γ-(dimethylalil)-p-cumaric acid, isolated from *Baccharis grisebachii* (Asteraceae) an endemic specie of Cuyo region, is an attractive metabolite due to its activity toward insects. So, the preparation of new derivatives from the mentioned phenylpropanoid is an interesting task in order to study structure-bioactivity correlations. Biocatalysis is an alternative to perform stereo and regioselective bioreactions difficult to assess by chemical methods. Enzymes from microorganisms constitute appropriate tools since they accept xenobiotics as substrates. A growing cell biotransformation procedure was performed by using *Aspergillus terreus* cultures. Biotransformation was daily followed by TLC, and after eight days, the product was extracted from the fermentation broth with ethyl acetate. The organic layers were concentrated and subjected to TLC prep. furnishing a chromane derivative as a sole biotransformation product. This process constitutes an excellent example of green chemistry since the p-cumaric acid derivative was completely metabolized into only one more polar compound by in an environmentally friendly process.

**93. HIGH CONTENT OF DHA AND OMEGA 3 FATTY ACIDS IN EGGS FROM LAYING HENS KEPT IN A NEW REARING METHOD**

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Llovera method (MLI) was used—placing 10% of discarded laying hens, without conventional feeding— to ecologically control flies in laying hen houses, so, the use of Plague Integrated Management (MIP) was disesteemed, and an additional economic benefit for the producer was obtained by the recovery of the productivity of discarded hens and the insecticide savings, what represents a factor of economic and ecologic importance.

With the aim of obtaining data helping to clarify what was observed and to verify the quality of eggs from caged and on floor birds, a chemical study of inorganic ions and total nitrogen of bedding constituents was made, grid samples (0,20 m<sup>2</sup> randomized portions taken from the cage hen house) were obtained. Lipid from caged hen eggs (J) and on floor hen ones from MLI hen house (P) and those from control cages from a hen house without this method (C) were also compared.

It was verified that birds incorporate the oligoelements from soil and faeces, except potassium taken from larvae.

J eggs have a low cholesterol content and a high presence of DHA and omega 3 poli-insaturated fatty acids and a lesser contribution of arachidonic acid, being very useful for human health. P eggs have an important nutritive value. The ethologic association of LLI could explain the metabolic change.

**94. SUBSTANCE P NEUROPEPTIDE ON CELIAC GANGLION MODULATES THE OVARIAN STEROIDOGENESIS RESPONSE IN RAT**

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We have previously shown that stimulation of celiac ganglion (CG) with Vasoactive Intestinal Peptide (VIP) in the ex vivo integrated system CG-superior ovarian nerve (SON)-ovary (O) regulates the ovarian progesterone release. In this work we study in the same system the effect of CG stimulation with 50 ng/ml Substance P (SP), a sensitive neuropeptide that is presents in the CG, on the ovarian Progesterone (P) and Androstenedione (A) release. Also, its possible relation with ovarian nitric oxide (NO) releasing and nerve growth factor (NGF) mRNA expression were determined. Holtzman cyclic rats on diestrus 2 (D2) were used. CG and O were placed in different cuvettes containing Krebs-Ringer buffer, and incubated in a metabolic bath. After SP addition to CG, samples from the ovarian cuvette were taken at 30, 60, 120 and 180 min, to measure P, A (by RIA) and NO (by Griess reaction) release. Basal values were obtained without SP addition. Ovarian NGF mRNA was determined by RT-PCR. SP on CG had a differential effect on the ovarian steroidogenesis since it significant decreased the A release and increased of P release ( $p < 0.001$ ). Those changes occurred at 30 min of incubation and were maintained until 180 min, compared with basal values. This ovarian response was not mediated by modifications in the ON release and expression of ovarian NGF mRNA, in contrast to the effect of VIP on CG previously showed by us.

**95. SEXUALLY TRANSMITTED INFECTIONS: MISSING CASES**

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It has been observed that a number of patients do not know they have a sexually transmitted infection because they do not pick up the laboratory result, which implies they do not receive adequate treatment.

To investigate the inability to diagnose STI due to the fact that patients do not pick up the laboratory results.

Retrospective study (2004 to 2006). Data obtained from the register books for HIV, hepatitis B and syphilis patients.

3241 patients who did not pick up the HIV results (6,62%); 2820 patients who did not pick up the hepatitis B results (4,11%); and 9394 patients who did not pick up the syphilis results (4,21%).

The results that were not collected were non-reactive for HIV and hepatitis B, while 4 syphilis tests turned out to be reactive ( $R=0,01$ ). These diseases must be reported by the health care provider, otherwise the treatment and monitoring of the patient is hindered, free provision of medicines is not possible, and adequate epidemiological actions cannot be taken. Both the community and the medical staff should involve in campaigns to raise the public's awareness of STI. This would translate into a decline of STI as well as of the legal-medical risk and the cost for the state.

**96. OXIDATIVE STRESS IN POSTMENOPAUSAL WOMEN WITH HYPERCHOLESTEROLEMIA**

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Estrogens exert a direct atheroprotective action on blood vessels, and indirect action on lipid metabolism. In menopause, the estradiol deficit predisposes to hypercholesterolemia, which originates endothelial dysfunction by increasing oxidative stress and reducing of vascular NO bioavailability enhancing the atherosclerotic process. The aim of this study was to determine whether the hypercholesterolemia in menopause induces oxidative stress, alters endothelial function and modifies paroxonasa enzyme activity (PON1) which has antioxidant capacity. 37 postmenopausal women with hypercholesterolemia (HPW) and 39 normocholesterolemic postmenopausal women (NPW) were studied. In hypercholesterolemic women: total cholesterol, LDL-c and TBARS levels were increased ( $P < 0.0001$ ), and the nitrite levels decreased ( $P < 0.0001$ ) in relation to NPW group. PON1 activity, HDL-c, estradiol and androstenedione levels were similar in both groups. Total cholesterol and LDL-c were positively correlate with TBARS ( $P < 0.0001$ ), and negatively with nitrite ( $P < 0.0001$ ;  $P = 0.001$ , respectively). TBARS and nitrite were inversely correlated ( $P = 0.006$ ). These results suggest that the increase of oxidative stress is associated to hypercholesterolemia and the decrease of NO to the oxidative stress imposed on the endothelium.

97.

**EFFECT OF THE ENVIRONMENTAL SIGNALS ON THE CORTEX-ADRENAL MEDULLA INTERACTION OF VISCACHA (*L.m.m.*). SEASONAL BIOCHEMICAL STUDY**Rodríguez H, Penissi A, Forneris M, Domínguez S, Scardapane L. *Cátedra de Histología y Embriología. UNSL.*

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In wildlife mammals, the adrenal gland plays a fundamental role in the adaptative process to adverse environmental conditions. There is evidence of the cortex-adrenal medulla interaction in this process. The aim of this work was to analyze the effect of the environmental signals on the gland integral functioning through biochemical studies. Adult male viscachas were captured in their habitats throughout the year, taken to the laboratory, anaesthetized and sacrificed. The adrenal gland was immediately extracted and processed by biochemical techniques. Results: Summer: Tissular cholesterol (mg/100mg)  $47.54 \pm 17.35$ . Plasmatic corticosterone (ng/ml)  $1.75 \pm 0.216$ . Tissular adrenaline (ng/g):  $747.58 \pm 42.9$ . Tissular noradrenaline (ng/g):  $126.71 \pm 12.9$ . Tissular dopamine (ng/g)  $22.38 \pm 3.2$ . Winter: Tissular cholesterol (mg/100mg)  $25.10 \pm 3.38$ . Plasmatic corticosterone (ng/ml)  $4.65 \pm 0.37$ . Tissular adrenaline (ng/g):  $515.28 \pm 59.9$ . Tissular noradrenalina (ng/g):  $75.42 \pm 10.2$ . Tissular dopamine (ng/g)  $12.58 \pm 1.6$ . During winter the plasmatic corticosterone increases and the tissular catecholamines decrease, demonstrating an important liberation into the blood circulation. These results indicate a stressing process in the animal physiological behaviour. The hydric and dietary stress might probably modulate the *Lagostomus* adaptative behaviour as a survival strategy.

98.

**PRELIMINARY STUDIES WITH HIPERCALORIC DIET ON YOUNG MALE WISTAR RATS**

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There is strong evidence that genetic factors contribute to the development of obesity in humans as well as laboratory animals. Another important factor leading to obesity is an increase in energy intake. Bibliographic data it can be observed that commercial diets with high content in sacharose and fats provided to normal Wistar rats produce obesity. The aim of this work is to evaluate the activity of the AIN-93G diet when its content of sacharose and fats are modified.

We worked with a lot of ten male Wistar rats (21 days old). The animals were separated in two groups: one control, fed with AIN 93G (CD), and a problem group was fed with the modified AIN-93G (containing 34,146% sucrose, 42% of calories from fat). The experience lasted 28 days. All animals were weighed and food intake was measured throughout the study. At the end of the feeding period, after an overnight fast, animals were anesthetized and sacrificed. Serum and diverse organs were isolated weighed, frozen in liquid nitrogen, and stored at  $-70^{\circ}\text{C}$ . Perirenal fat and epididymal fat were weighed.

Important changes on the weigh of epididymal and perirenal fats and total lipids of liver were observed ( $P < 0.01$ ). Although studies made on serum, such as lipids parameters, glucose, proteins, TBARS, do not show significant changes.

These results indicate that feeding of hipercaloric diet on young male Wistar rats produces changes on the lipidic metabolism.

99.

**STUDY OF ANTIOXIDANT DEFENSE PARAMETERS AND OXIDATIVE STRESS IN PLASMA AND BLOOD OF OBESE CHILDREN**

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The problem of the obesity in children and adolescents is a constant concern of the organisms of health. The increase of the prevalence of the obesity associates with a decrease of the quality of life and an increase of the cardiovascular risk.

The objective of our work was to determine: -parameters of oxidative stress in serum, - levels of enzymatic and non-enzymatic antioxidant parameters in plasma and hemolized blood and determine the concentration of nitric oxide. The controls were non obese children ( $n=10$ ) and 10 obese patients with an age of between 5 and 15 years.

As a marker of oxidative stress in serum TBARS were determined. Reduced glutathione (GSH) and non protein thiols (TNP) in plasma and hemolized blood were the parameters of non enzymatic defense. The activity of paroxonase was determined, in serum as enzymatic parameter of antioxidant defense. Nitric oxide (NO) was also determined in serum

The results showed a no significant increase of the TBARS in obese children respect to the control, as well as NPT and NO. GSH values decreases in the same manner as paroxonase.

We can suggest that obese children present a tendency to diminish the defense parameters while they showed a slight increase of the oxidant parameters.

100.

**NON-REVERSIBLE EFFECT ON MOTILITY AND VIABILITY OF AZORELLAN-17,13- $\beta$ -OLIDE ON HUMAN SPERM CELL**

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There is a great deal of interest in searching for a contraceptive method to inhibit the functions of the male gamete. The investigations about compounds of vegetal origin have aroused an increasing interest in the pharmaceutical industry, mostly because of the success in the treatment of several diseases and their possible applications as contraceptive agents. The objective of this work was to investigate the effects of following natural products: mulinolic acid, mulinenic acid and azorellan-17,13- $\beta$ -olide, diterpenes from *Azorella trifurcata* and *Mulinum spinosum*, on motility and viability of sperm cell. Human semen samples from healthy donors were used. After swim-up separation using GPM culture media, highly motile sperm cells were recovered. The concentration was adjusted. After adding the reagents (DMSO 1% as a control; 0.5, 1 and 2 mM of the three reagents tested) sperm motility was evaluated according to the WHO (1999) and sperm viability was evaluated by using the water test (Lin *et al.* 1999). Sperm motility and viability were examined under light microscopy (400x). The reversibility of the effect was assayed by washing with fresh GPM. Our compounds showed a stronger effect on sperm motility and viability. The reversibility of the effect was apparent when using mulinenic and mulinolic acid, but not with azorellan-17,13- $\beta$ -olide that showed a complete.

**101.**  
**SYPHILIS: MONITORING OF REACTIVE CASES**

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Epidemiologically, the control of the disease implies improving diagnosis methods and starting the right treatment in due time, as well as monitoring reactive cases effectively.

Both the efficacy of the treatment and the evolution of the disease in patients with syphilis were evaluated. In order to determine the variation of titers with the quantitative VDRL test, 56 out of 158 patients were selected (31 women and 25 men over 13 y.o. – 13 to 65 y.o.). The monitoring, which started in 1996, was performed during 10 years. The results obtained were the following: Women: 3 VDRL negative cases (2 to 5 years\*); 22 with persistent 1 to 4 dils titers (5 months to 10 years\*); 3 with a rise in their titer values after a marked drop (4 to 10 years\*); and 3 with significant titer values that did not return for control after 3 years. Men: 3 patients with non-reactive serology (6 months to 9 years\*); 19 patients with 1 to 4 dils titers (6 months to 10 years\*); 1 patient with persistent 32 dils titer (6 years to date\*); and 2 patients that had initial high titers, but stayed at 8 dils (6 to 9 months\*) and did not return for control.

Even when the role of the laboratory is essential for detecting and monitoring the disease, it is important that professionals work together in order to improve patients care, which will eventually lead to obtain information about the illness, the importance of the right treatment and its periodical control.

(\*The figures between brackets show the time periods ranging from the beginning of the treatment to the last documented control).

**102.**  
**EVALUATION OF NEPHROPATHY RISK PREDICTORS IN MEN WITH TYPE 2 DIABETES**

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In the last years there has been a notable increase of diabetic nephropathy. The poor metabolic control of patients with type 2 diabetes can accelerate this complication. Dyslipidemia and the levels of glycosylated hemoglobin A1c (HbA1c) and uric acid have been identified as nephropathy risk predictors. Considering these antecedents, our study was designed to evaluate the levels of glucose, total cholesterol, LDL cholesterol, triglycerides and uric acid and to investigate their relationship with HbA1c in type 2 diabetic men and poor glycemic control (HbA1c <sup>3</sup> 7%). In diabetic men the glucose and triglycerides concentrations were significantly higher ( $P \leq 0.0001$ ,  $P = 0.01$ , respectively) and the HDL cholesterol concentrations, significantly lower ( $P = 0.0009$ ) in relation to the control group. In the diabetic group, a significant positive correlation between HbA1c and glucose ( $P = 0.002$ ), and a negative correlation between HbA1c and uric acid ( $P = 0.006$ ) were found. The results suggest that the poor metabolic control of these patients predicts nephropathy risk, and the chronic hyperglycemia might induce an increase of the renal excretion of uric acid, which might be the cause of lack of hyperuricemia in this poor glycemic control group.

**103.**  
**EXPRESSION OF B1 AND A3 INTEGRIN SUBUNITS DURING PORCINE GESTATION**

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The integrins constitute a glycoprotein family composed by  $\alpha$  and  $\beta$  subunits that mediate the interactions between cytoskeleton and extracellular matrix. They participate in adhesion, migration, invasion and cell physiology control. The porcine placenta is epitheliochorial, non invasive and diffuse; therefore to analyze the presence of placental integrins would allow us to understand the molecular interactions that make possible porcine gestation. The aim of this study was to determine the presence of  $\beta 1$  and  $\alpha 3$  integrin subunits in placental tissues of 37, 60, 70, 80 days of gestation, at term and empty uterus. Indirect immunocytochemistry techniques were performed to detect them.  $\alpha 3$  and  $\beta 1$  were found positive in empty uterus epithelium.  $\beta 1$  appeared negative while  $\alpha 3$  marked positive, both in uterine glands. Integrin  $\beta 1$  showed positivity with high expression in early fetal trophoblastic villi, blood vessels and fetal connective fibres, becoming lower at the end of pregnancy. Subunit  $\alpha 3$  was expressed in maternal and fetal trophoblastic villi, and in blood vessels throughout the pregnancy. This results suggests a probably role of  $\beta 1$  and  $\alpha 3$  integrin subunits in the molecular events needed for a successful adhesion of fetal placenta, decreasing  $\beta 1$  throughout the porcine gestation.

**104.**  
**EVIDENCE THAT RATS SHOW PREFERENTIAL CHOICE OF EXPLORATORY RESPONSES WHEN EXPOSED TO NOVEL ENVIRONMENTS: PRELIMINARY EVIDENCE**

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Laterality as a function has been intensively studied in these recent years, after Broca's description for the first time of specific lateralized neural region for spoken language in the left brain hemisphere in humans. Animal models have had difficulties to be used due to an extended belief that laterality is a unique characteristic of human beings only. The objective of the present work was to design and test several models in order to evaluate possible lateralized spatial-dependent choices in exploration of novel environments in the rat. Three models for spatial choices in novel environments were developed: (1) the "T" Labyrinth (TL), the Multiple Double Choices Labyrinth (MDCL), and (3) the Double Vertical Holeboard (DVH). In the "TL" model, rats have to choose any of two identical light-protected shelters, disposed to the right or left side of the animal in order to avoid a high power light source. In the "MDCL" model, rats have to choose any of two open doors located to the right or the left of the animal in successive compartments during natural exploration. In the "DVH" model, rats have to walk into a corridor and to choose any of two hole-board walls to the right or the left of the animal during natural exploration. Twenty-three intact animals were selected and sequentially subjected to the three spatial decisions models only once and for no more than 3 min. Results showed that no laterality in preferential spatial choice was found in the "TL" and the "MDCL" models. However, in the "DVH" model rats showed a median walks significantly higher for the left wall than for the right wall ( $1 \pm 0.3$  walks versus  $0 \pm 0.2$  walks,  $p < 0.05$ ); walking and rearing median times significantly higher for the left wall than for the right wall ( $47.5 \pm 5.8$  Counts/3 min versus  $39.5 \pm 3.7$  Counts/3 min,  $p < 0.01$ ). In conclusion, results suggest the presence of laterality in some exploratory behaviors where spatial decisions are available for the animal.

**105.  
COMPARISON OF BEHAVIOURAL PATTERNS  
DISPLAYED BY FOUR DIFFERENT STRAINS OF RATS IN  
THE ENRICHED HOLE BOARD TEST**

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Genetic of behaviour studies differences between individual patterns despite environmental conditions. In previous studies we have observed strains differences in *Rattus norvegicus*. The aim of the present study is to measure behavioural patterns displayed in an enriched hole board test by four different strains of rats (Holtzman [H], Wistar [W], Atriche IPL [A] y Sprague Dawley [SD]). Male rats weighing 230-250 g (n=15) were used. The following parameters were considered: ambulatory movements, non ambulatory movements, number of movements, rearing, horizontal activity, number of fecal boli, sniffing, head dipping, total exploration and time of contact with the novel object. The A strain showed a significant increase of ambulatory movements when compared with H and SD (p<0.05) and W (p<0.01) and number of movements compared to W (p<0.01), and a higher number of fecal boli vs H and W (p<0.001). SD differed also of H (p<0.01) and W (p<0.001) in the number of fecal boli, that were increased. No differences were observed in the hole exploration. We conclude that these genetic differences could be interpreted as a desinhibitory pattern of A strain when compared with other groups.

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**106.  
DIFFERENTIAL AVERSIVE AND MNEMONIC COMPONENTS  
DISPLAYED BY FOUR DIFFERENT STRAINS OF  
RATS IN THE PASSIVE AVOIDANCE TASK**

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In previous studies we have proposed a passive avoidance task in which aversive-anxiogenic and mnemonic components were differentially measured. We studied here differences between individual patterns despite environmental conditions as a way to understand genetic elements of behaviour comparing different behavioural patterns observed in strains of *Rattus norvegicus* in a passive avoidance task. Males of four different strains of rats (Holtzman, Wistar, Atriche IPL y Sprague Dawley) were studied, weighing 230-250 g (n=15). The following parameters were considered: latency 1, latency 2, and the number of fecal boli expelled during training and experiment. No differences were observed in latency 1 and latency 2. Wistar strain rats exhibited a low number of fecal boli during test (p<0.05) when compared to Atriche IPL and Holtzman. We conclude that this genetic difference could be interpreted as a lower affective reactivity of this strain when compared with Atriche IPL and Holtzman, without differences in memory component of the test.

*Present study was supported with a grant of the Secretary of Science and Technology of the National University of Cuyo (06/J164).*

**107.  
WORKING MEMORY AND EXECUTIVE FUNCTIONS  
DISPLAYED IN DIFFERENT STRAINS OF RATS IN THE  
HOLE BOARD TEST**

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Hole board test is usually accepted as a working memory and executive functions test. In the genetic of behaviour comparisons between individual patterns despite environmental conditions are studied. The aim of the present study is to evaluate working memory and executive functions comparing behavioural patterns displayed by four different strains of *Rattus norvegicus* (Holtzman [H], Wistar [W], Atriche IPL [A] y Sprague Dawley [SD]). Male rats weighing 230-250 g (n=15) were used. The following parameters were considered: ambulatory movements, non ambulatory movements, number of movements, rearing, horizontal activity, number of fecal boli, sniffing, head dipping and total exploration. The A strain showed a significant increase of ambulatory movements when compared with H (p<0.001), SD (p<0.01) and W (p<0.001), and horizontal activity compared to W (p<0.001), H and SD (p<0.05), and a higher number of fecal boli vs H (p<0.01) and W (p<0.05). A showed also lower values in sniffing, head dipping and total exploration of hole when compared with the other groups. We conclude that these genetic differences could be interpreted as a desinhibitory pattern of A strain when compared with other groups, with prevalence of locomotor activity and a decrease in holes exploration.

*Present study was supported with a grant of the Secretary of Science and Technology of the Nat. University of Cuyo (06/J164).*

**108.  
EPIDIDYMAL SPERM MATURATION: PROTEINS  
ISOLATED FROM EPIDIDYMAL FLUID MEDIATE  
SPERM RE ASSOCIATION *IN VITRO***

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-In many mammals' species during their epididymal journey, sperm associated by their heads when they reach the Cauda, but not in the proximal regions of this organ. These associations, called Rosettes were previously characterized in Mouse and Rat. This suggests a maturational process, probably due to the milieu that contains several proteins secreted by the caudal epithelium. The physiological meaning of this behavior remains unclear.

-Chromatographic techniques were used to fractionate epididymal fluid proteins extracted from adult male Wistar Rats. All the fractions obtained were evaluated at different protein concentrations to re associated motile and isolated sperm *in vitro*.

-Fraction N°2, enriched in proteins with MW 153, 130, 85, 26,3 24 and 16 Kda and affinity for Concanavalin A was the most effective for re associate sperm in Rosettes after their incubation *in vitro*.

-Additional purifications and monoclonal antibodies raised against these proteins will led us to a better identification of the factors involved in Rosettes assembling *in vivo*, and clarify their biological importance in male reproduction.

**109. GLUTAMIC ACID: LOCATION AND FUNCTIONAL STUDIES IN SPERM – SPERMATOGENESIS - AND FALLOPIAN TUBE IN MOUSE**

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The Glutamic Acid (Glu) is well studied at the central nervous system (CNS), although Glu receptors (GluRc) and Glu functions have not been fully described in sperms or fallopian tubes (FT). We reported the presence of GluRc in sperm cell and Glu as a triggering molecule of acrosomal reaction (AR). Now GluRc location was also extending to FT and mouse's testis. The GluRc was detected during the spermatogenesis in specific stadium related to acrosomal development. On the other hand, the same antibodies in immunostained the epithelium of FT according to sexual cycle. Again superfusion assays (SA) to establish the release – uptake of Glu was definitive demonstrated at the CNS, but not at the FT. In FT is important because is the physiological place for induction of AR. Recently, SA in the FT was positive. Moreover, these interchange had also a relationship with the stadium of the female sexual cycle. The uptake and release was different within the female cycle showing high uptake during diestrus and releasing at the estrus. Results indicate that sperm possesses GluRc – progressively expressed during spermatogenesis -, Glu promote AR and Glu is secreted and capture by FT - following the sexual cycle – to promote high level of Glu at the fertilization place and moment.

**110. CHANGES IN RAT SPERMATOOZOA SPHINGOLIPIDS DURING EPIDIDYMAL MATURATION AND ACROSOME REACTION**

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During their epididymal transit spermatozoa undergo a series of changes -maturation and capacitated changes - before they can undergo the acrosome reaction (AR) that enable them to fertilize an egg. The mechanisms underlying these events have not yet been fully elucidated. In this work we focused on their effects on the amounts and fatty acid composition of the main sphingolipid class of mature rat spermatozoa. Sphingomyelin (SM) and ceramide (Cer), lipids that in previous work were shown to be typically rich in very long chain polyunsaturated fatty acids (VLCPUFA) in germinal cells from the rat testis, changed with epididymal maturation. The content of SM decreased but Cer increased, so the ratio Cer/SM increased from caput to cauda epididymidis, suggesting the activation of a sphingomyelinase. The proportion of SM-associated VLCPUFA such as 28:4n-6 tended to decrease in SM and to increase in Cer with sperm maturation. Capacitation was assessed by protein tyrosine phosphorylation assay and AR was induced by stimulation of spermatozoa with A23187/Ca<sup>2+</sup> in the presence of albumin. Capacitation resulted in a significant increase of SM and Cer, and both increased additionally in acrosome-reacted spermatozoa as did the male germ-cell specific sulfogalactolipid (seminolipid), SM and Cer being richer than those of the starting cells in VLCPUFA, both non-hydroxylated and 2-hydroxylated. The sphingolipid changes may work synergistically with protein phosphorylation to convert an initially stable to a fusion-competent membrane, capable of engaging in the AR.

**111. HUNTER SCHREGER BANDS ENAMEL**

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The undulant itinerary of the prisms of the enamel gives origin to secondary structures that are denominated Hunter Schreger Bands (HSB). Some authors sustain that they constitute an optic phenomenon, taken place by the change of direction of the prisms that appear both in transverse and longitudinal sections in adjacent bands. This was also evidenced using scanning electron microscopy (SEM). Studies on the enamel microstructure consider HSB as a specific type of enamel, characteristic of placental mammals. The purpose of the work was to determine the frequency of appearance of HSB in temporary human teeth using different amplifications to SEM. Twelve temporary exfoliated teeth were included in epoxy resin, worn away with sandpapers of decreasingly fine grain, both in longitudinal and transverse planes. Samples were treated with hydrochloric acid, cleaned with and ultrasound device and metalized before SEM observations. Of the total of studied samples bands were observed in 58.33% of the cuts both in under and larger amplification. In 83.3% of the longitudinal cuts bands appeared with under amplification and with more amplification, while in 17.7% remaining bands were not observed with any amplification. In transverse cuts bands were observed with under and larger amplification in 33.3% of the samples. We concluded HSB were observed more frequently longitudinal than transverse cuts to SEM. In certain cases HSB appear as longitudinal and transverse arrays of prisms in alternate form, while in other cases, a "soft" change in prism direction is evidenced in adjacent layers without the characteristic aspect of bands. Key words: enamel- temporary- MEB.

**112. TREATMENT OF THE DENTINARIAS WALLS WITH THE LASER OF Er. YAG.**

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The present work intends to demonstrate the effects and changes produced in the dentinarias walls by the irradiation with laser of Er. YAG using different powers and frequencies with the purpose of evaluating the elimination of organic and inorganic matter of the walls of the radicular conduit put under the action of the laser of Er. YAG.

80 superior central incisors were used, selected according to clinical and X-ray criterion, treated endodónticamente, marked, cut and metalized. Next the Er. YAG laser was applied to it and was observed in M.E.B.

The partial results obtained show a great difference when applying different powers.

**113. REDUCTION OF *Escherichia coli* O157:H7 IN LETTUCE LEAVES WASHED WITH DIFFERENT CHEMICAL AGENTS**

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Efforts are being performing on sanitation of fresh products to protect the consumer's health. The purpose of this study was to determine the efficacy of different chemical washes in reducing *Escherichia coli* O157:H7 contamination on fresh leaves of lettuce. A total of thirty leaves were analyzed. Two leaves per treatment in each experiment were submerged in a *E. coli* O157:H7 suspension at concentration  $1 \times 10^9$  cfu/ml. They were stored at 22°C overnight. Leaves were individually placed in sterile bags and treated during 1 min with 50 ml of: 1) sterile distilled water (DW), 2) 200 ppm chlorine (C), 3) 0.1 mg/ml benzalkonium chloride (BzC), and 4) 1% lactic acid (LA). They were rinsed with 100 ml DW for eliminating chemicals and were dried. Counts were performed on Trypticase Soy agar (TSA) and Sorbitol Mac Conkey agar (SMC). Counts of total mesophilic aerobes on noncontaminated leaves reached  $6.94 \pm 0.03$  log cfu/leave and the initial bacterial level on *E. coli* O157:H7 contaminated leaves was  $8.06 \pm 0.12$  cfu/leave. For this group, the following reductions: LA (1.70 log)  $\geq$  C (1.31 log)  $>$  BzC (0.39 log) = DW (0.18 log), were observed after washing. Viable *E. coli* O157:H7 was detected in washing and rinsing liquids. The structure of lettuce leaves seems to be important in the protection of *E. coli* O157:H7 from inactivation by chemical agents.

**114. ANTIBACTERIAL ACTIVITY OF PROLINE, HYDROXYPROLINE AND DERIVATIVES**

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With the purpose of synthesizing sphingolipids and their analogues and demonstrate their potential capacity as cytotoxic agent toward eukaryotic and prokaryotic cells, we have assayed a wide group of natural amino acids and their derivatives. Free amino acids and their BOC-amino acids (t-buthyl-oxo-carbonyl-amino acids) derivatives were evaluated for their antibacterial potential against two Gram positive and two Gram negative bacteria regarding to their well known cytotoxic activity against eukaryotic cells. Antibacterial activity was measured by determination of minimum inhibitory concentration (MIC) using the broth microdilution method. Results showed that free aminoacids (proline and hydroxyproline) were the most active against Gram positive and Gram negative bacteria with MICs lower than 125 mg/mL, nevertheless BOC-proline and BOC-hydroxyproline had a weakly activity compared with the free amino acids.

**115. DIFFERENTIAL ANTIBACTERIAL ACTIVITIES OF BLF DERIVED PEPTIDES**

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Bovine mastitis is the most prevalent and costly disease in dairy farm. Bovine milk lactoferrin (bLF) is an iron-binding glycoprotein that seems to play an important role in non-specific response against mastitis infections due to its bacteriostatic and bactericidal activities. Treatment of bLF with proteases is reported to yield a hydrolysate which, while unable to bind iron, remains active against gram negative and gram positive bacteria. In this work, we performed pepsin bLF digestions at acidic pH. Peptides were subsequently separated by size using differential dialysis, resulting in two fractions: one larger than 12kDa and the other smaller. Our results show that antibacterial activity against *S. aureus* is restricted to low molecular mass peptides, whereas *E. coli* growth inhibition seems to depend on high molecular mass peptides. In order to further characterize these peptides, we performed analytical cationic exchange chromatography at pH 7.0. Most of the short peptide fraction was unable to interact with cationic resins, indicating its neutral or basic nature. In contrast, the large peptide fraction showed a more extensive interaction with this resin, demonstrating its cationic composition. Our results show that different bLF derived peptides exhibit differential activities against *E. coli* and *S. aureus*. Since these peptide fractions also appear to have dissimilar biochemical properties, it seems feasible that two different mechanisms are responsible for biological activities of bLF peptides against gram (-) and gram (+) bacteria.

**116. INVESTIGATION OF *Salmonella* AND *enterohemorrhagic Escherichia coli* O157:H7 FROM RAW CHICKEN IN SAN LUIS, ARGENTINA**

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*Salmonella* and *E. coli* O157:H7 are causative agents of foodborne infections in humans. The prevalence of these microorganisms in cut raw chicken and chicken hamburgers acquired at different stores was evaluated. Twenty five grams of sample were homogenized in 225 ml of buffered peptone water (BPW) for 1 min and incubated at 37° C during 12 h for *Salmonella* enrichment. Subcultures were done in Tetrathionate and Rappaport-Vassiliadis broths at 42°C for 24 h, and subsequently isolated on Brilliant Green (BG), Salmonella-Shigella (SS) and Eosin Methylene Blue (EMB) agars. Lactose non-fermenting colonies from BG and EMB, and H<sub>2</sub>S producers from SS, were transferred to Buenos Aires modified agar (BAM) and confirmed by conventional biochemical tests and serological assays using polyvalent OSA and OSB antisera. Simultaneously, 10 ml of BPW were incubated at 42°C for 6 h, and streaked on Sorbitol Mac Conkey agar for *E. coli* O157:H7 recovery. Sorbitol non-fermenting colonies were subcultured on nutrient agar and Simmons Citrate agar for serological and biochemical confirmation. Eight *Salmonella* strains were recovered from 100 analyzed samples. No *E. coli* O157:H7 strains were detected. Good hygienic practices during food manipulations are recommended as preventive rule to avoid infections by these microorganisms.

**117. MICROBIOLOGICAL QUALITY OF DEHYDRATED SOUPS CONSUMED IN SAN LUIS**

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With the name of Soup, the elaborated nutritional conserve with broths is designated. *Clostridium perfringens* is an important pathogen agent which causes, among others, enteritis in humans and enterotoxemia in domestic animals. The aim of this work was to establish the microbiological quality of dehydrated soups expended in San Luis city. Fifty and nine samples of dehydrated soups were processed, acquired in trade of it expended the public in San Luis city. The following determinations were carried out: count of total aerobes in agar medium count, coliforms count in MacConkey broth and EC broth (MPN), count of *C. perfringens* in milk iron medium (MPN), determination of *C. perfringens* in agar triptosa sulfite cycloserine (TSCA) and determination of enterotoxigenic strains of *C. perfringens* by RPLA. Total aerobic counts in the 59 samples were 102-104 cfu/g. Coliforms were detected in an only sample. *C. perfringens* was detected in 19 (63,33%) samples. None enterotoxigenic strain was obtained. This 19 samples gave counts <math>\lt; 3.0\text{-}43\text{ C. perfringens/g}</math> by the MPN method, 3 samples surpasses the limits suggested by the Alimentary Code (CAA) (Art 442). The microbiological quality in general was good but the incidence of *C. perfringens* in this samples was low a potential risk exists, in the event of improper handling during the preparation of the foods.

**118. PROTEASE ACTIVITY OF CLOSTRIDIUM PERFRINGENS STRAINS ISOLATED IN SAN LUIS**

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The enterotoxin produced by *Clostridium perfringens* is the etiological agent of *C. perfringens* food poisoning in humans. Protease formation has been correlated with food spoilage and pathogenicity in a number of clostridia. The production of proteolytic enzymes by 10 *Clostridium* isolated of varying toxigenicity and origin was studied to determine if all isolated secreted proteases. The strains were isolated from meat foods, condiments and spices and dehydrated soups consumed in San Luis. Five of the strains were enterotoxigenic and five no enterotoxigenic. The bacteria were grown in thioglycolated broth at 37°C for 6 h. Cell-free supernatants were obtained from cultures by centrifugation to 10,000 x g, 10 min and assayed for proteolytic activity by using two methods: Brock method modified using 0,2% azocasein and the absorbance determination at 450 nm; and the Dominguez and Cejudo method modified using 1% azocasein and the absorbance determination at 366 nm. All *C. perfringens* strains analyzed produced extracellular protease. The enterotoxigenic strains showed bigger activity than the no enterotoxigenic strains. Comparing the two methods, the Dominguez and Cejudo method modified was the method of but easy application and where the biggest activities were shown. The protease activities of the 10 strains examined indicate a quantitative difference that could be related with the biggest pathogenicity of the enterotoxigenic strains.

**119. SURVIVAL OF *Clostridium septicum* INCUBATED WITH MOUSE PERITONEAL MACROPHAGES UNDER AEROBIC CONDITIONS**

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*Clostridium septicum* is a gram-positive, spore-forming anaerobic bacteria that causes malignant oedema (clostridial mionecrosis) in animals and many associated diseases in man. The importance of macrophages in the host defense against *C. septicum* infections is unknown. In this report we examined *C. septicum* interaction with macrophages *in vitro*. Mouse peritoneal macrophages were grown in DMEM cell culture medium with 10% fetal calf serum and incubated in 5% CO<sub>2</sub> at 37°C. *C. septicum* ATCC 10092 cells from log growth phase were added at a multiplicity of infections (MOI) of 5:1, 10:1 and 20:1 to aerobically treated macrophages. The macrophages were lysed at increasing times post-infection and the surviving bacteria were plated in OGY medium and incubated under anaerobic conditions. *C. septicum* ATCC strain survived at a rate of 0.001 to 0.004% of the original inoculum with no significant differences in bacteria viability between 3 and 24 h post infection. In the absence of macrophages, under aerobic conditions, the bacteria died before three hours. Since *C. septicum* persists despite being phagocytosed this action may be advantageous because the intracellular environment of the macrophage may provide a lower oxygen concentration than is found outside.

**120. NITRIC OXIDE LEVELS REGULATE MACROPHAGE COMMITMENT TO APOPTOSIS OR NECROSIS DURING *Clostridium septicum* INFECTION**

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*Clostridium septicum* is an anaerobic bacteria that causes gas gangrene or clostridial mionecrosis in animal, and infections in humans mainly associated with colon carcinoma. Once the infection begins, it rapidly spreads to healthy tissues and, if left untreated the disease is always fatal. A hallmark of clinical gas gangrene infections is the lack of phagocytic cells at the site of infection. Nitric oxide (NO) has toxic effects on pathogens but can also have adverse effects on host tissue. We have examined whether mouse macrophages increase production of NO during *C. septicum* infection *in vitro* and if these NO levels can induce apoptosis or necrosis. Mouse peritoneal macrophages were grown in DMEM cell culture medium with 10% fetal calf serum and incubated in 5% CO<sub>2</sub> at 37°C. *C. septicum* ATCC 12464 from log phase were added at a multiplicity of infections (MOI) of 2:1; 20:1 and 150:1 to macrophages. Apoptosis was detected by optic microscopy, Annexin V CY3 and DNA ladder. Evidence of increased NO production was provided by the levels of nitrite (Griess reaction). Macrophages added at low MOI clearly induced macrophage apoptosis and low NO production while macrophages that were co-cultured at high MOI and also have internalized a large number of bacteria produced higher NO levels and become susceptible to necrotic death.

**121. EFFECT OF TUMOR NECROSIS FACTOR RECEPTOR p55 DEFICIENCY ON THE RESPONSE OF PERITONEAL MACROPHAGES TO *Yersinia* antigens**

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We demonstrated that TNFRp55 has a protective role in *Yersinia enterocolitica* O:3 reactive arthritis (ReA). The objective was to study the role of TNFRp55 on the macrophage response to *Yersinia* arthritogenic antigens. Thioglycollate elicited peritoneal macrophages from wild-type and TNFRp55<sup>-/-</sup> C57BL/6 mice were obtained. For killing assays, adherent cells were infected with *Yersinia* (moi 1:100) and intracellular bacteria were counted at 0 and 90 min. Lipopolysaccharide (LPS) and outer membrane proteins (OMP) were prepared. Macrophages were stimulated with LPS O:3, O:5, O:8 (10 µg/ml) or OMP O:3 (50 µg/ml). Nitric oxide (NO) production, as nitrite, was measured in 6, 48 and 72 h supernatants using Griess reagent. Morphological changes of the cells were evaluated. TNFRp55<sup>-/-</sup> macrophages showed impaired intracellular *Yersinia* killing ( $p < 0.05$ ). The NO concentration was higher at 72 h ( $p < 0.01$ ) and lower with LPS O:3 ( $p < 0.05$ ). NO production was increased in TNFRp55<sup>-/-</sup> macrophages ( $p < 0.001$  and  $p < 0.003$  with LPS or OMP stimulation, respectively). Differential morphological changes were detected in wild-type and TNFRp55<sup>-/-</sup> macrophages, and after LPS or OMP stimulation. We concluded that TNFRp55 influences the response of macrophages to *Yersinia* antigens.

**122. IgA RESPONSE TO *Yersinia enterocolitica*: ROLE OF Yop H**

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The *Yersinia* outer protein (Yop) H is a tyrosine phosphatase that contributes to immune response evasion. Mutation in *yopH* of *Y. enterocolitica* has shown to result in attenuation. The objective was to study the role of Yop H on IgA response after oral infection with *Yersinia* in normal and immunodeficient mice. C57BL/6, TNFRp55<sup>-/-</sup>, IL-12p40<sup>-/-</sup> and IL-4<sup>-/-</sup> mice were infected with  $5 \times 10^8$  wild type (WAP) or Yop H deficient (*sycH*<sup>-</sup>) *Yersinia*. Feces were obtained after infection (day 1-53) and bacterial number was determined. *Yersinia*-specific IgA in feces was measured by ELISA. The number of specific IgA-secreting cells in the spleen and mesenteric lymph nodes (MLN) was studied by ELISPOT assay. The *sycH*<sup>-</sup> strain was attenuated in wild-type and immunodeficient mice. On day 32, the number of bacteria was significantly higher in feces from WAP-infected C57BL/6 mice ( $p < 0.0001$ ). *Yersinia*-specific IgA response was detected in feces of wild-type and knockout mice after *sycH*<sup>-</sup> infection. IgA-secreting cell number was significantly increased in MLN of WAP infected mice ( $p < 0.006$ ). However, significantly lower IgA level was detected in feces after WAP compared with *sycH*<sup>-</sup> infection ( $p < 0.05$ ). We concluded that *Yersinia sycH*<sup>-</sup> mutant induces IgA secretion. YopH could contribute to impair IgA transport in mucosa.

**123. MULTIPLEX PCR FOR DETECTION OF VIRULENCE GENES IN *Yersinia enterocolitica* FROM FOOD AND FAECAL SAMPLES IN SAN LUIS CITY**

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Of the six biotypes (B) of *Y. enterocolitica*, five (1B, 2, 3, 4 and 5) are considered pathogenic in humans and contain markers associated with virulence. These are located on the chromosome (*ail*, *inv*, *yst*, *htrA*) and on the pYV virulence plasmid (*yadA*, *virF*). Multiplex PCR assays for the detection of plasmid- and chromosome-borne virulence genes of *Y. enterocolitica* isolated from a variety of foods (skin chicken, bovine and pork meat) and human patients (faecal samples) in San Luis city were developed. Besides, the reference *Y. enterocolitica* strain W1024 B2 O:9 was assayed. Specific PCR primers were obtained from sequences available in the GeneBank database (GeneDoc and Primer 3 softwares). Each multiplex PCR assay was performed in 50 µl of reaction mixture containing 3 µl of DNA, 1mM of dNTP mix, 1.5 mM of Cl<sub>2</sub>Mg, 0.5 X reaction buffer, 10 pmol of each forward and reverse primer and 0.25 U of Taq DNA polymerase. The samples were amplified for 35 cycles, and each cycle consisted of 1.5 min at 94°C, 1.15 min at 58°C, and 1.5 min at 72°C. *Y. enterocolitica* B2 strains were positive for *virF* and *invA* genes, while *Y. enterocolitica* B1A strains were positive for *htrA* and *yst* genes. Multiplex PCR assays showed simultaneous amplification of virulence genes from different biotypes of *Y. enterocolitica* isolated from a variety of foods and human patients in San Luis city.

**124. ANTIMICROBIAL SUSCEPTIBILITY OF *Yersinia enterocolitica* ISOLATED FROM FOODS AND FAECAL SAMPLES IN SAN LUIS CITY**

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*Yersinia enterocolitica* is an important food-borne pathogen which causes human infection. It is transmitted by oral route through contaminated food and water. The pathogenicity of *Y. enterocolitica* has been mainly associated with the serogroups O:3, O:9, O:8 and O:5,27. Because of enterocolitis is autolimited, antibiotic therapy is only suggested for treatment of extraintestinal clinical manifestations. The antimicrobial susceptibility of *Y. enterocolitica* strains belonging to different bioserogroups isolated from foods and faecal samples in San Luis city was examined. A total of thirteen strains were tested by the standardized disc agar diffusion method (Kirby Bauer technique) against eight antimicrobial agents. All the strains were susceptible to amikacin, ciprofloxacin, gentamycin and trimethoprim-sulphametoxazole and resistant to rifampicin and erythromycin. *Y. enterocolitica* strains isolated in this study were susceptible to most of the antimicrobial agents commonly used in clinical reports. Our local susceptibility data might assist clinicians in choosing appropriate therapy for patients.

**125. GROWTH OF THE CIANOBACTERIUM *Nostoc minutum* IN THE PRESENCE OF ARSENIC**

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The occurrence of arsenic in natural water has received significant attention during recent years, since elevated arsenic concentrations can be toxic to humans. Southern zones of San Luis present arsenic contamination in drinking groundwater. Microorganisms show ample variations in their sensitivities to arsenic species ranging from inhibition of *Microcystis aeruginosa* at 7 ppm As (V) to unaffected growth of *Chlorella vulgaris* at 2000 ppm As (V). The tolerance of *Nostoc minutum* (Nm) to different concentrations of arsenic was studied in order to use it for bioaccumulation of the metalloid since it gave good results in the bioremoval of cadmium. A screening with different concentrations of As(V) from 5 to 1000 ppm in static cultures was done. Agitated cultures were grown in 250 ml flasks at 5 and 1000 ppm As (V) and compared with a control culture. Nm was isolated locally and cultivated in BW<sub>3</sub> medium under diazotrophic condition. Batch cultures were incubated at 30°C with continuous illumination (9,84 klx) and pH control for 14 days. Growth was estimated by dried weight determinations. The final biomass values were 2,29g/l and 3 g/l in the presence of 5 and 1000 ppm of As (V), respectively. The control culture reached 2,59 g/l at the same period. Optical microscopic observations showed no differences between arsenic and control cultures. Nm was able to grow at both high and low concentrations of As (V), with even enhancing effect showing promising results in remediation of water with elevated concentrations of arsenic.

**126. STUDY OF THE MICROBIAL GROWTH IN RECREATION WATERS**

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Samples of recreation waters extracted from the Independence lake of Rosario city were analysed in order to determine the microbial quality parameters. The recounts realised were aerobic bacteria, coliforms, presence of *Escherichia coli*, *Pseudomona auroginosa*, mhos and ambient yeast. The probes were done taking into account ambient temperature, temperature and pH of the water samples. A system for obtain the samples operating by siphon was designed. By this way there is not direct contact with water and it permits extract it from different levels. The water samples were taking in sterile plastic recipients as the corresponding norms suggest. The volume sample was about 500 ml. The recounts were realised during two years with the following results: coliforms bacteria from 130 NMP/100 ml to 2200 NMP/100ml (Lauril sulphate culture broth, 48 hs, 35°C), mesophilas aerobic bacteria from 170 UFC/ml to 2300 UFC/ml (APS, 24 hs 35°C). In all the cases the results were positive for *Escherichia Coli* presence and negative for *Pseudomona auroginosa*. From these results we can conclude that the developing of the microbial population is in constant augmentation, being favoured for see weed growth (which was manifested by the dark greenish colour) and the organic material accumulation which produces a high turbidity in the recreation medium.

**127. ANTIMICROBIAL ACTIVITY OF LACTIC ACID BACTERIA ISOLATED FROM REGIONAL GOAT MILK**

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Lactic Acid Bacteria (LAB) produce antimicrobial substances called bacteriocins which can be used as biopreservants in manufactured food. *Listeria monocytogenes* is a food pathogen resistant to several commonly used preservation methods such as cold, heat, high salt and antimicrobial activity of LAB isolated from other sources. Our objective was to isolate bacteriocin-producing LAB from goat milk collected in "La Blanquita", a regional goat milk products manufacturer, and test their ability to inhibit the growth of *Listeria monocytogenes*. Goat raw milk samples were refrigerated and spread-plated on MRS agar within 4 hs after collection and incubated at 25°C for 48-72 hs under anaerobiosis. Total amount of colonies was  $1,2 \times 10^4 \pm 0,4 \times 10^4$  CFU/ml of milk. Fourteen strains were Gram (+), catalase (-) and oxidase (-) characteristic of LAB. Microscopy revealed the presence of cocci, bacilli and coccibacilli. The antimicrobial activity of the fourteen LAB isolates were tested against the *Listeria monocytogenes* CLIP 74902 strain using the spot on the lawn assay. Three strains showed inhibitory activity against *Listeria monocytogenes*. Regional goat milk could be a natural source of microorganisms with antimicrobial activity against *Listeria monocytogenes*, and might have a potential application as biopreservant in food industry.

**128. DETECTION OF *Yersinia* sp IN MEAT FOODS BY NESTED-PCR AND CULTURE METHODS**

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Yersiniosis is a human disease widely distributed, caused by *Yersinia enterocolitica*. The present of *Y. enterocolitica* in chicken and porcine products at retail level in San Luis city, was investigated. The samples studied were: 17 pure pork sausages, 22 pork and beef sausages, 21 minced meat, 24 chicken carcasses and 9 chicken sausages. The samples were tested by nested PCR which target gene was *yadA* and by culture methods: 25g of the sample were homogenized with 225 ml tripticase soy broth (TSB) and then incubated 18 h 25°C. After that, two aliquots were analyzed: 1 ml was studied by PCR and 100 µl were transferred to 10 ml of Modified Rappaport broth (MRB), and incubated at 4°C during 21 days. After the enrichment, plate isolations were carried out on cefsulodin-irgasanovobiocin agar (CIN) and Mac Conkey agar (MC). The cultures were incubated during 48 h at 25°C. Four strains were isolated and bioserotyped as *Y. enterocolitica* B:2 O:9, *Y. enterocolitica* B1 A, *Y. intermedia* B:2 auto agglutinable (AA), and *Y. intermedia* B:2 AA. By nested PCR, the *yadA* positive samples were: 7 samples (41.17%) from pure pork sausage, 7 samples (31.81%) from beef and pork sausage, 12 samples (57.14%) from minced meat, 7 samples (30.43%) from chicken carcasses. The strain *Y. enterocolitica* B:2 O:9 was detected by nested-PCR and culture methods too. The nested-PCR was more sensitive to detect *Y. enterocolitica* from different foods than the culture methods.

**129.****CULTURE AND NESTED PCR IN DETECTION OF PATHOGENIC *Yersinia enterocolitica* STRAINS FROM GOAT'S CHEESE**

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Cheese can be cause of foodborne infections due to inadequate hygienic practices in its manipulation. The sensitivity of culture techniques and PCR for detecting pathogenic *Yersinia enterocolitica* strains in artificially contaminated goat's cheese was assessed. Samples of 25 g each were placed in 225 ml of trypticase soy broth, modified Rappaport broth and a nutrient formula developed in our laboratory (3 samples per broth). They were inoculated with each one of three virulence plasmid – bearing *Y. enterocolitica* strains: W1024 O:9 (Belgium, reference strain), B2 O:9 (local origin, egg-shell) and B3 O:3 (local origin, clinical sample), at  $1 \times 10^4$  cfu/g (high inoculum, HI) and  $1 \times 10^3$  cfu/g (low inoculum, LI), and cultured at 25°C. At 0, 3 and 18 h, aliquots were withdrawn for counts on trypticase soy and Mac Conkey or cefsulodin-irgasan-novobiocin agars and for nested PCR directed to *yadA* gene. At time 0, *Y. enterocolitica* was detected in HI and LI samples by PCR. After 3 h, seven HI samples were positive by PCR and two of them showed characteristic colonies. Five LI samples were also positive by PCR. At 18 h, results were positive by both methods. Pathogenic and proteolytic bacteria were not detected in natural microflora.

**130.****COMPARISON OF THE MICROPLATE METHOD AND WELL AGAR PLATES ASSAY TO QUANTIFY THE HEMOLYTIC ACTIVITY IN LISTERIA MONOCYTOGENES**

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The hemolytic activity (HA) is a fundamental criterion for the differentiation and identification of *Listeria* spp. *L. monocytogenes* produces an hemolysin termed listeriolysin and it is also a marker of pathogenicity. Consequently, several methods to quantify it have been developed. We propose a new approach to quantify *L. monocytogenes* HA and compare it with microtiter method (MM). *L. monocytogenes* CLIP 74902 was obtained from the Pasteur Institute, France. The inocula were prepared at different concentrations (107 to 1010 CFU/ml). MM: serial twofold inocula dilutions were made with saline solution in a microplate and a 3 % suspension of horse blood was added. The HA was expressed as complete hemolysis units (CHU: the reciprocal of the highest dilution at which 100 % hemolysis was observed). Agar plates assay (APA): Mueller Hinton agar containing 5% horse blood (MHAB) or the MHAB supplemented with 0.2% activated charcoal and 1 mM CaCl<sub>2</sub> (ABAC+Ca) were prepared. Wells were made by using drill bit and inoculated with different inocula. The HA was expressed as the ratio of halos diameter surrounding the well to its diameter (HAR). Microplates and agar plates were incubated at 37°C for 6h. A linear regression between HAs and log CFU/ml were calculated. There was a strong correlation in APA on MHAB:  $y = 0,1218x + 0,2089$  ( $R^2 = 0,9504$ ) and ABAC+Ca:  $y = 0,1464x - 0,084$  ( $R^2 = 0,9928$ ) while MM shown a poor linear relationship. Therefore, APA allowed the AH quantification overcoming the MM in the assay conditions.

**131.****EVALUATION OF HELICOBACTER PYLORI VIABILITY BY USING PLATE COUNTING AND FLUORESCENCE MICROSCOPY**

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The viability of *Helicobacter pylori* isolates must be maintained during transportation of the gastric biopsy samples to the laboratory, for optimal recovery rates. The aim of this study was to assess the viability of *H. pylori* in two different transport media by using plate counting and fluorescence microscopy. A house transport medium (HTM) and Mueller-Hinton agar (MHA) supplemented with 5% fetal calf serum as reference medium were used. Two clinical isolates were employed as a suspension in agar plug that resulted in an inoculum of 105 CFU/ml. The agar plug were stored at 4°C for up to 7 days and were processed for culture like gastric biopsy, were streaked on MHA supplemented with 7% horse blood at 24, 96h and 7 days and incubated under microaerophilic conditions. The recovery from agar plugs at 24 or 96 h was higher in HTM than that in reference medium ( $0.8-1 \times 10^3$  vs  $0.35-0.4 \times 10^3$  at 24h and 56-110 colonies vs 12-25 colonies at 96h ( $p \leq 0.05$ )). The LIVE/DEAD BacLight viability kit in fluorescence microscopy clearly differentiated live from dead bacteria when the samples were processed at 96h and 7 days. Incubation exceeding 5-7 days never resulted in additional isolates in either of the two clinical isolates. Our results indicate that the HTM permitted the recovery of *H. pylori* and could be proposed as transport medium alternative to minimize the loss of bacterial viability when the distances between laboratories are long.

**132.****LARREA DIVARICATA CAV: CROSS-REACTIONS WITH PROTEUS VULGARIS AND PSEUDOMONAS AERUGINOSA**

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*Larrea divaricata* Cav. (jarilla) is an important plant ethnobotanically it is found in the Northwest of Argentina. This plant is widely used in folk medicine, it is employed from the treatment of microbial infections, wounds, rheumatism, inflammation of the respiratory and intestinal tract, gastric disturbances, tumors, ecc. In agreement to the bibliography consulted they are very scarce reports referred to the immunogenic activity of *L. divaricata* Cav proteins. The aim of this work was the determination of IgG levels from a crude extract of *L. divaricata* Cav and *P. vulgaris* and *P. aeruginosa* whole-cell proteins by ELISA test. Crude extract proteins were concentrated and partly purified (PPCE) by using membrane concentrators (Centriplus Amicon) with a 10 kDa cut off. The determination of cross reaction between homologous and heterologous antigens, was carried out by using a mice polyclonal sera. Polyclonal sera were obtained by immunization with PPCE. The titers of IgG were 1/800, 1/500 and 1/300 with PPCE, *P. aeruginosa* and *P. vulgaris* respectively. There was not significant difference when titers of homologous and heterologous tests (with *P. aeruginosa*) were compared. These results would be indicating a high immunogenic homology between crude extract of *L. divaricata* Cav and *P. aeruginosa* whole-cell proteins. Therefore this work propose the study of immunogenic proteins from *L. divaricata* Cav. crude extract and their importance as good immunogenic potential candidates in the obtention of vaccines.

## 133.

**EFFECT OF *Vibrio cholerae* NON O1 AND *Listeria monocytogenes* ON THE TOXINOGENESIS OF *Clostridium argentinense***

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Biological interactions between *C. argentinense* and some aerobic bacteria, can exert profound effects on the growth and toxin production by *C. argentinense* when those microorganisms share their ecological niche. In the present work, toxin production of *C. argentinense* strain G89 in coculture with *V. cholerae* non O1 and *L. monocytogenes* respectively, was investigated. The cocultures were performed in an optimized medium (OM) previously described. One volume of 0,1 ml of each culture strains for assay, were cocultured with 0.1 ml *C. argentinense*, in 10 ml OM, and incubated anaerobically at 37°C, 96 h. The cocultures were centrifuged at 10,000 x g, 20 min at 4°C and the toxin titres in the supernatants were calculated by the Reed and Muench method. Simultaneously monocultures of each microorganisms were included as control. When *C. argentinense* was cocultured with *V. cholerae* non O1, the toxin level (1000 LD<sub>50</sub>/ml) was 10 times higher than the level of *C. argentinense* monocultures (100 LD<sub>50</sub>/ml). However, association with *L. monocytogenes* completely inhibited toxin production. In the control tubes, growth of *V. cholerae* and *L. monocytogenes* were observed. The synergic or inhibitory effects on the toxinogenesis of *C. argentinense* evidenced on the cocultures, may be also influenced by other factors which must be fully investigated in order to characterize completely the association observed.

## 134.

**ANAEROBIC GROWTH OF *Pseudomonas mendocina* ON GLUCOSE ENHANCES BOTULINIC TOXIN PRODUCTION BY *Clostridium argentinense***

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The level of botulinic toxin of *Clostridium argentinense* is influenced by the presence of other microorganism which can display an important metabiotic effect. *Pseudomonas mendocina*, an aerobic bacteria, due to its wide metabolic versatility can adapt to growth at low redox potentials. The objective of this study was to analyze if the production of botulinic toxin was stimulated by the presence of *P. mendocina* under anaerobic conditions. Cocultures of *C. argentinense*- *P. mendocina* were obtained in a microfermenter containing 700 ml of a rich culture medium with glucose as carbon source. Coculture biomass was estimated by dry weight determinations, glucose by an enzymatic method and the level of botulinic toxin by bioassay in mice. The results were compared with monocultures of *C. argentinense*. The cellular growth, specific toxicity and toxin productivity were 4,1, 4,3 and 17,5 times higher than that of monocultures. Glucose was totally consumed after 40 h in cocultures, while in monocultures 50% of the initial amount was not metabolized. The important synergic effect on botulinic toxic production in cocultures can be explained on two grounds: the elimination of a potential catabolite repression of toxin synthesis mediated by the total consumption of glucose and the presence of uronic acids produced by *Pseudomonas mendocina* that could serve as additional carbon sources of slow assimilation

## 135.

**SUPERNATANTS OF *Clostridium septicum* INDUCE MACROPHAGE APOPTOSIS**

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*Clostridium septicum* is a gram-positive, spore-forming anaerobic bacteria that causes clostridial mionecrosis in animals and humans. The fact that *C. septicum* has a very low infective doses and rapidly develop to a clinically stage indicate that this bacteria possesses defense mechanisms against phagocytic cells-mediated killing activities. In this work we evaluated the capability of extracellular proteins of *C. septicum* to induce apoptosis in macrophages. Mouse peritoneal macrophages were grown in DMEM cell culture medium with 10% fetal calf serum and incubated in 5% CO<sub>2</sub> at 37°C. Culture supernatants obtained at mid log phase from *C. septicum* ATCC 10092 were added to macrophages at 6, 20 and 50%. The viability of macrophages was evaluated by MTT assays. Apoptosis was detected by morphological changes observed by optic microscopy, Annexin V CY3 and DNA ladder. Supernatants induced apoptosis in macrophages at 6 and 20%. Early apoptosis was detected at 16 h of incubation by Annexin V CY3 and late apoptosis at 36 h by fragmentation of DNA (ladder). Higher concentrations exert a marked cytotoxic effect. Bacterial numbers are probably low at the beginning of the infection; therefore the evasion of the innate immune system seems to be important for the onset of mionecrosis.

## 136.

**CIRCADIAN RHYTHM OF BLOOD PRESSURE AND ARTERIAL PULSE: INFLUENCE OF CHRONOTYPES AND TIME OF DAY**

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The chronobiologists have recognized three chronotypes: Morning-types (Mt), Evening-types (Et) and Neither-types (Nt), based in the different behavior of physiological and psychological circadian variables. In this study, we investigated the influence of chronotypes and time of day on systolic and diastolic blood pressures (SP, DP) and arterial pulse (AP). The Morningness-Eveningness Questionnaire was used to categorize 35-health young male (mean age=22.66 years, SD=3.36) in: 8 Mt, 11 Et and 16 Nt. The SP, DP and AP were measured by a digital sphygmomanometer during the morning (9:00-10:00) and the evening (19:00-21:00). The data were analyzed by the general linear model multivariate test. AP was higher in Mt at the morning and in Et at the evening (p<0.05). The AP of Nt was equal in the morning and the evening. No difference was observed in DP and SP respect to the chronotypes and time of day. Nevertheless, SP and DP showed a tendency with similar results to AP. In general, the cardiovascular functions present circadian rhythmicity. However, the rhythmical variations may maintain the period, but it may modify the phase. In fact, the circadian functions of Mt exhibit a phase advance vs. Et, and Nt an intermediate position. Based in this concept and our results, we suggest that the circadian rhythm of AP and blood pressure may be influenced by individual chronotypes and time of day.

137.

**NITRIC OXIDE, PARAOXONASE AND OXIDATIVE STRESS IN SERUM OF AGUDE INFARCT MYOCARDIUM***Ciacara C, Santilan L, Bechem N<sup>1</sup>, Datto M<sup>2</sup>, Navarro P<sup>1</sup>, Gimenez MS.**Proyecto 8104. <sup>1</sup>Laboratorios Privados and <sup>2</sup>Hospital San Luis.*

The infarct of myocardium is produced by the obstruction of coronaries artery. Our aim was measure the parameters of oxidative stress: The substances reactive to tiobarbituric acid (TBARS) and antioxidant defense: Paraoxonase (PON-1) in fifteen adult patients with agude infarct of myocardium (AIM) hospitalized in different health centers. We also measured nitric oxide (NO) in serum. NO play important roles in cardioprotection. Inhibition of nitric oxide synthesis reduces infarct size. The marker enzymes of AIM were measured: Lactate dehydrogenase, creatine phosphokinase, and creatine phosphokinase MB using commercial kits. In all cases their values were significantly increased in relation to the controls. NO was measured by Griess reaction and PON-1 activity using phenyl acetate as substrate. The results shown that TBARS and NO increase and as protective antioxidant response increase PON-1. In all cases a positive correlation between the marker of AIM and the others parameters measured was observed.

138.

**FAS B-10, FAS C-20 AND TRAIL EXPRESSION DURING PORCINE PLACENTATION***Cristofolini A, Merkis C, Zubeldía D, Barroso F, Lloret M, Sanchis G, Vaquer V, Koncurat M.**Área Microscopía Electrónica, Univ Nac Río Cuarto. Río Cuarto 5800 Cba. Argentina. E-mail: acristofolini@ayv.unrc.edu.ar*

The apoptosis is a highly regulated and preserved physiological process that occurs in different cellular types and it is essential to the development of placentation in most mammals. The aim of this research was to study the expression of FAS B-10, FAS C-20, TRAIL and DNA's fragmentation by TUNEL in empty uterus and placental samples from different gestational periods, to determine cell apoptosis involvement in porcine placentation. Histological slides from porcine placenta of 30, 55, 114 days of gestation and empty uterus were used. In TUNEL technique ApopTag® kit and detection of apoptotic receptors by Santa Cruz, Inc. antibodies were used. High score was determined. Results: the expression of FAS B-10, FAS C-20 and TRAIL receptors at 30 days of gestation was of 250, 230 and 155, respectively. Therefore, at the beginning of gestation, FAS receptor and other members of the TNF R1 superfamily were involved. At 55 days, negative apoptosis through TRAIL way was detected. At term, the apoptotic process occurred mostly through FAS way. In TUNEL technique apoptotic cells were identified in placenta of different gestational periods. In conclusion, TRAIL way was not detected at 55 days of gestation. At term of pregnancy higher cellular remodeling was observed, which would be due to apoptotic FAS receptors.

139.

**BIOACTIVITY ACUTE TOXICITY TEST USING AS EXPERIMENTAL MODEL BACTERIA***Mascotti ML, Bisogno F, Kurina M, Giannini F.**Fac. de Qca, Bqca y Fcia. UNSL. E-mail: fagian@unsl.edu.ar*

The potential bioactivity of a compound can be associated with its toxicity, taking into account the noxious effect that drugs presents toward living organisms. This evaluation can be done using multicellular organisms as crustaceans, rotifers, fishes, amphibians and even for more advanced studies mammalian; but those assays are very expensive, requires important quantities of drugs and even are very conflictive because of animals law's protection.

We present in this work the development of a bioassay requiring bacteria as experimental model, specifically *Lactobacillus acidophilus* and *Bacillus subtilis*. Commercial lyophilized bacteria are suitable for this assay.

As a primal stage of this work we standardized conditions that direct the assay as amount of initial inocula, optimal incubation time, culture media and positive controls. The obtained results allow us to present it as a valid, fast, economic, reproducible tool, easy to handle assay, requiring low amount of tested compounds, in order to measure potential drug bioactivity and toxicity.

140.

**DISTINCTION BETWEEN *Clostridium botulinum* STRAINS ISOLATED FROM INFANT BOTULISM CASES AND FROM OTHER ORIGINS***Barzola CP, Lúquez C, Sagua MD, Fernández RA.**Área Microbiología, Fac Cs Médicas, UNCuyo, 5500 Mendoza. E-mail: rferrand@fcm.uncu.edu.ar*

Infant botulism (IB) is caused by botulinum neurotoxin (BoNT). Most cases are due to *Clostridium botulinum*, however specific characteristics of these strains are unknown. The present work aim was to determine antimicrobial sensibility and biochemical characteristics of *C. botulinum* isolated from IB cases, and ganglioside binding and haemagglutinin (HA) activity of its toxin. We studied 27 type A strains: 13 from IB cases and 14 from other origins. Minimal Inhibitory Concentration (MIC) was determined for ampicillin (AMC), clindamycin (CLI) and metronidazole (MTZ). Biochemical characterization was performed by usual tests for anaerobe bacteria. Ganglioside binding was determined by immunostaining Thin Layer Chromatography (TLC) and haemagglutinin activity by a microtitration method. AMC, CLI and MTZ MIC values were between 0.06 - 2 µg/mL, 16 - 32 µg/mL and 0.25 - 64 µg/mL, respectively. According to biochemical tests all strains corresponded to Group I (proteolytic) and produced HA negative BoNT. All BoNT bind to GD1a and GM1 receptors. Binding to others gangliosides was relatively specific for each BoNT. These results suggest that antimicrobial susceptibility, biochemical characteristics and HA activity of BoNT does not present significative differences to characterize these strains. For this purpose ganglioside binding could be used.

**141. ANTAGONISM OF LACTIC BACTERIAS AND TWO STRAINS CAUSING BOTULISM**

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Some scientific publications evidence the LB (lactic bacteria) antagonistic activity on Cb (*Clostridium botulinum*). This microorganism, Cb, is reason of foodborne botulism that could be present in package homemade foods or "fresh-like" minimally processed foods and of human infant botulism. Increase in outbreaks cases of foodborne botulism in Argentina was reported in 2006 for ANLIS-Malbrán, whereas infant botulism is the major frequency in the world. In this work was select LB select from fermented vegetables to screen them for their influence in the development of two (A-Jardinera and A-BLMza 81) strains isolated of one foodboorne outbreak and the other for one infant botulism case both of Mendoza. Isolation of BL was for streak in surface fermented vegetables broth onto MRS (Man, Rogosa, and Sharpe) agar. Chopped-meat medium was used for Cb. The growth of Cb in presence of each LB was done employing the modified diffusion agar (Cb in surface and one strike of LB), to measure inhibition Fleming's modified diffusion test was used. Therefore isolated cultures as inhibition cultures were incubated in anaerobic medium to 35°C. Four strains were selected which produced a greater zones inhibition. Three of the selected ones, resistant to bile and NaCl which inhibited both Cb strains, there are: *Lb.paracasei ssp paracasei*, *Lb.plantarum* and *Lb brevis*. The results suggest your possible use in starters and functionals foods.

**142. EFFECTS OF *Chlamydia trachomatis* INFECTION ON INTERCELLULAR UNIONS OF EPITHELIAL CELLS IN CULTURE**

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The cadherin/catenin complex serves as an important structural component of adherens junctions in epithelial cells. While studying the effects of the bacterial pathogen *Chlamydia trachomatis* on human cervical epithelial cells in culture, we observed that *C. trachomatis* caused the epithelial cells to separate from each other without detaching from their growing surface. The objective of the present study was to determine if this effect might involve the disruption of the cadherin/catenin complex. HeLa cells were infected with *C. trachomatis*. Cadherin-like immunoreactive materials and beta-catenin were visualized by immunofluorescence. In noninfected cells, N-cadherin and beta-catenin were colocalized at the intercellular junctional complexes. By contrast, the infected cells showed a marked loss of both N-cadherin and beta-catenin labeling from the junctional complexes and the concomitant appearance of intense beta-catenin labeling associated with the chlamydial inclusion. These results indicate that *C. trachomatis* causes the breakdown of the N-cadherin/beta-catenin complex and that the organism can sequester beta-catenin within the chlamydial inclusion. This could represent an important mechanism by which *C. trachomatis* alters epithelial cell function

**143. BACTERIAL PLAQUE CONTROL AS PREVENTION OF PERIODONTAL ILLNESS IN ADOLESCENTS**

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In periodontal illness. Gingivitis periodontitis and bacterian plaque are ethiological factors. In adolescents, hormonal changes predispose to gingivals alterations as inflammation and bleeding, many times with painful symptomatology. This situation prevent a correct brushing of the dental pieces and contribute to the settling of the bacterian plaque- In a subsequent condition the periodontal ligament will be affected. The object of the present work is to determine the presence of the bacterian plaque to point preventive actions to control the periodontal illness in adolescents. A seventy five adolescents, from polimodal level of a school in Ensenada city, were examined during 2005. There was achieved in two stages during the year. In the first one from march to april, was determined the incidence of the bacterian plaque with developing reactives. The result was 80% of incidence. Preventive actions were to teach the effective brushing techniques. In the second stage from September to October, the results were confronted with de same sample. The result was a decrease of 35% in the incidence of the bacterian plaque. The conclusion of the proceeding was that the correct brushing, certainly diminish the incidence of the bacterian plaque.

*Key words.* Gingivitis. Plaque. Incidence. Periodontitis.

**144. LOCATION, ISOLATION AND CHARACTERIZATION OF PROTEIN WITH THIOL GROUPS DETECTED DURING MATURATION IN SPERM**

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Oocyte fertilization involve maturate and capacitated sperm. The sperm support maturation during the epididymal transit and capacitation during the stadium in the female genital tract. Changes in thiol oxidation, to disulfide bonds, in sperm proteins are verified during epididymal trip. Instead, the capacitation status is assumed by an increment in tyrosine phosphorylated sperm protein (p-Y). Using sonication and ultracentrifugation in sucrose gradient we fractionated and isolate sperm head and tails from mature vs. immature or capacitated vs. non-capacitated sperm cell. Them incubation of fractions with fluorescence monobromobimane (mbb) permitted thiol proteins stain. The cell location of these proteins was established in sperm head or tail by microscopy and molecular weight by standard SDS-Page. Parallel gel was also run to perform an immune-blot for p-Y. These gels were used to compare the thiol or/and p-Y amount. Some samples from the sucrose gradient were incubated with 1% Triton X100 and soluble vs non-soluble proteins were also analyzed with the protocol above mention. Phosphorilated and mbb marked proteins were coincident in mature and capacitated sperm and were isolated in soluble fraction of sperm. Results indicate that same proteins are stabilized during maturation by thiol changes and phosphorilated during capacitation.

145.

**ADMINISTRATION OF NEUROTRANSMITTERS IN THE AUTONOMIC GANGLIA MODIFIED THE TESTIS HORMONAL SECRETION IN AN *IN VITRO* SYSTEM***Cavicchia JC, Palmada N, Fóscolo M, Sosa M.**Inst.de Histología y Embriología (IHEM). Fac. de Ciencias Médicas U.N.Cuyo; and <sup>1</sup>LABIR U.N.S.L. E-mail: jccavic@fcm.uncu.edu.ar*

Early evidences indicate that acetylcholine administered to the inferior mesenteric plexus (IMP) inhibits testosterone ( $T_2$ ) secretion from the testis using an *in vitro* system in which we used two separate containers, one for the testis and the other for the plexus and both interconnected by the spermatic nerve. In our new experiments we administered another neurotransmitter, nor epinephrine (NE) in two autonomic nerve ganglia, the IMP and the superior mesenteric ganglion (SMG). The  $T_2$  liberation in the testis compartment was measured by RIA. Data were compared by Student's t test. Results: (Mean (ng/mg testis/ml)  $\pm$  SEM). When NE was added in the IMP container  $T_2$  release underwent a progressive significant increment ( $p < 0.001$ ) while with the SMG we observed no significant changes. These results indicate that the neural ganglionic stimulation clearly participates in the hormonal release from the testis, which depends of the neurotransmitter type and the ganglion involved. The present results make it evident that not only the well known classical hypothalamus hypophysis axis but also that the peripheral nervous system via the autonomic ganglia is directly involved in the hormonal control of the testis.

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146.

**POLYPOID CELLS CONTAINING AN ENDOCYANOBIONT IN THE MIDGUT GLAND OF THE APPLESNAIL *Pomacea canaliculata****Cueto JA, Vega IA, Koch E, Castro-Vazquez A.**Lab. of Physiology, IHEM (FCM/UNCuyo-CONICET), Mendoza Argentina. E-mail cueto.juan@fcm.uncu.edu.ar*

*Pomacea canaliculata* (Caenogastropoda, Ampullariidae) is a South American freshwater snail which lodges a cyanobacterium in epithelial cells of its midgut gland. Two morphotypes of this cyanobacterium (identified as C and K morphs) are each one associated to a specific epithelial cell type in the gland. C morphs are contained within "columnar", probably digestive cells bearing regular size nuclei, while the K morphs are associated to "pyramidal" cells bearing large nuclei, with two or more nucleoli, and a conspicuous development of the rough endoplasmic reticulum. Electron microscopy studies suggested that pyramidal cells contribute to the generation of the thick protein cover of K corpuscles. In the current work we wanted to establish if the larger nuclear size might be indicating a greater ploidy of pyramidal cells, which may mean a larger extent of protein expression by these cells. Squash, paraformaldehyde-fixed preparations of adult glands were subjected to the Feulgen reaction to establish, through image cytometry, the relative DNA quantity present in the different nuclei. The obtained histograms showed the existence in the midgut gland of *P. canaliculata* of several cell populations with different DNA-ploidy (2, 4, 8 and 16 C). Polyploid nuclei (i.e., those showing either 4, 8 or 16 C) where the larger ones, and with prominent nucleoli. Feulgen-treated embryos' squashes also indicated definite polyploid cells populations, i.e., at a time when K corpuscles have not yet colonized the midgut gland.

147.

**ELECTROPHYSIOLOGICAL ACTIONS OF TAMOXIFEN DURING ISCHEMIA AND ITS EFFECT UPON REPERFUSION ARRHYTHMIAS***Diez ER, Ponce Zumino AZ, Carrión A, Dupanlou ML.**Fac Cs Med, Univ Nac Cuyo. 5500 Mendoza.**E-mail: aponce@fcm.uncu.edu.ar*

According to recent reports tamoxifen (TX) is an effective antioxidant that protects membranes against oxidative damage and also blocks several ionic currents. We try to investigate if these abilities of TX may lead to a cardioprotective effect on incidence and duration of reperfusion arrhythmias (RA) in perfused isolated rat hearts. The protocol consisted in a 15 min preischemic (PI) period, 10 min occlusion (O) of the left coronary artery and subsequent 15 min of reperfusion. Action potentials (AP) and ECG were recorded; also coronary flow (CF) and total antioxidant capacity (TAC) were determined. We performed 3 series of 10 experiments each: control, (C); tamoxifen 1  $\mu$ M, (TX1) and tamoxifen 2  $\mu$ M, (TX2). Data were statistically analyzed with ANOVA I and  $\chi^2$ . Results demonstrated that TX reduces the incidence of RA from 70% in C to 30% in TX1 and TX2. TAC values (as % of ABTS inhibition) were 25.57, 41.59 and 42.37 for C, TX1 and TX2 respectively ( $p < 0.0001$ ). During PI and O TX1 showed lengthening of AP duration. AP amplitude, heart rate and CF did not show changes. These data suggest that the protection observed with TX may be due to its antioxidant action and that the few changes in electrophysiologic variables were a consequence of its blocking action upon  $I_{kr}$  and  $I_{CaL}$  currents.

148.

**NUTRACEUTICAL ANTIOXIDANT CAPACITY IN PERFUSED HEARTS***Dupanlou ML, Ponce Zumino AZ, Miatello RM.**FCMédicas, UNCuyo and IMBECU-CONICET, Mendoza.*

The usefulness of antioxidant nutraceutical (AN) consumption has been postulated in cardiovascular diseases prevention. The aim was to examine changes in total antioxidant capacity (TAC) in heart tissue after perfusion of isolated hearts (Langendorff) with modified Krebs-Henseleit solution containing AN.

TAC was analyzed in left ventricular tissue homogenates by using Katalinic modified technique, by reaction with 2,2'-azinobis-(3-ethyl-benzathiozoline-6-sulfonic acid (ABTS+), compared with an ascorbic acid pattern and expressed as percentage of ABTS+ inhibition. Results are expressed as mean  $\pm$  sem and processed by ANOVA followed by Bonferroni post test.

TAC in control hearts (n=11) was 22.8  $\pm$  0.8%. Wine solution (0.75% v/v) perfused hearts (n=9) increased their TAC to 31  $\pm$  0.7% ( $p < 0.01$ ). Perfusion solution containing aqueous garlic extract (20 and 40 mg/L) increased TAC to 27.83  $\pm$  1% (n=10;  $p < 0.01$ ) and 38  $\pm$  3% (n=7;  $p < 0.001$ ), respectively.

Obtained data suggest that cardiac tissue antioxidants capacity could increase when nutraceuticals were administered and are consistent with the hypothesis that postulate the beneficial effect of AN consumption in the prevention of cardiovascular disease.

**149. URIC ACID AND ALLANTOIN IN AN EXPERIMENTAL MODEL OF STRESS**

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The South American applesnail *Pomacea canaliculata* accumulates uric acid in large and ubiquitous stores throughout its body. This snail may undergo a period of seasonal dormancy (called "estivation") when the limnotopes they inhabit dry up, and uric acid may serve an antioxidant role when dormancy is interrupted and a reperfusion oxidative stress follows. In the current study we found that uric acid content of the snail significantly increased during estivation, and that it dropped significantly within 24 hours after raising from dormancy; uric acid is not excreted in this species, and therefore, the decrease in uric acid stores should be explained by alternative mechanisms. We have also found that allantoin (an oxidation product of uric acid) is accumulated during estivation, and its accumulation diminishes 24 hours after reactivation. Notably, allantoin excretion increased after reactivation, while the excretion of urea and ammonia decreased, suggesting that uric acid was indeed acting as an antioxidant compound during reactivation. However, the reactivation-induced decrease of the snail's total uric acid content cannot be quantitatively explained by its transformation into allantoin since the excreted amounts are very low if compared to the tissue amounts. Hence, other fates for uric acid should be looked for in future studies. However, regarding the possible role of uric acid as an antioxidant compound, it should be emphasized that its antioxidant activity should be restricted to the comparatively small circulating fraction of this purine, and that its transformation into allantoin should probably occur only there.

**150. EXPRESSION OF MYOSIN ISOTYPES AND MORPHOLOGY OF PERITUBULAR MYOID CELLS DURING TESTIS DEVELOPMENT**

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During rat testis development, seminiferous tubules (ST) growth to achieve the adults diameter. Peritubular myoid cells (PMCs), that surround ST, express the cytoskeletal markers of true smooth muscle ( $\alpha$ -isoactin and PMC-myosin). With the aim to know the patten of PMC differentiation, the expression of PMC-myosin isotypes and the morphology of PMCs during rat development were analysed. PMC myosin isotypes were purified and analyzed in 4% Urea-SDS-PAGE. To determinate the patten of PMC contraction, STs were stimulated to contraction with Endotelin 1 (E-1). PMC's morphology (x,y axes and surface) was assayed with Scion Image I Program from AgNO<sub>3</sub> stained STs. PMCs of 12 days old rats expressed 3 isotypes of PMC- myosin: 205 (A), 200 (B) and 195 (C) kDa respectively, where the relative percent of expression was: A 20; B 20; C 60. PMCs of 16-27 year old rats expressed: A 33; B 32, C 35. Meanwhile rats of 43-90 days old expressed: A 45; B 45, C 10. PMC's x, y axes form 16 year old rats were:  $19,2 \pm 2$ ;  $18,9 \pm 3$   $\mu\text{m}$  respectively and the surface was of  $540 \pm 59$   $\mu\text{m}^2$ . PMC's x, y axes from ST contracted with E-1 showed:  $18,3 \pm 5$ ;  $19,1 \pm 3$   $\mu\text{m}$  respectively, with a surface of  $507 \pm 90$   $\mu\text{m}^2$ . PMC's x, y axes from 43-90 year old rats were of  $34,8 \pm 5$ ;  $26,1 \pm 5,5$   $\mu\text{m}$  respectively with a surface of  $810,5 \pm 185$   $\mu\text{m}^2$ . PMC's x, y axes of E-1 ST contracted, were of  $28,2 \pm 3,5$ ;  $26,7 \pm 4,5$   $\mu\text{m}$  respectively with a surface of  $539,4 \pm 145$   $\mu\text{m}^2$ . PMCs present an active cytodifferentiation during ST development.

**151. DISINHIBITORY-LIKE EFFECT INDUCED BY INJECTION OF CHLORAZEPATE DIPOTASSIUM WITHIN THE MEDIAL PREFRONTAL CORTEX OF MALE RATS IN THE PLUS MAZE TEST**

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The medial pre-frontal cortex (mPFC), like amygdala (Amy) and ventral hippocampus (Hip), projects to the Nucleus Accumbens Septi (Acc) through glutamatergic pathways. The mPFC projections have been implicated in goal directed behaviors. Glutamatergic transmission within Acc modulates these effects. We studied here behavioral changes induced in the plus maze by the injection of chlorazepate dipotassium within one of the origin nucleus of Acc afferences, mPFC. Rats bilaterally implanted by stereotaxic surgery were injected with saline (1  $\mu\text{l}$  each side, n=15-18), or chlorazepate dipotassium (1 or 2  $\mu\text{g}$  in 1  $\mu\text{l}$ , n=15-18). The drug injection induced a decrease in time per entry ( $p < 0.05$  for both doses) when compared to its control group. We conclude that GABAergic blockade within mPFC leads to behavioral stereotyped entries to the open arm when injected within the mPFC.

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**152. ANXIOLYTIC EFFECT INDUCED BY INJECTION OF CHLORAZEPATE DIPOTASSIUM WITHIN THE AMYGDALA OF MALE RATS IN THE PLUS MAZE TEST**

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The amygdala (Amy), like medial pre-frontal cortex (mPFC) and ventral hippocampus (Hip), projects to the Nucleus Accumbens Septi (Acc) through glutamatergic pathways. The Amy is a structure that have been implicated in anxiety. Glutamatergic transmission within Acc modulates these effects. We studied here behavioral changes induced in the plus maze by the injection of chlorazepate dipotassium within origin nucleus of Acc afferences, Amy in this case. Rats bilaterally implanted by stereotaxic surgery were injected with saline (1  $\mu\text{l}$  each side, n=15-18), or chlorazepate dipotassium (1 or 2  $\mu\text{g}$  in 1  $\mu\text{l}$ , n=15-18). The drug injection induced an increase in time spent in the open arm ( $p < 0.01$ , 2  $\mu\text{g}$ ), open arm entries and open/closed arm quotient ( $p < 0.05$ , 2  $\mu\text{g}$ ), and extreme arrivals ( $p < 0.05$  for both doses). We conclude that GABAergic blockade within Amy induced an anxiolytic-like effect, with several characteristic behavioural patterns.

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153.

**BEHAVIOURAL PROFILE INDUCED BY INJECTION OF CHLORAZEPATE DIPOTASSIUM WITHIN THE HIPPOCAMPUS OF MALE RATS IN THE PLUS MAZE TEST**

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The ventral hippocampus (Hip), like medial pre-frontal cortex (mPFC) and amygdala (Amy), projects to the Nucleus Accumbens Septi (Acc) through glutamatergic pathways. Glutamatergic transmission within Acc modulates the effects of these projections, mainly Hip, that has a gating effect on Acc afferences. We studied here behavioral changes induced in the plus maze by the injection of chlorazepate dipotassium within origin nucleus of Acc afferences, Hip in this case. Rats bilaterally implanted by stereotaxic surgery were injected with saline (1  $\mu$ l each side, n=15-18), or chlorazepate dipotassium (1 or 2  $\mu$ g in 1  $\mu$ l, n=15-18). The drug injection induced an increase in the time spent in the open arm in saline controls, that were decreased by the lower but not the higher dose ( $p < 0.05$ ), like open arm entries ( $p < 0.05$ ). The open/closed arm entries, starting also with high control values, were decreased by both drug doses ( $p < 0.05$ ). We conclude that GABAergic blockade within Hip leads to an interference with hippocampal modulation of anxiety and environment recognition.

*Present study was supported with a grant of the Secretary of Science and Technology of the National University of Cuyo (06/J164).*

154.

**SUPPLEMENTAL ANTIOXIDANT INTAKE AND PROSTATE CANCER RISK IN A COHORT OF MEN IN MENDOZA**

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Prostate cancer (CAP) is one of the most commonly diagnosed, yet the etiology of this disease is largely unknown. There is evidence that antioxidants, may help prevent some forms of cancer. The objective is to describe a male population with high risk of CAP. A sample of 20 men from 50 to 75 years old with high risk of CAP was selected for the study. The research included an initial and final clinical day in which a digital rectal examination, a Prostate Serum Antigen (PSA) dosage, a transrectal ultrasound and a biopsy were carried out, a nutritional interview where a detailed dietary history and anthropometric measurements were done. Plasma samples of men diagnosed with prostate cancer, and a healthy control group, were analyzed for PSA level, malonyldialdehyde (MDA) and total antioxidant content in serum (TAS). The average age of the patients was 59 years old. Average basal values in control and patient groups were:

**PSA:** Control:  $1,05 \pm 0,4$  mmol/L; Patients:  $1,53 \pm 0,38$  mmol/L.

**TAS:** Control:  $5,1$  ng/mL  $\pm 0,3$ ; Patients:  $12,77$  ng/mL  $\pm 0,8$

**MDA:** Control:  $0,65 \pm 0,22$   $\mu$ mol/L; Patients:  $0,92 \pm 0,25$   $\mu$ mol/L.

A significative rise of PSA was observed, while TAS and MDA were diminished. Comparing the blood levels of antioxidants in cases and controls, we conclude that antioxidants may help to protect against prostate cancer.

155.

**A MULTIDIMENSIONAL VIEW TO THE TEACHING NATURAL SCIENCE**

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In the different subjects on the field of natural science, it is being more and more frequent to find teachers who feel a real frustration due to the less satisfactory results in the teaching of disciplines as Chemistry, Physics, Biology. They use to propose methodologic changes such as incorporating more laboratory work practicum or reducing the number of topics to develop during the attendance of the programme; which in a way, as a result of this issue, they do a self evaluation on how they are teaching the contents of the subject and this brings about to ask what is the best methodology to avoid the frustration of students as well as teachers at school. Therefore, it is here where the concept of scientific and technologic alphabetization emerges, which means to take into account the teaching of sciences from a multidimensional view to obtain better results. The most important thing is to work in the implication of science and technology in the natural –and psycho socio cultural- environment, as to say, to promote the relationship Science- Technology- Society. That way, students are interested in the science curriculum and get to understand and attach importance to learning its disciplinary contents. The experience carried out with positive results is centred in the teaching of Chemistry related to Biology at Polimodal level bearing in mind basic aspects of the issues of two cores: environment and health and the link between them, pollution (water, ground, air, food pollution, among others). We consider that this is a motivating teaching strategy.

156.

**CYTOPROTECTIVE EFFECT OF LOSARTAN IN RENAL PROXIMAL TUBULES (PT) FROM SPONTANEOUSLY HYPERTENSIVE RATS (SHR): ROLE OF HSP70**

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Caveolin proteins function to recruit lipids and proteins to caveolae, for participation in intracellular trafficking of cellular components and operation in signal transduction. Caveolin 1 (cav1) is required for normal renal AT<sub>1</sub>-receptor expression. We have demonstrated cav1 and HSP70 interaction after losartan (Los) administration in SHR cortex. In the present study, we evaluated HSP70 involvement on the cytoprotective effect of Los in SHR. Methods: Four groups were studied: SHR, SHRLos and WKY with/without Los. Cav1 and HSP70 were evaluated by western blot in microdissected PT, immunofluorescence (IF) of both proteins in renal cortex. Coimmunoprecipitation (CIP) of cav1/HSP70 in absence/ presence of HSP70 was performed. TUNEL, caspase 3 activity and mRNA of Bax/Bcl<sub>2</sub>, NADH oxidase activity (fluorescence). Results: Los decreased BP near controls values in SHR compared to SHR+H<sub>2</sub>O associated with decreased AT<sub>1</sub> expression. Cav1 and HSP70 were demonstrated in membrane fractions from isolated PT of SHRLos. Increased immunoreaction was shown for both proteins by IF in membranes from PT. CIP of cav1 and HSP70 was demonstrated in cortex membranes in SHRLos. Conversely, absence of HSP70 expression was shown in membrane fractions from SHR. Decreased NADH oxidase activity near controls was demonstrated in membrane fraction of SHRLos vs SHR. No differences in apoptosis induction were found among groups. From these results, the translocation of HSP70 to membrane fraction in PT after Los administration, might exert a cytoprotective effect by decreasing oxidative stress in SHR.

**157.**  
**CONDILOMAS OF ORAL MUCOSA . PROTO-ONCOGEN C-MYC**

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Annual incidence of condyloma acuminata has been reported in oral mucosa that revealed the clinical and histological features of HPV infection. Numerous reports exist with respect to the prevalence of HPV in the oral cavity. Annual incidence of condylomata acuminata lesions has been reported to be as high as 106.5 per 100.000 (about 0,1%) of the entire population in association with premalignant conditions. With the purpose to study genotypification and amplification of proto-oncogen c-myc on oral condiloma acuminata. It was considered 15 samples with histopathological diagnoses of condyloma. For typification was applied PCR technic-LIS-SSCP. Co-amplification with reference locus beta-globine technic was applied. Control specimens were choosen. HPV+ 73,3%, the prevalent types were 6 and 11. *C-myc* amplification: 33,3%. Control group amplification: 10%. It could suggest that c-myc amplification could identifying on proliferativa, no invasive lesions. According to international publications, HPV+ low risk subtypes are the most prevalent on condylomata, meanwhile c-myc proto-oncogen could be an useful biomarker. This conclusions brings us outstanding challenges and the necessity of further investigations with greater number of samples.

*Histotechnologist: Del Viso S.*

**158.**  
**ORAL SQUAMOUS CELL CARCINOMAS HPV+ . PROTO-ONCOGEN C-MYC**

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Alterations at the cellular genome level affecting functions and expression on control and cell differentiation, are considered crucial. Proto oncogen *C-myc* appears to be related with the development of progression with cancer associated with Human Papilloma Virus infection. Our purpose was to study detection, typification and amplification of *c-myc* on squamous cell carcinomas. 16 specimens of oral carcinomas and 21 of cervix cancer were selected. It was performed genotypification of HPV by PCR-LIS-SSCP. Reference Gene Technic using as reference gene (onecopy) Betaglobine was applied to study *c-myc*. Electrophoresis lecture of amplification was performed by poliacrilamida mingles. We have obtained the followed results: oral squamous cell carcinomas HPV+ 68,8%. The most frequent genotype was HPV16 (54,5%). Amplification of *c-myc* by PCR-RG: 12,5%. About cervical carcinomas, 100% of them were HPV+. Prevalent type was 16 (67%) and *c-myc* amplification: 34,3%. Our results agreed with international reports in order to remark HPV16 prevalence. *C-myc* seems to be a biomarker of great value and showed overexpression on cervical cancer. It would be necessary to improve this investigation.

*Histotechnologist: Del Viso S.*

**159.**  
**ELECTROPHORETIC CHARACTERIZATION OF THE 900 KDA TYPE A BOTULINUM NEUROTOXIN**

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The type A botulinum neurotoxin (NTBo), produced by *Clostridium botulinum*, is one of seven antigenic types recognized so far. This toxin is synthesized as a native simple chain, where the toxic fraction (S), of approximately 150 kDa, associates with non toxic haemagglutinin subcomponent and with other non toxic non haemagglutinin one by covalent bonds. All these components may be associated, forming three different protein complexes of approximately 900, 500 and 300 kDa (named LL, L and M respectively). The aim of this work was to characterize the LL NTBo by electrophoresis and what is the sensitivity of the complex to chemical and physical agents.

The toxin was produced and purified from an A Hall strain-stock prototype of *Clostridium botulinum*, cultured in liquid yeast trypticase medium. Purification was carried out by acid precipitation followed by dialysis. Samples were submitted to different temperatures and the products were analyzed by SDS-PAGE on 6% acrylamide gels, under reducing or non reducing conditions. We observed that the integrity of the complex LL is affected by high temperature, since the gels were depleted of 900 kDa band. Moreover, reducing conditions induced changes in electrophoretic mobility. We conclude that 900 kDa NTBo is highly sensitive to physical and chemical agents, and we should take in account this behavior for ulterior manipulations of the toxin. The study will be extended to other possible conditions that may affect the protein complex.

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