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### **Abstracts from the XXII Annual Scientific Meeting**

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**December 2 - 4, 2004  
Merlo, San Luis, ARGENTINA**



### 1. **CAMPYLOBACTER-ASSOCIATED REACTIVE ARTHRITIS: A REGIONAL STUDY IN SYNOVIAL LIQUIDS**

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Reactive arthritis is an aseptic synovitis that can appear after primary *Campylobacter* infection. The immunological mechanisms of synovitis involve chronic inflammation by antigens deposited in the articulation and autoimmune response to articular components. The objective of the present study was to investigate *Campylobacter* antibodies in synovial liquids (SL) of patients with arthropathies from San Luis. Twenty SL from patients with rheumatoid arthritis (RA), 5 with spondyloarthropathies and 20 with arthrosis (negative controls) were studied. The antibody determination was done by ELISA, using as antigen *Campylobacter* cultures disrupted by sonication. The cutoff point was calculated as the mean + two standard deviations of the optical densities of negative controls. *C. jejuni* specific immunoglobulin response was detected in 3 SL of patients with RA, and *C. jejuni* specific IgA response was detected in 2 SL. According with the results we concluded that *Campylobacter* infection could be associated with arthropathies in San Luis.

### 3. **CYTOPROTECTOR EFFECT OF *Plantago major* AGAINST RATS GASTRIC MUCOSA INJURY BY ACETYLSALICYLIC ACID**

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The objective of this work is to show that the injury produced by acetylsalicylic acid (AAS) on rat gastric mucosa can be prevented by gavage of *Plantago major* (Pm) 10% infusion. Wistar female rats (200-250g) were divided in two groups (each n=5). Group I received 1 mL of water, and 1 h later 1 mL AAS (300mg/kg). Group II received 1mL 10 % Pm infusion followed 1h later by 1 mL AAS (300 mg/kg). After another hour, animals were killed, blood was drawn and the stomach removed and homogenized in buffer phosphate (pH 7). Spectrophotometrical determinations as reactive oxygen species (ERO) and malonyldialdehyde (MDA) as lipoperoxidation index were used. Antioxidant indicators were catalase (CAT) and glutathione reductase (GSHred) Unpaired Student *t* test ( $P < 0.05$ ) was used to compare both groups. Results are mean  $\pm$  SEM. Homogenate: ERO (nmol/min) Group I  $3.05 \pm 0.23$ ; Group II  $1.82 \pm 0.07$ . MDA ( $\mu\text{mol/L}$ ): Group I  $32.38 \pm 0.89$ ; Group II  $20.09 \pm 0.78$ . Blood: CAT (pmol/mg protein) Group I  $2.75 \pm 0.29$ ; Group II  $4.16 \pm 0.46$ . GSH red (mg/L) Group I  $51.19 \pm 1.20$ ; Group II  $56.18 \pm 1.08$ . Plasma: ERO (nmol/min) Group I  $1.08 \pm 0.08$ ; Group II  $0.68 \pm 0.02$ . These results indicate that *Plantago major* 10% infusion has a cytoprotector effect on gastric mucosa exposed to injury by high concentration of acetylsalicylic acid.

### 2. **BIOCHEMICAL AND PHARMACOLOGICAL PRODUCTS OBTAINED FROM *Plantago major* PROJECTED ONTO ITS GASTRIC CYTOPROTECTIVE ACTIVITY**

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This study assessed the cytoprotector and antioxidant effects of products from *Plantago major* (Pm) on challenged rat gastric mucosa. An extract was obtained by three extractions with cold ethanol and a 250 mg/kg aqueous solution was prepared. Solutions were administered by gavage with a 1-h interval between them. Experiments were performed on 4 lots (L; each n = 5) of Wistar male rats (200 – 250 g). L 1: 1 mL water and 1 mL water. L 2: 1 mL water and 1 mL ethanol. L 3: 1mL water 1 mL Pm extract. L 4: 1 mL Pm extract and 1mL ethanol. One hour after the second solution animals were killed, blood was drawn and the stomach was removed. Spectrophotometrical determinations of reactive oxygen species (ERO) and malonyldialdehyde (MDA) as lipoperoxidation index were used. Antioxidant indicators were catalase (CAT) and superoxide dismutase (SOD). ERO (nmol/min) L1  $0.40 \pm 0.10$ ; L2  $1.26 \pm 0.01$ ; L3  $0.45 \pm 0.04$ ; L4  $0.48 \pm 0.02$ . MDA ( $\mu\text{mol/L}$ ) L1  $0.39 \pm 0.11$ ; L2  $1.30 \pm 0.30$ ; L3  $0.61 \pm$ ; L4  $0.56 \pm 0.30$ . CAT (pmol/mg protein): L1  $2.9 \pm 0.36$ ; L2  $1.13 \pm 0.13$ ; L4  $2.24 \pm 0.36$ . SOD (U/mL): L1  $233.75 \pm 15.12$ ; L2  $124.00 \pm 4.76$ ; L3  $233.00 \pm 60.00$ ; L4  $230.00 \pm 14.00$ . The gastric mucosa was examined and the ulcer index (IU) was obtained for L3 ( $4.8 \pm 0.12$ ) and L4 ( $1.00 \pm 0.20$ ). Results were contrasted with ANOVA1 test and Bonferroni's post test. Products obtained from *Plantago major* have antioxidant and cytoprotector effect on gastric mucosa exposed to ethanol.

### 4. **RAPID ADULTERANT SCREENING IN *Hedera helix* BASED COUGH SYRUPS**

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Several plant extracts are used in the local market as active constituents of pharmaceutical formulations. Adulterations with synthetic drugs are common problems in herbal medicines and this can potentially cause serious adverse effects. In order to contribute with the commercialization of phytotherapeutic products, different *Hedera helix* based syrups and their ingredients were tested for the presence of adulterants. The aim of this study was to develop a fast method to analyze bromhexine, codeine and noscapine present in phytotherapeutic products by capillary electrophoresis. The experimental conditions which yielded the best performance were: background electrolyte (BGE) sodium tetraborate 20 mM, pH 8.5; applied voltage 25 kV, capillary temperature 25°C, sample injection 0.5 Psi, 5 seconds. The detection was performed at 205 and 250 nm. This technique was validated for linearity, precision, accuracy, LOD and LOQ and was applied to a glycolic extract and two different commercial syrups. The present methodology seems to be suitable for the adulterant screening of antitussive drugs in based *Hedera helix* phytopharmaceutical products.

**5. COMPARISON BETWEEN PROTEINS EXTRACTED FROM DIFFERENT SEASONAL REPRODUCTIVE STAGES IN *Lagostomus maximus maximus* (VISCACHA)**

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The viscacha, a seasonal rodent, is native of the Pampas and Mesopotamia areas of Argentina. The male exhibits an annual reproductive cycle characterized by a short period of gonadal regression in July-August (winter), a recovery period during September-November (Spring) and a period of gonadal activity during January-May (Summer-Autumn). The aim of this work was to determine the different protein expression in capacitated sperm. Sperm were collected from epididymis cauda in HECM-3 medium, and capacitated in the same medium during different periods (5, 7 and 11 hs). Proteins were obtained according to Laemmli and were separated by 10% SDS Polyacrylamide gel electrophoresis. The number of sperm in each determination was equivalent. Significant changes associated with seasonal cycles were found. The most intensive bands were observed in samples obtained from May (active period) comparing with the samples corresponding to August (regressed period) and September (recovery period). This seasonal variation in protein expression is correlated with the seasonal morphological changes observed in the epididymis of viscacha and the decrease of sperm number during the gonadal regression.

**7. IRRITANT ACTIVITY OF *Euphorbia schickendantzii* AND *Colliguaja integerrima* (EUPHORBIACEAE): A HISTOLOGICAL STUDY**

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*Euphorbia schickendantzii* and *Colliguaja integerrima* are commonly known as "pichoa" and "colihuai", respectively. Many species of this family are used in folkloric medicine as drugs for medicinal preparations (Wiriyachitra *et al.*, 1985). The objective of this work was to investigate the irritant activity of the infusions of *E. schickendantzii* and *C. integerrima* on rabbit skin (OECD, 2000) through a histological study. Sterile gauze patches were soaked with the infusions and they were fixed on the dorsal part of albin rabbits of both sexes, which were previously shaved. Readings took place at 1, 4 and 24 h. Both infusions showed irritant activity on the skin (Index of primary cutaneous irritation in the rabbit: moderately (*E. schickendantzii*.) and lightly irritant (*C. integerrima*) (OECD, 2000). Skin was processed and fixed for light microscopy study. Infusions of both plants showed irritant activity according to macroscopic study. The histological study indicated few scaly alterations with lost of epithelium continuity. Latter studies will be necessary to determinate the reversibility of those injuries.

**6. A KINETIC STUDY OF BACTERIOSTATIC ACTION OF CHALCONES AGAINST *Enterobacter sp.***

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Flavonoids are biologically active compounds. Among them, chalcones show bacteriostatic activity. Minimal inhibitory concentrations (MIC) of 2',4'-(OH)<sub>2</sub>-chalcone, 2',3-(OH)<sub>2</sub>-chalcone and 2',4-(OH)<sub>2</sub>-chalcone against *Enterobacter sp* were determined. A kinetic-turbidimetric method was developed for calculating the number of CFU/mL. A Rosi 1000 incubator, with constant stirring (35 °C, 180 rpm) and nutritive broth were employed. Aliquots were extracted at 20 min intervals during 5 h, recording transmittance measures (T) at 720 nm with a BIO-RAD SmartSpect Plus spectrophotometer. T values were related to the number of N<sub>t</sub> as follows:  $\ln N_t = 40.22 - 34.29.T$ . The equation of microbial growth is  $\ln N_t = \mu \cdot t + \ln N_0$  where, t: time in min; N<sub>0</sub>: cfu/mL at t = 0; N<sub>t</sub>: cfu/mL at the t = t and  $\mu$ : specific growth rate in 1/min. Specific growth rates with increasing drug concentrations were obtained from the curve of  $\ln N_t$  vs t in the exponential phase. The equation  $\mu = \mu_T - k_1 \cdot C$ , was used to calculate MIC values: MIC<sub>2',4'-(OH)2</sub> = 137.36 µg/mL, MIC<sub>2',3-(OH)2</sub> = 110.61 µg/mL and MIC<sub>2',4-(OH)2</sub> = 106.36 µg/mL. These values are higher than those previously obtained against *Escherichia coli* ATCC 25 922. This is probably because *E. coli* has more porins on its surface.

**8. EFFECT OF CHRONIC EXPOSURE TO CADMIUM ON ULTRASTRUCTURE OF TESTIS AND PROSTATE**

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Cadmium (Cd) is a toxic element, and an important environmental contaminant. Based on previous studies showing Cd-induced non-proliferative changes in the prostate and an involuntional state, we assessed Cd effect on testis ultrastructure. Male Wistar rats were divided in a Cd group, which received 15 ppm of Cd in drinking water for 12 wk and a control group (Co) which received water without Cd. Portions of prostate and testis were processed by conventional electronic microscope (EM) techniques. Additionally prostate samples (100 mg) were homogenized in the presence of protease inhibitors and protein (40 µg) was separated in an 8% SDS-PAGE, transferred to PVDF membranes and incubated with an antibody against androgen receptors. The EM study showed on Cd-exposed testis nuclear changes compatible with apoptosis. The EM study of prostate showed a higher activity on control cells, as suggested specially by the higher number of storage vesicles, while Cd-exposed prostate exocrine cells had smaller number of supranuclear vesicles and their contain appeared be different than in controls. Results allowed us to confirm the involution state as well as an alteration on the testis due to exposition to Cd. Perhaps this damage in prostate is, in part, because of an endocrine malfunction, as suggested by lower androgen receptor expression in Cd-exposed prostate.

### 9. BIOCHEMICAL MARKERS IN HEPATITIS A

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During 2004 the cases of hepatitis A were increased in the population of San Luis city, IgM anti-HAV (ELISA) being detected in 238 patients. A descriptive study was performed to allow checking the behavior of the biochemical markers: bilirubin, transaminase (ALT and AST), alkaline phosphatase (ALK) and prothrombin percentage (PP). In the studied population there was a preponderance of masculine sex (54.6%). Mean age was 10.5 years (SD = 7.21), range 1 to 41 years, median 8 years. The prevalence of affected children reflects the absence of immunity against the virus in this population group. The alterations in the hepatic determinations are within those usually found in this illness: elevation up to 950 and 1110 U/L of AST and ALT, respectively, in 60% of the cases; the values of FAL also increased significantly; the concentration of total bilirubin was rarely above 70 mg/L with an increase of direct bilirubin; PP diminished in 20% of the patients, reaching 43% in only two of them. No patient presented a fulminant strain. We continue patient follow up with checks every 2 months to study the evolution of the markers. It is concluded that the diagnosis of hepatitis A in this population is of easy management in the laboratory and the increase of bilirubin, ALT, AST and FAL and the decrease of PP in outbreak situations can serve as an epidemic strategy for hepatitis A diagnosis.

### 11. PHYSIOGNOMIC TYPES OF VEGETATION OF THE "BAJO LAS SALADAS" (SAN LUIS)

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With the objective of studying the physiognomic types of vegetation of the "Bajo las saladas", located at 33° 37' of south latitude and 65° 24' of west latitude, to a height of 505 m ASL, information was gathered combining photointerpretation procedures in cabinet with field corroboration. The studied sector belongs to the "chaco-pampeana" plain from the physiographic point of view, while its climate unit is defined as dry cold and the great landscape is a plain sandy dunes on the alluvial plain of the "Quinto" river. The landscape corresponds to a depression or saline lowland that includes a basin of scarce slope that, born in the north sector of Villa Mercedes city goes toward the east, then takes a southeast course definitively until its outlet in the "Quinto" river. During its itinerary it forms different fresh and salty wetlands. It develops three physiognomic types with different vegetable communities in each one. 1) The "monte" with a community of "chañar" accompanied by other species. 2) The "arbustal halófilo bajo" with two communities: "zampa" and "jume", and 3) the "estepa halófila" with three communities: "jume + pelo de chancho", "pelo de chancho" and "junco". We conclude that the methodology has been efficient for the delimitation of the physiognomic types of vegetation.

### 10. IMPROVED IMMUNOCAPACITY OF *Clostridium septicum* SONICATED VACCINES

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*Clostridium septicum* is one of the agents causing malignant edema with high mortality in many animal species. This microorganism produces lethal alpha toxin, considered the principal antigen protector. At present, two types of commercial *C. septicum* vaccines, toxoid or whole formalinized cultures (WFC), are available for preventing this disease. It is well established that *C. septicum* produces lethal antigen only in low titre. In addition the immunogenicity of toxic filtrates is weak, which results in poor antibody response in animals. The aim of this study was to compare the immunoprotective capacity of sonicated cultures (SC) with commercial vaccines. To obtain different antigens *C. septicum* ATCC 12464 was cultured under anaerobic conditions. The protective capacity was evaluated by protection tests in mice; immunogenicity by ELISA and the identification of immunoreactive bands by Western blot. SC elicited a higher protective capacity and immunogenicity ( $p \leq 0.05$ ) than the other antigen preparations. Two bands of relative molecular masses of 46 kDa (alpha-prototoxin) and 42 kDa (active alpha-toxin) of extracellular filtrates were recognized by sera immunized with SC, WFC and toxoide. These proteins were also recognized by sera immunized with SC and toxoid when they reacted with sonicated cellular antigens. The results indicate that the contribution of cellular alpha-toxin liberated by sonication remarkably increases the protective capacity of the *C. septicum* vaccines.

### 12. EGG WHITE LYSOZYME PURIFICATION USING YEAST CELL PREPARATIONS AS AFFINITY PARTICLES

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In the ultrafiltration affinity purification method, the membranes used have a pore size large enough to allow all protein molecules in the sample to pass through the pore. If an affinity particle is added to the system, the target protein bound to the ligand will then be selectively retained in one side of the membrane, whereas all other molecules will pass through. In this study, affinity particles from yeast cells modified by chemicals and with the Cibacron blue 3GA ligand molecule bound to the cell wall were prepared. The binding of lysozyme on these particles and washing/elution steps were investigated. The adsorption capacity was 110-120 mg lysozyme/mL wet particles, determined by adsorption isotherm assays. Washing with Tris-HCl buffer (50 mmol/L Tris-HCl/50 mmol/L NaCl, pH 8) and elution of the enzyme by increasing the ionic strength with 2 N NaCl gave suitable results. Analytical scale experiments with egg white showed, by gel electrophoresis (SDS-PAGE), lysozyme of high purity and appropriate specific activity by Shugar's method. The affinity particles obtained by yeast cell preparations have high lysozyme adsorption capacity and adequate chemical/physical properties for large scale use in processes of ultrafiltration affinity purification.

**13. EFFECT OF SESQUITERPENE LACTONES ON CULTURED *Leishmania mexicana***

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*Leishmania mexicana* is the etiological agent of a severe leishmaniasis that affects to millions of people in the world. This parasite can be easily cultured in liquid medium at room temperature and cycle between the two well know stages; amastigote and promastigote. In this work we made a screening of natural compounds to evaluate a possible use against *L. mexicana*. The parasites were cultured in Diamond Medium at room temperature. The sesquiterpene lactones dehydroleucodine (DhL), mexicanine (MXC) and helenaline (HLN), purified from leaves of native plants, were added to the parasite cultures, at different concentrations, and we evaluated the effect on proliferation, and viability of the cells. The three lactones inhibited cell proliferation, although MXC and HLN exerted such effect at lower concentrations than DhL (from 1ug/ml) and mostly by a deleterious action. This effect was also stronger than that on *Trypanosoma cruzi* reported previously. We also observed that the infection of Vero cells with treated parasites (DhL 5ug/ml, MXC 1ug/ml and HLN 0,5ug/ml) was decreased (from 20 to 3 parasites/cell). From these results we concluded that sesquiterpene lactones appear as possible agents against *L. mexicana* and they became attractive since are abundant in the nature and exert their action at lower concentration than other known compounds.

**15. ORAL PALEOPATHOLOGY PROFILE IN EXTINCT POPULATIONS OF THE PRESENT TERRITORY OF THE PROVINCE OF SALTA (ARGENTINA)**

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Studies of oral paleopathology are important for the reconstruction of diet and nutrition, and include a contribution to the study of the environmental pressure that are exposed. In the present study a register by standardized methods of the pathologies found in the collection catalogued like Pampa Grande (Salta, Argentina) deposited in the Museum of La Plata was made. From 96 pieces were selected 15 cranium and 19 calvarium and were registered by age, sex, teething, maxilar and dental group. From the whole of dental pieces, 75 were lost pre mortem, 371 post mortem and 39 not erupted. From 289 teeth studied, the findings were: 45 caries (16%), 6 coronary fractures (2%), 6 hypoplasias (2%) in adults of both sexes. Occlusal surface wear is more moderate in the front sector being the mode for men =4 and for women =.2 Smith index. In the back sector the greater degree microwear is observed in the first molar of men mode = 16 and in women mode = 14, in the second molar mode=8. and the third molar mode=4 Scott index. The alveolar bony reabsorption showed predominance of moderate forms. Microwear characteristics and the pathologies found confirm the nutritional habits and the putative nutritional stress.

**14. IN VITRO TISSUE CULTURE OF *Larrea cuneifolia***

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In the species of the genus *Larrea* a lignane, the nordihydroguaiaretic acid, occurs in concentrations from 10 to 15% of the dry weight of leaves and stems. This compound possesses antioxidative activity and the capacity of inhibiting the growth of numerous types of human cancer both *in vivo* and *in vitro*. Chemopreventive activity has been demonstrated in carcinogenic models in rats induced chemically and for UV light. With the purpose of achieving the establishment *in vitro* of *Larrea cuneifolia* plants stock, as initial stage for later studies of production of the secondary metabolite, the environmental parameters settled down, necessary nutritional and aseptic aspects to achieve high and constant *in vitro* multiplication rate. The callus formation was observed in solid medium (agar 6 g/L), inorganic nutrient composition of Murashige and Skoog and  $\alpha$ -naphthaleneacetic acid (2 mg/L). The following step is to begin the cell suspensions cultures. Is necessary to determine the kinetics of cell growth, as well as the extraction, characterization and quantification of the metabolite of interest, optimizing the conditions to the selection and maintenance of cells lines producers.

**16. NATURAL RUBBER LATEX PROTEINS FROM FIG TREE (*Ficus carica*)**

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Natural rubber latex is a processed plant product. Over 99% of the world's supply is derived from the latex or the milky cytosol of the tree *Hevea brasiliensis*. Latex is produced by specialized lactifer cells and is composed of many different molecules, including lipids, phospholipids and proteins. The proteins are responsible for allergic sensitization predisposing to IgE-mediated reactions. There are 200 other latex-producing plant species. In this work, latex from the fig tree (*Ficus carica*) was utilized. The characterization of the latex proteins was performed by electrophoresis. The samples were collected from the leaves of the tree in small tubes containing ammonia to prevent degradation. Total protein was measured and the stain intensity from the different protein fractions was used to calculate their relative amounts. A mesh of known molecular weight proteins was employed to standardize the method. About twenty different proteins were detected in the latex preparation by electrophoresis. Under the present experimental conditions, *Ficus carica* latex proteins are negatively charged, i.e., behave as anions.

**17. VALIDATION OF AN EXERCISE PROGRAM FOR CONSERVATION OF BONE MASS IN POSTMENOPAUSAL WOMEN**

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Although physical exercise is deemed to help women to avoid postmenopausal bone loss, no clear consensus exist regarding optimal exercise schedule, particularly for the hip. The bone mineral density (BMD) of the lumbar spine and hip was measured with dual X-ray absorptiometry (DXA; Lunar DPX-L) in postmenopausal women participating in an exercise program (n = 14, compliance > 80%) before and after 2 years, and in sedentary postmenopausal women (n = 14) at a similar time interval. Exclusion criteria for both groups were intake of calcium supplements or drugs known to affect BMD, heavy smoking, or alcohol consumption. Height, weight, bone mass index, age, time since menopause and interval between DXA measurements were not significantly different between the groups. Mean differences of changes in BMD at the end of the study (active vs. sedentary) were for lumbar spine + 3.8% (P = 0.04), for femoral neck + 7.1% (P = 0.004) and for trochanter + 6.1% (P = 0.005). Multiple regression analysis showed that, besides the first DXA value, the only variable that consistently correlated with the second DXA value was physical activity status. Therefore, the program was effective for avoiding postmenopausal bone loss.

**19. MORPHOLOGICAL STUDIES AND DOMESTICATION OF HERBACEOUS NATIVE FABACEAE SPECIES FROM CENTRAL ARGENTINA**

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There is a lack of forage species that can be cultivated in low nitrogen content soils and semiarid regions. This work aims at domesticating native Fabaceae species to be used as forage crops in areas with those characteristics. Species of *Adesmia* and *Vicia* were studied. The environment where they grow was characterized. Seeds were collected and their germination rate was determined. Seeds, fruits, pollen grains, stems, leaves and roots were included, cut and stained for anatomical studies. The analyzed species were seeded in 2 x 4 m plots where a randomized block design with three replications was applied. Three populations of *Adesmia bicolor*, *A. muricata*, *A. macrostachya* and *Vicia pampicola* are detected. These populations usually grow in loam to clay loam and sandy loam soils. The low germination rate is explained by the presence of sclereids in the seed teguments. Roots and stolons have high starch content. While the species of *Vicia* have dehiscent elastic fruits, the species of *Adesmia* have indehiscent ones. Characteristics of apertures, shape, AMB and size of pollen grains allow to differentiate among species. Although these studies showed that the analyzed species have potential as forage crops, they have a limitant low germination rate.

**18. EFFECT OF *Avena sativa*, *Escholtzia californica* AND *Matricaria chamomilla* ASSOCIATION ON THE NERVOUS CENTRAL SYSTEM IN OPEN FIELD TEST**

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Sixty male Sprague Dawley rats, weighing between 180 and 300 g, body weight were tested. Rats were grouped at random in 6 groups of 10 animals each. Group I: control; Group II: Syrup placebo, 0.5 mL/100g; Group III: Syrup of *Avena sativa*, *Escholtzia californica* and *Matricaria chamomilla* previous single 0.5mL/100g; Group IV: Syrup of *Avena sativa*, *Escholtzia californica* and *Matricaria chamomilla* previous sequential 0.5mL/100g; Group V: Diazepam, in syrup, at dose 4 mg/kg at volume 0.5mL/ 100g and Group VI: Amphetamine, in syrup, at dose 10 mg/kg and volume 0.5 mL/ 100g. All drugs and syrups were administered orally. Fifteen minutes after each administration, rats were placed on the board of open field, observing motor variables (walked squares and biped positions) and emotives variables (number of pellets defecated, numbers and duration of grooming). From the analysis of results, it is concluded that syrup single and sequential administration modifies emotional tension in the variables number and duration of grooming, similar to what happens with tranquilizing drugs. Thus for a better effect in all the variables in study it is recommended to increase the dose and administer the syrups in study for a longer period of time.

**20. *Aspergillus niger* EPOXIDE HYDROLASE AS AN ALTERNATIVE MODEL TO STUDY HUMAN CHOLESTEROL EPOXIDE HYDROLASE**

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Colestan-5-epoxi-3 $\beta$ -ol and its metabolite colestán-3 $\beta$ ,5 $\alpha$ ,6 $\beta$ -triol are not only involved in atherogenic processes but also in mutagenic and citotoxic activities. *A. niger* epoxide hydrolase shows structural and mechanistic analogies with human microsomal epoxide hydrolase. A fungal model using whole cell cultures of *A. niger* ATCC 11394 has been developed in order to mimic human metabolism. A two- step assay using batch culture system was performed. Firstly, ten 125ml batch containing 30ml of Czapeck medium, were inoculated with *A. niger* spores and submitted to rotatory agitation (180 rpm) for 24hs at 28°C. Then pellets were transferred to ten batches with fresh medium and cultured at the same conditions for 24hs. The substrate, 10 mg of colestán-5-epoxi-3 $\beta$ -ol, was dissolved in 20  $\mu$ L of EtOH and added to each batch. After a 14 days incubation the bioconversion products were checked by TLC, purified and characterized by  $^1\text{H}$ ,  $^{13}\text{C}$  NMR and MS techniques. This model results in a cheaper, simpler, easier-to-handle and less risky alternative system than the use of mammalian tissue cultures.

**21. SPERM CAPACITATION: STRUCTURAL CHANGES IN MEMBRANE MICRODOMAINS (RAFT)**

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Membrane microdomains containing cholesterol and sphingolipids conform structures with specific functions in sperm (e.g. cholesterol loss has been involved in sperm capacitation). The exact distribution of raft lipids during capacitation is unclear, though. We searched these changes during mouse sperm capacitation and its effects in fertilization. Sperm were incubated in HMB medium (capacitation) 60' or PBS buffer (control) 10', 37°C and 5% CO<sub>2</sub>. Later were fixed and marked: cholesterol by filipin III (FIII) and sphingolipids (GM1) by fraction  $\beta$  cholera toxin ( $\beta$ CT). FIII is self-fluorescent and  $\beta$ CT is conjugated with Alexa Fluor®. Samples were observed in a fluorescence microscope. Sperm (capacitated and control) showed different fluorescence standards with FIII and  $\beta$ CT, probably due to a lipidic redistribution on sperm surface. In fecundation *in vitro* assay, we blocked lipids raft preincubating sperm with  $\beta$ CT (GM1) 15'. Then, sperm were coincubated with Hamster oocyte, and the sperm bounded were counted. First observations showed a binding decrement in sperm blocked with  $\beta$ CT (GM1) comparing with the control (not blocked). These results suggest that GM1 (Sphingolipid), a classic raft component, and its redistribution after capacitation are involved in sperm – oocyte binding.

**23. CHARACTERIZATION AND CLASSIFICATION OF SINGLE CROSS HYBRIDS OF MAIZE WITH SSR MARKERS**

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The characterization of cultivars has important practical applications in plant breeding for identification of commercial genotypes and for assessment of the genetic relationship. Molecular markers allow analysis of an extensive number of genetic *loci* and the findings of high levels of polymorphism. In this work the polymorphic microsatellites (SSR) were utilized in order to characterize commercial hybrids of maize and to ascertain similarities among genotypes according to their breeder sources. To characterize 19 single cross hybrids of maize belonging to four private breeders and from INTA Castelar, we analyzed 17 SSR producing 61 polymorphic bands. Genetic distance estimates, which were obtained by the simple concordance index, ranged from 0,00 to 0,50. With the distance matrix a dendrogram was constructed based on the UPGMA method. This dendrogram clearly showed three groups with 9, 7 and 2 genotypes respectively, and a unitary group. The genetic relationships among hybrids were confirmed with the principal coordinate analysis. The level of polymorphism and the repeatability of banding patterns confirm that SSR may be used as a complementary criterion to distinguish single cross hybrids of maize. Our results showed that the SSR allow to differentiate single cross hybrids even when they have a close genetic relationship. Cultivar-specific markers, such as SSR offers an alternative for the identification of cultivar and evaluation of genetic stability of hybrids as well as for the protection of plant breeders' rights.

**22. ROLE OF EXPANSINS IN THE RHIZOBIA-LEGUME SYMBIOSIS**

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Many legumes respond to rhizobia inoculation by developing new organs, known as root nodules. Among the cell wall proteins studied to date, expansins are unique in their ability to induce immediate cell wall extension *in vitro* and cell expansion *in vivo*. This capacity to extend the cell wall is lost upon denaturation of wall protein. Gene expression analyses with gene specific probes have shown that expansin genes are differentially regulated by developmental, hormonal and environmental signals. We demonstrate previously that the expression of a *Melilotus alba* expansin gene is up-regulated during the interaction with *Sinorhizobium meliloti*. Here we describe the effect of phytohormones on expansin gene expression during the development of the nitrogen-fixing symbiosis with rhizobia. Increased accumulation of expansin gene transcripts was observed in roots treated with cytokinin, however neither northern nor RT-PCR analysis appeared to be sensitive enough to detect expansin gene expression in untreated versus treated roots at early time points after treatment with auxin. Additionally, expansin transcripts were found in the nodule cells that are undergoing growth, including the cells of nodule meristem, but not reliable within root hair. As shown for other plant tissues, our results suggest that expansins in legume correlates with proliferation and expansion activities of nodule tissues.

**24. CARCASS CHARACTERISTICS IN KIDS FROM CREOLE AND ANGLO NUBIAN BREEDS**

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Caprine production in Southern Cordoba is mainly focused towards kid crop production. The objective was to characterize the carcass of Creole and Anglo Nubian kids from the southern Córdoba. Ten female and male Creole and ten male Anglo Nubian kids, between 10 to 12 kg of BW and 45 to 60 days of age were used in this study. Carcass evaluation was done by determining live and carcass weight, carcass length, perimeters, and width. A T-student test was used to compare the data between female (FC) and male (MC) Creole breed and between Creole male and Anglo Nubian male (MAN). BW and carcass yield (CY) did not show significant differences between FC and MC, but there were significant differences ( $p > 0.001$ ) between MC and MAN for these two variables (11.07 kg, 11.74 kg and 10.34 kg of BW and 49.4%, 48.5% and 53.1% CY for FC, MC and MAN, respectively). Front quarters showed significant differences ( $p < 0.05$ ) for muscular tissues between MC (68.31%) and MAN (65.46%). The heaviest section of the carcass, for both breeds, were the hind quarters with, 825.14 g, 910.90 g and 809.90 g for FC, MC and MAN, respectively.

**25. THE REFURBISHING OF AN ULTRAPHOT I, IN ORDER TO ADD ACTUAL LIGHT MICROSCOPY TECHNIQUES-**

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The Ultraphot I is a universal microscope developed during 1937 covering all possible light microscope techniques that were in use at that time. It was provided with a unique compact illumination system allowing the correct use of the Köhler principle either in epi- or trans-illumination, fluorescence images included. Nevertheless a small disadvantage was the 2.1 factor always added when the classical objectives (160mm [tube length: t.l.] corrected), either for subjective observation or photo-micrography. Stand number 6939, was refurbish changing tube lenses (ground in Argentina) so that it can be used with ancient finite t. l. corrected objectives as well as the infinite t. l. corrected common in all offered microscopes nowadays, only a 1x factor is now present. The complete illumination system was preserved and provided with different light sources. The possibility of digital image analysis and confocal microscopy is considered.

**27. EFFECT OF *IN VITRO* CADMIUM INTOXICATION ON INOSITOL PHOSPHATE LABELLING OF RAT ANTERIOR PITUITARY LOBE**

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In an *in vivo* experimental model, we have previously shown that cadmium (Cd) intoxication induces alteration of phospholipid (PL) contents and reduction of phospholipase D activity in rat anterior pituitary lobe (APL). In this work we studied in an *in vitro* model the effect of Cd on the inositol phosphates (InsP) formation in dispersed cells of APL from adult male Wistar rats. Cells were obtained by trypsin/DNase treatment and mechanical disruption in Dulbecco's modified Eagle's medium at 37°C, in 95% O<sub>2</sub>: 5% CO<sub>2</sub> atmosphere. Cells (10<sup>6</sup>) were incubated with 1μCi myo-[2-<sup>3</sup>H]inositol for 60 min. After washing, Li<sup>+</sup> (5 mmol/L) was added 10 min before stimulation with Cd solution (10 μmol/L) for 30 min. Control cells were not exposed to Cd. Labelled InsP were assayed with Dowex 1-X8 anion-exchange resin columns and elution with Na formate/Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub> and NH<sub>4</sub>formate/formic acid. Results of individual InsP were calculated as percentage of radioactivity in total InsP. In Cd-treated cells an increase of total InsP, InsP<sub>1</sub> and glycerophosphoinositol was observed (p < 0.005). Labelling of IP<sub>3</sub> was decreased (p < 0.01) suggesting that Cd might interfere with phospholipase C activity. Phosphoinositides and InsP may be involved in the mechanisms of Cd intoxication in APL.

**26. ENZYMATIC ACTIVITY IN *Phymaturus punae* (SQUAMATA)**

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Several studies examined how digestive enzyme characteristics of species matches with contrasting diets in the field. Enzyme activity and its pattern along the small intestine gives important information about the quality and quantity of substrate to process. So far among omnivore and carnivore lizards we have not find a clear effect of the diet on the intestinal digestive enzymes. *P. punae* is and strict herbivore species; thus we predicted that its intestinal carbohydrazase activity should be higher when compared to insectivore/omnivore lizard species. We present here a preliminary test of this prediction studying four individuals of this species captured in Catamarca (Argentina). We assayed aminopeptidase-N, maltase and sucrase activities in homogenates of their small intestines. The pattern of aminopeptidase-N, maltase and sucrase activities were: proximal = medial > distal. Maltase and sucrase activities were tenfold higher than those reported for omnivore and insectivore lizard species. However, the level of aminopeptidase-N was comparable to other lizards with more protein based food habits. These results give partial support to our prediction. Nonetheless, we caution that few species have been studied to date and a phylogenetic effect cannot be ruled out.

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**28. GROUP B STREPTOCOCCI CARRIER DETECTION IN PREGNANT WOMEN**

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Group B streptococci (*Streptococcus agalactiae*) is a major cause of neonatal bacterial infection. The anorectal carriage of group B streptococci (GBS) in pregnant women is important, due its possibility to colonize the genital tract. Newborns might be infected by intrapartum transmission of GBS or *in utero* by an ascendant way, with a vertical transmission rate of about 50%. Parturient carriers of GBS were searched by collecting samples on swabs from the vagina and rectum from 35 – 38 wk pregnant women, between 17 June and 8 Oct 2004. The swabs were submersed in Todd – Hewitt broth supplemented with colistin and nalidixic acid and incubated at 37 °C for 24 h. After that time, all the tubes were plated on blood agar and incubated at 37°C for 24 – 48 h. β – Hemolytic colonies that were catalase (-), PYR (-), TMS (R), BC (R), growth in 6.5% NaCl broth (-) and sodium hippurate (+) were identified as GBS and then confirmed by serology. Nine out of 95 samples cultured were positive for GBS (9.5%). These data agree with those found in the literature (10% - 30%). The rapid and accurate identification of this organism in pregnant women helps to decrease the high mortality and morbidity associated with GBS in newborns.

**29. RESISTANCE OF *Helicobacter pylori* TO TETRACYCLINE IN SAN LUIS**

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For treatment of *H. pylori* infections, tetracycline-based triple or quadruple therapies are often used as a second-line treatment. Until the turn of the century only a few reports were published on spontaneous tetracycline (Tet) resistance. However, an increase in the incidence of Tet resistance has been reported in the last 2 years. The aim of this study was to determine the prevalence of tetracycline-resistant *H. pylori* isolated from symptomatic adult patients from San Luis. Tet MIC for sixty-two clinical isolates was determined by agar dilution method according to the NCCLS recommendations. *H. pylori* NCTC 11638 was used as control. Antibiotic was included in Mueller Hinton agar supplemented with 7% horse blood as two-fold dilutions from 128 to 0.008 mg/L. Resistance to Tet was present when MIC = 1 mg/L. Four tetracycline-resistant strains were determined in this study. Interestingly, all of the Tet-resistant isolates exhibited cross-resistance to metronidazole. Several authors have suggested that there may be a progressive acquisition of resistance where metronidazole resistance may be required before Tet resistance can develop, but not vice versa. In *H. pylori*, metronidazole is converted to an active, mutagenic compound that could increase mutations in the 16S rRNA genes responsible for Tet resistance in *H. pylori*. Emergence of cross-resistant strains among clinical isolates represents a major threat to current *H. pylori* eradication therapies.

**31. THE KARYOTYPE OF *Caesalpinia mimosifolia* (FABACEAE)**

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*Caesalpinia mimosifolia* (Fabaceae), considered endemic of Argentina, is a defenseless and glandulous shrub of 0.50-2 m of height. Continuing with the study presented in a previous analysis, the objective of this work was to make the karyotype of this species in order to establish phylogenetic relationships with the other native species of the genus *Caesalpinia* distributed in San Luis. Chromosomal counts were performed on meristematic root cells of radical apices. Seeds of *C. mimosifolia* were collected from the Sierra de Las Quijadas National Park and germinated in laboratory. The young roots were treated, fixed and stained. The selected apices were "squashed" and permanent slides were prepared with Euparal. 20 metaphasic plates were analyzed and the diploid number of  $2n=24$  was determined. This results agree with the basic number of the genus  $x=12$  and with those of other species of *Caesalpinia* inhabiting in San Luis. The karyotype is composed for 8 m pairs and 4 sm pairs. These results show similarities with *C. gilliesii* and *C. paraguariensis* in both the basic number, karyotype formula and the presence of satellites and cells poliploy ( $2n = 48$ ), and differences in the size of the chromosomes. Therefore, it may be suggested that during their diversification no chromosomal rearrangements have taken place, although occult structural changes could have occurred.

**30. KARYOTYPES SYMMETRY ANALYSIS OF *Caesalpinia gilliesii*, *C. mimosifolia* AND *C. paraguariensis* (FABACEAE)**

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The composition of a karyotype can be expressed by means of its degree of symmetry or asymmetry. A symmetrical karyotype is one in which the chromosomes are all of approximately the same size, and have median or submedian centromeres. These karyotypes are considered to be more primitive than asymmetrical karyotypes. The degree of symmetry can be measured using the  $A_1$  Index, that depends on differences among chromosomes in the karyotype and the  $A_2$  Index that expresses morphological differences in chromosomes. The objective of this work was to analyze karyotype symmetry of *Caesalpinia gilliesii*, *C. mimosifolia* and *C. paraguariensis* using  $A_1$  and  $A_2$  indexes, in order to classify and compare them with each other and with other related species. The classification of Stebbins was employed to determine categories of symmetry. The results indicate differences in  $A_1$  between *C. paraguariensis* and the other two species and a scarce variation in the size of the chromosomes of *C. gilliesii* and *C. paraguariensis*, regarding the values of  $A_2$ . The three taxa possess constant and moderately symmetrical karyotypes and are included in the 2A and 2B Stebbins categories. This characteristic is common in the subfamily and reflects the tendency of perennial and woody plants, of having stable and less diversified karyotypes.

**32. MANNOSE-6-PHOSPHATE RECEPTOR EXPRESSION IN RAT EPIDIDYMIS IS ALTERED BY ANTIANDROGENIC (FLUTAMIDE) TREATMENT**

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The epididymis provides the proper environment for maturation of spermatozoa during their transport through the epididymal duct. Epididymal function and integrity is maintained by normal androgen levels, i.e., testosterone and its active metabolite dehydrotestosterone. The epididymal epithelium synthesizes and secretes, among several proteins, important amounts of lysosomal enzymes. In other cell types, the sorting and transport of lysosomal enzymes is regulated by mannose-6-phosphate receptors (MPRs) that recognize this mannosylated residue on the enzyme molecule. Two types of MPRs have been described so far, the cation-dependent (CD-MPR) and cation-independent (CI-MPR) mannose-6-phosphate receptor. When the enzyme-receptor system is altered, lysosomal enzymes are mostly secreted to the extracellular medium. We wondered if the secretion of lysosomal enzymes in epididymis is related to MPRs expression. By Western blot analyses, we found that in rat epididymis both MPRs are expressed at levels higher than liver and this expression increases by the treatment with the antiandrogenic drug flutamide. From these results we postulate that the increase of MPRs expression by the treatment may be related to a higher intracellular retention and/or a participation in autophagic processes. Further studies on possible redistribution of MPR and lysosomal enzyme by the treatment are carried out at present.

**33. PREGNANOLONE ANXIOTICITY IS PRIMED BY HORMONAL STATUS IN FEMALE BUT NOT MALE RATS**

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Neurosteroids are steroids synthesized and metabolized in several brain areas, e.g. cortex, hypothalamus and pituitary. It has been reported that although neurosteroid exposure may influence both the pharmacological properties of the GABA<sub>A</sub> receptor and the manifestation of anxiety in both sexes, the effects of neurosteroids may be modulated in a sex-specific manner. To test this hypothesis this study compared the behavioral effects of pregnanolone regarding different hormonal status and sex. Adult Sprague-Dawley male rats, intact and castrated, and females at 15th day of pregnancy were used (n=8 animal/group). Pregnanolone 6 μM and Krebs solution were injected i.c.v. Anxiety (total time spent exploring the open arms: TOA) and locomotion activity (number of total arm entries: TLA) were tested on an elevated plus-maze. Our results showed that TOA was significantly shorter in treated pregnant females ( $p < 0.05$ , Student's *t* test). On the other hand, there was no difference between control and treated males. Accordingly, there was a significant increase of TLA in treated pregnant animals ( $p < 0.05$ , Student's *t* test). We were unable to detect a comparable effect in males. From our results we conclude that progesterone was probably responsible of priming the pregnanolone effect, and that this priming was sex-dependent.

**35. ALLOPREGNANOLONE STIMULATES <sup>3</sup>H-GLUTAMATE RELEASE IN FEMALE RAT MEDIAL BASAL HYPOTHALAMUS *IN VITRO***

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A variety of steroids can be synthesized in the rat brain independently of peripheral glandular sources. Such steroids formed within the brain from cholesterol are defined as neurosteroids. Allopregnanolone (All) is a neurosteroid that modulates ionotropic receptors like NMDA and GABA<sub>A</sub>. The present work investigated the modulation of All on the release of glutamate (Glu) and its relation with the GABAergic system from medial basal hypothalamus (MBH). Ovariectomized, estrogen and progesterone primed adult Sprague-Dawley rats were used. The effect of All was evaluated by means of K<sup>+</sup>-evoked tritium Glu (<sup>3</sup>H-Glu) release from MBH slices. The slices were exposed to All 6 μmol/L, the GABA<sub>A</sub> antagonist bicuculline (Bic) 9.8 μmol/L, All + Bic or KRBG (control). All stimulated <sup>3</sup>H-Glu release in comparison with the control group ( $112 \pm 18$  vs  $27 \pm 3$ ;  $p < 0.001$ ). Addition of Bic inhibited the effect of All ( $62 \pm 8$  vs  $112 \pm 18$ ;  $p < 0.001$ ). Bic plus All did not modify the inhibitory effect of Bic (ANOVA 1, Student-Newman-Keuls test). We conclude that All induces changes in the NMDA excitatory system, with a positive modulation. This effect was dependent of integrity of the GABAergic system.

**34. EXPRESSION OF MARKERS FOR APOPTOSIS IN MAMMARY GLANDS OF HYPERTHYROID RATS ON DAY 14 OF LACTATION**

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We have shown that hyperthyroidism (HT) produces precocious mammary involution in lactating rats, with decreased serum Prl and mammary aP2 and SREBP1c expression, increased mammary expression of SGP2, number of adipocytes and greater proportion of collapsed alveoli. We studied the expression of several markers of involution and adipogenesis on midlactating HT rats. T<sub>4</sub>-treated (10 μg per 100 g bw/day or vehicle-treated rats (Co) were mated 2 wk after the start of treatment and sacrificed on day 14 of lactation. We measured by RT-PCR the abundance of p53, PPARγ (peroxisome proliferator-activated receptor γ), ADRP (adipocyte differentiation-related protein), AIF (apoptosis inducing factor), PKCα (Protein Kinase C α) and RPr1 (Long Pr1 receptor) mRNAs. Aliquots of the reverse transcription reaction mix cDNA were amplified with specific primers for each gene. The β-actin gene served as internal control. HT increased ADRP (HT:  $0.851 \pm 0.05$ , Co:  $0.688 \pm 0.06$ ), PKCα (HT:  $0.947 \pm 0.11$ , Co:  $0.704 \pm 0.04$ ); and decreased PPARγ (HT:  $0.87 \pm 0.17$ , Co:  $2.79 \pm 0.7$ ) and RPr1 (HT:  $1.108 \pm 0.16$ , Co:  $1.52 \pm 0.14$ ). The greater number of adipocytes observed may be associated with increased expression of ADRP. The variations in the expression of apoptotic and survival factors may represent attempts of the tissue to maintain its function during active lactation in the presence of the involution stimuli produced by the HT state and reduced circulating prolactin.

**36. CHRONIC CANNULATION OF THE SMALL INTESTINE OF PIGEONS (*Columba livia*): A TECHNIQUE TO ASSESS BIOAVAILABILITY**

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Many *in situ* and *in vitro* techniques have been developed to study intestinal absorption processes. *In vivo* techniques have the advantage to reproduce conditions close to a normal situation. Our aim was to develop an *in vivo* method to study intestinal absorption of solutes in intact pigeons (*Columba livia*). To achieve our objective we installed chronically a cannula in the proximal section of the small intestine, near the pyloric valve. The procedure started by anesthetizing birds with a mix of Ketamine/xylazine. Then a small incision was cutted in the abdomen and, a modified nasogastric catheter (K-33) was inserted in the proximal part of the small intestine of the pigeons. A 21G catheter was introduced in the nasogastric catheter to avoid the entrance of substances from the lumen. Recuperation after surgery was very fast and no apparent body mass reduction was apparent. Animals consumed food and water within a day. The cannula was expelled spontaneously by pigeons around two months after surgery. The method is simple, inexpensive and useful for nutrition research. Particularly, it was useful to determine bioavailability of solutes in intact animals without influence of anaesthesia.  
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**37. MYCOLOGICAL DIAGNOSIS OF SUPERFICIAL SKIN LESIONS IN SAN LUIS CITY**

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Superficial infections of the skin and its appendages are the most frequent causes of dermatological consultation. Most of them are caused by fungi. Our purpose was to perform a retrospective study from November 2002 to September 2004 to determine the frequency of superficial mycosis and its etiological agents. A total of 446 samples of male and female patients, were obtained by scraping. Direct examinations with 20% KOH and methylene blue were performed. In the cases of suspected pityriasis, Sabouraud glucose, lactrimel agar and DTM were seeded. The identification of etiological agents was performed by macro- and micromorphological studies and biochemical tests. Of the 446 processed samples, 234 (52.46%) were positive and 212 (47.54%) negative by direct examination and/or culturing. The most frequent result was *non-Candida albicans yeast* (56, 23.9%), followed by *Malassezia spp* (34, 14.5%), *Trichophyton spp* (33, 14.1%), *Trichophyton rubrum* (32, 13.7%), *Trichophyton mentagrophytes* (29, 12.4%), *Microsporium canis* (21, 9%), *Candida albicans* (19, 8.1%), *Trichophyton tonsurans* (4, 1.7%), *Epidermophyton floccosum* (2, 0.8%), *Microsporium gypseum* (2, 0.8%), *Fusarium* (1, 0.4%) and *Aspergillum* (1, 0.4%). The most frequent lesions were dermatomycosis, caused by *non-Candida albicans yeast* and *Malassezia spp* and dermatophytosis, caused by *Trichophyton spp*.

**39. HYDROLYTIC CAPACITY OF DIGESTIVE ENZYMES IN DUCK CAECA (*Anas platyrhynchos*)**

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Chickens and geese have caecal absorption activity of amino acids together with sugar and protein digestion. Enzyme breakdown activities by the caeca of ducks were measured. We predicted that the digestion of proteins and carbohydrates will be related to the level of these substrates in the diet. To test this prediction, we fed two groups of adult ducks during 15 days with either a high carbohydrate or high protein diet. At the end of treatment activities of maltase and aminopeptidase-N were assayed. Diet did not influence aminopeptidase-N activity but had an effect on maltase activity ( $p < 0.05$ ). Maltase activity exhibited a positive correlation with the amount of carbohydrates in the diet. The contribution of the maltase and aminopeptidase-N of the caeca to the total hydrolysis of carbohydrates and peptides was relatively small, 3.45% and 6.57% respectively. Nonetheless, the specific activities of the aminopeptidase-N reached values near those of the small intestine and, for maltase, half of the levels found in the small intestine. Thus, there is a moderate cecal contribution to carbohydrate and protein hydrolysis in these birds, although higher than for chickens (1.66% for maltase and 4.73% for aminopeptidase-N). In conclusion, the levels of specific activity attained for both enzymes suggest that the caeca may play an important role in digestion in the duck.

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**38. TEMPORAL AND SPATIAL VARIATIONS OF THE AVIAN COMMUNITIES OF THE FOREST OF CALDÉN, PARQUE LURO, LA PAMPA, ARGENTINA**

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An avian community is largely affected by the structure of the habitat and seasonal changes. We studied the species composition and seasonality of the avian communities in two environments with different plant composition and structure: open (BA) and closed (BD) forest of *Prosopis caldenia* (Parque Luro). Data were collected from 12 transects (250x30m) during autumn, winter and spring of 1998. We recorded 53 bird species, 41% of species cited for the phytogeographic region "El Caldén". Avian richness (S) and abundance (N) was higher in BD (N=1773; S=47) than in BA (N=1170; S=38). Avian diversity (Shannon index; t-Hutchenson,  $p < 0.05$ ) was also higher in BD than in BA during autumn and winter, but not in spring. Seasonal habitat diversity of BA was low in winter and, during spring, for BD. Bird species were assigned to five trophic guilds. Omnivores were only present during spring and winter. Richness of BD in terrestrial granivores (GT), flying insectivores (FI) and terrestrial insectivores was increased during spring. The abundance of arboreal herbivores was higher in BD than BA. In summary, temporal and spatial variations were registered in avian communities present in the open and closed forest of Caldén. In addition, the community composition is not stable during the year: Different species assemblages are present in each season, the annual resident and the seasonal species.

**40. AMILORIDE-SENSITIVE SHORT-CIRCUIT CURRENT AND OXYGEN CONSUMPTION IN COLON EPITHELIUM FROM CHRONICALLY HYPOXIC RATS**

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At sea level, rat distal colon epithelium has no amiloride-sensitive electrogenic  $\text{Na}^+$  absorption unless serum aldosterone is above normal. Surprisingly, an amiloride-sensitive short-circuit current (Isc) develops in rats submitted to chronic hypoxia, even when they have very low serum aldosterone. Blood samples and isolated mucosa preparations were obtained from adult rats submitted to hypoxia (air pressure = 400 mmHg) for 10 days. Preparations ( $n = 10$ ) were mounted in an Ussing chamber modified for determination of oxygen consumption rate ( $\text{QO}_2$ ) and kept at 37°C. Isc and  $\text{QO}_2$  were measured during basal conditions and after addition of amiloride (0.1 mmol/L). Serum aldosterone was about 25% of basal ( $0.38 \pm 0.16$  nmol/L). Amiloride lowered both Isc ( $2.42 \pm 0.23 \mu\text{mol}\cdot\text{h}^{-1}\cdot\text{cm}^{-2}$  vs  $3.26 \pm 0.31 \mu\text{mol}\cdot\text{h}^{-1}\cdot\text{cm}^{-2}$ ;  $P = 0.0011$ ) and  $\text{QO}_2$  ( $2.92 \pm 0.09 \mu\text{mol}\cdot\text{h}^{-1}\cdot\text{cm}^{-2}$  vs  $3.26 \pm 0.07 \mu\text{mol}\cdot\text{h}^{-1}\cdot\text{cm}^{-2}$ ;  $P = 0.0003$ ). A significant correlation was found between reduction of Isc and of  $\text{QO}_2$  by linear regression ( $r^2 = 0.64$ ;  $P = 0.0054$ ). The amiloride-sensitive component accounted for about 25% of total Isc and 10% of total  $\text{QO}_2$ . Neither Isc nor  $\text{QO}_2$  were affected by amiloride in  $\text{Na}^+$ -free solution. Thus, while lowering serum aldosterone, chronic hypoxia induces electrogenic  $\text{Na}^+$  transport in this tissue.

**41. MASS SELECTION FOR SEVERAL CHARACTERS IN COLONIES OF THE UNRC APIARY**

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Due to the recent importance of beekeeping in Argentina, the began in July 2003 a breeding program to increase honeybee production. The apiary was made up with 14 hives belonging to the University plus 15 nuclei from Mieleles del Sur hives and 2 from San Luis. In December 10 nuclei with fertilized queens of Tasinato breeder and 9 more from Mieleles Santiagueñas breeding company were added. Thus, the assay population was made of 50 families. During 2003 management, feeding and varroa control were made the apiary being also used for teaching activities. Durante the summer of 2003-2004 the following characters were recorded: number of combs with bees and with brood, and sanity, temperament and hygienic habit giving values from 5 to 1 in decreasing merit order. It was also measured varroa prevalence and acaricide efficiency using bottom hive traps and counting dead mites. During spring 2004 periodic evaluations were repeated. Mass selection of queens was applied. From selected colonies larvae were grafted and best daughter queens reared to return to the initial number of families in the apiary.

**43. MITOCHONDRIA: ULTRA-STRUCTURAL CHARACTERISTICS OF THE DIFFERENT MORPHOTYPES IN BOVINE EMBRYOS PRODUCED *IN VITRO***

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The environment, in which the *in vitro* produced (IVP) embryos develop, induces morphological, physiological and biochemical changes. Some of them affect mitochondria's. Environment-caused mitochondria alterations reflect improper metabolic function and turn out into increased sensibility to cryopreservation, as well as low rates of pregnancy and birth (Fair *et al.*, 2001). IVP embryos show reduced mitochondria volume and present mature and immature forms, as well as vacuolated and hooded mitochondria types (Crosier *et al.*, 2000). The last two types use to come associated with serum-supplemented culture media (Dorland *et al.*, 1994). In determining the mitochondria morphotypes of IVP embryos cultured in serum-free and serum-supplemented media through different developmental stages (between two-cells and morulae), we particularly observed, by transmission electron microscopy, the "hooded" type. Some authors associate it with abnormal shapes (Shamsuddin & R-Martinez, 1994; Abe *et al.*, 1999) while others view it among the normal forms (Brackett *et al.*, 1980; Rivera *et al.*, 2003). Research work in course is quantifying the surfaces covered by this and the other morph types in an effort to relate it causally with lipid accumulation. The observed dependence on culture conditions shows that better specifying them decreases the observed sub-cellular alterations, probably reducing their influence on the future growth of embryos and foetuses.

**42. LIPID DISTRIBUTION IN BLASTOMERS OF BOVINE EMBRYOS PRODUCED *IN VITRO*: PRELIMINARY OBSERVATIONS**

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The culture conditions of *in vitro* production (IVP) technique induce cellular alterations that evidence incorrect metabolic operation and decrease survival capacity. This appears crucial insofar as embryo quality plays a key role in the embryo's resistance to cryopreservation and its use on animal production. Lipid droplet cover area is greater in *in vivo* embryos than in IVP embryos. Also, some authors affirms that embryos cultured in serum supplemented media (SSM) shows more droplets than those cultured in serum free media (SFM). Lipid droplets mainly consist of triglycerides, whose absolute amount are invariable on IVP embryos cultured in SFM, but increase more than twice between 9-cell stages and blastocyst on SSM. We observed bovine lipid-droplets and cytoplasmic-vesicles distributions of IVP embryos cultured in SFM and SSM at different developmental stages, through semi-thin section treated with toluidine blue and embryos stained by Sudan. Assessing the relationships between lipid droplets and different morphotypes of mitochondria in different embryo-stage cells on serum-free and serum-supplemented media will allow to detect the ultra-structural conditions -which mirror the metabolic ones- at the beginning of lipid accumulation.

**44. DIAGNOSIS OF HEPATITIS B IN PREGNANT WOMEN**

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Only a small proportion of acute infections caused by hepatitis B virus (HBV) can be identified on clinical bases. Between the years 1996 and 2003 5,467 pregnant women attended for HIV test and 3,497 for HBsAg (ELISA techniques). Results were analyzed according to the table.

Years	HIV		HBV	
	Total	Prevalence	Total	Prevalence
1996	612	0	6	0
1997	623	0	31	0
1998	548	0	424	0.94
1999	672	0	323	0
2000	734	0	515	0.19
2001	745	0.26	731	0.13
2002	742	0.13	718	0
2003	791	0	749	0

Results suggest that during the 90s, the high infectivity degree of the HBV was not adequately considered. This was gradually reverted from 2000 on. Because of better awareness, and despite the increased prescription of HIV testing, that of hepatitis B was more marked through the years. The prevalence of HBV was 0.97-1.18-1.21-1.23-1.02-1.10-1.00-0.91, respectively. The current strategy is early detection of the pregnant carrier woman and thus to detect appropriately the infected newborn, in order to indicate the specific treatment. Chronic HBV infection affects approximately 90% of the infants pre- or perinatally infected.

**45. ANTIFUNGAL ACTIVITY OF DECOCTIONS OF PLANTS USED IN POPULAR MEDICINE IN ARGENTINA**

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Many plants display antimicrobial activity. Prevalence of fungal infections, especially in immunosuppressed patients, and treatment cost justify a search for alternative treatments. This work studied the growth inhibition of several fungi by different plant decoctions (D). The aerial parts, leaves and seeds were dried and powdered and decoctions obtained (5% w/v). The activities of *Larrea divaricata* (jarilla), *Ganaphalium gaudichaudianum* (marcela), *Baccharis trimera* (carqueja), *Schinus areira* (aguaribay), *Schinus terebenthifolius* (pimienta rosa), *Xanthium spinosum* (cepa caballo), *Lippia turbinata* (poleo), *Coryza bonariensis* (yerba carniceira), *Thelesperma megapotamicum* (té indio) and *Jodina rhombifolia* (peje) were evaluated on *Aspergillus niger*, *Penicillium notatum*, *Candida albicans* and *Saccharomyces cereviceae*. The strains were kept at 4°C in glucose agar Sabouraud medium. *C. albicans* and *S. cerevisiae* cultures of 24 h and 8-day cultures of *A. niger* and *P. notatum* incubated at 28°C were used. The inoculum consisted of 10<sup>6</sup> yeasts or conidia/mL. Plates containing 12.5, 25, 50, 100, 150, 250 mg/mL of each D were inoculated with each microorganism. After a 4-day incubation the MIC was determined. Jarilla and carqueja showed inhibition on *S. cerevisiae* at 100 mg/mL ( $p \leq 0.01$ ). The other extracts showed different inhibition activities on *S. cerevisiae* growth ( $p \leq 0.01$ ). No D caused inhibition of *A. niger*, *P. notatum* or *C. albicans* growth. Our results showed that *S. cerevisiae* was the microorganism more effectively inhibited by the extracts tested.

**47. Larrea divaricata CAV (JARILLA) AQUEOUS EXTRACTS INDUCE APOPTOSIS OF MOUSE MACROPHAGES**

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Apoptosis (programmed cell death) can be activated by several factors like cytokines, nitric oxide, etc. The aim of this work was to study the effect of decoction (D) and infusion (I) of *Larrea divaricata* Cav (jarilla) on mouse macrophages (MØ). The experiments were programmed using I and D extracts (5% w/v) obtained from the aerial parts of the jarilla. MØ were incubated in an atmosphere of 5% CO<sub>2</sub> with 1, 2, 4, and 8 mg/mL of each extract for 1.5 h at 37°C. Afterwards the MØ were stained with Giemsa (G) and ethidium bromide/acridine orange (EB/AO). MØ treated with the extracts and stained with G present the following alterations compared with controls: decrease in nuclear size, intense picnosis, nuclear fragmentation and citoplasmatic retraction. Chromatin lumps were observed next to the nuclear membrane and apoptotic bodies were seen in the cytoplasm with the EB/AO technique. With D extract, 91.40% and 3.50% of cells were observed in apoptosis and necrosis respectively. Similar results were obtained with I: 97.3% and 2.7% of apoptosis and necrosis. The results indicate that the treatment with aqueous extracts of jarilla produces MØ cell death through both necrosis and apoptosis.

**46. ANTIFUNGAL ACTIVITY OF ETHANOLIC EXTRACTS OF THREE PLANTS USED IN POPULAR MEDICINE IN ARGENTINA**

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This work evaluated the antifungal activity of ethanolic extracts (Ee) of *Ganaphalium gaudichaudianum* D.C (marcela), *Larrea divaricata* Cav. (jarilla) and *Baccharis trimera* (carqueja). Dry material (5 g) was added to 100 mL of 96% ethanol. After 48 h the extract was dried, weighed, dissolved in ethanol, and tested against *Aspergillus niger*, *Penicillium notatum*, *Candida albicans* and *Saccharomyces cerevisiae*. They were kept at 4°C in test tubes containing glucose agar Sabouraud. *C. albicans* and *S. cerevisiae* cultures of 24 h and 8-day cultures of *A. niger* and *P. notatum* incubated at 28°C were used. Plates with different concentrations of Ee were inoculated with each species (10<sup>6</sup> yeast or conidia/mL) and were incubated for 4 days. Growth inhibition was evaluated 24 h later for *C. albicans* and *S. cerevisiae* and 96 h later for *A. niger* and *P. notatum*. Growth of *S. cereviceae* was inhibited by all Ee used. No significant inhibition was observed for *A. niger* and *P. notatum* by any extract. *C. albicans* growth was inhibited by jarilla. Different grades of growth inhibition were observed on *C. albicans* (20 mg/mL) and *S. cerevisiae* (2.5 mg/mL) using Ee of jarilla. Ee of marcela and carqueja only inhibited *S. cerevisiae* growth. Our results showed that fungal growth was more effectively inhibited by Ee of jarilla.

**48. Clostridium botulinum SPORE LOAD IN SOIL OF CHUBUT AND RIO NEGRO, ARGENTINA**

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*Clostridium botulinum* (Cb) spores are widely distributed in nature. They occur in both cultivated and forest soils, and can often be found on the environmental dust, many agricultural products, honey and medicine plants. Frequency of positive soil samples for Cb and the spore load can be considered two important parameters for botulism transmission in its forms: foodborne botulism, infant botulism, and wound botulism. Fourty nine soil samples from Chubut (25) and Rio Negro (24) were proceeded for detection of Cb, typing of neurotoxin (BoTN) and estimation of Cb viable spore concentration (VSC) with the most probable number (MPN) method. **Chubut:** Positive results were obtained in 4/25 (15.66%) samples. VSC per gram of soil (MNP/g): 84% (21/25) with less than 0.11 (0.02-0.21)\* MNP/g; 4% (1/25) with 0.12 (0.04-0.41)\*MNP/g; 8% (2/25) with 0.33 (0.10-1.09) and 4% (1/25) with 1.83 (0.55-6.05)\* MNP/g. BoTN types: A 50% (2/4), B 25% (1/4) and E 25% (1/4). **Rio Negro:** Positive results were obtained in 4/24 (16,66%) samples. VSC: 83,33% (21/24) with less than 0.11 (0.02-0.21)\* MNP/g.; and the other with 0.12 (0.04-0.41)\*MNP/g; 0.26 (0.08-0.86)\*; 0.73 (0.22-2.41)\* and 1.83 (0.35-3.81)\* MNP/g. BoTN types: A 50% (2/4), B 50% (2/4) [\*95% confidence limits] Results are similar in both provinces but they are lower than those obtained in other regions of Argentina, with high incidence of human botulism.

49.

**PREVALENT DIAGNOSES IN OUTPATIENTS IN A SAN LUIS CITY HOSPITAL***De Pauw M, Calderón C, Maggini V, Pelzer L, Rapisarda M\*, Valsecia M<sup>§</sup>**Farmacol, Univ Nac San Luis, 5700 San Luis; \*Droguería Provincial; <sup>§</sup>Farmacol, Univ Nac NE. E-mail: ccal@unsl.edu.ar*

An observational, cross-sectional, retrospective study was performed on Hospital del Sur outpatients, assessing prevalent diagnoses and distribution by patient and specialty. Diagnoses were collected during June 2004 and classified by ICD-10. Patients were classified by age, sex and consulted specialty. Results(%): Sex: female(F) 57.5, male (M) 42.5; Age: <15: 36.5, >14: 62.3; Age and Sex: <15: F 47.4, M 52.6, >14: F 63.2, M 36.8. Diagnoses: Respiratory (J) 35.7, abnormal symptoms (R) 12.5, digestive(K) 8.4, osteomuscular (M) 8.1, endocrine(E) 6.22, circulatory (I) 4.8, genitourinary (N) 4.4, infections(A,B) 3.7. Specialty: Emergency room (G) 43.8, generalist (Gen) 28.6, pediatrics 18.0, diabetology 7.8. Age and diagnoses: >14: J (31.8), M (12.3), K (11.0), R (10.4), I (7.8), E (6.6), N (5.2), A,B (4.2); <15: J (42.5), R (14.4), K (3.7), A,B (9.9), E (5.0). Prevalent Diagnoses: >14: influenza (8.5), dorsalgia (7.9), arterial hypertension (7.1), bronchitis (6.7), diabetes (6.4), pharyngitis (6.0); <15: bronchitis (12.7), pharyngitis (10.1), cold (6.5), fever (6.3), emesis (5.4), gastroenteritis (4.9). Conclusions: Distribution by age showed a preponderance of adults. Distribution by sex showed a higher proportion of females among adults, but similar for both sexes in children. Most consulted specialties were G and Gen. Prevalent diagnoses were J, R, K, and M. Respiratory diseases were the most frequent in children, while influenza and some chronic illnesses were seen more often in adults.

51.

**EFFECT OF CADMIUM EXPOSURE ON LIPID METABOLISM IN RAT HEART***Domeniconi MA, Pellegrini F, Gimenez MS, Larregle E**Bioquím Molec, Univ Nac San Luis, 5700 San Luis. E-mail: adomeniconi@hotmail.com*

It is known that cadmium (Cd) is an environmental toxicant that causes different disease in human according to the doses and exposition time of it. Previous results obtained in our laboratory showed that 15 ppm of Cd, as CdCl<sub>2</sub>, administrated in the drinking water for two months, causes alteration in the lipid amount in liver, kidney, intestine, adipose tissue and serum of Wistar male rat. In order to determine if the increase of serum triglycerides observed in these Cd treated rats is associated to changes in the lipid content of the heart, the amount of different lipids and proteins in heart was measured. Adult rats were separated in two groups: control group and Cd exposed (15 ppm in drinking water for two months) group. Heart lipid were separated by TLC and then quantified: Triglycerides (TG) by Sardesai and Manning's method, total (TC), free (FC) and esterified cholesterol (EC) by Zack's method, total phospholipids (TP) (according to Rausser *et al.*) and proteins by Biuret method. The results showed that TG, FC, EC, TP and proteins were not modified in Cd treated rats. Considering that lipid content of other organs is affected, these results could indicate that the heart develops a protective mechanism, probably associated to alterations in lipid uptake from serum.

50.

**EFFECT OF *IN VITRO* STIMULATION OF COELIAC GANGLION ON THE FUNCTION OF POLYCYSTIC OVARY IN PREPUBERTAL RATS***Delgado SM, Forneris ML, Oliveros L**Fac Quím Bioquím Farm, Univ Nac San Luis, 5700 San Luis. E-mail: mforner@unsl.edu.ar*

In the left coeliac ganglion-superior ovarian nerve-ovary (CG-SON-O) integrated system isolated from prepubertal rats, we found more nitric oxide (NO) and less androstenedione (A) ovarian release when the CG is stimulated with acetylcholine. In this work we studied, with the same system, the effect of norepinephrine (NE, 10<sup>-6</sup> mol/L) added to CG on ovarian A and NO release in polycystic (PCO) rats. PCO was induced with sc injection of 1 mg estradiol valerate -or vehicle for controls (C)- in 4-day old rats. At 30-day old PCO and C rats were separated in two groups each. The CG-SON-O system was isolated and CG and O incubated in separate cuvettes. The CG was treated with NE or vehicle and samples were taken from the ovarian cuvette at 15, 30, 60, and 120 min to measure A (by RIA) and NO (Griess reactive). In C, release of NO decreased while A did not change with time; both were increased with CG NE stimulation (p < 0.001). In PCO, NO and A showed no change with time. Only A was increased (p < 0.01) after CG NE stimulation. The different steroidogenic ovarian response indicates that the CG is sensible to NE in both C and PCO. The increase of A and NO from C rats after NE stimulation, while in PCO only A increased, suggests that other factors are involved in the PCO ovarian response.

52.

**EVALUATION OF *Akodon molinae* AS A WILD RESERVOIR FOR *Trypanosoma cruzi****Doña RD, Calendino J, Caviedes-Vidal E**Unidad Invest Cs Biol, Univ Nac San Luis, 5700 San Luis. E-mail: rdona@unsl.edu.ar*

*Akodon molinae*, a sigmodontine rodent of wide geographical distribution in Argentina, is a putative wild reservoir of *Trypanosoma cruzi*, the etiological agent of Chagas disease. The hypothesis was tested in Beazley (San Luis, Argentina), a region where this rodent is abundant. We searched for trypanosomes by (a) microscopical examination of fresh blood samples. Morphology and biometry were assessed from parasite photomicrographies from blood preparations stained with May-Grünwald Giemsa. Criteria for data analysis were: parasite grouping; shape and size of the parasite; flagellum length; size and location of the kinetoplast, and size and location of the nucleus. (b) DNA extraction and PCR using primers #121 y #122 that amplify kADN of *T. cruzi* y *T. rangeli*. Out of 77 individuals captured, 21 (27.3%) exhibited parasitemia with trypanosomes. Three types of kinetoplastids were distinguished: 1) with a body length (BL) range between 16-24µm, and a nucleus located (NL) in central third of the body (N=11/77); 2) BL range 16-24µm + NL in the distal third of the body (N=5/77); 3) Non detectable by microscopy, but detectable by PCR (N=2/77). These results suggest that *A. molinae* has a relatively low incidence in the wild cycle of Chagas disease (2.3% of infection by *T. cruzi*) actually lower than for other trypanosomatides.

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**53. USE OF PCR TO DETECT CHAGAS DISEASE DURING CHILDHOOD: A COMPARATIVE APPROACH**

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Chagas disease is endemic in Cuyo region. San Luis Health Agencies have monitored during several years the incidence of this disease in children, reporting indexes of 1.57 to 2.43% for children of age 5 to 9 years old. The importance of a precocious detection is because this disease can be completely cured when it is treated before 14 years old. The aim of our study was to assess the performance of new molecular technology (e.g. using Polymerase Chain Reactions PCR) as a tool for epidemiological detection of the Chagas disease. PCR may be useful in those cases where the parasites are in the blood circulation. Another advantage is that it is a direct determination method. To evaluate this method we screened a sample of 108 children (age: 6-15 years old) enrolled in a school of Juana Koslay (San Luis). Blood samples were obtained by vein puncture without anticoagulants. Sera were assayed using two serological methods, HAI (indirect hemagglutination) and ELISA tests. On the remnant coagulum, a PCR assay was performed using 2 primers (#121 & #122) that amplify differentially kDNA from *Trypanosoma cruzi* and *T. rangeli*. Serological tests (titres > 1/32) showed two positive children, whereas by PCR only one of those cases was detected. We conclude that molecular tests have a relative importance in epidemiological screening, since it requires large blood samples, technicians with high expertise and instruments and reagents of elevated cost compared to traditional serological methods.

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**55. BIOLOGICAL ACTIVITY OF ETHYL ACETATE EXTRACTS FROM SOIL FILAMENTOUS FUNGI**

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Soil samples were collected on August 2003 in 25 de Mayo district, San Juan Province (Argentina) to perform a screening for filamentous fungi able to produce bioactive extracts. Fungi were isolated by standard microbiological methods and were identified by Dra. S. N. Chulze, UNRC. Ten fungi strains were selected for preparative scale fermentation in different suitable media. A standard two-stage fermentation protocol was employed for scale-up. Fermented culture medium was successively extracted (three times each) with ethyl acetate. After filtration, combined extracts were evaporated to dryness, in order to obtain ethyl acetate extracts. Assays: Antibacterial activity was evaluated by agar dilution method using Müeller-Hinton agar medium for Gram (+) and Gram (-) bacteria. Free radical scavenging effect of fungi extracts was measured by decoloration of 1,1-diphenyl-2-picrylhydrazyl radical (DPPH). Inhibition of germination assay was carried out using *Lactuca sativa* L. and *Phalaris canariensis* L. seeds. Results: *Aspergillus japonicus*, *Fusarium moniliforme*, *Penicillium minioluteum* and *P. purpurogenum* extracts showed a DPPH decoloration > 60% at 100 µg/mL. *F. moniliforme*, *F. oxysporum* and *Aspergillus awamori* extracts showed a 100% germination inhibition of *L. sativa* seeds at 500 µg/mL. *P. chrysogenum* showed higher antibacterial activity against methicillin resistant-*Staphylococcus aureus* at 500 µg/mL. The bioactive extracts will be studied further to isolate the active metabolites.

**54. IMPACT OF IL-12 DEFICIENCY IN EARLY STAGE OF *Yersinia enterocolitica* O:3 ARTHRITIS**

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Interleukin 12 (IL-12) is a cytokine which plays an important role in early innate resistance to bacterial infection. *Yersinia enterocolitica* causes reactive arthritis (ReA) as a complication of the gastrointestinal infection. *Y. enterocolitica* O:3 is the most frequent *Yersinia* arthritogenic serotype in humans. This work studied the impact of IL-12 deficiency on early stage of *Y. enterocolitica* O:3 arthritis. IL-12 knockout (IL12<sup>-/-</sup>) mice and normal C57BL/6 mice were orogastrically infected with  $7 \times 10^8$  UFC of *Y. enterocolitica* O:3. Mice were sacrificed at day 3 postinfection for examination of histopathology of joints and bacterial clearance. Spleen cell proliferation in response to *Yersinia* lipopolisaccharide (LPS), concanavalin A (ConA) or heat-killed yersinia (HKY) were studied. Histological analysis showed histopathological changes in the joints of IL-12<sup>-/-</sup> mice. In these animals, dilation of the joint cavity, luminal disorganization and desquamation of the synovial membrane, decrease in synoviocyte number, and slight mononuclear infiltration were observed. Joint pathology in IL-12<sup>-/-</sup> correlated with higher bacterial load in the spleen, Peyer's patches and mesenteric lymphoid nodes. Impaired proliferation in response to ConA, LPS or HKY was observed in IL-12<sup>-/-</sup>. This study indicates that IL-12 controls the severity of *Yersinia*-induced arthritis and plays a role in early protective host responses after *Y. enterocolitica* O:3 infection.

**56. PHARMACOKINETIC AND BIOAVAILABILITY OF CEFTIOFUR IN GOATS**

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Pharmacokinetic parameters of ceftiofur sodium were determined following intravenous (EV) and intramuscular (IM) 2.2 mg/kg single dose, in a crossed design study in adult non pregnant goat females, (n = 6). Plasmatic levels were determined by microbiological assay using *Providencia alcalifaciens*. The data of ceftiofur plasmatic concentrations were analyzed by non compartmental model. The EV parameters were: average life of elimination ( $t^*$ ) =  $1.63 \pm 0.04$  h and volume of distribution (Vd) =  $0.38 \pm 0.19$  L/Kg. Values did not significantly differ from those obtained from the IM test ( $P > 0.05$ ) and are similar to those in other animal species. In the IM test maximum concentration (C<sub>máx</sub>) was  $3.6 \pm 0.5$  µg/mL with a maximum time (t<sub>max</sub>) =  $0.5 \pm 0.3$  h and  $97.6 \pm 0.1\%$  bioavailability. Results indicate that IM administration provides fast absorption and optimal bioavailability, generating plasmatic levels that exceed the MIC<sub>90</sub> for sensible respiratory pathogens, and lasting for 12 h. Considering these results and the MIC<sub>90</sub> ( $\leq 0.06$  µg/mL) for bacteria like *Pasteurella haemolytica* and *multocida*, one sets out for a 1.3 mg/Kg IM dose every 12 h, whose predicted effectiveness (t > CIM) is about 77.8%.

**57. CONSERVATION IN THE COLOR DISCRIMINATION LEARNING: ACTION OF AP7 AND NBQX IN THE NUCLEUS ACCUMBENS SEPTI**

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The effect on conservation in discriminative learning was examined using 2-amino-7-phosphonoheptanoic acid (AP7) and 1,2,3,4-tetrahydro-6-nitro-2,3-dioxo-benzo[F]quinoxaline-7-sulfonamide (NBQX), compounds known to alter neurotransmitter status in the nucleus accumbens septi (Acc). Young adult pigeons (n = 38) supported with a restriction of 20% of their weight were used: 24 in the experimental group (12 each in AP7 and NBQX) and 14 as controls. They performed tasks of color discrimination learning in an experimental room of 2x2x2 m and food is placed as positive reward in one ("assigned color") of 4 troughs of color (red, yellow, blue and green). Experimental stages: **1-Color discrimination learning** (criterion: correct moves in 80% of all visits) followed by stereotaxic surgery for implanting cannulae in Acc. **2-Test** without reinforcement; experimental groups were injected with either AP7 or NBQX and control group received saline. **3-Retraining** (with reinforcement). Later histological control. **I AP7.** Color discrimination learning did not show significant differences between experimental and control groups. Tests showed significant differences: **Exploration**  $p < 0.006$ ; **Quietude**  $p \leq 0.001$ . They did not demonstrate conducts of tension - emotion. Retraining showed significant differences: **Exploration**  $p \leq 0.03$ , in meeting 1 and  $p \leq 0.05$  in meeting 2. **II NBQX.** Experiment showed no significant differences between experimental and control groups in meetings of learning, test or retraining.

**59. DIFFERENT ENRICHMENT MEDIA IN THE RECOVERY OF *Yersinia enterocolitica* O:9 AND O:5 FROM PORK SAUSAGES**

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Different enrichment techniques and culture media have been designed for recovering *Yersinia enterocolitica*, a well known enteropathogen for humans. The performance of 4 enrichment techniques and 2 plating media in the recovery of serotypes O:9 and O:5 of *Y. enterocolitica* inoculated in pork sausages was studied. Samples were divided in: nonsterilized/noninoculated (NS/NI), sterilized/inoculated (S/I) and nonsterilized/inoculated (NS/I) groups. They were suspended in phosphate buffered saline (PBS) added with 1% sorbitol and 0.15% bile and enriched according to the following techniques: a) Irgasan-ticarcillin-carbenicillin broth (ITC) at 22°C for 3 days, b) trypticase soy broth (TSB) at 22°C for 24 h followed by modified Rappaport broth (MRB) at 22°C for 4 days, c) TSB at 22°C for 24 h followed bile-oxalate-sorbose broth (BOS) at 22°C for 5 days, and d) PBS at 4°C for 21 days. Cultures were surface-spread on trypticase soy agar (TSA), Mac Conkey agar (MC) and Irgasan-novobiocin agar (IN). Based on the increases of microbial counts and the incubation period, TSC-MRB-MC is proposed for recovery of both *Y. enterocolitica* serotypes in S/I group. TSC-BOS-MC or TSC-BOS-IN is suggested for recovery of these serogroups in NS/I groups.

**58. INTESTINAL PASSIVE ABSORPTION OF HYDROSOLUBLE COMPOUNDS IN *Galea musteloides***

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We have demonstrated that intestinal absorption of glucose by the small intestine in birds occurs mostly through the paracellular pathway (75-100%). In contrast, studies in mammals report that paracellular glucose absorption is small (15-25%). However, all mammalian data have been obtained in anesthetized animals using perfusion techniques. In this study we measured intestinal absorption in "intact" animals (*G. musteloides*) and estimated the contribution of the paracellular (PC) and transcellular (TC) route for nutrients absorption. Non metabolizable sugars were used: 3-O-methyl-D-glucose (3OMG) (PM 192) as a marker for both routes (TC+PC) and L-rhamnose (PM 182) and cellobiose (PM 342) for the paracellular pathway. Sugars were administered orally (VO) and injected intramuscularly (IM) to the animals (n = 5, body mass = 229.33 ± 26.9 g). Plasma sugar concentrations were measured in serial blood samples after both VO and IM administration using HPLC/Fluorescence. The amount of sugars absorbed (FA) was calculated from areas under the post-absorption and post-injection plasma curves ( $AUC_{VO}$  and  $AUC_{IM}$ ) with a classical pharmacokinetic equation  $FA = [AUC_{VO}] / [AUC_{IM}]$ . FA was: 68.02 ± 16.34, 16.30 ± 4.61 and 7.92 ± 1.45 (mean ± SEM) for 3OMG, L-rhamnose and cellobiose respectively. In these mammals, the contribution of the paracellular route is smaller than in birds of similar body size.

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**60. ASSOCIATION BETWEEN CYANOPHYTA ALGAE AND ROOTS OF *Prosopis caldenia* BURKART "CALDÉN"**

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There are numerous studies on associations between microorganisms (micorrizae and bacteria) and Fabaceae, but there are scarce reports on associations between blue-green algae and roots of Fabaceae. In this work transverse courts were performed at different heights in roots of *Prosopis caldenia* Burkart with secondary growth as of little plants of four months of life, obtained by cultivation *in vitro*. The little plants were obtained from seeds of a population of *Prosopis caldenia* located near Villa Mercedes (S.L.) With light microscopy, within the cortical cells radical the presence of algae was detected, corresponding to the division Cyanophyta. Differential stainings were made to rule out fungi (as for example *Rhizobium*) and bacteria. Microphotographs were obtained and drawings of the studied material were prepared. These results are the beginning of an exhaustive investigation on the subject.

**61. PROTEOMICS OF ARGININE KINASE (AK) FROM *T. cruzi*: THREE-DIMENSIONAL X-RAY STRUCTURE AT 2.0 Å RESOLUTION**

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Arginine kinase buffers cellular ATP levels by catalysing reversible phosphoryl transfer between ATP and arginine. *Trypanosoma cruzi* arginine kinase is not present in mammalian tissues, making it a suitable target for rational drug design of chemotherapeutic agents against Chagas disease and other parasitic diseases caused by related organisms such as *T. brucei*. The structure at 2.0 Å of the native apo-enzyme refined to an R-factor of 0.23 including about 200 water molecules. The enzyme has 357 residues which build into two domains. Domain 1 belong to the alpha class with an orthogonal bundle architecture. Domain 2 is an Alpha Beta 2-layer sandwich.

**63. PHYTOCHEMICAL STUDY OF *Monttea aphylla* (SCROPHULARIACEAE), I. ETHEREAL EXTRACT**

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The aphyllous shrub *Monttea aphylla* (Miers) Benth. & Hook. is known under the vernacular names "ala de loro", "pata de loro", "retamo" or "matasebo". Its dark green stems and branches are spinescents, with 2-3 axillar tubulous and bluish flowers; its fruit is a pubescent capsule surrounded by the persistent calyx. This native shrub grows in the Monte phytogeographical province in Argentina. It is popularly used in infusions by their antispasmodic and hepatoprotector properties. A phytochemical study was carried out to determine the secondary metabolites of the plant. Aerial dry parts (140 g) were powdered and extracted (first with petroleum ether, then with dichloromethane, ethyl acetate and finally with methanol) at ambient temperature. The ethereal extract (9.5 g) was eluted with petroleum ether-ethyl acetate in increasing concentrations (10%, 20%, 30%, 40%, 50%, 60%, 80% and 100%). The collected fractions were purified by successive crystallizations and recrystallizations. The main fraction was obtained from the mixture of petroleum ether-ethyl acetate (50:50). The structural elucidation of the main compound was carried out through H<sup>1</sup> NMR and C<sup>13</sup> NMR. The spectral data, in comparison with the literature, reveal the presence of the flavone *apigenine*.

**62. TESTOSTERONE ACTS ON SPLENOCYTES MODIFYING THE STEROIDOGENIC ACTION OF THEIR SECRETIONS ON ADULT RAT OVARY**

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Steroidogenesis of rat polycystic ovary (PCO) is under neuroimmune regulation by splenocyte (S) secretions through the neural connection involving the ovary, coeliac ganglion and spleen. Here, we study the effect of secretions of S treated with testosterone (T) on progesterone (P), androstenedione (A) and estradiol (E) release from control (C) and PCO ovaries. PCO was induced in 60-day old rats by injection of 2 mg estradiol valerate. After 2 months C and PCO S (1 x 10<sup>6</sup> cells) were cultured in RPMI medium. Control S were treated by 24 h with T 10<sup>-6</sup> mol/L to create an androgenic environment as PCO does, and T 10<sup>-6</sup> mol/L plus androgen receptor antagonist flutamide (F) 10<sup>-4</sup> mol/L. All secretions were used to stimulate C and PCO ovaries. P release from C ovaries incubated with those secretions was not modified, while that from PCO ovaries, incubated with secretions of PCO S, increased (p < 0.001). A and E release from C ovaries treated with secretions of PCO and C+T S increased; with secretions of C+T+F S decreased, while an opposite effect was observed from PCO ovaries. Results suggest that T modifies splenocyte secretions via androgen receptor and S steroidogenic activity of PCO rats can be regulated peripherally by the hormonal environment. The fact that splenocyte secretions can also be regulated by neural way could contribute to PCO etiopathogeny.

**64. PHYTOCHEMICAL STUDY AND ANTI-INFLAMMATORY ACTION OF *Minthostachys mollis* (LAMIACEAE)**

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*Minthostachys mollis* (Kunth) Griseb., popularly known as "peperina" or "peperina cordobesa", grows in the central-western Argentina (Cuyo region). It is an Argentinian official drug (FNA ed. VI), used in infusions because their antispasmodic and aromatics properties. We evaluated in mice the anti-inflammatory properties of one compound isolated from this plant. Phytochemical separations yielded *oleanolic acid* (IR and NMR spectra were confronted with bibliographics ones). Rockland mice (25-30 g) of both sexes were divided into 3 groups (control, reference and test), and carrageenan-induced paw edema was determined. Mice were injected sc with 0.05 mL of carrageenan type IV (3,5% w/v) into the subplantar region of the left hind paw. Phenylbutazone (80 mg/kg), the reference drug, oleanolic acid (75 mg/kg) and saline solution (control) were all administered ip 1 h before the carrageenan injection. Paw volume was measured before injecting carrageenan and 1, 3, 5 and 7 h later. Percentage inhibition by each compound was calculated respect to its vehicle- treated control group. Oleanolic acid produced an inhibition of the edema of 39% at 3 h and 40% at 5 h (P < 0.05). This effect is in agreement with the inhibition of inflammation mediators by triterpene compounds described by others authors.

**65. CHARACTERIZATION OF INTERSTITIAL CELLS OF CAJAL IN THE BOWEL OF THE PIG (*Sus scrofa*)**

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The interstitial cells of Cajal (ICC) play an important role in the control of the gastrointestinal motility. ICC have been described in different mammals. In the present work, ICC were searched in the bowel of pig using polyclonal anti c-kit antibodies in immunohistochemical assays. Vimentin and desmin intermediate filaments were also determined using monoclonal antibodies. ICC were positive to c-kit and vimentin antigens but negative for desmin. The c-kit positive cells were found in the tunica muscularis. ICC were observed within the region of the myenteric plexus, closely associated with ganglia and smooth muscle cells in the small and large intestine. Other ICC populations were found freely distributed in clusters in circular (ICC-CM) and longitudinal (ICC-LM) muscle layers, varying in their amount throughout the different segments of the intestine. The small intestine seems to have larger quantities of ICC than the colon. This distribution seems to be similar to those reported for other studied species.

**67. STUDY OF ACUTE TOXICITY OF *Baccharis polifolia* GRISEB IN MICE**

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*Baccharis polifolia* Griseb., popularly known as "quincha mali", has been used in folk medicine for gastrointestinal disorders. Its acute toxicity was assessed in mice. Plant material was defatted using n-hexane twice. The solvent was evaporated and the material was extracted twice with boiling ethanol (EtOH). After several liquid-liquid fractionation EtOH extract was recovered. Mice were fasted for 4 h and given increasing oral doses of EtOH extract. It was administered to five groups of 6 mice each (3 male and 3 female); one group served as control. The doses studied were 5 to 2000 mg/kg body weight. Animals were observed for 7 consecutive days to record deaths or toxic symptoms. The effects on behavioral responses were investigated using an actograph. Serum glutamate oxaloacetate (GOT) and glutamate piruvate (GPT) transaminases were determined spectrophotometrically. Even at the highest dose, no animal treated with EtOH extract showed symptoms of toxicity. There were no signs or symptoms of restlessness, respiratory distress, diarrhea, convulsions, or coma. The relative wet weight of lungs, heart, liver, spleen and kidneys were not significantly different from controls. EtOH extract did not induce change on spontaneous activity in mice. Serum GOT and GPT values of treated groups were not statistically different from controls. In conclusion, under the present experimental conditions, *B. polifolia* showed no toxicity.

**66. STEROIDS IN THE MALE REPRODUCTIVE SYSTEM OF THE AMPULLARIID SNAIL *Pomacea canaliculata***

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Reports on the existence of steroid hormones and their receptors in mollusks are rather scanty. In *P. canaliculata*, only testosterone in the testis, and estradiol and progesterone in the ovary and uterine gland have been reported (although the method was not described). We determined the concentration (mg/g tissue) of free and sterified cholesterol through thin layer chromatography and colorimetry in the midgut gland, testis, prostate, penile complex, and the proximal and distal parts of the penile sheath of *P. canaliculata*. Progesterone, androstenedione, testosterone and estradiol concentrations were measured using specific and sensitive radioimmunoassays. The midgut gland showed the highest (0.27) sterified/free cholesterol ratio, while the other tissues showed sterified/free ratios of 0.05 or less, suggesting that only the midgut gland is able to accumulate cholesterol for steroid synthesis. We did not detect either testosterone or estradiol in any case. No other steroids were detected in testis either, while low mean progesterone levels (18-46 ng/100 mg tissue) were present in the other studied tissues, except for the distal part of the penile sheath. Androstenedione (230-752 pg/100 mg tissue) was detected in all tissues but the testis. We conclude that (1) the midgut gland may be the main steroid producing organ in this snail; (2) both progesterone and androstenedione may be accomplishing the androtrophic function; and (3) these hormones may also originate in the accessory male organs (i.e., not in testis), where they would act locally.

**68. PLASMA PHARMACOKINETIC OF BALOFLOXACIN IN CALVES**

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Balofloxacin is a fluoroquinolone with broad antibacterial spectrum against aerobic bacteria and mycoplasma, without photosensitization effects in animals. The aim of the present study was to determine the plasma pharmacokinetics of balofloxacin following intravenous and subcutaneous single dose (5mg/kg) administration to calves in a cross-over design. 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) was used. Main intravenous pharmacokinetic parameters were:  $t_{1/2} = 2.36 \pm 1.1$  h; Clearance =  $10.23 \pm 3.40$  mL/min/kg;  $V_d$  area =  $1.86 \pm 0.31$  L/kg;  $AUC_{\infty} = 551.3 \pm 247.0$   $\mu$ g/min/mL. These parameters did not differ significantly from those obtained with subcutaneous administration ( $P > 0.05$ ) with the exception of  $t_{1/2}$  and  $V_d$  area. Both values were larger than those obtained on intravenous administration. The parameters of absorption were:  $C_{max} = 1.38 \pm 0.48$   $\mu$ g/mL and  $t_{max} = 51.26 \pm 12.10$  min. With the exception of the parameters mentioned, the calves pharmacokinetic profile is similar to those of goats.

**69. PLASMA PHARMACOKINETICS OF BALOFLOXACIN IN GOATS**

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Balofloxacin is a fluoroquinolone with broad antibacterial spectrum against aerobic bacteria and mycoplasmae, without photosensitization effects in animals. The aim of the present study was to determine the plasma pharmacokinetics of balofloxacin following intravenous and subcutaneous single dose (5 mg/kg) administration to goats in a cross-over design. Balofloxacin concentrations in plasma were determined using a validated HPLC method with isocratic elution in reversal phase (18 C-column, and fluorescence detection). A non-compartmental pharmacokinetic data analysis program (PK-solution) was used. Principal intravenous pharmacokinetic parameters were:  $t_{1/2} = 2.8 \pm 0.9$  h; Clearance =  $7.4 \pm 2.6$  mL/min/kg; Vd area =  $1.8 \pm 0.9$  L/kg;  $AUC_{\infty} = 748.7 \pm 262$  µg/min/mL. These parameters did not differ significantly from those obtained with subcutaneous administration ( $P > 0.05$ ) with the exception of  $AUC_{\infty}$ . The parameters of absorption were:  $C_{\max} = 1.56 \pm 0.6$  µg/mL,  $t_{\max} = 42.5 \pm 38.2$  min, and plasma bioavailability = 75%. In goats, the balofloxacin plasma kinetic profile is similar with intravenous and subcutaneous administration.

**71. CHOLESTEROL LEVEL AND HMG-CoA EXPRESSION IN AORTA OF VITAMIN A DEFICIENT RATS**

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Accumulation of oxidatively modified low-density lipoprotein (oxLDL) in the vascular wall is a characteristic feature of atherosclerosis. oxLDL can be taken up into monocytes, vascular smooth muscle cells, and endothelial cells. It is known that antioxidants reduce oxidative modification of LDL and attenuate or prevent the decrease in endothelial function. Previously, we demonstrated that vitamin A deficiency induces oxidative stress and increases cholesterol content in rat aorta. This work aimed at determining if the cholesterol increment is linked to its endogenous synthesis. Wistar male rats (21 day-old) were fed during 3 months with either vitamin A- free diet (-A) or the same diet plus 8 mg of retinol palmitate/kg of diet (+A). Vitamin A deficiency was confirmed in plasma by HPLC. To evaluate aortic cholesterol synthesis, [ $^{14}$ C]- acetate incorporation into non-saponifiable lipid fraction and mRNA expression of HMG-CoA were determined. In -A rat aorta [ $^{14}$ C]- acetate incorporation into cholesterol and mRNA expression of HMG-CoA did not change compared with +A rat aorta. The increase of cholesterol level observed in -A in relation to +A aorta ( $2.109 \pm 0.31$  vs.  $1.73 \pm 0.21$ ,  $p < 0.05$ ) is not due to its *in situ* endogenous production. The prooxidant environment caused by vitamin A deficiency can induce LDL oxidation that could lead to increased aortic LDL uptake.

**70. CENTRAL ADRENERGIC STIMULATION OF PROGESTERONE RELEASE FROM COELIAC GANGLION-SUPERIOR OVARIAN NERVE-OVARY SYSTEM**

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Results obtained in our laboratory have shown that a central  $\beta$ -adrenergic stimulus decreases progesterone (P) release from the ovary on day 2 of dioestrus (D2). In the present study we evaluated the ovarian function in the *in vitro* system, coeliac ganglion-superior ovarian nerve-ovary (CG-SON-O) isolated from 4 groups of virgin Holtzman rats (220 g) 5 min after injection in the right lateral ventricle (icv) with: I) 5 µL ascorbic acid (vehicle), II) 5 µg epinephrine (Epi), III) 5 µg isoproterenol, and IV) the CG of the CG-SON-O system from rats with Epi icv injection was stimulated with norepinephrine (NEpi)  $10^{-6}$  mol/L. The CG and O, joined by the SON, were incubated in different compartments in a metabolic bath. Samples from the O compartment were collected at 15, 30, 60, 120 and 180 min to measure P release (ng/mg ovary) by RIA. The icv injection of Epi increased ovarian P release until 120 min ( $p < 0.005$ ) but that of isoproterenol did not induce any change compared with basal values. In group IV, NEpi in CG increased ovarian P release compared with II. Probably times shorter than those used here must be considered to observe the central effect of Epi on ovarian P release.

**72. CHROMOSOMAL ANOMALIES IN PIGS VACCINATED AGAINST CLASSIC SWINE FEVER**

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The genotoxic potential of the vaccine against Classic Swine Fever (CSF) was evaluated. In Argentina the disease is controlled with attenuated live virus vaccine (China strain). Materials and methods for the cytogenetic analysis were: 1) experimental pigs from official control tests of vaccines made by the Animal Health Service (SENASA); 2) pigs from private farms where immunization is applied; and 3) an *in vitro* system using lymphocyte cultures from non vaccinated pigs. To quantify the effect of vaccine on DNA the Single Cell Gel Electrophoresis (SCGE) was used. Results in the group of animals or lymphocyte cultures exposed to CSF vaccine showed its clastogenic effect. Monochromatid and isochromatid breaks, trirradial and quadrirradial configurations, cells with multiple chromosome abnormalities and pulverization were induced. In all cases the frequency of cells with chromosomal anomalies was related to the dose, with minor lesions at lower dose and severe damage at higher doses. The effect of several vaccine brands tested was the same. The SCGE test proved the direct effect of vaccine virus on DNA fragmentation that, at cytological level, would correlate with chromosome pulverization. At the highest dose, 30% of comet morphology indicated lymphocyte apoptosis. In conclusion, results confirm the genotoxic capacity of living vaccine against CSF used currently in Argentina.

**73. PIGMENTS IN THE PINEAL GLAND OF VISCACHA (*Lagostomus maximus maximus*)**

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The presence of pigments (melanin, lipofuscin, hemosiderin) has been reported in the pineal gland of several mammals, but its biological relevancy is not yet established. The present research was carried out to study the pigments in the viscacha pineal gland. Adult males and females were caught, anesthetized and sacrificed. The pineal body were quickly removed and processed for light or electron microscopy. The following histochemical methods were applied: Masson-Fontana, Schmorl and DOPA-oxidase. The pigments were also studied at electron microscopy level. The histochemical and ultrastructural results showed the presence of melanin granules in the viscacha pineal gland. This pigment was evident in females and very scarce in males. The occurrence of lipofuscin was also observed, but no difference between both sexes was found. The pineal melanin may be explained by its embryogenesis and phylogeny. The pineal gland is a neuroectodermal organ as others structures containing melanin (skin and retina). Likewise, the melanin may be a cellular rest reflecting the primitive photoreceptive nature of the gland. On other hand, the different amount of melanin between both sexes may be due to the capability of estrogens and progesterone of inducing biosynthesis of melanin. Lipofuscin are considered pigments of aging process. Its occurrence in the viscacha pineal gland can be related to the animal age.

**75. URIC ACID AND URATE CELLS DISTRIBUTION IN THE NEOTROPICAL APPLE-SNAIL *Pomacea canaliculata* (Caenogastropoda, Ampullariidae)**

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We studied the correlation between tissue concentration of uric acid ( $\mu\text{g}$  of uric acid per g of tissue dry mass) and the topographical distribution of crystalloid-containing cells ("urate cells") in organs of *P. canaliculata*. Basal concentrations of uric acid (range of mean values: 0.2-2.2  $\mu\text{g}/\text{g}$ ) were only found in those organs devoid of urate cells (propodium, posterior kidney, uterine gland), while high concentrations (range: 33.8-198.7  $\mu\text{g}/\text{g}$ ) were found in those with numerous urate cells arranged in typical perivascular sleeves (midgut gland, testis, lung, and the coiled part of the intestine). Also, high uric acid concentrations were correlated with conglomerates of urate cells that were not integrated to typical perivascular tissue, and that were found in (1) the intestinal mucosa, particularly in the typhlosole of the coiled intestine; (2) the mesentery; and (3) the aortic ampulla. In the latter place, urate cells were organized in conspicuous subendothelial nodules. Besides those locations, the anterior kidney and the gill showed intermediate uric acid concentrations (range: 6.7-9.0  $\mu\text{g}/\text{g}$ ), which were correlated with the presence of urate cells in the sleeves surrounding the adjoining vessels but not in the organs themselves. This study is part of a broader program aimed to disclose the physiological significance of uric acid storage in this snail.

**74. CHARACTERIZATION OF INTRACELLULAR CRYSTALLOID BODIES IN THE MIDGUT GLAND OF THE AMPULLARIID SNAIL *Pomacea canaliculata***

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*P. canaliculata* shows sleeves of a specialized tissue surrounding arteries of the midgut gland. This tissue is formed mainly by "radiated cells" (large vacuolated cells, bearing small nuclei without nucleoli) which are arranged around the vascular muscular layer. Smaller "satellite cells", with a scarce cytoplasm and clear nucleolated nuclei, are found together with some nerve endings and muscle fibers on the external surface of these sleeves. Radiated cells are full of crystalloid spheres (about 5  $\mu\text{m}$  diameter) showing a complex inner fibrillar structure. Crystalloid corpuscles, and pigmented C and K corpuscles present in midgut gland alveoli, were isolated from gland homogenates. Uric acid, urea, ammonia, protein and calcium were determined in both gland homogenates and corpuscular fractions. Uric acid was the major non-protein nitrogen compound of the midgut gland and was concentrated in crystalloid corpuscles (accounting for 53% of corpuscular dry mass), but was not detected in pigmented corpuscles. Calcium accounted for only 0.6% of crystalloid dry mass. Protein was another significant component of crystalloid bodies (32% of dry mass). Ammonia, followed by urea, was the main nitrogen excretory product in the soluble fraction of excreta, while protein predominated in the particulate excretory fraction. Uric acid stored in such specialized tissue may serve as a free radical scavenger and antioxidant compound, a function that may be particularly important when the snail arises from seasonal dormancy.

**76. COMPARATIVE STUDY OF DIRECT CRYSTALLIZATION AGAINST  $\text{CuSO}_4$  PRECIPITATION IN EGG WHITE LYSOZYME PURIFICATION**

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The egg white lysozyme is an enzyme that splits the (1-4) linkages between N-acetylglucosamine and N-acetylmuramic acid of cell walls of Gram positive bacteria. Lysozyme purification methods have been reported for many years. The classical laboratory and commercial procedure involves direct crystallization at pH 9.5 by addition of 5% sodium chloride. Several re-solubilisations and re-crystallizations must be performed to obtain a high purity enzyme preparation (60-80%). Chromatography methods are very expensive. The crystallization method was studied in this work and compared with the  $\text{CuSO}_4$  precipitation. The two methods were performed using fresh egg white. The protein fractions obtained were evaluated by SDS-PAGE with 13% of acrylamide and the gels stained with Coomassie Brilliant Blue. The protein concentrations were evaluated from its absorbances at 260 and 280nm. The enzyme activity from different fractions was evaluated according to Chang *et al.*, 1986. The results of the  $\text{CuSO}_4$  precipitation showed that the obtained lysozyme had a high degree of purity. Crystal obtained in the first crystallization step showed activity values of around 13000 units and a high degree of purity. In general the two methods produced lysozyme of a similar activity but precipitation with  $\text{CuSO}_4$  proved to be a faster method.

**77. COMPARATIVE STUDY OF SERIC LIPIDS ON DIABETES TYPE 2 AND FIRST DEGREE RELATIVES**

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Among the risk factors of Type 2 diabetes mellitus, there is the family background; to have a diabetic first degree relative gives a 40% risk of suffering the disease. This work studied the lipidic profile of first degree relatives and compared it that of the diabetic patient. Serum of 60 patients and its first degree relatives were obtained. The following determinations were performed: blood glucose, triglycerides (TG), Total (TC), c-HDL and c-LDL- cholesterol. All determinations were done using commercial kits from Wiener. Body mass index and the ratio waist/hip were also calculated. We found that there were significant differences on blood glucose, TG and CT. There were no significant differences on c-HDL and c-LDL. We did not find variations on the body mass index and the ratio waist/hip. The atherogenic index or Castelli index (TC/c-HDL) did not show significant modifications. We were unable to find a correlation between serum lipids and diabetic status that would allow predicting the disease.

**79. ENTEROPARASITOSIS IN CHILDREN OF JUANA KOSLAY HOME FROM SAN LUIS CITY**

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Intestinal parasitosis represent a world-wide public health problem, and their prevalence vary from region to region in accordance to social and cultural factors. The purpose of this work was to study the enteroparasites prevalence in healthy children aged between 1- and 8-year old, from *Juana Koslay Home* of San Luis City. For this purpose, 50 samples were collected in three consecutive days and were studied by means of coprological analysis using Carles-Barhelemy and Faust enrichment techniques and Graham modified method. The overall prevalence of intestinal parasitism was 78%. The reported parasites and their prevalence are as follows: *Enterobius vermicularis* (48%), *Giardia duodenalis* (24%), *Entamoeba coli* (2%), *Ascaris lumbricoides* (2%), *Hymenolepis nana* (2%). These results show a strong correlation between basic sanitary conditions, the epidemiological role of children home and the prevalence of parasites, as we had previously demonstrated in other studies carried out in children of San Luis City.

**78. EFFECTS OF THREE STRESS TREATMENTS ON MATERNAL BEHAVIOR IN RATS**

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The effect of neonatal stress (NS), maternal stress during the lactation period (MAS) and NS plus MAS on maternal behavior were analyzed. Control rats (C) were not stressed. Maternal behavior was scored with the Meaney's test on days 6 and 7 after delivery in 40 min diurnal and nocturnal observations. Nine behavioral components were grouped in 3 behavioral categories: (1) Lactation posture behavior, (2) pups cleaning behavior and (3) pups retrieval behavior. The posture behavior scores of group NS were lower than scores of the other groups in diurnal tests (Dunn's test  $p < 0.01$ ) but higher in nocturnal tests. Between-groups differences in pups cleaning behavior were not found in diurnal tests but NS and MAS groups displayed higher scores in nocturnal tests. A trend for higher scores of pups retrieval behavior was found in NS group exposed to diurnal tests; these reached significance in nocturnal observations ( $p < 0.05$  compared to C). Dams were retested on day 7 after a 4 hs isolation of litters. Lactation posture behaviors in C, NS and MAS groups was stimulated by litter separation (Mann Whitney test  $p < 0.05$ ) but not in the NS plus MAS group. Consistently, body weight gain of C, NS and MAS groups after suckling was positive whereas this was negative in the NS plus MAS group. When a stress stimulation was performed before returning litters to their dams all maternal behaviors were lower than C in the NS plus MAS group. Results show that the combined SN and MAS treatment causes deleterous effects on maternal behavior of rats.

**80. EFFECT OF CADMIUM EXPOSURE ON LIPID METABOLISM IN RAT TESTIS**

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Cadmium (Cd) is an environmental toxicant implicated in human prostate carcinogenesis. The mechanism of its toxicity is far from fully understood. This study evaluates the effect of exposure to an oral non-carcinogenic Cd doses on different testicular lipid variables. Adult Wistar male rats were separated in three groups: a control group and two Cd-exposed groups that received 15 ppm (Cd15) and 100 ppm (Cd100) in the drinking water for 3 months. Testis content of triglycerides (TG) (Sardesai and Manning's method), total (TC), free (FC) and esterified cholesterol (EC) (Zack's method), total phospholipids (TP) (according to Rausser *et al*) and proteins (Biuret method) were measured. Diacylglycerol-acyl transferase -2 (DGAT2) was determined by RT-PCR. The bands were quantified using the software program Scion Image from NIH and were normalized using the values obtained for  $\beta$ -actin. Results of lipid contents showed a decrease of TG in Cd15 and Cd100 ( $p < 0.05$ ); an increase of TP in Cd15 ( $p < 0.005$ ) and Cd100 ( $p < 0.05$ ). The levels of FC and EC were not modified in Cd15 compared with control, but those of Cd100 increased significantly ( $p < 0.05$  and  $p < 0.005$ , respectively). The content of DGAT2 mRNA did not change compared with control. Cd exposure for 3 months induces significant changes in testicular lipid metabolism with Cd at both 15 and 100 ppm.

**81. NUCLEAR PORE CHANGES IN APOPTOTIC CELLS DURING POST-PARTUM CORPUS LUTEUM REGRESSION IN THE RAT**

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In a previous paper we have described the apoptotic nuclear pore changes during ovary cyclic regressions. TUNEL (TdT-mediated dUTP nick-end labeling) and thin-sections observations encouraged us to study the apoptotic nuclear membrane in the corpus luteum after delivery in the rat using freeze-fracture techniques. Fixation and cryoprotection were done according to a routine procedure. Fracture and replicas were performed in a Balzers BAF 301 apparatus. Platinum-carbon replicas were examined in a Zeiss 900 electron microscope. Pores were counted using a conventional morphometric analysis. Changes in nuclear pores begin in an atypical form. They display an intense reduction in number showing an even distribution on the nuclear envelope although chromatin does not aggregate in sharp zones separated themselves from pore-free membranous areas characteristic of the usual apoptotic model. Thin-sections indicate, coincident with the freeze-fracture finding, small irregular aggregates of marginated chromatin condensations. After nuclear fragmentation and micronuclear formation pores behave in the usual manner. The present results support in this model the hypothesis that nuclear pore complexes are dynamic structures which migrate toward nuclear membrane areas devoid of chromatin aggregations that might block the nuclear-cytoplasmic transport in such areas.

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**83. BIPLLOT ANALYSIS OF MAIZE SEED QUALITY WITH MECHANICAL DAMAGE**

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Seed quality is affected by the mechanical damage caused in the seed coat and in the internal tissue during seed conditioning process. Susceptibility to mechanical damage depends on the genetic and environmental factors. The aim of this work was to determine the magnitude of the genetic, environmental and genotype-environment interaction effects on the susceptibility of maize seeds to mechanical damage. For this work 9 inbred lines were cultivated during 2002/03 in two locations. Each seed caliber-location combination was treated as an environment. The seeds were artificially damaged and evaluated by mean of the fast green test, which quantifies the percentage of damaged seeds and classifies them into three levels. The study of the genotype-environment interaction was made with the SREG model and its biplot. The analyzed trait at the different levels was significantly affected by the effect of genotypes, environments and genotype-environment interaction, except in level 1, in which the genotype-environment interaction was not significant. Regarding the three fast green levels, it was possible to observe that the environments were subdivided into three subgroups. Line 2 was the most tolerant to impacts, but it was the least stable. Line 8 showed the least susceptibility in the embryonic axis in all environments and it was the most stable. The results showed that identification of stable genotypes could be important for improving seed quality.

**82. DETECTION OF *Bacteroides forsythus* IN ATHEROSCLEROTIC LESIONS**

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Recent epidemiological studies have established that periodontal bacterial pathogens initially found in gingival tissues might enter the blood stream and subsequently localize in atheromatous plaques. In this study 26 patients affected by periodontal disease were examined. A typical oral flora compatible with the most common oral pathogens such as *Actinobacillus actinomy-cetemcomitans*, *Porphyromona gingivalis* and *Bacteroides forsythus* were observed by light microscopy and transmission or scanning electron microscopy. Five atherosclerotic plaques obtained from patients who were submitted to surgical operations were examined for the presence of the above mentioned bacteria using specific oligonucleotide primers in polymerase chain reaction (PCR) assays. Forty percent of the atheromas yet examined were positive for *B. forsythus* meanwhile all of them were negative for the other two bacteria. We conclude that the periodontal pathogen *B. forsythus* was found with high incidence in atherosclerotic lesions where, like the other reported infectious microorganisms, they may play a role in the development and progression of atherosclerosis.

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**84. ENDOPROTEASES OF *Amaranthus* SEED**

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We analyzed the properties of endoproteases in *Amaranthus* (*Amaranthus mantegazzianus* Pass cv Don Juan) seeds and activity changes during imbibition. Proteolytic activities are essential for mobilization and reutilization of protein reserves during germination and seedling development. The globulin fraction of seed protein showed a rapid degradation, while in the albumin and glutelin fractions the decrease was slower. By ionic exchange in a column of carboximethyl cellulose (CMC) of a protein extract of dry seeds, we observed 2 main peaks of endoprotease activity, using azocasein as substrate. In seeds imbibed to 28°C for 2 days, an additional peak is observed. Each peak gives a distinct band in polyacrylamide-gelatin electrophoresis. On the other hand, the endoprotease activity of crude extract of dry seeds showed an optimum pH of 5-5.5, while in extracts of seeds imbibed for 2 days, the optimum pH was 6.5-7. Simultaneously a remarkable increase in the endoprotease activity was observed. This increment was not inhibited by cycloheximide, suggesting that *de novo* enzyme synthesis was not involved. In dry seeds, PHMB and N-ethylmaleimide (cysteine proteinase reagents) were inhibitory. During imbibition, this was maintained, and appeared further a sensibility to EDTA. In conclusion, endoprotease activity is present in dry seeds (possibly cysteine-proteinases), and further another activity, with a neutral optimum pH, which continuous increase was observed through the studied period (4 days). Inhibition by EDTA suggests that this endoprotease belongs to the metalloproteinase group.

**85. DIFFERENTIAL STRESS RESPONSE IN SPRAGUE DAWLEY (SD), WISTAR (W) AND OFA hr/hr (O) FEMALE RATS**

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Sensitivity to stress depends upon genetic and environmental factors. In the present work we studied the stress response of female rats of three strain on oestrus day using two tests: a) 1 min. exposure to a white noise, recording motor and exploratory activity before, during and after stressor application. b) Restraint stress followed by plus-maze, to evaluate the ansiogenic component. In the last test only W and O were observed. a) W and SD showed similar activity scores; O was hyperactive. Freezing response was exaggerated in W, while O showed the lowest score. W and SD pre-stress head-dipping scores fell to near 0 after stress; O exhibited a low score all the time. Pre-stress scores of novel object exploration were similar in the three groups, but the stress-induced impairment of exploration was lower in the O group. b) In the closed arm, immobility basal scores were higher in O, but restraint stress lowered immobility. Basal and post-stress rearing were lower in O. Performance in the open arm was similar in W and O. The opposed effects in immobility scores of O in each testing condition may be due to motivational contextual cues. In O basal hole exploration is almost blocked, while novel object exploration is similar to the other groups. Stress response was lower in the O group, while W rats showed the highest sensitivity to the noise stress. No differences in ansiogenic components were found. Low circulating prolactin and hyperfunctioning DA systems could be the basis of the differences seen in the O group.

**87. MIDGUT GLAND DEVELOPMENT IN THE AMPULLARIID SNAIL *Pomacea canaliculata* AND THE ORIGIN OF INTRACELLULAR PIGMENTED CORPUSCLES**

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Pigmented, presumably symbiotic elements (called C and K corpuscles) are present in the midgut gland and feces of juvenile *Pomacea canaliculata* since days 3-5 after hatching. Because they originate within specific alveolar cells we wanted to trace the origin of these cells and of the corpuscles within. The mouth, the archenteron and the anal papilla can be already distinguished in trochophora larvae (2 days after egg-laying). One day later, veliger larvae show a round, pink-colored archenteron resulting from the accumulation of egg albumen within giant cells (up to 100 µm diameter) forming the organ's wall. About 5 days after egg-laying, the reddish midgut divides into an anterior and a posterior lobe (included in the upper head and the developing protoconch). By day 6 the anterior lobe has been reduced and the only the posterior lobe remains, from which the midgut gland, style sac and stomach differentiate. After day 11 the cells that will form the alveoli appear as patches among giant cells. The digestive gland around day 7 post hatching is similar to that of the adult in that giant cells have disappeared and the alveolar epithelium is composed by columnar cells of the adult type, with C corpuscles being freed towards the lumen. Large cells with multiple nucleoli are also present in the alveoli. However, no K corpuscles can be recognized. This evidence supports the view that C corpuscles are associated with alveolar columnar cells since early in life, while K corpuscles develop only later.

**86. BIOACTIVITY OF CLERODANE DITERPENES FROM *Baccharis flabellata* HOOK & ARN VAR. *flabellata* AGAINST *Tribolium castaneum* HERBST**

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Clerodane diterpenes are a large group of diterpenes with an interesting insect-feeding deterrent bioactivity. The antifeedant activity of a group of these compounds, isolated in our laboratory, against *Tenebrio molitor* L., lead us to test them and their derivatives using another test model, in order to extend the conclusions of previous studies. We continue studying the effect of new clerodanes isolated from *Baccharis flabellata* Hook & Arn var. *flabellata*, and their derivatives, against stored grain insect *Tribolium castaneum* Herbst. Comparison among experimental results and a computer - assisted conformational and electronic study (MMFF/AM1), allows to confirm the previously postulated relationship between bioactivity and the distance from furane oxygen to the carbon atom that can be attacked in an Michael addition reaction type.

**88. BIOCHEMICAL AND GENETIC FACTORS INFLUENCING HUMAN HYPERTENSION**

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Angiotensin II (Ang II) exerts a central role in blood pressure control by interacting with AT<sub>1</sub> receptors. Ang II type 1 receptor polymorphism A<sup>1166</sup>C has been related to hypertension. In control and hypertensive patients from San Luis, a search for the presence of A<sup>1166</sup>C polymorphism and biochemical parameters altered was performed. Patients were selected carefully in order to include only those with essential hypertension. The hypertensive (HT) group showed elevated blood pressure and increased values of total cholesterol, LDL cholesterol and triglycerides (TG). The body mass index was also increased in HT patients. The presence of the polymorphism was studied by mismatch PCR and RFLP. Two alleles are recognized, the A and the C allele. The A<sup>1166</sup>C polymorphism introduces an additional DdeI recognition site on the C allele. Of the total number of subjects, only 5 were heterozygotic for the AC genotype (86% of the population), all of them belonging to the HT group. We did not found the homozygote genotype CC in the patients studied. The frequency of the C allele was 0.10 on the HT group and 0.07 for the total population studied. Detection of the A<sup>1166</sup>C polymorphism might be useful as a predictive factor in patients with a familiar history of hypertension.

**89. PROTEIN PATTERNS IN BIOLOGICAL FLUIDS STUDIED BY CAPILLARY ELECTROPHORESIS**

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Most diagnostic applications of traditional electrophoretic methods are directed to studying protein abnormalities in different biological fluids. Clinical assays require accurate, precise and reproducible results in the minimum of time. Protein quantitation should be accurate and reproducible in several conditions such as multiple myeloma, sclerosis, liver diseases and rheumatoid arthritis. We have carried out a capillary zone electrophoresis (CZE) technique for the determination of poorly separated monoclonal serum proteins by agarose gel electrophoresis (AGE). Serum samples were diluted 50-fold in running buffer. Separation was achieved using borate buffer 50 mmol/L, pH 9.6 at 25°C, 25 kV. Detection was performed at 200 nm. Proteins were separated in less than 15 min. Some cases have been reported in which a small monoclonal protein (M-protein) cannot be detected by conventional cellulose acetate membrane electrophoresis. The proposed method seems to be a helpful technique for the determination of poorly separated serum proteins by AGE. The results proved to be powerful in diagnosis of certain disease states and monitoring of dyscrasias in routine laboratory practice.

**91. EFFECT OF CADMIUM ON NON-ENZYMATIC AND ENZYMIC ANTIOXIDANT DEFENSE SYSTEM IN RAT LIVER**

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Recent research indicates that cadmium (Cd) induces oxidative damage in cells; however, the mechanism of the oxidative stress induced by this metal is still unclear. The effect of cadmium on antioxidant parameters as Glutathione total (Gt), reduced (GSH) and oxidized (GSSG), non-protein thiols (NPT) and glutathione reductase (GR) was studied. Previous results obtained in our laboratory showed an increase of TBARS, and a decrease of glutathione peroxidase, superoxide dismutase and catalase in liver of rats treated with Cd. Wistar male rats (180 g) were treated with 15 ppm of cadmium in tap water. After 2 months of treatment, the rats were sacrificed, the livers were obtained and the biochemical determinations were carried out. Results: GR showed an increase ( $p < 0.006$ ) and the others parameters: Gt, GSH, GSSG and NPT showed a significant decrease ( $p < 0.004$ ,  $p < 0.002$ ,  $p < 0.0003$  and  $p < 0.02$ ) respectively in treated rats respect to the controls. We can suggest that cadmium alters the non enzymatic and enzymatic antioxidant system; depletion of GSH increases the susceptibility of cells to free radical induced toxicity.

**90. QUANTITATIVE DETERMINATION OF NEPHROLITHIASIS URINARY MARKERS BY CAPILLARY ELECTROPHORESIS**

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The determination of citrate and oxalate in urine is an important diagnostic tool for nephrolithiasis disease. Capillary electrophoresis (CE) offers several advantages over the traditional clinical method in terms of fastness, cost, reproducibility and sample throughput. The clinical data were obtained by a capillary electrophoresis method and were compared to the enzymatic traditional method. Oxalate and citrate were measured in urine with minimum sample pretreatment, just acidification and centrifugation. Detection was performed at 200 nm. Separation was achieved using phosphoric acid adjusted to pH 6 with NaOH, constant voltage of -10 kV, capillary temperature 25°C, sample injection 0.5 Psi, 5 s. Oxalate and citrate were separated in less than 20 min. The response was linear within the range 200-1000 mg/L for citrate and 10-200 mg/L for oxalate. Limits of detection were 0.80 mg/L for oxalate and 30.0 mg/L for citrate. The CE methodology was validated in terms of reproducibility, precision, accuracy, linearity and ruggedness. Results were in agreement with those obtained by the traditional method.

**92. cAMP IS A SECOND MESSENGER INVOLVED IN SIGNALING ALONG THE PHAGOCYTOTIC PATHWAY**

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Phagocytosis is a process by which macrophages internalize large extracellular particles. Phagosomes fuse and exchange materials with endosomes, Golgi-derived vesicles and lysosomes. By these events, phagosomes mature and acquire degradative ability. Phagocytosis is a highly regulated process but the signaling involved still remains unclear. The objective of this work was to unravel the participation of cAMP along the phagocytic pathway. We used a butyryl derivate of cAMP that can cross the plasma membrane and *St. aureus* opsonized with radiolabelled antibodies as phagocytic particles. Macrophage cell-associated radioactivity was taken as an index of phagocytosis. Our results showed that the increase on cAMP intracellular levels stimulates particle engulfment. Similar results were obtained when we used forskolin, an activator of adenylyl cyclase, enzyme that produces cAMP from ATP. The addition of IBMX, an inhibitor of phosphodiesterases, prevents cAMP hydrolysis, and as a consequence augments cAMP effects. Regarding to phagosome maturation, the rise in cAMP intracellular levels reduces particle degradation. These results suggest that cAMP, acting as a second messenger, regulates phagocytosis and trafficking along the phagocytic pathway.

**93. MORPHOLOGICAL CHANGES PRODUCED BY ENDOTHELIN-1 IN RAT TESTIS MYOID CELLS**

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Spermatozoa in the tubular lumen are transported to the rete testis by the contractile activity of myoid cells (MC). MC form a layer that surrounds the seminiferous tubules (ST). MC have a hexagonal shape that can be visualized by silver stain. We analyzed the morphological changes of ST and MC when the ST was stimulated to contract with endothelin-1 (ET-1). Isolated rat ST in MEM medium were treated without or with 50 nmol/L ET-1 for 20 s at 32°C. ST were fixed and stained with 1% silver nitrate. ST were visualized with a magnifying glass at 20 X and recorded with Paint Shop Pro 6 software. The ST diameter and area, and axis of the MC were quantified with Scion Image software. Control ST diameter was  $235.25 \pm 6.19 \mu\text{m}$  along the entire axis of the tubule, while treated ST showed a diameter varying from 200.83  $\mu\text{m}$  to 251.67  $\mu\text{m}$  depending on the measuring position. Surface was  $810.48 \pm 185.8 \mu\text{m}^2$  for control and  $539.36 \pm 144.7 \mu\text{m}^2$  for treated MC. Longitudinal axis was  $34.79 \pm 5.2 \mu\text{m}$  and  $28.15 \pm 3.5 \mu\text{m}$  and transverse axis was  $26.1 \pm 5.5 \mu\text{m}$  and  $26.7 \pm 4.5 \mu\text{m}$  for control and treated MC respectively. From these results, we conclude that ST contract in segments and the MC of these segments contract oriented longitudinally to the ST axis.

**95. STATISTICAL ASSESSMENT OF VAGINAL PATHOGENS**

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The microbiology of the female genital tract is complex. It is therefore fundamental to distinguish between pathogenic organisms from harmless colonizing germs. The most common vaginal infections are bacterial vaginosis, trichomoniasis and candidiasis. A statistical assessment of the most frequent vagina pathogens was done in this work. Pathogens were extracted from vaginal discharge of fertile women attending the Braulio Moyano Hospital, and collected over a period of 17 consecutive months from its opening in May 2003 to September 2004. Samples (n = 59) were obtained with endocervical swabs and were characterized by microscopic analysis, Gram staining and culture in Agar Sabouraud. Results: *Gardnerella vaginalis* was isolated in 33.9% of samples, *Candida albicans* in 15.3%, *trichomonas vaginalis* in 5.05%, *Corynebacterium* spp in 37.3% while normal flora was found in 16.9%. The results revealed a remarkable dominance of bacterial vaginosis (*G. vaginalis*) compared with other pathogens causing vaginal infections, while 37.3% of patients harbored *Corynebacterium* sp. without any other pathogen responsible for leucorrhoea. A combination of two different pathogens, *Gardnerella vaginalis* and *Candida albicans*, or *Trichomonas vaginalis* and *Candida albicans*, were detected in 5 of the 59 patients.

**94. EFFECT OF ACID CONTAMINATION ON THE LICHEN *Usnea* sp. IN LABORATORY CONDITIONS**

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Lichens are used as indicators of contamination. *Usnea* sp. is considered a good bioindicator because it is not present in contaminated areas. SO<sub>2</sub> is regarded as the principal contaminant affecting *Usnea* physiology. The objective of the present study was to determine the effect of acid contamination on *Usnea* sp. The lichens were collected from a natural place (El Trapiche, San Luis). Four groups were sprayed each 4 days with the acid solution. The control was sprayed with distilled water. The laboratory conditions were 20°C, 40% relative humidity. Concentration of chlorophyll and pheofitins were measured before and after spraying. The extraction of chlorophyll was made with DMSO. Phenological aspect was evaluated. The results do not show differences (ANOVA) in chlorophyll concentration between the initial condition ( $2.98 \pm 0.77 \text{ mg/L}$ ) and control ( $3.57 \pm 1.22 \text{ mg/L}$ ) or acid treatment ( $2.87 \pm 0.59 \text{ mg/L}$ ) (P = 0.2418). The pheofitins concentrations showed differences; respectively  $1.22 \pm 0.12$ ;  $1.06 \pm 0.10$  and  $1.19 \pm 0.08$  with acid treatment (P = 0.0483). In all cases the color of lichens sprayed with acid solution was modified from green toward the brown. The results show morphological and physiological effects of acid in *Usnea* sp.

**96. ACTION OF LYSOZYME, NISIN AND EDTA AGAINST *Yersinia enterocolitica* IN ORANGE JUICE**

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*Yersinia enterocolitica* is a food-borne human pathogen able to survive under low pHs and temperatures, conditions present in refrigerated orange juice. Nisin, lysozyme and EDTA are harmless compounds that could be used as antimicrobials in food. The aim of this work was study the action of nisin, lysozyme and EDTA, alone and combined, against *Y. enterocolitica* in orange juice. Commercial pasteurized orange juice without pulp and laboratory prepared orange juice with pulp (199 mL) were used. The inoculum was prepared with *Y. enterocolitica* W1024 O:9 pYV(+) to a final concentration of 10<sup>7</sup> CFU/mL. Inoculated samples were treated with 100 IU/mL nisin, 2400 IU/mL lysozyme and 20 mmol/L EDTA. Samples without addition of antimicrobials were used as control. Once the compound was added the juice was incubated at 22°C and samples were taken at 0 and 20 min, 6 and 24 h. One mL of juice was serially diluted with 0.1% peptone water (PW) and 0.1 mL of an appropriate dilution was spread onto Mac Conkey (MC) agar and incubated 48 h at 22°C. In the commercial orange juice, no growth was observed at 6 h of incubation when EDTA alone and combined with nisin or lysozyme were used. In the laboratory prepared juice, EDTA alone was the best treatment because no colonies were observed at 24 h. These compounds, alone or combined, are useful to inhibit *Y. enterocolitica* in orange juice and could be used in industry to decontaminate them.

97.

**PHARMACOKINETIC PARAMETERS OF ENROFLOXACIN IN SHEEP***Lüders C, Velasco M, Betancourt L, Garcia H, Errecalde C, Prieto G**Farmacol, Fac Agron Vet, Univ Nac Río Cuarto, Río Cuarto 5800 Córdoba. E-mail: cluders@ayv.unrc.edu.ar*

Enrofloxacin (5mg/kg) kinetics were determined in 6 sheep after intravenous (IV) and subcutaneous (SC) administration. Serum concentrations were obtained by microbiologic method, using *Bacillus subtilis* ATCC 6633 as the test microorganism. Serum concentrations best fitted a two compartment model and one compartment model for the endovenous and subcutaneous routes, respectively. The pharmacokinetics parameters were obtained by the pharmacokinetic program Resid (Ritchel, 1975). The parameters ( $X \pm S.D.$ ) for IV are:  $T_{1/2\alpha} = 0.11 \pm 0.07$  h,  $T_{1/2\beta} = 2.39 \pm 0.33$  h,  $Vd_{ss} = 0.74 \pm 0.05$  L/kg,  $Cl_{total} = 3.90 \pm 0.61$  mL/min/kg.; and for SC are:  $T_{1/2ab} = 0.65 \pm 0.29$  h,  $T_{1/2} = 3.09 \pm 1.43$  h,  $Vd = 1.08 \pm 0.60$  L/kg,  $Cl_{total} = 3.96 \pm 0.57$  mL/min/kg,  $C_{max} = 2.72 \pm 0.49$   $\mu$ g/mL,  $T_{max} = 1.78 \pm 0.52$  h,  $F = 84\%$ . The results are in agreement with those described by other authors. This antibiotic proved to reach effective concentrations in serum that overcome the MIC (minimal inhibitory concentrations) for most of the pathogens of veterinary interest in sheep. The pharmacokinetics parameters contribute to determine the appropriate dosage of this antibiotic in sheep.

99.

**EVALUATION OF THREE METHODS FOR SEQUENTIAL ISOLATION OF AVIAN INTESTINAL EPITHELIAL CELLS***Mac Donal O, Chediack JG, Caviedes-Vidal E**Unidad Invest Cs Biol, Univ Nac San Luis, 5700 San Luis. E-mail: macdonal@unsl.edu.ar*

We evaluated three methods (mechanical, enzymatic and by chelating agents) for isolation of intestinal epithelial cells. We selected Feral pigeons (*Columba livia*) as the biological model. We used birds of 1 to 30 days old. Cells were isolated by mechanical, enzymatic and chelating methods in two steps and characterized by morphology, cell number, viability, microphotographs and the activities of alkaline phosphatase (AP), a villus cell marker and sucrase- isomaltase (SI). Intestinal epithelial mass was markedly greater with the enzymatic and chelating procedures than with the mechanical method. In all methods high yields of cells and high viability in both steps were obtained. AP and SI activities were constant along villus- crypt axis with each method. All processes produced a high number of intestinal epithelial cells with viability around 50%. As it happens in chickens, enzyme activities were not different along the intestinal villus-crypt axis, Therefore, unlike mammals, avian enterocytes have a similar pattern of AP and SI expression.

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98.

**PHARMACOKINETIC PARAMETERS OF ENROFLOXACIN IN 8-MONTH OLD FOALS AFTER INTRAMUSCULAR ADMINISTRATION***Lüders C, Salas M, Betancourt L, Garcia H, Errecalde C, Prieto G**Farmacol, Fac Agron Vet, Univ Nac Río Cuarto, Río Cuarto 5800 Córdoba. E-mail: cluders@ayv.unrc.edu.ar*

Enrofloxacin kinetics were determined in 6 foals after intramuscular administration (5 mg/Kg) in the neck area. Serum concentrations were obtained by a microbiologic method, using *Bacillus subtilis* ATCC 6633 as the test microorganism. Serum concentrations best fitted a one compartment model. The pharmacokinetic parameters were obtained by the pharmacokinetic program Resid of Ritchel (1975). The parameters obtained were (mean  $\pm$  sd):  $t_{1/2a} = 1.027 \pm 0.284$  h,  $C_{max} = 0.694 \pm 0.158$   $\mu$ g/mL,  $T_{max} = 3.027 \pm 0.382$  h,  $Vd/F = 5.268 \pm 1.641$  L/kg,  $Cl_{total} = 10.557 \pm 2.358$  mL/min/Kg,  $t_{1/2e} = 5.740 \pm 1.204$  h. and  $T_{lag} = 0.048 \pm 0.040$  h. These results are in agreement with those reported by other authors. The drug showed a slow absorption from the injection site and reached effective concentrations in serum, being able to achieve levels above the CIM for most of the pathogens of veterinary interest in foals. The obtained pharmacokinetic parameters contribute to determine the appropriate dosage of this antibiotic in foals.

100.

**ENDOGENOUS NITRIC OXIDE ROLE IN RENAL NEONATAL APOPTOSIS***Manucha W, Carrizo L, Ruete C, Vallés P**Área Fisiopatol, Fac Cs Med; Univ Nac Cuyo-IMBECU CONICET, 5500 Mendoza. E-mail: wmanucha@fcm.uncu.edu.ar*

Unilateral ureteral obstruction (UUO) is associated with apoptosis, through a mechanism not yet completely elucidated. Angiotensin II and nitric oxide (NO) have been involved in apoptosis activation. In a model of neonatal UUO, we studied whether endogenous NO is involved in renal apoptosis induction. Newborn rats were subjected to UUO (n:10) within the first 48 h of life. After 5 and 14 days, kidneys were removed. Results: Decreased expression of  $AT_2$  receptor at 14 day relief vs 5 day relief in control cortex (CC):  $0.497 \pm 0.2$  vs  $0.993 \pm 0.5$  ( $p < 0.01$ ) and obstructed cortex (OC)  $0.566 \pm 0.3$  vs  $0.974 \pm 0.3$  ( $p < 0.01$ ) (RT-PCR). Increase in the number of apoptotic cells in cortical collecting ducts (CCD) and proximal tubules (PT) in OC related to CC (TUNEL), and near two fold increase Bax/Bcl<sub>2</sub> ratio after 14 days of obstruction (RT-PCR). Decreased iNOS expression in OC ( $p < 0.01$ ). On the contrary, slight increase of tubular apoptotic cells in OC and Bax/Bcl<sub>2</sub> ratio with simultaneous increase in iNOS expression related to control:  $1.253 \pm 0.07$  vs  $0.767 \pm 0.08$  ( $p < 0.01$ ) after 5 days of obstruction. Increased TGF $\alpha$  expression was shown after 14 days of obstruction (RT-PCR). The present study suggests a protective role of NO for apoptosis in neonatal renal obstruction including upregulation of Bcl<sub>2</sub> as a mechanism from mitochondrial pathway.

**101. EFFECTS OF ACETYSALICYLIC ACID IN CLINICAL BIOCHEMISTRY**

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In the medical literature a continuous update of databases containing important clinical interferences and biological effects of drugs on biochemical research appears. Drug effects on laboratory test results can be also found in several Clinical Guides and different databases for Laboratory Tests. This work aimed at assessing acetylsalicylic acid (ASA) effects on clinical laboratory tests. We continuously reevaluate and update the content from the database that is available as network versions for the PC and the computer version of some books. This has allowed us to start our review on ASA. According to our survey of available data, ASA produces the following biological effects: hyper- or hypoglycaemia, hypoprothrombinemia, increases serum aminotransferase activities (SGOT and SGPT) and serum urea; decreases serum lactate dehydrogenase activity (LDH), serum albumine and bilirubin; it also may cause anemia, leukopenia, thrombocytopenia, and dose-dependently alters plasma urate concentration. Moreover, ASA also interferes with analytical methods for serum albumine and LDH determination. Problems related to interferences and biological parameters of drugs should be revised continuously. Knowledge of drug effects on laboratory tests is very important for proper interpretation of clinical biochemistry results.

**103. IMMUNOSENSOR FOR RAPID QUANTIFICATION OF HUMAN SERUM IgG ANTIBODIES AGAINST *Helicobacter pylori***

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*Helicobacter pylori* is a bacterium that invades the gastric epithelium, causing chronic gastritis, peptic ulcers and gastric cancer. In this study we report an amperometric immunosensor with rotation, incorporated into a flow injection system, for rapid and sensitive quantification of human serum immunoglobulin G (IgG) antibodies against *H. pylori*. Antibodies in the serum sample are allowed to react immunologically with purified *H. pylori* antigens immobilized on a rotating disk. The bound antibodies are quantified by horseradish peroxidase (HRP) enzyme-labeled second antibodies specific for human IgG. In the presence of hydrogen peroxide, HRP catalyzes the oxidation of hydroquinone to p-benzoquinone. The electrochemical reduction back to hydroquinone is detected on a glassy carbon electrode surface at -0.15 V. The electrochemical detection can be performed within 1 min, and the analysis time does not exceed 30 min. Calculated detection limits for the proposed method and standard ELISA method are 0.6 and 1.9 U/mL, respectively. Thus, the amperometric immunosensor showed higher sensitivity and speed than the standard spectrophotometric detection ELISA method. The immunosensor is able to operate as a fast, selective, and sensitive detection unit when it is incorporated into a flow injection system.

**102. DETERMINATION OF ASCORBIC ACID USING AN ENZY-MATIC ROTATING BIOREACTOR INCORPORATED INTO A FLOW INJECTION SYSTEM**

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L-Ascorbic acid (AA) is important in processes of oxidation and reduction in humans, participating in several metabolic reactions. AA has been used for prevention and treatment of the common cold, mental diseases, infertility, cancer, and AIDS. Thus, the determination of AA content is particularly important in the pharmaceutical and food industry, where it is widely used as an antioxidant. In this work, an enzymatic rotating bioreactor for determining AA in pharmaceutical formulations was tested. Horseradish peroxidase (HRP), immobilized on a rotating disk, in presence of hydrogen peroxide catalyzes the oxidation of hydroquinone to p-benzoquinone, whose electrochemical reduction back to hydroquinone is detected on glass carbon electrode surface at -0.15 V. When AA is added to the solution, this acid reduces chemically p-benzoquinone to hydroquinone and acts as mediator of HRP, decreasing the peak current obtained proportionally to the increase of its concentration. The recovery of AA ranged from 99.09 to 101.10%. This method may be used to determine AA concentration in the range 12 nmol/L–3.5 µmol/L ( $r = 0.998$ ). The determination of AA was possible with a limit of detection of 6 nmol/L, processing 25 samples per hour. The method was successfully applied for analysis of AA in pharmaceutical formulations.

**104. INVASION DANGER? A POPULATION OF *Cenchrus ciliaris* IN THE RIVERBANKS OF THE RÍO SECO, SAN LUIS (ARGENTINA)**

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During December 2002 a “pasto salinas” or “buffel grass” (*Cenchrus ciliaris* L) population was detected at the riverbanks of the Río Seco (San Luis, Argentina). The buffel grass population was composed by a group of vigorous individuals growing in form of a discontinuous patch. In order to identify the invasion capacity of this species, the patch was visited and the area of the patch evaluated during December 2002 and 2003. The area occupied by the species increased from 7.5 m<sup>2</sup> in 2002 to 250 m<sup>2</sup> in 2003, although its coverage (30 ± 5.5 % in 2002 and 34.5 ± 15 % in 2003) was not significantly different ( $X^2 = 2.3$ ,  $p = 0.1294$ ). This taxon shows the following characteristics: 1. It presents resistance to the fire. 2. It produces a great quantity of seeds. 3. It possesses a history of documented invasion. 3. Two other invader species of the genus are present in the region: *Cenchrus pauciflorus* and *C. myosuroides*. 4. It has vegetative reproduction through short rhizomes. 5. The environmental characteristics of the origin area of the species agree with those of San Luis. These characteristics, and the preliminary results obtained in this work, would be indicating the invasion capacity of *Cenchrus ciliaris* in the region.

**105.**  
**EFFECT OF CADMIUM ON ANTIOXIDANT DEFENSE SYSTEM, IN DM 4800 SOYBEAN (*Glycine max L*)**

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The relationship between short time cadmium toxicity and the production of oxidative stress in plant cells was studied. Cadmium toxicity on DM 4800 RR soybean was evaluated by determining the non-enzymatic and enzymatic antioxidant parameters, ion superoxide and peroxide in soybean leaves. Grown plants, were exposed without roots on the 10<sup>th</sup> day of adaptation to hydroponic conditions in Hoagland's solution to intoxication with cadmium (40 µM) during 0, 4, 6 and 24 hours. Total glutathione (Gt), glutathione disulfide (GSSG), reduced glutathione (GSH), catalase (CAT), superoxide dismutase (SOD) and peroxide (H<sub>2</sub>O<sub>2</sub>), were determined in the first pair of leaves. Results: Gt and GSSG did not show any significative changes, GSH decreased (p< 0.05) respect to the control without root after 24 h of treatment. Ion superoxide increased in 4 h and 6 h with Cd 40 µM. It was parallel with SOD activity decrease. Also peroxide increased during the first time and it was correlated with CAT activity decrease. We can suggest that cadmium alters the non-enzymatic and enzymatic antioxidant system, in soybean leaves in a short time curve, demonstrating a signaling role of AOS and GSH in plant defense responses.

**107.**  
**ISOLATION OF GLYCOPROTEINS INVOLVED IN SPERM ASSOCIATIONS DURING EPIDIDYMAL TRANSIT**

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In several mammalian species, sperm associate conforming structures with characteristic morphology during epididymal maturation. In mouse and rat, these associations (rosettes) consist in 10-15 sperm joined by their heads. They appear only when sperm reach the distal segment of the epididymis (Tail). This suggests an environmental influence, probably due to sperm - epididymal secretion interactions. In previous studies we described morphologically and ultrastructurally this phenomenon in those species. We noted that an electron-dense material associated sperm heads. Glycoproteins recognized by Concanavalin A (Con A) were involved in the sperm association. Rat epididymal fluid (EF) isolated from adult males by epididymal perfusion with PBS was fractionated in a gel chromatography Sephacryl S200 column and 4 fractions were obtained. Each one of them was tested by co-incubation at 37°C with isolated and motile rat sperm to recreate *in vitro* the epididymal sperm association (Rosettes). First observations showed that Fractions 1 and 2 (with highest MW), were able to reassociate motile sperm *in vitro*. These fractions will be further purified by affinity chromatography (Con A column). These group of proteins (containing α-D- glucose/mannose residues) retained by Con A will be tested to check whether they retain sperm-associating ability.

**106.**  
**MYCORRHIZAL STATUS OF SIERRA DE LAS QUIJADAS NATIONAL PARK FLORA (SAN LUIS, ARGENTINA), II. BROMELIACEAE'S HOSTS**

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Roots of most plants are associated with glomalean fungi by vesicular-arbuscular mycorrhizas (VAM). Hence, many plants are asymptotically colonized by ascomycetous dark septate (DS) fungi. These symbiotic associations improve host nutrition and survival or have no effects on the host. Epiphytic vascular plants are VAM and mainly widespread in tropical humid forest. Bromeliaceae have epiphytic (E) and terrestrial (T) species and its mycorrhizal status is poorly studied. "Sierra de las Quijadas" National Park is an arid area at NW of San Luis province. There are 45 families of vascular plants, and Bromeliaceae has 9 species. The mycorrhizal status in E and T Bromeliaceae into the Park was studied. Six Bromeliaceae taxa (2 T and 4 E) were collected in summer and autumn; in each sample, roots were washed, fixed, clarified, stained, placed onto slides and observed under microscope to detect the symbiotic relationship. Terrestrial Bromeliaceae were VAM and epiphytes were DS colonized. DS association of epiphytic Bromeliaceae differed from the VAM found in cloud forest, environmental features would be determined to the type of association formed because DS are mutualistic in very harsh ecosystems. Terrestrial species were VAM as some Bromeliaceae members according to the arid conditions of the Park and the higher spore availability in the soil.

**108.**  
**PRODUCTION AND QUALITY OF THE *Trichloris crinita* SEED UNDER NATURAL RANGE CONDITIONS**

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*Trichloris crinita* is a summer perennial forage grass, which is a native component of the natural range of the arid and semi-arid Argentinian region. The aim of this work was to determine the quality and production of seeds from native populations seed in Mendoza and San Luis. The sampling of inflorescences was carried in the field. The weight of the seed per inflorescence, the weight of 1000 seeds and germination percentage were determined. The seeds were incubated in plastic trays of 10 x 14 cm with germination paper in cultivation camera at 30:20°C and 8:16 (light darkness). Comparison of means was performed with Duncan's Multiple Ranges Test with GLM of SAS. Yields were obtained from 0.665 to 3.168 g of seed for plant, weight of 1000 seeds of 0.20 to 0.24 g with germination percentage of 20 to 40%. Significant differences were detected among the plants, the inflorescences and places.

**109.**  
**CULTURE OF *Lippia turbinata* (GRISEB) (VERBENACEAE) “POLEO” IN DIFFERENT IRRIGATION CONDITIONS**

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The poleo (leaves and flowers) is classified under the Food Argentine Code for the preparation of drinks by distillation, infusion and softened. There is submitted to a commercial system clearly extractive, which might drive to a dangerous decrease of its population. The green yield - dry in leaves is 12% (Schiuma, 1997) in plants that grow spontaneously. This work has for objective determine the yield of dry matter in plants cultivated in conditions of irrigation and dryness. The plants obtained of seed (mericarpo) were tried after 10 months of culture. Measured up length and number of branches and cut to 10 cm of the soil. The vegetable material removed was weighed and dried during 48 h, to a temperature of 60 °C. The obtained data average were: 4.63 branches, 81 cm of height and 28,50% of dry matter in plants with watering and 2.73 branches, 71 cm of height and 28,14% of dry matter in plants cultivated in unirrigated land. Although the test will be repeated, this information suggests an important morpho-physiological adjustment of this species to changing environmental conditions, like, for example, water availability.

**111.**  
**HEAVY METAL CONTAMINATION OF *Odontesthes bonariensis* IN LA FLORIDA, (SAN LUIS, ARGENTINA)**

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Lead and cadmium water concentrations in La Florida are over EPA admissible values. Since there is no background information about fish contamination in this region, the aims of this work were to monitor Pb and Cd in organs of the silverside (*O. bonariensis*) and to analyze the risk for human consumption. Contamination was detected in gills (Gi), liver (L), intestine (I), estomach (E), gonads (G), muscle (M) and vertebra (V) of fish of age 1 to 6 years old by AAE-GF. The minimum and maximum levels expressed in ng/g for each organ were: Gi = 86.85 – 581.23; L = 928.89 – 6961.37; I = 213.75 – 1278.62; E = 120.40 – 671.05; G = 883.30 – 23134.56; M = 23.90 – 434.68 and V = 917.33 – 3719.66 for Pb, and Gi = 28.94 – 151.21; L = 254.45 – 2158.31; I = 45.54 – 250.38, E = 65.91 – 384.50; G = 381.12 – 12328.5; M = 13.34 – 142.05 and V = 207.21 – 2548.92 for Cd. These values are the first data for heavy metal reported for silversides in this region. Pb and Cd total concentration tended to increase proportionally with body size. Concentrations of both toxicants were beneath SENASA recommended limits for human consumption.

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**110.**  
**ASPECTS RELATED TO THE GERMINATION OF *Lippia turbinata* (Griseb) “poleo” (VERBENACEAE) IN SAN LUIS, ARGENTINA**

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Irrational use of medicinal native plants of the Center - West of Argentina has endangered the survival of numerous useful species (del Vitto *et al.*, 2003). The “poleo” *L. turbinata* presents “secrets benefits” (Karlin *et al.*, 1994) in the field of the fitomedicina and the nourishing industry. In April, 2003 mericarpos obtained of plants that grow in the Department The Capital (San Luis) were harvested. Tests were performed every two months, with temperatures that they ranged between 18 and 25 °C, in conditions of light and darkness, and there decided the variation of the germinative power (PG) and the germinative value (VG), across the time. “Seeds” were placed by treatment (n=150) in Petri’s boxes, with absorbent paper and distilled water. There was verified that as age increases a trend for increase is observed in the PG and is eliminating the need of light. In accidental form, a test at 30°C showed a response different from the observed one at low temperatures, namely that VG and PG did not show significant differences with light an without it. Therefore, new experiences will be performed by at temperatures above 25°C. The percentages of germination, relatively low, might be a consequence of the employment of fruits (mericarpos) with defective seeds due to bad pollination, auto-pollination, climatic or other factors.

**112.**  
**INFLUENCE OF A POLAR VEHICLE IN THE PERMEATION OF SALICYLIC ACID**

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The physicochemical variables that control salicylic acid (SA) delivery through membranes (Viledon 2430 and 2431, poly vinylidifluoride (PVDF) and egg shell membrane), its modulation and the permeation parameters were determined. The vehicle employed was isopropanol and the receptor medium was phosphate buffer. The experiments were carried out using the Microette system with four Franz diffusion vertical cells. The quantity of drug released was determined by spectrophotometry. The values obtained for  $J_m$ , P and D are in the Table:

Membrane	$\Delta X$ $\times 10^4$ (cm)	vehicle	$J_m \times 10^7$ (g/cm <sup>2</sup> .s)	$P \times 10^6$ (cm/s)	$D \times 10^8$ (cm <sup>2</sup> /s)
Vil. 2430	134.8	IP- IP	6.199	2.718	3.665
Vil. 2431	133.8	IP- IP	7.460	3.2711	4.377
PVDF	280.0	IP- IP	4.573	2..078	5.819
egg shell	198.0	IP- IP	4.147	1.884	3.732

$\Delta X$ : membrane thickness;  $J_m$ : diffusional flux; P: permeation and D: diffusion coefficients.

The values obtained were similar for the different membranes, this allows to infer that the vehicle (IP) does not alter their properties. On the other hand, these parameters are significantly different from those obtained in our previous experiments using PG, which is a consequence of the distinct interaction between the vehicle, the active principle and the membranes.

**113. SERUM INTERLEUKIN-10 LEVELS IN HIV-INFECTED PATIENTS TREATED AND UNTREATED WITH HIGHLY ACTIVE ANTIRETROVIRAL THERAPY**

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Infection with HIV is known to cause an immune dysfunction with changes in cytokine levels. In this study we measured serum interleukin-10 (IL10) in 26 HIV-infected patients naive of antiretroviral treatment and in 43 patients receiving highly active antiretroviral therapy (HAART). Healthy blood donors (n = 20) served as controls. When patients were stratified according to CD4+ cell count, the IL-10 levels (in pg/mL) were significantly higher in patients with < 200 CD4+/ $\mu$ L ( $9.6 \pm 0.6$ ,  $p < 0.05$ ) compared to patients with  $\geq 200$  CD4+/ $\mu$ L ( $7.2 \pm 0.9$ ) and controls ( $7.8 \pm 0.4$ ). A negative correlation was found between the IL-10 levels and the CD4+ cells counts ( $r = -0.53$ ,  $p < 0.05$ ). No correlation was observed between IL-10 levels and plasma viral load, due to the wide range of variability in the number of HIV copies/mL present in the different patients. In patients with HAART, the IL-10 levels in those with < 200 CD4+/ $\mu$ L ( $8.8 \pm 1.2$ ) or  $\geq 200$  CD4+/ $\mu$ L ( $7.3 \pm 1.5$ ) were similar to those of the control group ( $7.8 \pm 0.4$ ). These findings indicate that modifications in serum IL10 levels in HIV-infected patients naive of antiretroviral treatment are associated to progression of immunological damage, and suggest an imbalance of cytokines with an involvement of type-2 cytokine in latter stages of HIV infection. These variations were improved by antiretroviral therapy.

**115. Chlamydia trachomatis INFECTION IN SYMPTOMATIC WOMEN IN MENDOZA**

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Screening women for lower genital tract infection with *Chlamydia trachomatis* (Ct) is important in the prevention of pelvic inflammatory disease, ectopic pregnancy and infertility. The mean ages of women was  $28 \pm 1$  yr. Cervical specimens were collected, stored in transport medium at 4°C, inoculated onto McCoy cells and overlaid with growth medium. Chlamydial inclusions were detected with iodine staining, genus-specific antichlamydial lipopolysaccharide (LPS) or anti-MOMP monoclonal antibodies conjugated with fluorescein. The relationship between active chlamydial replication and clinical manifestations was assessed by using the number of inclusion-forming units produced in cell tissue culture. A polymerase chain reaction (PCR)-based assay for detection of the chlamydial major outer membrane protein gene was compared with culture. Increased numbers of inclusions produced in culture were associated with acute inflammation and most clinical signs of chlamydial infection. Multivariate regression analysis was used to assess the relationship between inclusion-forming unit count and clinical manifestations. Inclusion forming units counts increased with greater quantity of cervical exudate and greater number of PMN ( $P < 0.001$ ). The isolate rate of Ct by cell culture was 86% for MOMP detection and 92% for LPS. The data suggest that active chlamydial replication is associated with high inclusions counts. The sensitivity of the LPS antibody detection immunofluorescent method in cell culture is not statistically significant compared with the MOMP antibody assay.

**114. COMPARATIVE PROFILES OF Hedera helix FORMULATIONS BY CAPILLARY ELECTROPHORESIS**

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*Hedera helix* formulations present in the Argentinian market are used for the treatment of respiratory diseases. The safety, quality and efficacy of herbal medicines and their derivatives is an important issue for health professionals. Chemical fingerprinting is a helpful tool that should be included as a quality control method in order to confirm the adequacy of plant material used for the manufacturing of a product. A commercial syrup and two different extracts of *Hedera helix* leaves (glycolic and fluid) were analyzed. Different profiles of the samples under study were obtained by capillary electrophoresis (CE). The background electrolytes (BGEs) used were buffer sodium tetraborate 20 mmol/L, pH 9.0 and buffer acetic acid – sodium acetate 11 mmol/L, pH 5.0. The obtained electrophoretic profiles were compared with the aim of investigating the diversity of constituents present in the different preparations. Electrophoretic profiles can be one step towards ensuring quality in phytopharmaceuticals products, complementing the already established analytical techniques.

**116. Rab11-FIP3, THE Rab11-FAMILY INTERACTING PROTEIN 3, IS ASSOCIATED TO PHAGOSOMAL MEMBRANES**

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Rab11-FIP3 is a recently described protein that belongs to the class III Rab11-family binding proteins. It is also known as Arfophilin and Eferin. Rab11-FIP3 interacts with both Rab11 and ADP-ribosylation factor (ARF). It has been localized to the cleavage furrow during cytokinesis. We sought to investigate the Rab11-FIP3 subcellular localization in RAW macrophages. By immunofluorescence using a specific antibody, we found that the endogenous protein is predominantly membrane-bound and displays a punctate vesicular pattern throughout the cytoplasm. Similar results were obtained by confocal microscopy when we overexpressed Rab11-FIP3 fused to the green fluorescent protein. We have observed that Rab11-FIP3 colocalizes with Rab11 in the perinuclear endosomal compartment. Interestingly, we found that Rab11-FIP3 is associated as discrete patches to early phagosomal membranes. Overexpression of Rab11-FIP3<sup>244-756</sup> a truncated form containing the Rab binding domain remains membrane-bound and associated to Rab11-positive structures. Our results suggest that Rab11-FIP3 by forming mutually exclusive complexes with Rab11, could serve a role in cellular processes that require the delivery of large amounts of membrane such as phagocytosis and plasma membrane recycling.

### 117. ERYTHROCYTES PARAMETERS IN HEALTHY NEONATAL BABIES

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The objective of this study was to establish referential values of different erythrocytes parameters from healthy neonatal babies born on time among our population. The sample included 98 healthy neonates (46 girls) born by normal delivery or by caesarean with 47 weeks of gestation. The parameters resulted from the analyses of capillary anticoagulant blood extracted during the first 24 hours after the child birth in the hematology analyzer (Cell Dynn 1400). The reticulocytes count was carried out in blood extensions containing supravital coloring matter. The obtained values are showed in the following chart:

Parameter	Mean $\pm$ DS	percentiles 5 & 95
Erythrocytes (x 10 <sup>12</sup> /L)	4.98 $\pm$ 0,47	4.11 to 5.73
Hemoglobin (gr/dL)	17.38 $\pm$ 1.74	14.10 to 20.30
Hematocrit (L/L)	0.48 $\pm$ 0.05	0.38 to 0.53
VCM (fL)	97 $\pm$ 4	91 to 103
HCM (pg)	34.8 $\pm$ 1.6	31 to 37
CHCM (gr/dL)	35.7 $\pm$ 1,1	33.8 to 37.3
Reticulocytes (%)	3.4 $\pm$ 1.2	1.8 to 5.9
Reticulocytes (x 10 <sup>12</sup> /L)	170 $\pm$ 67	87 to 292

There were no relevant differences among the analyzed parameters between female and male babies, between type of delivery or time of blood extraction after the child birth. As there were no reference values among our population, this study contributed with those data, which agree with values found in the literature. It also established a hemoglobin cut value to detect the presence of anemia in neonates.

### 119. *Acacia visco* METHANOLIC EXTRACT AND *Larrea divaricata* ETHANOLIC EXTRACT ACTIVITY ON GASTRIC ULCER BY NECROTIZING AGENTS

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Antioxidant activity has been previously determined in methanolic extract of *Acacia visco* and *Larrea divaricata* leaves (Biocell Vol 27 Supp.II 2003). Here we investigate the antiulcer effect of ethanolic extract of *Larrea divaricata* leaves (*LdEEI*) and methanolic extract of *Acacia visco* leaves (*AvMEI*) on gastric ulcer induced in rat by necrotizing agents (Robert *et al.*, 1979). Wistar rats were fasted, water *ad libitum*, divided into seven groups and orally treated as follows: G1 (*AvMEI*) and G2 (*LdEEI*): 200 mg/Kg respectively, after one hour NaOH 0.2N. G3 (*AvMEI*) and G4 (*LdEEI*) 200 mg/Kg respectively, after one hour NaCl 25%. G5 (*AvMEI*) and G6 (*LdEEI*) 300 mg/Kg respectively, G7 (*LdEEI*) 400 mg/Kg, after one hour HCl 0.6 N. Animals were sacrificed 60 min later and the degree of gastric mucosa evaluated according to Marazzi-Uberti and Turba scale, expressed as ulcer index (UI). Each group had its respective ulcer control and reference group (omeprazol 20 mg/kg). The inhibition percentage was calculated with respect the control (100% damage). Damage inhibition was 100% for G1 and G2, 67% and 73% for G3 and G4 respectively, 97% for G5 and G6 and 100% for G7. The preventive action of *AvMEI* and *LdEEI* could be attributed to the presence of compounds with free radical scavenger effect.

### 118. EFFECT OF *Acacia visco* METHANOLIC EXTRACT IN ADJUVANT ARTHRITIS

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In this work we investigated the effect of the *Acacia visco* methanolic extract from leaves (*AvMEI*) and bark (*AvMEb*) in arthritis by adjuvant-carrageenan induced inflammation (Mizushima *et al.*, 1972). Wistar rats were i.d. injected with 0.1 mL Freund's complete at the tail base and, six days later, orally treated on a daily basis for 28 days with 0.4% Carboxymethylcellulose (vehicule) in saline (Group 1, inflammation control); 200 mg/Kg of *AvMEI* and *AvMEb* (Groups 2 and 3, respectively); and 80 mg/Kg of phenylbutazone (Group 4, reference). One hour later, all groups were injected with 0.1 ml/rat of carrageenan type IV w/v 2% in saline, in the hind paw subplantar region. Pedal oedema was determined with a plethymometer at 3 and 5 h (acute phase) and discontinued from day 7 to 28 (chronic phase), and compared to the control. The arthrogram score was obtained. Body, spleen and thymus weight were determined. Inhibition was 80 and 66% by *AvMEI* and 73 and 71% by *AvMEb*, at 3 and 5h respectively. In the chronic phase, *AvMEI* and *AvMEb* inhibited the oedema and reduced the arthrogram score. *AvMEI* caused no body, spleen and thymus weight differences, while thymus weight showed no differences with *AvMEb*. *AvMEI* and *AvMEb* antiinflammatory effect might be related with antioxidant capacity and to the presence of triterpenes in *AvMEb*. The lack of lymphoid organs weight variation suggests mechanisms unrelated with specific immunity.

### 120. ACTIVITY OF *Larrea divaricata* METHANOLIC EXTRACT IN ADJUVANT ARTHRITIS

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Nordihydroguaiaretic acid (NDGA) has been isolated from *Larrea divaricata* leaves (Waller *et al.*, 1945). The purpose of this work was to investigate the effect of methanolic extract from *Larrea divaricata* leaves (*LdME*) in arthritis by the adjuvant-carrageenan induced inflammation method (Mizushima *et al.*, 1972). Wistar rats were i.d. injected with 0.1 mL Freund's complete at the tail base; Six days later, they were orally treated as follows on a daily basis. G1 (inflammation control): 0.4% Carboxymethylcellulose (vehicle) in saline; G2: *LdME* 200 mg/Kg; G3 (reference): phenylbutazone 80 mg/Kg. One hour later, all groups were injected with 0.1 ml/rat of carrageenan type IV w/v 2% in saline, in the hind paw subplantar region. Pedal edema was determined with a plethymometer at 3 and 5 h (acute phase) and discontinued from day 7 to 28 (chronic phase) and compared to the control. The arthrogram score was obtained. Body weight, spleen and thymus were determined. Edema inhibition was 79 and 83% at 3 and 5h, respectively. In the chronic phase *LdME* led to highest inhibition on days 22 and 28 (58 and 67%, respectively), and reduced the arthrogram score. No significant changes in lymphoid organs weight were observed. The effective anti-inflammatory action of *LdME* might be related with the free radical scavenging capacity of NDGA. The lack of lymphoid organs weight variation suggests mechanisms unrelated to specific immunity.

**121.  
SUPERIOR OVARIAN NERVE TRANSECTION PRODUCES  $\beta_2$ -RECEPTORS CHANGES IN UTERUS DURING RAT PROESTRUS AND DIESTRUS**

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Rodent uterus and vagina, target organs of ovarian hormones, show marked histological changes during the estrous cycle. The objective of this study was to determine if superior ovarian nerve (SON) transection (SON-t) affects the density of  $\beta_2$ -adrenergic receptors in different uterine regions in proestrus (PE) and diestrus (D1). Five virgin Holtzman rats were used per each group, namely control (sham) and experimental (SON-t) groups. The uteri were cut into three portions.  $\beta_2$ -Receptors were determined with  $I^{125}$ ciano-pindolol. Student's *t*-test and ANOVA were used for statistical analysis, at a significance level of  $p < 0.05$ . Results are expressed in fmol/mg ovary (mean  $\pm$  SEM). In both PE and D1, SONt increased  $\beta_2$ -receptors in the three portions ( $p < 0.001$ ) compared to sham group. When the groups PE and D1 were compared, the latter presented a lower density ( $p < 0.05$ ). The uterine innervation by SON showed important regional variations. It is possible that uterine innervation density may be regulated anterogradely by SON or neurotrophic factors projecting from coeliac ganglion, origin of the postganglionic sympathetic nerves arriving to the uterine horns.

**123.  
EVALUATION OF ANTIDIARRHEAL ACTIVITY AND ACUTE TOXICITY OF *Aristolochia argentina* DECOCTION**

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The roots of *Aristolochia argentina* are used in folk medicine as antidiarrheal and antihemorrhoidal. The antidiarrheal activity and acute toxicity effects of the decoction of *A. argentina* was assessed in mice. Decoction (10%) of the roots was prepared. Effect of decoction on normal defaecation was evaluated. Diarrhea was induced by administering castor oil orally to mice. The test group received decoction 30 min before castor oil. Acute toxicity: The control group received water and the test group received decoction (500 to 6000 mg/kg). Animals were observed carefully every 2 days to record toxic manifestations, and their body masses were measured. After the 7-day experimental period mice were sacrificed. The organs were observed macroscopically and the relative weights (organ/body) were determined. The results of the decoction effect on normal defaecation showed that normal defaecation was inhibited by 47%. Decoction reduced the total and wet number of faeces ( $p < 0.001$ ). No toxic symptoms or deaths occurred in each decoction group. The general conditions of all mice were also normal. There were no significant alterations in body weight during the experiment. The relative weights of kidney, liver, lung and heart were not statistically different from control. The decoction of the roots of *A. argentina* showed significant antidiarrheal activity and did not present acute toxic effects, providing scientific support to the traditional use of this plant.

**122.  
CYTOPROTECTION BY *Baccharis polifolia* ON DIETHYL-DITHIOCARBAMATE-INDUCED GASTRIC ULCERS AND CHRONIC ULCERS IN RATS**

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*Baccharis polifolia* Griseb is used in folk medicine for gastrointestinal disorders. This work evaluated the effect of *B. polifolia* on diethyldithiocarbamate-induced antral ulcers and chronic ulcers in rats. Twenty four h before the experiments, Wistar rats were fasted. The pylorus was ligated. Diethyldithiocarbamate (800 mg/kg) was given sc and 1 mL oral dose of 0.1 N HCl was then given. The rats were killed 5 h later. The ulcer index was expressed as the area of the antral ulcer (mm<sup>2</sup>). *B. polifolia* ethanolic extract (250 mg/kg) prevents the formation of gastric mucosal lesions ( $p < 0.001$  vs. control). Induction of chronic gastric lesions was studied according to the methods of Takagi *et al.* (1969) and Albelda *et al.* (1973) employing acetic acid as ulcerogenic agent. A solution of acetic acid was injected below the gastric serosa of the rats. Animals were administered orally *B. polifolia* extract (250 mg/kg, 1 mL) during 3 days. On the 7<sup>th</sup> day after acetic acid injection, animals were killed. The ulceration grade was determined by area (mm<sup>2</sup>). Oral administration of *B. polifolia* extract decreased the ulceration size ( $p < 0.01$  vs control). The experimental results demonstrate that *B. polifolia* protects the gastric mucosa against lesions induced by diethyldithiocarbamate and acetic acid. These facts support the use of *B. polifolia* to treat digestive disorders in traditional medicine.

**124.  
LIPIDIC PROFILE OF RAT LUNG: EFFECT OF INHALATION OF A CORTICOSTEROID**

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Corticosteroids are used in respiratory diseases such as asthma primarily because of their antiinflammatory properties. In this work, we studied the effect of the synthetic corticosteroid, budesonide, at three different doses (low [LD], therapeutic [TD] and high [HD]), on the lipidic profile of male rat lung. In order to observe if there were modifications on the lipidic profile in lung, male Wistar rats were separated in four groups, which inhaled: group I, saline solution (Group I, control), and three different doses of budesonide (groups II, III and IV) for 5 min, twice a day, during 15 days. Total lipids (TL), proteins, triglycerides (TG), total (TC), free (FC) and esterified (EC) cholesterol, phospholipids and phosphatidil choline (PC) were determined. We found that TL, proteins and TG did not change with any of the different doses. TC increased with the therapeutic dose, while FC increased with the high dose and EC decreased with the low dose and increased with the TD. Regarding PL and PC, there was a decrease with LD and an increase with TD and HD. These results show that budesonide modifies the lipids of the rat lung in a dose-dependent manner.

125.

**EFFECT OF CADMIUM ON NADPH-GENERATING ENZYMES IN DM 4800 SOYBEAN (*Glycine max.L*)**

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The role of the NADPH-generating enzymes, isocitrate dehydrogenase (ICD), malic dehydrogenase (MD) and glucose 6-phosphate dehydrogenase (G6PD) in the antioxidative response of soybean roots to cadmium contamination was studied. Grown plants were exposed on the 10<sup>th</sup> day of adaptation to hydroponic conditions in Hoagland's solution to intoxication with cadmium (10 µmol/L) during 0, 4, 6 and 24 h. The enzymatic activity of ICD, MD and G6PD was determined in soybean roots. Results: ICD with 6 h of treatment showed a significant decrease ( $p < 0.04$ ) but increased after 24 h ( $p < 0.03$ ). MD increased at 4h ( $p < 0.001$ ) but decreased after 6 h ( $p < 0.01$ ). G6PD only showed a significant increase at 24 h of treatment ( $p < 0.001$ ). We suggest that cadmium alters NADPH production, indicating an essential role of this cofactor to maintain the reduction equivalents of the GSH- GSSG system as part of the antioxidant defense responses.

127.

**PHARMACOBOTANICAL AND PHYTOCHEMICAL STUDIES ON "CARQUEJAS" (*Baccharis*, Asterac.) FROM CUYO, ARGENTINA**

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Five aphyllous (or nearly so) plant species of *Baccharis* (Asteraceae: Asteroideae: Baccharineae) so-called "carquejas" are used in folk medicine of Cuyo as hepatic and diuretic remedies: (1) *B. crispata* Spreng., (2) *B. articulata* (Lam.) Pers., (3) *B. trimera* (Less.) DC., (4) *B. sagittalis* (Less.) DC. and (5) *B. triangularis* Hauman. Morphological, anatomical, micrographical and phyto-chemical studies were carried out tending to define adequately the drugs and to achieve an effective quality control in commercial samples. Two of them (2 and 4) show the bioform chamaephyte or low nanophanerophyte, with the lowest stomata number and stomata ratio. The three others are mainly geophytes, from which (5) shows the highest values in stomata, vein-islets and vein-termination numbers, and (1) and (3) present intermediate ones (parameters measured on stems wing-surface, for analogy with the leaf structure). All species yielded mainly flavones, diterpenes and volatile terpenes. (1) and (3) are very similar, but (3) lacks the butenolides. (2) contains acastepine, circimaritine, salvigenine, barti-culidiol malonate, bacchotricuneatine A,  $\beta$ -guaiene,  $\delta$ -cadinene and aromadendrene. From (4), marrubiagenine and two new diterpene glycosides were isolated, while (5) yielded an *ent*-neo-clerodane dilactone and two flavones (luteolin and 7-O-methyl-luteolin). Noteworthy, the stereochemistry of the isolated diterpenes was different. Usually the genus exhibit *ent*-neo-clero-dane type diterpenes with an A/B ring *trans* fusion; significant, (4) showed an A/B *cis* stereochemistry in all isolated diterpenes.

126.

**EFFECT OF THE INHALATION OF A  $\beta$ -ADRENERGIC AGONIST ON THE LIPIDIC PROFILE OF RAT LUNG**

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$\beta$ -Adrenergic agonists produce bronchodilatation and vasodilatation in chronic illnesses such as asthma. We studied the effect of the agonist salbutamol (SAL) inhaled at three different doses: low (LD), therapeutic (TD) and high (HD). Male Wistar rats were separated in four groups. Three groups inhaled SAL in the three different doses and the control group (Co) inhaled saline solution for 5 min, twice a day during 15 days. Bronchoalveolar lavages were made in order to study the lipidic profile of the lung surfactant (LS). The determinations done in total lung (TL) and LS are: total lipids (TL), triglycerides (TG), total (TC), esterified (EC) and free (FC) cholesterol, total phospholipids (TP) and proteins (P). TG and P did not show variations neither in TL nor in LS; in LS, TL increased significantly with LD and TD when compared to Co. FC decreased with LD and HD, and EC increased with LD. TP decreased with LD and increased with TD and HD while phosphatidylcholine (PC) only increased with HD. In TL, TC decreased with LD and TD, FC decreased in LD and TD while it increased in HD. EC decreased with LD; TP and PC decreased with LD and increased with TD and HD. These results show that SAL modifies cholesterol and phospholipid contents in TL and LS in a inhaled dose-dependent manner.

128.

**BONE MARROW EXAMINATION IN AIDS PATIENTS**

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HIV infection is associated with multiple hematological abnormalities in peripheral blood, as well as in the bone marrow. In this work, we evaluated cytomorphological characteristic and iron deposit in bone marrow from AIDS patients. Peripheral blood and sternal bone marrow aspirates smears were obtained from 31 patients (28 with opportunistic infections and 3 with neoplasias). It was determined that: blood cell count (autoanalyzer), CD4<sup>+</sup> T lymphocytes (flow cytometer), cellular differential of bone marrow, stainable iron in bone marrow macrophages and sideroblast count (Prussian blue reaction). All patients were severely immunodepressed and cytopenia in peripheral blood was detected in 93% of patients (35% with pancytopenia, 42% with bicytopenia and 16% with unicytopenia). The most striking abnormality in bone marrow was: hypercellularity in 68% of cases with granulocytic hiperplasia (14%), erythroid hiperplasia (43%) or myeloid/erythroid ratio normal (43%). Dysplastic characteristic of erythroid and granulocytic series associated with eosinophilia, plasmocytosis or lymphoid infiltration was observed in all cases. In 13% of cases the bone marrow was hypocellular. Features of opportunistic infection were detected in 81% of cases. Macrophages containing an excess storage of iron were observed in 74% of cases with normal sideroblast. These findings demonstrate that bone marrow of AIDS patients shows a cytological particularity and its evaluation provides useful information for demonstrate the stage of HIV disease and for the diagnosis of its associated complications.

**129. DETECTION OF STEC (SHIGA TOXIN *Escherichia coli*) IN CATTLE IN MENDOZA**

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Shiga toxin *Escherichia coli* is an important cause of infectious diarrhea and diarrhea associated hemolytic uremic syndrome (HUS). Direct or indirect exposure to bovine or human feces account for many cases. The aim of this project was to determine the prevalence of STEC in intestinal contents and carcasses of cattle in a slaughterhouse in Mendoza. In the period from March 2003 to September 2004, 58 animals were studied. One fecal swab from the colon of each animal were collected and 55 samples of different zones on the carcasses were obtained. Bacterial colonies were grown on Mac Conkey agar incubating for 24 h at 37°C. An aliquots of confluent growth was processed for DNA extraction. All the *E.coli* isolates were screened for the presence of the genetic sequences of stx1, stx2 and eae by PCR. 23% of Stx strains were isolated from fecal swabs, while 12% Stx were obtained from the carcasses. The eae gene was identified in 12,5% of fecal samples and in 2% of carcasses. According to this findings and considering that there is no vaccine or effective treatments against STEC or their induced diseases, the identification of risk factors is very important to implement preventive strategies.

**131. REACTIVE OXYGEN SUBSTANCES AND REPERFUSION ARRHYTHMIAS IN ISOLATED RAT HEARTS**

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Experimental reperfusion arrhythmias (RA) are attributed to calcium overload that starts during the ischemic period and increases at the moment of reperfusion through the explosive production of reactive oxygen substances. The effect of two antioxidant and scavenger agents, melatonin, 5-methoxy-N-acetyl-tryptamine, and tiron, 4,5-dihydroxy-1,3-benzenedisulfonic acid, on the RA was studied. Ventricular action potentials and electrograms were recorded in spontaneously beating rat hearts, before, during 10 min of coronary occlusion and 15 min of reperfusion (R). Four series of experiments (N=10 each) were performed: 1) control, perfusion with Krebs-Henseleit(K-H) solution without antioxidant (C). 2) K-H plus 10µmol/L of melatonin (MEL10). 3) K-H plus 20µmol/L (MEL20) and 4) K-H plus tiron 10mmol/L (TI10). Electrophysiological variables were analyzed by ANOVA and the incidence of RA was evaluated by X<sup>2</sup>test. Sustained RA decreased from 70% in C to 30% in MEL10, 10% in MEL20 and 10% in TI10 (p<0.01). In all the 4 series 30% of hearts developed RA but they recovered sinus rhythm between 1 and 14 min of R. 40% of hearts in MEL10 and 60% in MEL20 and TI10 did not develop RA (p = 0.0296). No treatment presented significant differences in the electrophysiological variables measured, so the antiarrhythmic effects of melatonin and tiron are not attributable to variations in cellular electrical activity but to their antioxidant and scavenger activities.

**130. ANTI-PROLIFERATIVE ACTIVITY OF 2H-DhL, A DEHYDROLEUCODINE DERIVATE WITH AN INACTIVATED  $\alpha,\beta$ -UNSATURATED  $\gamma$ -LACTONE RING**

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Dehydroleucodine (DhL) is formed by three rings. A  $\gamma$ -lactone ring is connected to a 7-membered ring and that is fused with a 5-membered ring. It is accepted that the biological activity of DhL is related to the  $\gamma$ -lactone ring since it has a methylene group in the  $\alpha$ -position. The  $\beta$ -carbon of the methylene group acts as a Michael's acceptor. Work performed in our lab showed that DhL inhibits the growth of budding yeast. Here, we analyzed which chemical group is responsible for the effect. Two DhL analogs were used: 11-13dihydroDhL (2H-DhL) and 2-3,9-10,11-13hexahydroDhL (6H-DhL).  $2 \times 10^6$  yeast (grown in stationary phase) were incubated in YPD medium for 39 hours. Cultures were treated with 0 or 1mM DhL, 2H-DhL and 6H-DhL. The number of yeast at each time of treatment ( $\times 10^6$ ) is shown in the table:

Hours	Control	DhL	2H-DhL	6H-DhL
0	2.1 ± 0.1	2.3 ± 0.1	1.9 ± 0.1	2.2 ± 0.03
16	23 ± 1.3	7.9 ± 0.4	20 ± 1.1	22.4 ± 0.7
27	66 ± 5	22 ± 1.1	51 ± 0.5	56 ± 0.9
39	86 ± 2	47 ± 0.4	64 ± 5	70 ± 6

Taking the DhL induced inhibitory effect as 100%, 2H-DhL inhibits by 50% and 6H-DhL 0%. We conclude that 2H-DhL has a moderate degree of activity, showing that the  $\alpha,\beta$ -unsaturated group in the  $\gamma$ -lactone ring is not the only chemical group participating in the inhibitory effect.

**132. PLASMA DISPOSITION OF CEPHALOTIN IN CALVES FOLLOWING ENDOVENOUS AND INTRAMUSCULAR INJECTION**

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The aim of this work was the determination of the kinetic profile of cephalotin in weaning calves following endovenous and intramuscular injection. Healthy Argentine Holando bull calves, of 46.6 ± 3.9 kg weight, 32.5 ± 16.6 days age, in identical handling conditions were used. Once conformed groups A (n = 6) and B (n = 5), each animal received a 40 mg/kg endovenous and intramuscular single dose, respectively. Plasma concentrations were determined by microbiological assay, using *Bacillus subtilis* BGA. Data were analyzed by means of software RESID being interpreted like a monocompartmental model. Obtained values for endovenous route were: elimination half life ( $t_{1/2}$ ) = 0.6 ± 0.12 h; mean residence time (MRT) = 0.64 ± 0.15 h, volume of distribution (Vd) = 2.74 ± 0.39 L/kg, Clearance (Cl) = 71.35 ± 13.99 mL/min/kg. Meanwhile for intramuscular administration  $t_{1/2}$  = 0.86 ± 0.19 h; MRT = 1.38 ± 0.21 h, Vd = 9.18 ± 5.68 L/kg, Cl = 80.11 ± 41.51 mL/min/kg. The kinetic parameters are similar to those in other domestic animals and humans, except for the high Vd as a possible consequence of the greater volume of corporal water in young animals and the tendency of the antimicrobial to spread in the interstice.

## 133.

**EFFECT OF DEHYDROLEUCODINE ON MIGRATION OF RAT MESENTERIC SMOOTH MUSCLE CELLS INDUCED BY ANGIOTENSIN II**

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Vascular smooth muscle cells (SMC) migration is an important step in neointimal formation in several cardiovascular diseases. We have previously observed that Angiotensin II (Ang II) induces *in vitro* aortic SMC migration. In addition, we have found that dehydroleucodine (DhL), a sesquiterpene lactone isolated from a medicinal herb grown in Argentina, inhibits Ang II-induced proliferation and migration of aortic SMC in culture. We studied DhL effect on Ang II-induced migration of rat SMC obtained from mesenteric resistance vessels. SMC migration was assessed by the scrape-wound migration assay, in the presence of OH-urea to avoid cell proliferation. Cells were incubated 24 and 48 h in DMEM with 0.1% fetal bovine serum (FBS), Ang II (100 nmol/L) or Ang II+DhL 2 $\mu$ mol/L. Results (mean  $\pm$  SEM) are expressed as % of the control group (0.1% FBS). Statistical significance was assessed by ANOVA.

	Control	Ang II	Ang II+ DhL
24h (n =14)	100 $\pm$ 12	339 $\pm$ 40 *	125 $\pm$ 18 •
48h (n =14)	100 $\pm$ 13	251 $\pm$ 21 *	98 $\pm$ 9 •

\*P<0.001 vs.control; •P<0.001 vs. Ang II

DhL effect on vascular SMC behavior could support its therapeutic use in vascular remodeling present in cardiovascular diseases.

## 135.

**EFFECT OF CHRONIC GARLIC ADMINISTRATION IN A MODEL OF INSULIN RESISTANCE**

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The main objective was to observe the effects of chronic aqueous garlic extract (G) gavage administration in an experimental model of metabolic syndrome in rats (FFR). Male Wistar rats were assigned to 6 groups (n=6 in each): 1) Control (C); 2) C+G125 mg/Kg/d; 3) C+G250mg/Kg/d; 4) FFR: 10% fructose in drinking water; 5) FFR+G125 and 6) FFR+G250. After a 6 weeks experimental period, variations in weight ( $\Delta$ W-g), systolic blood pressure (SBP-mmHg), glucose tolerance test (GTT-mmol/L.90min) and aortic NAD(P)H oxidase activity (NA) (cpm/mg) were evaluated. Data were processed by ANOVA and Newman-Keuls post-test (Table).

Group	C	C+G125	C+G250	FFR	F+G125	F+G250
$\Delta$ W	141 $\pm$ 3	142 $\pm$ 6	160 $\pm$ 2*	172 $\pm$ 6*	146 $\pm$ 4#	154 $\pm$ 1#
SBP	115 $\pm$ 1	112 $\pm$ 2	110 $\pm$ 2	133 $\pm$ 1*	120 $\pm$ 2#	108 $\pm$ 1#
GTT	1295 $\pm$ 18	1152 $\pm$ 64	1167 $\pm$ 67	1774 $\pm$ 63*	1333 $\pm$ 34#	1460 $\pm$ 31#
NA	46 $\pm$ 7	45 $\pm$ 13	43 $\pm$ 11	229 $\pm$ 45*	63 $\pm$ 17#	63 $\pm$ 6#

Mean  $\pm$  sem. \* P < 0.01 vs C; # P < 0.01 vs FFR

Rats from FFR group increased their weight and systolic pressure, and showed glucose intolerance and an increment in NAD(P)H oxidase activity, an important enzyme producing superoxide. These changes were reverted by the administration of garlic, suggesting an useful role of natural antioxidants in the prevention of some of the cardiovascular and metabolic alterations associated to this pathology.

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## 134.

**FREE POLYAMINE LEVELS IN SALINIZED SEEDLINGS OF *Prosopis strombulifera***

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Alterations in polyamine levels – especially putrescine (*Put*) – in salt stressed plant tissues have been reported. Endogenous polyamines in halophytes have not been previously studied; therefore this work assessed them in the halophyte, *Prosopis strombulifera*. Seedlings were grown hidroponically in Hoagland solutions with addition of NaCl (50 mmol/L) and Na<sub>2</sub>SO<sub>4</sub> (38 mmol/L) for the respective monosaline treatments and isoosmotic mixtures of both salts for bisaline treatments. Salt pulses were added every 48 h until final osmotic potentials of -1, -1.88 and -2.6 MPa. Control plants were grown in Hoagland 25%. HPLC and fluorescence spectrophotometry were used to separate and quantify free polyamines as their dansyl derivatives. *Put* content in leaves increased considerably with all salt treatments, but mainly in the bisaline one. Spermidine (*Spd*) increased with all sulphate-containing treatments, being accompanied by cadaverine (*Cad*) and 1,3 diaminopropano (*Dap*) (uncommon polyamines in plants) at the higher salinities, in coincidence with the appearance of toxic symptoms in the plants. This was not observed in NaCl treatments. Spermine (*Spm*) increased only in salt mixtures at the highest concentration. In roots, *Cad* and *Dap* were also detected in response to high salinity (-2,6 MPa, NaCl 700 mmol/L and Na<sub>2</sub>SO<sub>4</sub> 538 mmol/L). *Put* levels also increased with salinity but less than in leaves, while *Spd* and *Spm* decreased with salinity, probably due to catabolism or conjugation.

## 136.

**EFFECT OF “ENERGY DRINKS” ON RAT DIURETIC ACTIVITY**

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Caffeine, taurine, vitamin and mineral- containing drinks have been on the European and American market for about a decade. They are commonly called “energy drinks” and are widely consumed by young people. This study investigated the effect of an energy drink on diuretic activity of the rat. The method by Lipschitz *et al.* (1943) was followed. Wistar rats weighing 200-250 g were divided into two groups: experimental (n:10) and control (n:10), deprived or not of water and food for 18 h prior to the experiments. They were administered at a volume of 50 mL/Kg by intragastric route energy drink in treated rats, while control group received only saline. Animals were placed in individual metabolism cages and urine output was recorded at 60 min intervals for 6 h. The urinary volumetric excretion (UVE) was calculated. The physical-chemical and biochemical urine parameters were determined. The energy drink produced significant decreases in UVE in two different conditions studied (One-way ANOVA, P < 0.0001). Significant differences in urine analysis, such as protein, glucose, pH, density and macroscopic aspect were found. In conclusion, this energy drink modified diuretic activity. Further research is necessary to know the mechanisms of these actions.

**137. DEVELOPMENT OF A SIMPLE TEST TO ASSESS THE BIOFILM FORMATION IN RHIZOBIA**

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Symbiotic nitrogen fixation by rhizobia-legume couple is a process of ecological and agricultural importance. The study of the establishment of rhizobia inoculated in legume roots, their colonization, and nodule occupancy requires the use of adequate techniques. The objectives in this research were to visualize and to quantify the *Sinorhizobium meliloti* biofilm formation on artificial substrates, where basically the biofilms were grown on microtiter-plate over a short time. The experimental methods were based on the ability of bacteria to form biofilms on polyvinylchloride or polystyrene surfaces. When the formation of the biofilm was monitored over an 18 h period, *S. meliloti* formed a biofilm mainly at the interface between the air and the liquid medium. The biofilm formation was not significantly different between the strains Rm1021 and Rm2011, two closely related strains of *S. meliloti* that are capable of forming nitrogen-fixing nodules in *Medicago* hosts. The biofilm was greater when the bacteria were incubated in the minimal media. In rich media, bacteria grew better but failed to attach to the surface, instead forming pellets at the bottom of the well. It appears that a nutritionally limited environment increases the transition from planktonic to a sessile mode of life and the subsequent development of the biofilm. The results indicate that microtiter-plate test can provide a convenient abiotic surface for studying rhizobia biofilm.

**139. CORTICOADRENAL RESPONSE TO ENVIRONMENTAL STRESSORS IN WILD RODENT *Lagostomus maximus maximus***

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In subtropical habitats, regulation of the energy available for survival is important for the species because it constitutes the limiting factor. Over the cold season, wild animals are stressed by low temperatures, and water and dietary restrictions. Stress increases energy requirements, since it disrupts homeostatic balance and energy is required to reestablish it. During this disturbance, glucocorticoids are released and energy is mobilized to reestablish the balance. This work presents the results of the morphological and biochemical seasonal study of corticoadrenal fascicular region of the wild rodent *Lagostomus maximus maximus*. Adult male viscachas were captured, anesthetized and sacrificed. The adrenal glands were quickly removed and processed for morphometrical studies. Cholesterol was determined by colorimetry, and corticosterone by a specific radioimmunoassay. In summer, cells had lax chromatin nuclei and abundant lipid drops. Nuclear volume was  $286.4 \pm 14.72 \mu\text{m}^3$ . Cholesterol:  $47.54 \pm 17.35 \text{ mg}/100 \text{ mg}$ . Plasma corticosterone:  $1.755 \pm 0.216 \text{ ng}/\text{mL}$ . In winter, cells had scant lipid drops and nuclei with condensed chromatin. Cholesterol:  $25.10 \pm 3.81 \text{ mg}/100 \text{ mg}$ . Plasma corticosterone:  $4.650 \pm 0.369 \text{ ng}/\text{mL}$ . These results suggest that glucocorticoids participate actively in increasing availability of metabolic energy, as a part of a general mechanism of adaptation to extreme conditions of survival.

**138. INTERACTION BETWEEN CHROMAFFIN AND GLIAL CELLS IN ADRENAL MEDULLA OF VISCACHA (*Lagostomus maximus maximus*)**

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New evidence provides valuable information about the participation of glial cells in chromaffin cell catecholamine secretion. In this process, calcium ions play an important role. It has been shown that there is an intense ionic traffic between both types of cells. Also, glial cells take an active part in calcium metabolism, regulating levels of the ion and indirectly, the synthesis and release of catecholamines. This background information induced us to study glial population of *Lagostomus* adrenal medulla and its morphologic relationship with the chromaffin population. The animals were captured throughout the year, transported to the animal facilities, anesthetized and sacrificed. The adrenal gland was processed by immunohistochemistry using antiserum antiprotein S-100, a specific marker. Through the morphological and immunohistochemical study, it was found that there are glial cells in different regions of adrenal medulla, mainly in the basal zone of chromaffin cells, which constitute glomerular structures around blood capillaries. Cytoplasmic extensions of glial cells penetrate into chromaffin cells and make contact with the basal membrane of the capillary endothelium. The relationship among chromaffin cells, capillaries and glial cells suggests that they intervene actively in adrenal medulla metabolism.

**140. ORGANOCHLORINE CONTAMINATION IN *Odontesthes bonariensis* FROM FIVE WATER RESERVOIRS OF SAN LUIS, ARGENTINA**

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No information is available about organochlorine pesticide (OCPs) contamination in the fauna of the water reservoirs of the center region of San Luis. Thus, the aim of this study was to survey (OCPs) pollution in five water reservoirs of San Luis, La Florida, Esteban Agüero, Potrero de los Funes, Villa General Roca y Cruz de Piedra. We selected silverside (*O. bonariensis*) as a model, since this fish is a secondary consumer occupying a high position in the food chain and is ate by humans. OCPs were assayed by GC-ECD. Samples from all five water reservoirs exhibited OCPs detectable levels. The range of values (min-max) for each OCPs family in ng/g fat were: 1) HCH and isomers:  $\alpha$ ,  $\beta$ ,  $\gamma$  and  $\delta$ : not detected (ND) - 76.74 2) DDT and methoxichlor: ND-154.4 3) the cyclo diene group: a) chlordane: ND-96.25 b) aldrin, endrin, dieldrin and endosulfan: ND-219.26. In summary, this is the first report OCPs contamination of silversides in water reservoirs from the center region of San Luis. Supported by FONCYT, CyT UNSL to ECV.

**141. EFFECTS OF NITROGEN FERTILIZER ON *Poa ligularis* IN THE SEMI-ARID REGION**

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To quantify the effect of a nitrogen fertilizer on *Poa ligularis* dry matter production and its impact in different cattle systems in the Semi-arid Region., 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 and harvested monthly at dry matter yields. The obtained results, according to the statistical analysis ANOVA, indicate that there are significant differences among the treatments and between cuttings. The evaluated parameter (accumulated production) has evidenced that more investigation is needed for evaluating the impact in grassland forage production.

**143. LIPIDS LEVELS IN THE FIRST TRIMESTER OF PREGNANCY**

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Recent investigations have shown that cardiovascular diseases (CVD) are by no means confined to stressed and overweight middle aged men living in developed countries. Rather, CVD also threat women and children worldwide. In 2004, 159 women in their first trimester of pregnancy were interviewed in relation to CVD, in oral and voluntary form, before determination of serum cholesterol, HDL-cholesterol, LDL-cholesterol and triglycerides. Their age in years (mean  $\pm$  sb) was  $24.0 \pm 6.2$ ; range 15 to 42.

g / L	Chol	HDL-cho	LDL-cho	TG
<b>MEAN</b>	1.53	0.480	0.973	0.857
<b>SD</b>	0.21	0.047	0.236	0.380
<b>Median</b>	1.5	0.49	0.9	0.8
<b>Range</b>	1.0 – 2.8	0.35 – 0.59	0.51–1.96	0.3 – 2.6

Of pregnant women with values differing from those recommended in the report of the III Panel of Experts, there were 9 (5.7%) with cholesterol  $\geq 2.0$  g/L; 12 (7.5%) with HDL-cholesterol  $\leq 0.40$  g/L; 13 (8.2%) with LDL-cholesterol  $> 1.3$  g/L and 7 (4.4%) with triglycerides  $> 1.6$  g/L. Most women with high cholesterol (6/9 = 67%), low HDL-cholesterol (8/12 = 67%), high LDL-cholesterol (8/13 = 62%) and high triglycerides (5/7 = 71%) were below 25-year old. Unawareness of lipid status is a major problem regarding increased CVD risk. Hypercholesterolemia is a risk factor that should be controlled or, still better, prevented from an early age.

**142. SCREENING OF HUMAN BRUCELLOSIS BY SERO-DIAGNOSIS IN SAN LUIS CITY AND RURAL POPULATION**

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Brucellosis is primarily an animal disease that, under natural conditions, is transmitted to humans causing impact in public health. Between the years 2002 at 2004 seroepidemiological investigation were carried out in order to assess its prevalence. Out of 1,999 individuals studied, 772 were male (39%), and 584 from rural populations (29%). Ages: 389 were younger than 15 years (19.5%), 1,409 were between 15 and 64 years (70.5%) and 201 were older (10%). The diagnostic methods were Rose Bengal (RB) and plate agglutination test (PAT) for screening and the confirmation method was tube agglutination test (TAT). In 99% of those patients interviewed, antibodies were not detected. Of the 20 positive sera at the screening, 13 (65%) were confirmed by TAT technique. The seroprevalence was of 0.35% for people from urban areas and 1.3% for the rural population. This information should contribute, among other purposes, to make an optimal planning for prevention and control programs of this endemic disease in San Luis province.

**144. FLAVONOID ACTIVITY ON EXPERIMENTAL ARTHRITIS**

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Flavonoids are compounds of scientific and therapeutic interest. The efficacy of different flavonoids on experimental arthritis was assessed. Wistar rats (120-150 g) were divided into groups of 6 animals each. Experimental arthritis was developed through 0,1 mL Freund adjuvant administration in the tail base. Six days later, 0,1 mL 2% carrageenan was injected into the subplantar zone of left paw of each animal. Three groups received hesperidin, rutin and quercetin from day 6 to 30 and other 3 groups received from day 6 to 16 morin, genkwanin and 7-O-methylsudachitin (80 mg/kg ip). Reference groups received phenylbutazone (80 mg/kg ip) or indomethacin (6 mg/kg ip). A control group received only saline. Edema was measured by plethysmography daily until day 10 and discontinued until day 16 or 30 and was calculated as the volume difference between both hind paws. Each group was compared with control group in order to obtain the inhibition percentage. Flavonoids inhibited the inflammation between 30 to 73%, while phenylbutazone did that between 27-54% and indomethacin between 37 to 50%. Rutin and 7-O-methylsudachitin exhibited activity throughout the experiment, while the other compounds showed efficacy with interruptions and the reference drugs were only effective until day 9. Thus, flavonoids showed similar or higher antiarthritic activity, and in some cases longer lasting, than reference drugs.

**145. DIETARY ZINC DEFICIENCY-INDUCED CHANGES IN LUNG LIPID METABOLISM**

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Zinc deficiency is a widespread problem. Zn essential roles in many aspects of the metabolism (Vallee and Falchuk, 1993), includes the activity of more than 300 enzymes, the structure of many proteins, and control of genetic expression. Zn chronic deficiency modified lipid parameters, especially phospholipids and cholesterol (Gómez *et al.*, 2002). The present study was conducted to investigate the effects of Zn deficiency on the expression of lipidic enzymes such as ACC, LPL, HMG-CoA R, GPAT and SREBP<sub>2</sub> in rat lung. Wistar male rats (200 ± 20 g) were fed with AIN-93 diet (zinc-deficient, ZD) or with 30 mg Zn/ kg (Control, Co) during 2 months. Total RNA was isolated from lung by using TRIzol. 1 µg of RNA was transcribed to cDNA at 42°C using random hexamers as primers and RT-MMLV (Moloney Murine Leukemia Virus). Aliquots of 2 µL of cDNA were used in the amplifications by PCR using specific primers. Beta actin was used as an internal control. We detected a significant increase in ACC (p < 0.01), LPL (p < 0.005) and SREBP<sub>2</sub> (p < 0.01). HMG-CoA R (p < 0.005) decreased significantly, while GPAT did not change. From the results of this study, it can be concluded that Zn deficiency produces numerous alterations on lipids metabolism in lung.

**147. RAT CARDIOMYOCYTE TRANSMEMBRANE IONIC CURRENT IN HIGH K<sup>+</sup> IN WHOLE-CELL PATCH-CLAMP**

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Resting transmembrane potential (TP) is mainly K<sup>+</sup>-dependent. When intra- and extracellular K<sup>+</sup> concentrations are equal to each other, TP is reduced and varies depending on other ions present. Total ionic current (TIC) recorded under these circumstances is due to the presence of ions other than K<sup>+</sup>. Adult rat hearts were excised, cannulated and perfused with a Lagendorff-type apparatus at 38°C. In the last phase collagenase was added. The heart was cut with scissors and myocytes were harvested, recalcified and seeded on Petri dishes, which were put on the stage of an inverted microscope for microelectrode recording under whole-cell configuration (Pclamp 6, Axon Instruments). Records obtained in control conditions were compared with those in high K<sup>+</sup> (KCl 150 mmol/L). Mean ± SEM (n = 6) were 2.60 ± 0.20 MΩ (electrode resistance), 2.60 ± 0.20 GΩ (seal resistance), 8.44 ± 0.74 µm<sup>2</sup> (surface by geometric estimate) and 9.01 ± 0.08 µm<sup>2</sup> (surface by capacitance estimate). Baseline TP was -68.35 ± 1.31 mV and TIC -9.45 ± 3.37 pA. After addition of KCl, TP was -11.80 ± 7.40 mV and TIC -5.46 ± 2.52 pA. A fast inward current averaging -11.16 ± 7.94 pA was recorded with depolarization to -60 mV under control conditions, but not in high K<sup>+</sup>. This current is presumably carried by Na<sup>+</sup> ions.

**146. NEONATAL UNILATERAL URETERAL OBSTRUCTION: ROLE OF CASPASE-3 IN APOPTOSIS INDUCTION**

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Unilateral ureteral obstruction (UUO) is characterized by renal atrophy and tissue loss, which is mediated by renal apoptosis. There exist two main apoptosis cascades, activated by extrinsic or intrinsic stimuli. In the cell-external stimuli pathway, the intracellular signal transduction to the nucleus is carried out by caspases. We studied whether induced apoptosis in renal obstruction is mediated through the caspase cascade. Newborn female rats were subjected to complete left UUO (n = 8) within the first 48 hours of life. After 5 and 14 days of obstruction, their kidneys were removed. Apoptotic cells (AC) were identified with TUNEL. Quantification of AC was assessed by using an image analyzer. Hence a total area of 0.35 mm<sup>2</sup> was counted. The numbers were expressed as AC per mm<sup>2</sup> ± SEM. Caspase-3 protein expression was evaluated by Western blotting (WB) and immunohistochemistry. WB analysis demonstrated that 32kDa procaspase-3 protein was increased two fold related to control after 5 days of obstruction. Downregulation of procaspase-3 protein due to its cleavage, 1.20 fold decrease, was observed after 14 days of obstruction. Simultaneously, quantification of AC showed a significant increase of nearly five fold in cortical and medullary collecting ducts of obstructed kidney (OK) after 14 days compared to OK after 5 days. Our results suggest that by prolonging neonatal unilateral kidney obstruction, apoptosis is induced via a caspase dependent pathway.

**148. CHRONIC HYPOXIA INDUCES COEXPRESSION OF ELECTROGENIC SODIUM ABSORPTION AND CHLORIDE SECRETION IN RAT DISTAL COLON**

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In normal rat distal colon epithelium, electrogenic Na<sup>+</sup> absorption and Cl<sup>-</sup> secretion are reciprocally related, probably by a cross-talk between Cl<sup>-</sup> and Na<sup>+</sup> apical channels. Whether this is so chronic hypoxia was assessed. induces a has no amiloride-sensitive electrogenic Na<sup>+</sup> absorption unless serum aldosterone is above normal. Adult rats submitted to hypoxia (air pressure = 400 mmHg) for 10 days were killed to obtain isolated mucosa preparations. Tissues (n = 10) were mounted in an Ussing chamber modified for determination of oxygen consumption rate (QO<sub>2</sub>) and kept at 37°C. Isc and QO<sub>2</sub> were measured during basal conditions and after addition of amiloride (0.1 mmol/L) and the chloride-blocker channel diphenylamine-2-carboxylate (DPC, 0,1 mmol/L). DPC alone (n = 10) lowered both Isc (2.40 ± 0.24 µmol.h<sup>-1</sup>.cm<sup>-2</sup> vs 3.27 ± 0.33 µmol.h<sup>-1</sup>.cm<sup>-2</sup>; P < 0.0001) and QO<sub>2</sub> (2.90 ± 0.1 µmol.h<sup>-1</sup>.cm<sup>-2</sup> vs 3.25 ± 0.08 µmol.h<sup>-1</sup>.cm<sup>-2</sup>; P < 0.0001). Simultaneous addition of amiloride and DPC (n = 10) lowered both Isc and QO<sub>2</sub> (Δ Isc = -1.63 ± 0.11 µmol.h<sup>-1</sup>.cm<sup>-2</sup> and Δ QO<sub>2</sub> = -0.62 ± 0.06 µmol.h<sup>-1</sup>.cm<sup>-2</sup>; both P < 0.0001). Linear regression showed a significant correlation between reduction of Isc and of QO<sub>2</sub> (r<sup>2</sup> = 0.70; P = 0.0025). It is concluded that chronic hypoxia somehow disrupts cross-talk between apical Na<sup>+</sup> channels and Cl<sup>-</sup> channels, allowing both Na<sup>+</sup> and Cl<sup>-</sup> electrogenic transport to coexist and to contribute to Isc, jointly demanding about 20% of total QO<sub>2</sub>.

**149.**  
**DISTRIBUTION OF CERVICAL-VAGINAL PATHOGENS IN PATIENTS OF PUBLIC AND PRIVATE LABORATORIES IN CONCARÁN (SAN LUIS)**

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Cervical-vaginal samples (n = 1559) were studied with the same protocol of investigation directed to recuperate *Neisseria gonorrhoeae*, *Trichomonas vaginalis*, Gamm complex, *Candida albicans* and other fungal infections. In 834 samples (53.5%) at least one infectious agent was detected, distributed as follows: Gamm complex 395 (47.4%); *C. albicans* 212 (25.4%); mixed infections 116 (13.9%); *T. vaginalis* 99 (11.9%); non *C. albicans* ferments 10 (1.2%); *N. gonorrhoeae* 2 (0.2%). In mixed infections the associations were: *T. vaginalis*-Gamm 66 (56.9%); *C. albicans*-Gamm 16 (39.7%); *T. vaginalis*-*C. albicans* 2 (1.7%); *T. vaginalis*-*C. albicans*-Gamm 2 (1.7%). We conclude that: 1-The low level of detection of *N. gonorrhoeae* suggests that we must improve its search. 2-The presence of the Gamm complex in 509 of the positive samples (61%) reflects the necessity of routine examination of pregnant women. 3-The search of other pathogenic agents should be incorporated because, in many samples, an important inflammatory response was found while there no infectious agent was identified. 4-The high percentage of recovery of sexually transmitted agents shows the necessity of implementing a preventive programme against sexually transmitted infections.

**151.**  
**INDOMETHACIN LOWERS SHORT-CIRCUIT CURRENT AND OXYGEN CONSUMPTION IN COLON EPITHELIUM FROM CHRONICALLY HYPOXIC RATS**

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Cyclooxygenase inhibition (COI) reduces short-circuit current (Isc) in normal rat distal colon. We have previously shown that it also lowers oxygen consumption rate (QO<sub>2</sub>) and that reductions in Isc and QO<sub>2</sub> are linearly correlated. This work aimed at testing the effect of COI in colonic epithelium from animals submitted to chronic hypoxia. Adult rats were submitted to hypoxia (air pressure = 400 mmHg) for 10 days and killed. Isolated mucosa preparations (n = 15) were mounted in an Ussing chamber modified for determination of oxygen consumption rate (QO<sub>2</sub>) and kept at 37 °C. Isc and QO<sub>2</sub> were measured during basal conditions and after addition of indomethacin (30 µmol/L). COI reduced both Isc (2.26 ± 0.14 µmol.h<sup>-1</sup>.cm<sup>-2</sup> vs 3.04 ± 0.13 µmol.h<sup>-1</sup>.cm<sup>-2</sup>, P < 0.0001) and QO<sub>2</sub> (3.00 ± 0.10 µmol.h<sup>-1</sup>.cm<sup>-2</sup> vs 3.38 ± 0.13 µmol.h<sup>-1</sup>.cm<sup>-2</sup>; P < 0.0001). As in tissues from normal rats, reduction of Isc and QO<sub>2</sub> were linearly related (r<sup>2</sup> = 0.69; P = 0.0001). Indomethacin slightly lowered QO<sub>2</sub> but not Isc in the presence of chloride secretion blockers, indicating that the effect on Isc only partially accounts for the reduction in QO<sub>2</sub> induced by COI in tissues from hypoxic rats.

**150.**  
**5-EPI-ICETEXANE AFFECTS THE PROLIFERATION OF *Trypanosoma cruzi* BY MULTIPLE MOLECULAR MECHANISMS**

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*Trypanosoma cruzi* is the etiological agent of chagas' disease. In cultures, these parasites cycle between different forms of the flagellate epimastigote, and scarcely differentiate to the infective trypomastigote. Since decades, several natural compounds have been used as trypanocidal agents at the acute phase of the disease. Quinones have shown to be effective against the parasite, although their use is still restricted because a cytotoxic effect on the host cells. We previously demonstrated that an hydroxylated quinone, 5-epi-icetexane (ICTX), purified from *Salvia gilliesi*, exhibited an antiproliferative effect on cultured *T.cruzi* epimastigotes, even at very low concentrations. The present work was addressed to understand the mechanism of that antiproliferative effect. The growth of the parasites was synchronized at G1 phase by treatment with hydroxyurea (HU) for 24 hr, and ICTX was added at different time points after removal of HU. We observed that ICTX is able to arrest the cell cycle of the parasites at the time corresponding to S phase, and at lesser extent when added at G2 phase. We also evaluated if expression of particular proteins was affected by the drug, and observed no differences in the protein patterns. However, proteins in the range of 30-70 kDa exhibited higher phosphorylation in serine and tyrosine residues than those in the controls. Among these proteins, p53 appeared as a target for the action of ICTX, as shown by a Western blot analysis. We conclude that ICTX exerts an antiproliferative effect on *T.cruzi* by inducing phosphorylation of certain proteins that regulate the cell cycle of the parasites.

**152.**  
**CAPILLARY ELECTROPHORESIS METHOD FOR DISSOLUTION TEST IN A DICLOFENAC AND PARACETAMOL PHARMACEUTICAL FORMULATION**

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Dissolution test is considered one of the most important quality control tests performed on pharmaceutical dosage forms, because it can provide valuable information about biological availability of a drug as well as batch-to-batch reproducibility. The analysis of test dissolution samples is usually carried out using UV spectrophotometry and HPLC. Capillary electrophoresis (CE) has advantages over chromatographic methods in terms of rapid method development, reducing operative cost and increased simplicity. This study developed a method for the simultaneous determination of diclofenac and paracetamol in tablets after a dissolution test. Final electrophoretic conditions were: background electrolyte (BGE) sodium tetraborate 20 mmol/L, pH 9.20; applied voltage 30 kV, capillary temperature 25°C, sample injection 0.5 Psi, 5 s. The detection was performed at 212 and 255 nm. The BGE gave baseline separation with good resolution, short migration times (less than 4 min), high reproducibility and accuracy. The present method can be applied in the routine analysis in the pharmaceutical laboratory.

**153.**  
**INITIAL CHARACTERIZATION OF GLUTAMATE RECEPTORS IN MOUSE SPERM**

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Glutamic acid (GA) is well-known excitatory neurotransmitters. Recently was involved in triggering the Acrosomal Reaction in mouse and human sperm. More over the signal pathway involved in this novel signal transduction system was characterized. But the receptor/s involved in sperm are not characterized to this moment. In this way, two receptors (Glu-R), Ionotropics (AMPA/Kainico, NMDA) and Metabotropics, had been described at the CNS. Using western blot and immune cytochemistry methods we try to identify Glu-R in mouse sperm. The following Antibodies were used: Anti metabotropic - mGluR2/3 (mGluR2 and mGluR3) and mGluR5/1 (mGluR5 and mGluR1á) - and NMDA - pan-NMDA (NR1, for splice variants) and NMDAR-2C (NMDAR-2A and NMDAR-2B). Homogenates from cerebral cortex (CC), hipocampus (Hipo) (as controls) and mouse sperm were used as Glu-R source. Two proteins bands in sperm homogenates were detected with anti mGlu-R NMDAR2C, that were coincident with CC and Hipo; and two bands with anti mGluR 5/1 also expressed in CC and Hipo. Subtype NMDAR 1 was detected in CC but not in sperm. Immuno cytochemistry techniques permit to locate NMDAR-2C at the dorsal edge of sperm head and mGlu-R over the acrosomal region. More effort must be done to identify the Glu-R but subtypes of them were identifying here by two independent methods. Results presented here support the idea that GA could trigger the acrosomal reaction in mouse sperm.

**155.**  
**Tanacetum balsamita L. ESSENTIAL OIL AS A POTENTIAL GRAIN PROTECTIVE AGAINST *Tribolium castaneum* HERBST AND *Ulmoides dermestoides* FAIRM**

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The effects of essential oils extracted from aromatics plants on insects have been widely investigated. They possess a multifaceted pest control properties, which include toxic, repellent, ovicidal and antifeedant effects. As part of a program aimed to study the effects of essential oils against pest insects, we have investigated the bioactivity of *T. balsamita* essential oil. We have determined contact toxicity, repellency and alterations in the nutritional indices on *T. castaneum* and *U. dermestoides*. *T. balsamita* essential oil presented certain activity on the model insects used here. In the toxicity test, *U. dermestoides* was more sensible than *T. castaneum*, presenting the lowest LD<sub>50</sub> values. Both insects were highly repelled by the essential oil. The results suggest that *T. balsamita* essential oil could be considered a potential alternative to control pest insects of stored products.

**154.**  
**ANTI-INFLAMMATORY ACTIVITY OF *Cyclolepis genistoides* IN MICE**

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The plant genus *Cyclolepis* (*Asteraceae*, *Multisieae*) is endemic in Argentina, *Cyclolepis genistoides* being the only species that grows in the Northeastern and Southern salty areas of this country. It is used in popular medicine in infusions, mainly for its diuretic and antirheumatic actions and also for renal and hepatic colics. In previous assays we showed that a 10% *C. genistoides* infusion has diuretic activity in rats. In this study we tested the anti-inflammatory properties of two compounds isolated from this plant through phytochemical study. The method for testing acute inflammation was the carrageenan-induced paw edema (Susgishita *et al.*) in Rockland mice. The isolated compounds from *C. genistoides*, among others, were oleanolic acid and deacylcynaropicrin determined by <sup>1</sup>H <sup>13</sup>C NMR studies. Phenylbutazone as the reference drug (80mg/kg) and the compounds (75 mg/kg) were all administered i.p 1 h before the carrageenan 3.5% injection. The volume of paw edema was measured by plethysmometry. Statistical analysis was performed with ANOVA and Dunnett's test, at significant level of P < 0.05. Compared with controls, oleanolic acid inhibited edema by 39% and deacylcynaropicrin by 32%. These effects are accounted for by the inhibition of inflammation mediators elicited by carrageenan as others authors reported for flavonoids and triterpenes.

**156.**  
**EFFECT OF DEHYDROLEUCODINE ON THE FERTILIZING ABILITY OF MOUSE CAPACITATED SPERMATOZOA**

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It has been shown that sesquiterpene lactones act *in vitro* to inhibit a group of steroidogenic enzymes, the aromatases. These enzymes transform testosterone into estradiol, and are localized in Leydig cells and in cells of the seminiferous epithelium. We tested the effect of dehydroleucodine (DhL), a sesquiterpene lactone isolated from matico, a medicinal herb widely distributed in the Cuyo area, on the male tract of adult male Balb C strain mice. Mice were treated with 0.7 mg of DhL/kg body weight per day for 30 days. After the treatment, spermatozoa from the cauda epididymis were obtained and capacitated with HAM-F10 medium supplemented with 25 mg/ml of serum albumin and 25 mM of bicarbonate; incubated at 37 °C in 5% CO<sub>2</sub> atmosphere for 60 minutes. Oocytes were obtained from female adult hamsters in estrus. The zona pellucida of the oocytes was removed with 1% hyaluronidase, and were incubated with capacitated spermatozoa at 37°C in 5% CO<sub>2</sub> atmosphere for 30 minutes and fixed with 5% glutaraldehyde. After analysis by inverted microscope, the control group showed 2.6 ± 0.6 spermatozoa per oocyte; and the treated group showed 7.2 ± 2.7 spermatozoa per oocyte. From these results we conclude that DhL is increasing the affinity of capacitated spermatozoa for zona pellucida-free oocytes.

**157.**  
**EVALUATION OF HERBICIDE PHYTOXICITY IN SORGHUM, PUMPKIN AND ALFALFA**

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The herbicide waste previously used can affect the following cultivation. The aim of this paper was to evaluate the phytotoxic effect of four herbicides on sorghum, pumpkin and alfalfa cultivation. The sandy soil was pulverized with 13% humidity with commercial dose recommended by the Argentinean phytosanitary's guide: atrazine, 2,4-D, clorimuron, presides and control. A randomized block design was used with three repetitions. Plastic trays were used with 180 grams of soil in field capacity. They were incubated in a germination camera at 30:20°C and 8:16 (light-darkness). Comparison of means was performed with Duncan's Multiple Ranges test with GLM of SAS. Phytotoxicity was evaluated as the difference with the abnormal and dead seedling control. The phytotoxicity produced in sorghum was from 15 to 35%, in pumpkin from 39 to 95% and in alfalfa from 29 to 44%.

**159.**  
**PRODUCTION AND QUALITY OF THE *Tetrachne dregei* SEEDS UNDER NITROGEN FERTILIZER CONDITIONS**

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*Tetrachne dregei* is a subtropical species, a component of natural range in South Africa. It is a perennial summer grass, considered promissory for the cattle bovine. The aims of these paper was to determine the quality and production seed with four nitrogen fertilizer: 50, 100, 150 and 200 kg/ha. A randomized block design was used with three repetitions. The spikelets yielded per plants, the cariopsis percentage per spikelet, the cariopsis yielded per plant and germination percentage were determined. The seeds were incubated in plastic trays of 10 x 14 cm with germination paper in cultivation camera at 30:20°C and 8:16 (light darkness). The test of Duncan's multiple range ( $p \leq 0.05$ ), from the GLM of SAS was runned on the results which show a statistically significant among the treatments. The range of cariopsis yields were obtained from 2.9 to 8.4 grams per plant with germination percentage of 40 to 80.

**158.**  
**GROWTH AND DEVELOPMENT OF SEED OF *Panicum coloratum* KLEIN VERDE**

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*Panicum coloratum* Klein Verde. is a perennial summer grass, considered promissory for the cattle bovine systems of semi-arid region. The aim of this work was to determine the maximum moments for seed quantity and quality. The cluster sampling was carried out in each plant every 3 and 4 days during 70 days starting from 06/12/2002. The weight of the seed per cluster (WSC), the weight of 1000 seeds (W1000S) and germination percentage (GP) were determined. Design of blocks at random with three repetitions with measures repeated in time. The seed were incubated in plastic trays of 10 x 14 cm, with germination paper in cultivation camera at 30:20°C and 8:16 (light- darkness). Comparison of means was performed with the test of Duncan's Multiple Ranges with GLM of SAS. Significant differences were detected at the 17 and 52 days with the maximum values: W1000S 1 g, PG 90 and WSC 300 mg.

**160.**  
**MORPHOLOGICAL DESCRIPTION OF PIGS WITH CHROMOSOMAL ALTERATIONS**

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Eight cases of swine intersexes found in farms near Río Cuarto were selected for description. Six of them were females with hernia inguinalis. Lymphocyte XX/XY chromosomal chimaerism, frequently found in this phenotype, was registered in four. Of the two non chimaeric females one had bilateral hernia. This one, and three other females were morphologically studied. The one with bilateral hernia had one abdominal testis and a mature ovary on the left side of the body, and inside the right side of the hernia, an ovotestis, being therefore, hermaphrodite, this agree with the norm of presenting the XX cell line only. Those having unilateral herniae had structures of the two sexes such as vaginal vestibule, vulvar orifice, uterine horns, vaginal rings, inguinal canal, cremaster muscle, and vestiges of Muller and Wolff ducts, agreeing with the porcine freemartin pattern, although one of them was not chimaeric. The remaining two animals were phenotypically males. Only one was chimaeric. Both had two testes but without penis and presented small vulvae with well developed clitoris. After necropsy of one of them it was corroborated nonfunctionality of testes and full size vagina. Ovaries were not recognized. The eight cases of intersexuality studied could be thus classified: five porcine freemartins, two pseudohermaphrodite males, and one hermaphrodite.

161.

**HEMOLYTIC ACTIVITY OF *Listeria monocytogenes* AT DIFFERENT PH AND TEMPERATURE**

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Listeriolysin (LLO), produced by *L. monocytogenes* (*Lm*) strains, is a 58 kDa hemolysin. LLO is an essential virulence factor, necessary for the intracellular survival of these bacteria. The intracellular environment might impose stress conditions similar to heat shock on invading bacteria. This work studied the effect of heat shock and pH on LLO hemolytic activity (HA). *Lm* CLIP 74902 strain was grown in CASO (casein peptone-soymeal peptone) broth at pH 5.5 and at pH 7.2, and incubated at 37 °C for 6 h with shaking. Cultures were centrifuged and pellets resuspended in 100 mL CASO broth supplemented with 0.5% yeast extract at pH 5.5 and 7.2, respectively. Both were preheated at 48°C and 37°C, and held for 1 h. Cells were centrifuged, resuspended in 5 mL EDTA, and sonicated for releasing total intracellular proteins (IP). The supernatant obtained was mixed with chilled acetone to precipitate extracellular proteins (EP). A quantitative plate hemolysis assay was performed with 50 µL of protein sample and 100 µL of freshly prepared 3% HRBC. Plates were incubated for 6 h. The HA was determined by reading absorbancies at 545 nm. Assays were triplicated. IP-HA at 48°C and pH 7.2 was higher than at 5.5, while at 37°C they were similar at both pH. EP-HA at 48°C and pH 5.5 was higher than at 7.2, but similar in both pH at 37°C. Previous reports have described that pH 7.2 at 48°C had higher intracellular HA. Most likely, excreted LLO has accumulated in the cells during heat-shock.

163.

**CHARACTERIZATION OF DNA PRESENT IN PUTATIVE SYMBIOTIC CORPUSCLES WITHIN THE MIDGUT GLAND OF THE AMPULLARIID SNAIL *Pomacea canaliculata***

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Adult individuals of *P. canaliculata* bear two types of pigmented corpuscles (namely, C and K corpuscles) in their midgut glands. The first ones are round, brownish/greenish bodies delimited by a thick wall (about 14 µm width), while the second are club-shaped, dark brown, multilamellar bodies (about 35 µm length, 14 µm width). On the basis of their morphology and DNA content we have proposed that they may be morphs of a cyanobacterial symbiont that accounts for about 10% of the glandular mass. Mean corpuscular DNA content was  $57 \pm 9$  fg (N=11) in C corpuscles. The DNA/protein ratio was 7-fold higher in C than in K corpuscles (Student's test,  $P < 0.05$ ) and was not different to that of *Escherichia coli* and *Synechococcus sp.* cells (ANOVA I, Newman-Keuls test). A 1500 bp sequence corresponding to the gene encoding for bacterial 16S rRNA was amplified (PCR) using DNA extracted from isolated C corpuscles as template. The amplified DNA was cloned and about 500 bp on each of both ends were sequenced. Seven different sequences were found: one of them was akin to the cyanobacterial genus *Microcystis* (morphologically similar to C corpuscles), while the other were related to cocci and rod-like bacteria (presumably normal inhabitants of the snail's gut, whose lumen communicates freely with the major midgut gland's ducts). Hybridization studies are being conducted for further testing the apparently symbiotic nature of these corpuscles.

162.

**ANDROGENIC GANGLIONIC INFLUENCE ON OVARIAN PHYSIOLOGY AT THE END OF PREGNANCY IN RATS**

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Previously, we have shown that androstenedione ( $A_2$ ) in coeliac ganglion modifies ovarian progesterone (P) release from the coeliac ganglion-superior ovarian nerve-ovary (CG-SON-O) system of rats at the end of pregnancy. In the present work we studied in that system whether the  $A_2$  effect is mediated by androgen receptors of CG and its relation with nitric oxide (NO) release from the ovary. The system CG-SON-O was incubated in Krebs Ringer-glucose-albumin (0.1mg/mL) at 37°C, keeping CG and O connected by the SON, in separate cuvettes. The CG was stimulated with  $A_2$   $10^{-6}$  mol/L ( $A_2$ )<sub>g</sub>, the androgen receptor antagonist flutamide  $10^{-4}$  mol/L (Flu)<sub>g</sub> and both ( $A_2$ +Flu)<sub>g</sub>, to measure P at 30, 60 and 120 min (by RIA) and NO (Griess reactive). Controls were not stimulated. Student's *t* test and ANOVA were used, at  $p < 0.05$  level. Results were: Control P (ng/mg ovary; mean  $\pm$  SEM) at 30 min,  $0.032 \pm 0.003$ ; at 60 min,  $0.042 \pm 0.005$ ; at 120 min,  $0.039 \pm 0.008$ . P release was increased at all times compared with control with ( $A_2$ )<sub>g</sub> and (Flu)<sub>g</sub> ( $p < 0.01$  and  $p < 0.05$ , respectively). In addition, the ( $A_2$ )<sub>g</sub> effect on P release was stronger than those of (Flu)<sub>g</sub> and ( $A_2$ +Flu)<sub>g</sub> ( $p < 0.05$ ). Control NO release (nmol/mg ovary; mean  $\pm$  SEM) at 30 min,  $2.54 \pm 0.77$ ; at 60 min,  $2.00 \pm 0.50$ ; at 120 min  $1.98 \pm 0.50$ . ( $A_2$ )<sub>g</sub> increased NO release compared with control, (Flu)<sub>g</sub> and ( $A_2$ +Flu)<sub>g</sub> ( $p < 0.01$ ). The results suggest that CG androgen receptors participate in the ovarian physiologic response.

164.

**CHOLINERGIC STIMULATION IN SUPERIOR MESENTERIC GANGLION ON RELEASE OF OVARIAN STEROIDS IN PROESTRUS IN THE RAT**

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Besides the classical neuroendocrine system hypothalamus-hypophysis-gonads, there is currently evidence for the existence of a direct neural control between gonads and central nervous system. Knowing that the autonomic nervous system stimulates ovarian steroid synthesis, the present work assessed whether the presence of acetylcholine in the mesenteric ganglion cuvette, in an integrated *in vitro* system **MG-OVP-O**, modifies ovarian steroids release in proestrus (PE). Experiments were carried out in left and right system. Six adult virgin Holtzman rats for group and buffer Krebs-Ringer pH 7.4 in metabolic bath were used. Androstenedione ( $A_2$ ), estradiol ( $E_2$ ) and progesterone (P) were determined by RIA. Student's *t* tests were used for analysis, at  $p < 0.05$  level.  $A_2$  significantly increased for the whole time tested  $p < 0.01$ , and  $E_2$  only significantly increased its release at the latter times, 60 ( $p < 0.01$ ) and 120 min ( $p < 0.001$ ), while no significant changes were found in P release. These results show that neural stimulation is important in ovarian physiology, which depends on physiologic status, making it evident that neural function via OVP may be directly involved in regulation of steroid secretion. This shows a peripheral neural regulatory link between sympathetic postganglionic efferents and ovary via OVP.

**165.**  
**HYGIENIC AND SANITARY QUALITY OF "CHORIZOS" (SAUSAGES) IN SAN LUIS**

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Multiple factors contribute to microbial contamination of "chorizos" (sausages). The hygienic and sanitary quality of 75 samples of chorizos stored at  $-18^{\circ}\text{C}$  for 28 days was studied. Analyses were performed at days 0, 7, 14, 21 and 28. Samples of 25 g each were processed for: i) total mesophilic aerobe (TMA) counts on PCA, and ii) MPN of total (CT) and fecal (CF) coliforms by the multiple-tube fermentation technique. *Escherichia coli* was identified by growth on EMB agar and biochemical tests. Other samples were enriched by standardized techniques for isolation of: iii) *Salmonella*, iv) *Pseudomonas aeruginosa*, v) *Yersinia enterocolitica* and vi) *E. coli* O157:H7. Decreases of TMA and CT populations ( $p < 0.05$ ) were observed while CF (*E. coli* non O157:H7) remained invariable along storage. Seven *Salmonella anatum*, one *Salmonella enteritidis*, eleven *P. aeruginosa* and three *Y. enterocolitica* B1A O:7,8-8-8,19 strains were isolated. No *E. coli* O157:H7 strain was recovered. Freezing contributed to the control of bacterial proliferation but it was ineffective in the total elimination of microorganisms. While *P. aeruginosa* is a spoilage microorganism, presence of coliforms and *E. coli* was related to the deficient quality of these chorizos. *S. anatum* and *S. enteritidis* have been linked to foodborne outbreaks in other regions. *Y. enterocolitica* B1A strains might cause gastroenteritis in humans despite the absence of recognised virulence determinants.

**167.**  
**ROOTING IN VITRO IN *Prosopis caldenia* USING *Agrobacterium rhizogenes***

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During the last 35 years the demand for high quality hard wood has increased, making the wood of the *Prosopis caldenia* Burk one of the potential products of the arid lands of lives unitary price. The objective of the present work was to evaluate the interactions between *Prosopis caldenia* and *Agrobacterium rhizogenes* to induce and to improve the formation of roots *in vitro*. *Agrobacterium rhizogenes* was inoculated in nodal sections, cultivated in presence and absence of  $\alpha$ -naphthalenacetic acid and indole-3-butyric acid. It was used the complete Murashige and Skoog medium, suplementado with 2% sucrose, ascorbic acid (2 mg/L), gibberellic acid (1 mg/L). The regulators of growth used in different concentrations. The number of roots average for nodal sections was of three and the percentage of rooting was 90%, when the segments were inoculated with *Agrobacterium rhizogenes* combined with indole-3-butyric acid (3 mg/L). The explants were cultivated to a temperature of  $25 \pm 2^{\circ}\text{C}$  and 16 hs light / 8 h darkness. It is hoped to achieve to high survival rates of the plantlets during acclimatization.

**166.**  
**MICROPROPAGATION OF *Acacia praecox* AND *Acacia aroma***

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*Acacia praecox* and *Acacia aroma* are species with beautiful flowers, which confer an important ornamental potential. Micropropagation of this species from nodal segments containing two axillary buds was investigated. Shoot generation from explants of eight-month-old plants was readily achieved in Murashige and Skoog medium plus 30 g/L sucrose and supplemented with growth regulators. Further multiplication and elongation of regenerated shoots were obtained by subculture in a fresh medium of similar composition. The experimental design in all experiments was fully randomized. Data were subjected to analysis of variance and differences between means were tested using Tuckey test. Percentages were analyzed by arcsine test. The best result was obtained with indole-3-butyric acid (0,1 g/L), N<sup>6</sup>-benzylaminopurine (1 g/L) and gibberellic acid (0.1 g/L). The plants propagated by tissue culture did not show any visual morphological abnormality and satisfactory regeneration of abundant plants can be obtained within a short period.

**168.**  
**HUMAN ATRIUM CARDIOMYOCYTE ISOLATION FOR PATCH-CLAMP RECORDING**

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Electrophysiological studies in human myocardial cells are hindered by difficulties in isolating viable cells. A protocol kindly provided by Dr. Anthony J. Workman (Glasgow University, U.K.) for human cardiomyocyte isolation was tested. With informed consent, samples of atrial tissue routinely discarded during cardiac surgery were obtained and transported in tissue storing solution (TSS) at  $0^{\circ}\text{C}$ . On arrival to the laboratory, weight and dimensions were measured. Tissue was cut into pieces in TSS at  $37^{\circ}\text{C}$ , filtered, washed and put in  $\text{O}_2$ -saturated solution with nitrilotriacetic acid. After 3 min, pieces were incubated in  $\text{O}_2$ -saturated solution with protease XXIV (4 U/mL) for 45 min, and then in collagenase (1.35 mg/mL) shaking for 15 min at ambient  $\text{PO}_2$ . The latter step was repeated twice for 15 and 20 min. Afterwards the material was centrifuged (2 min, 500 rpm). The pellet was extracted and resuspended in BK solution, centrifuged again (2 min, 600 rpm), resuspended in low calcium. Cells were seeded in Petri dishes with solution containing 1 mmol/L  $\text{Ca}^{2+}$ . Results from 10 experiments showed a good yield of viable cells and in pilot experiments records were obtained from some of them. This method will be applied to a comparative study of human hearts with sinusal rhythm and with atrial fibrillation.

**169. SCIENTIFIC RESEARCH: EMULATING A SCIENTIFIC WORK IN 9<sup>TH</sup> LEVEL OF EGB 3 TO MOTIVATE THE STUDY OF SCIENCES**

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A scientific research work emulating those conducted at the National University of San Luis was carried out through the interdisciplinary work of the areas of Chemistry, Physics, Biology, Language, Physical Education and Tutorials. The purpose of this experience was to stimulate the study of sciences in students of 9<sup>th</sup> level of EGB 3 of a university dependant school. The research started in the classroom with the bibliographic search related to the future experiments. The experimental work consisted in the study of the water and soils of Nogolí, a town in San Luis, where the students went camping. Techniques of sample collection, pH measurements, temperature measurements, etc. were carried out using different devices such as digital pH-meters and pH specifying tapes. Assays such as dissolved oxygen, carbonates, nitrates, etc. to specify the waters and soils quality were also done in the school laboratory. The final work was exposed in the educational institution as a poster similar to those presented in scientific congresses. The results demonstrated that this experimental activity aroused great interest in the students and that the emulation of a scientific research work allowed them to learn about the researcher's job, knocking down the barriers between popular beliefs and the reality of sciences.

**171. CHARACTERIZATION OF ENTEROPATHOGENIC AND ENTEROAGGREGATIVE *E.coli* ISOLATED FROM Sporadic Cases of Diarrhea in Mendoza**

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There are six categories of *E.coli* that cause diarrhea; between them, EPEC (Enteropathogenic *E.coli*) and EAEC (Enteraggregative *E.coli*) are important pathogens in children less than 2 years old. Clinical laboratories diagnostics *E.coli* only by biochemical and serological tests. However, EPEC and EAEC possesses specific virulence markers, for their identification. 100 strains of *E.coli* isolated from children with acute diarrhea, serogrouping as EPEC, were studied by PCR for AAF/I, aggR, EAST1 genes (markers of EAEC), bfp and eae genes (specifics for EPEC). 36% of the previously EPEC strains, carried a diarrhea related gene. 86% of them were EPEC, 10% EAEC and 7% presented only the EAST1 gene. According to this findings, specific detection methods are recommended in order to the identification of this pathogenic agents.

**170. ROLE OF PROSTAGLANDINS IN GASTROPROTECTIVE ACTIVITY OF *Artemisia douglasiana***

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*Artemisia douglasiana* Besser, commonly known as "matico", is used in folk medicine as a cytoprotective agent against peptic ulcer. This work evaluated the role of prostaglandins (PG) in the gastroprotection induced by *A. douglasiana*. Additionally, we investigated the gastroprotective activity of *A. douglasiana* on chronic ulcers. Induction of chronic gastric lesions was performed according to the methods of Takagi *et al.* (1969) and Albelda *et al.* (1973) employing acetic acid as ulcerogenic agent. A solution of acetic acid was injected below the gastric serosa of the rats. Previously, the animals were administered orally with *A. douglasiana* 10% infusion during 3 days. On the 7<sup>th</sup> day after acetic acid injection, animals were killed. The ulceration grade was determined by area (mm<sup>2</sup>). Administration of *A. douglasiana* decreased the ulceration size (Student's *t*-test:  $p < 0.001$  vs. control). The experiment for the PG study was performed according to the method of Robert *et al.* (1979). Absolute ethanol was employed as the necrotizing agent. Indomethacin was given as PG synthesis inhibitor (10 mg/kg, sc). Indomethacin pretreatment resulted in a significant reduction of the cytoprotective action of *A. douglasiana* (ANOVA and Tukey-Kramer post-test;  $p < 0.001$ ). These results support participation of endogenous PG in *A. douglasiana* protection against ethanol damage.

**172. SUBCLINICAL HYPOTHYROIDISM IN A UNIVERSITY STUDENT POPULATION: TSH LEVELS AND ANSWER TIME IN AN INTELLECTUAL TASK**

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Hypothyroidism has an incidence between 5.0 and 13.2% in Latin America, according to the studied population. In subclinical hypothyroidism (SH), thyrotropin (TSH) levels are increased while triiodothyronine (T3) and thyroxine (T4) levels are normal. This work assessed the prevalence of SH in university students and the performance in the Raven's Progressive Matrices test (RPM) of people with increased TSH levels (SH). In 59 voluntary students (17- to 31-year old; 45 females) TSH, T3 and T4 were determined by RIA, and heart frequency, arterial pressure and the RPM were recorded. Statistical analysis was performed with Student's *t* test. Sixteen students (27.12%) had values of TSH between 4 and 10  $\mu$ UI/mL but T3 and T4 levels within the normal range (SH group). TSH levels were 0.30 and 3.99  $\mu$ UI/mL in the euthyroid group. The SH group had lower levels of T4 and longer RPM answer times compared with euthyroids ( $p < 0.0001$  and 0.047 respectively). T3 levels, age, heart frequency, arterial pressure and RPM score were within normal range in SH and euthyroid group, and no difference was detected in any of those variables. The results suggest that the prevalence of SH is high in this population and that values of increased TSH levels (SH) are associated with delayed answers in intellectual tasks as the RPM.

**173.**  
**SPERM MODIFICATIONS IN *Galea mustelloides* DURING EPIDIDYMAL TRANSIT AND GONADAL ANNUAL CYCLE**

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In different mammalian species, during epididymal maturation, sperm support several changes. But more conspicuous changes could verify in mammals with stational rhythms. To characterize sperm changes in *Galea's* sperm we processed epididymal sperm and epididymal tissues for electron microscopy (transmissio: TEM, and scanning: SEM) alone or combined with cytochemical reactions for acid phosphatase, polysaccharides (Ps), adenylate cyclase (AC) and cholesterol (Cho). In spring-summer, SEM and TEM studies show sperm in a close contact with the epididymal epithelium - especially with stereocilium at the epididymal cauda region. Ps – probably glycoprotein – and AC were located at the acrosomal region of sperm head (ARSH). Digitonine – an ultrastructural Cho marker - was also located at the ARSH but vary from scarcely to abundant during the epididymal maturation. Accompanying the changes, head was also modified from flat to “spoon” like aspect. On the other hand, the regressive period show a progressive destruction of gametes with increment in acid phosphatase activity at the epididymal lumen. We suggest that epididymis function as the place for: sperm maturation -indicated by sperm changes- and destruction, as a conservative answer of this specie.

**175.**  
**CHOLESTEROL AND URIC ACID NORMAL VALUES DETERMINATION IN JUAN DOMINGO PERÓN POLICLINIC PATIENTS**

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Normal laboratory values accepted as standard have been determined starting from studies carried out in racial, cultural and environmentally different populations. Therefore each laboratory should manage its own population reference values. The aim of this work was determine cholesterol and uric acid laboratory standard values from Juan Domingo Perón Policlínica patients. Observational, transverse and descriptive study was realized in four men and women groups: 0-20, 21-40, 41-60 and >60 years. A RA 1000 autoanalyzer and Bayer reactives were used. Cholesterol mean values were in mg%: 144.2±34.3, 171.6±48.6, 194.1±52.5, 188.2±38.3 for men and 162.4±44.3, 181.9±53.1, 194.7 ±44.2, 201.5±44.8 for women. Uric acid mean values were in mg%: 3.2±1.4, 4.3±1.8, 5.4±1.5, 5.2±1.8 for men and 2.9±1.3, 3.2±1.2, 3.7±1.2, 4.1±1.5 for women. Population cholesterol values were within no risk cardiovascular disease rank. Uric acid values were below standard population levels with normal protein intake.

**174.**  
**THE SOLANACEAE FAMILY IN THE PROVINCE OF SAN LUIS (ARGENTINA)**

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The Solanaceae family comprises 33 genera and 328 species in Argentina. The aim of this study is to contribute to the identification of the adventitious species growing in San Luis. ZULOAGA (1999) cited 25 species, grouped into 13 genera, for San Luis. In the herbarium of the “Facultad de Ingeniería y Ciencias Económico-Sociales” of the Universidad Nacional de San Luis (VMA), there appear 25 species, grouped into 9 genera. In the herbarium (VMSL) of the INTA San Luis, 33 species, grouped into 13 genera, have been registered.

To date, the conducted studies show that 39 species, grouped into 13 genera, have been recorded for San Luis: *Capsicum baccatum*, *Cestrum parqui*, *Datura ferox*, *Grabowskia duplicata*, *Jaborosa microcarpum*, *Leptoglossis linifolia*, *Lycium chilense*, *L. elongatum*, *L. ciliatum*, *L. gilliesianum*, *L. tenuispinosum*, *Nicotiana plum-baginifolia*, *N. nodiflora*, *Nierembergia aristata*, *N. linariaefolia*, *Petunia axillaris*, *P. parviflora*, *Physalis viscosa*, *P. mendocina*, *Salpicroa organifolia*, *Solanum eleagnifolium*, *S. chacoense*, *S. stuckertii*, *S. hieronymii*, *S. hastatlobum*, *S. sisymbriifolium*, *S. triflorum*, *S. nitidivaccatum*, *S. atriplicifolium*, *S. pygmaeum*, *S. sublobatum*, *S. juvenale*, *S. incisum*.

**176.**  
**PHARMACOBOTANICAL CHARACTERIZATION OF *CLEMATIS MONTEVIDENSIS* VAR. *MONTEVIDENSIS* DRUG (RANUNCULACEAE)**

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The plant so-called “barba de viejo”, “cabello de ángel”, “bejuco” or “loconte” (*Clematis montevidensis* Spreng. var. *montevidensis*) is a rhizomatous vine up to 3-4 m high, widely distributed in Argentinian central-western region, where its aerial parts are used in folk medicine because their rubefacient to vesicant properties. Recent tests have demonstrated that the drug acts as a strong irritant as well as a moderate diuretic. Their morphological, anatomical, and micrographical characters were studied with the aim to give an adequate definition of the drug and to contribute to their effective quality control. The leaves are glabrescent, bijugous with 3-5 asymmetric leaflets and haptotropic petiole and petiolules. The dorsiventral mesophyll shows cells provided with druses and fatty drops. The stomata are anisocytic and the pubescence is scarcely distributed, with claviform glandular and falcated non glandular trichomes. The stems are glabrescent, terete to striate/subcostate and with an eustele. The style is very accrescent (up to 10 cm long), feathery at maturity. The quantitative micrographic parameters are: 11.16 ± 1.52 stomata.mm<sup>-2</sup> from lower leaf-surface; 1 ± 0.05 stomata.mm<sup>-2</sup> from upper leaf-surface; stomatal ratio lower/upper leaf-surface 11.16; stomatal index 2.44-2.56 (upper leaf-surface) and 16.39-22.22 (lower leaf-surface); palisade ratio 4.57 ± 1.01; 7.91 ± 2.16 vein-termination.mm<sup>-2</sup> and 4.33 ± 1.37 vein-islets.mm<sup>-2</sup>.

177.

**MELISSA OFFICINALIS VS. NEPETA CATARIA (LAMIAC.) FROM HERBAL MEDICINES IN CUYO, ARGENTINA**

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During routine pharmacobotanic quality control carried out on samples of herbal medicines labelled "Melisa" marketed in Cuyo, it has been detected the adulteration (or partial substitution) of the official drug *Melissa officinalis* L. (1) (FNA IV) with another aromatic Lamiaceae, *Nepeta cataria* L. (2). There have been pointed out pharmacobotanical and micrographics differential characters to warn about incidental effects because of their diverse chemical composition and not at all concordant applications (1). "melisa", "toronjil" or "lemon balm" is cultivated or adventive in Cuyo; it possess aldehydes, terpenes, alcohols, tannins, phenolic acids, flavonoids; their leaves and flowered summits are used as carminative, digestive and sedative; their essential oil possess antimicrobial (on viruses, bacteria & fungi) and spasmolytic properties. (2), "menta de los gatos", "nepeta", "nébeda", "catnip" is an adventive rich in essential oils (*carvacrol*, *thymol*), *nepetalactone* and *nepetalic acid*; leaves and summits are used because of their digestive, sedative, antispasmodic and pectoral effects; popularly is mistakeable with (1) because of their appearance, but the two entities can be differentiated by some characters as follows:

Character	<i>M. officinalis</i>	<i>N. cataria</i>
Prevailing scents	pleasant (citronella)	strong (camphorated)
Inflorescence	axillar	apical
Indument	shortly pubescent	villosus
Palisade ratio	2.75-4.5	8.25-13.5
Vein-termination.mm <sup>2</sup>	3.1±1.27	1.57±1.06

178.

**STUDY OF THE CI-MPR IN LIVER OF NEWBORN AND ADULT RATS.**

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The cation-independent mannose-6-phosphate receptor (CI-MPR) has been widely studied since it can recognize several types of ligands. A body of evidence demonstrated that CI-MPR can also exist as homodimer in cell membranes, although it is still controversial if this feature is related to its binding capability. We previously demonstrated that CI-MPR expression in rat liver decreases progressively from fetuses to adults. However, the K<sub>d</sub> values indicated that the affinity for mannose-6-phosphate bearing ligands is higher at late stages of development. Since that, we wondered if this fact is related to the dimer/monomer (D/M) ratio in the membranes at different ages. In this study we explored this hypothesis by stabilizing the pre-existing dimers in the membranes at 4°C, using the irreversible cross-linker Disuccinimidyl suberate, in newborn and adult rats. The membrane proteins were then solubilized with TritonX-100 and loaded to a phosphomannan affinity matrix. The CI-MPR bound to the column were eluted with 5 mM of mannose-6-phosphate, and analyzed by SDS-PAGE. We observed that dimers and monomers were bound to the column and the ratio D/M was similar at the ages studied. We also confirmed that dimeric forms are stabilized by disulfide bonds. Interestingly, we found two bands around 300 kDa (monomeric) in both ages, which are stabilized by the cross-linker, indicating that CI-MPR may interact with other small membrane proteins (judged by the vicinity of the bands). We concluded that the difference in CI-MPR kDa values between adult and newborn rats is not due to the D/M ratio, but possibly to the presence of neighbor proteins in the membranes.

<b>A</b>						
Abdón A	1		Borkowski EJ	86	Corchs J	16
Abud M	2, 3		Bosco H	72	Correa S	28
Acerbo M	57		Bozzini JP	25, 42, 43	Cortiñas TI	10
Acosta J	4		Bragagnolo L	58	Crenna M	41
Aguilar C	12		Brigada AM	26	Crespo EM	106
Aguilar CF	61, 76		Bruno M	15	Crocco M	42, 43
Aguilera C	54		Burgos MH	150, 173	Cruz F	26
Aguilera Merlo C	5		Buxton N	33	Cruzado M	131, 133, 135
Ahumanda F	18				Cuitiño MJ	171
Aiassa J	23		<b>C</b>			
Alberio RH	42, 43		Caballero PA	48		
Aliendro O	37		Cabrera R	33, 35	<b>D</b>	
Aller J	43		Cabrillana ME	107	Damiani MT	92, 116
Aller JF	42		Calderón C	49	Daquino EB	9, 44, 142, 143
Alvarez E	124, 126		Calderoni AM	27	Davicino RC	45, 46, 47
Alvarez MA	6		Calendino J	52	de Jong LIT	48
Alvarez ME	7, 114		Calero I	39	de Rosas J	21
Alvarez SM	8, 71, 80, 145		Camargo G	28, 29	de Rosas JC	156
Alvarez Rogel J	11		Campos L	72	De Bortoli MA	70, 172
Alzari P	61		Cangiano MA	30, 31	De Gea G	24
Ampuero VE	79		Caniglia LF	82	De Pauw M	49
Anton R	58, 111, 140		Cargnelutti D	54	De Vito IE	102, 103
Ardanáz C	155		Carra G	40	Debattista NB	6, 112
Arrieta OR	168		Carra GE	148, 151	De garbo E	129
Arrieta RD	9, 44, 142, 143		Carreño G	72	De garbo SM	48
Azar N	169		Carrión A	131	Del Vitto LA	127, 176, 177
			Carrizo L	100, 146	Delgado S	162
<b>B</b>			Carvelli L	32, 178	Delgado SM	50
Balmaceda M	11		Casais M	162	Dequino C	169
Balmaceda VR	10		Casali YA	45, 46, 47	Di Genaro S	1, 54, 161
Banchio E	22		Cassán F	134	Di Renzo M	23
Barbeito CG	65		Casteller G	33	Di Renzo MA	83
Barbini NB	165		Castro T	152	Diangelo ME	44
Barbosa OA	11		Castro D	54	Díaz D	23
Barrera G	12, 76		Castro-Vazquez A	66, 74, 75, 163	Domeniconi MA	51
Barrera P	13		Castro-Vazquez AJ	87	Dominguez S	5, 73, 138
Barrionuevo A	14		Caula C	113	Donadel O	155
Basconsuelo S	19		Cavicchia JC	81, 82	Doña RD	52, 53
Batista S	15		Caviedes-Vidal E	26, 36, 38, 39, 52, 53, 58, 99, 111, 140		
Becerra R	158				<b>E</b>	
Bernardi A	54		Ceci ML	34	Elías C	124, 126
Berón AA	119		Cejudo C	2	Eliçabe J	54
Bertoldi MV	93		Celedon V	35	Enamorado MF	55
Bertolo R	17		Chediack J	58	Errecalde C	56, 68, 69, 97, 98, 132
Bertoluzzo SM	16		Chediack JG	36, 99	Escudero ME	59, 165
Bertoluzzo MG	16		Chetcoff A	42, 43	Etchegoren J	150
Betancourt L	18, 97, 98		Chierichetti B	95		
Biaggio VS	145		Ciacera C	37	<b>F</b>	
Bianco C	19		Cianchino V	4, 152	Fachinelli CC	57
Bianco CA	11		Cicarelli AS	48	Faricelli M	19
Bianco F	178		Cicarelli MA	48	Faricelli ME	83
Bianco MI	48		Cid FD	36, 38	Farrando M	168
Biole F	169		Cifunte DA	127	Fasulo SV	36
Bisogno FB	20		Ciminari E	26	Fasulo V	58
Boarelli P	21		Ciminari ME	39	Favier GI	59
Boarelli PV	107		Cincunegui LM	40, 148, 151	Favier L	114
Bogino P	22, 137		Ciuffo GM	88	Feresin GE	55
Bonamico N	23		Codazi L	77	Fernández C	19
Bonamico NC	83		Colombino M	157	Fernández D	93
Bonardello N	37		Colombo MI	92, 116	Fernández E	104
Bonvillani A	24		Conill A	82	Fernandez EA	109, 110
			Cooke P	113	Fernandez MC	60
					Fernandez P	61

Fernández RA	48	Grivel D	24	Luna C	105
Filippa V	138	Grosso M	19	Luna V	134
Flores J	17	Guardia T	118, 119, 120, 144	<b>M</b>	
Fogal T	73	Guardia Calderón CE	118, 119, 120	Mac Donal O	99
Forneris M	62	Guembe G	81	Maceda JJ	38
Forneris ML	50	Gutiérrez L	173	Maggini V	49
Fornes M	21	Gutiérrez P	11	Magnoli A	69
Fornes MW	5, 8, 107, 153, 173	Gutierrez Y	54	Majul R	29
Fóscolo M	81, 82	Guzmán AMS	59, 96, 165	Maldonado AM	84
Fourcade G	28	Guzmán AS	10	Malpassi R	19
Franzoni L	84	<b>H</b>		Manucha W	100, 146
Frasinelli C	19	Hampp E	19	María A	67, 101, 122, 170
Freire V	24	Hasuoka R	28, 37	María AM	7
Fuentes L	101, 136	Hidalgo MF	82	María AOM	2
Funes M	159	<b>I</b>		Mariano MI	42, 43
Funes S	39	Ibáñez J	40	Márquez SG	65
Furlán Z	19, 60	Ibáñez JE	147, 148, 151	Martinez E	109
Fusco M	123, 154	Ibáñez M	23	Martinez L	4, 89, 114, 152
Fusco MR	63, 64	Ibáñez MA	83	Martinez LD	90
<b>G</b>		Ingrassia M	41	Martinez Grassi E	175
Gallardo S	3	Irazoqui T	175	Martínez O	19
Galmarini C	135	Isola MC	84	Mattana C	1
Galotta JM	65	Ituarte LME	147, 168	Melegatti P	41
Gamarra-Luquez C	66	<b>J</b>		Mercado SE	174
Gambarte AJ	168	Jahn G	85	Messina GA	102, 103
Garaguso P	25	Jahn GA	34	Miatello R	131, 133, 135
García E	67, 122	Jáuregui EA	169	Micalizzi B	45, 46, 47
García EE	150	Jimenez-Ortiz V	13	Minisini A	16
García FP	149	Jofré G	32, 178	Miranda A	37
García H	97, 98, 132	Juan Hikawczuk VE	86	Moglia MM	104
García M	2, 3, 155	Juárez A	64, 101, 154	Mohamed F	7, 138
García Aseff S	67, 136	Juárez AO	144	Molina A	91, 105, 125
García Ovando H	56, 68, 69	<b>K</b>		Molina G	19
Gargiulo P	57	Karasov WH	58	Molina H	131
Garraza MH	70	Kenny V	83	Molina MG	106
Gatica LV	71, 79, 80	Koch E	87	Monclus M	21
Genghini R	41, 72, 160	Kraus T	19	Monclus MA	5, 107
Gianello JC	127	Kurina Sanz MB	20	Mora S	108
Gil E	73, 172	<b>L</b>		Morán JR	57
Gimenez IT	37	Laciar A	1, 161	Morandini M	24
Giménez MS	8, 27, 51, 71, 80 91, 124, 126, 145	Lapierre AV	88, 89, 90	Moriconi JI	109, 110
Giordano OS	20, 86, 127, 155	Larregle E	51, 91, 105	Muñoz N	105
Giordano W	22, 137	Ledeza C	53	<b>N</b>	
Giraud-Billoud M	74, 75, 163	Leiva N	92, 116	Nafissi G	157
Giuliani F	35	Leyes A	169	Navarro Becerra N	111, 140
Gómez A	12	Leyton CM	93	Nieto M	13, 67, 122, 150
Gómez M	166	Lijteroff R	94	Nuñez S	167
Gomez MR	4, 89, 90, 114, 152	Llanes A	134	<b>O</b>	
Gomez N	54	López JR	88	Ojeda A	115
Gómez NN	8, 80, 145	López L	133	Ojeda MS	77, 124, 126
Gómez P	29, 95	López LA	93, 130, 156	Olivella MS	112
Gómez Barroso JA	76	López Verrilli MA	86	Oliveros L	50, 62
Gonzalez C	105	Lopresti R	95	Oliveros LB	27, 70, 71
González E	135	Lucero Estrada CSM	96	Olsina R	114
Gonzalez I	77	Lüders C	18, 56, 97, 98, 132	Olsina RA	102, 103
González LE	79	Luders Post C	68, 69	Orden AO	20
Gonzalez UA	80	Lugo MA	106	Ordoñez G	108
González Jatuff A	78			Orozco JHA	129
González Jatuff AS	85			Orsilles M	113, 128
Gouiric S	55				

Orsina R	4	Risler N	131, 133, 135	Suhaiman L	156
Ortega C	114, 152	Rivarola C	3	<b>T</b>	
Ortiz A	115	Rivelli Antonelli J	22, 137	Tamashiro H	1
Ortiz AM	129, 171	Rivera-L P	63, 64	Tapia A	55
<b>P</b>		Robles S	64, 154	Téllez-Iñón MT	150
Pallavicini Y	174	Roby L	33	Terenti O	108, 157, 158, 159
Palmada NA	82	Rodríguez F	158	Terragno R	165
Pappano NB	6, 112	Rodríguez FA	11	Téves MR	176
Pavarotti M	92, 116	Rodríguez G	172	Tiranti I	41, 72, 160
Paveglia D	117	Rodríguez GB	79	Tonn C	13, 150
Pedemera AM	118, 119, 120	Rodríguez H	138, 139	Tonn CE	86, 127, 155
Pellarin C	121	Rodríguez J	12	Toranzo HH	9, 142
Pellegrini F	51, 91	Rodríguez JA	76	Torrecilla NM	57
Pelzer L	49, 67, 122, 123, 154, 170	Rodríguez N	111, 140	Torres D	3
Pelzer LE	118, 119, 120, 144	Rodríguez Echandía EL	78, 85	Torres J	28
Peralta C	122, 170	Romero MB	141	<b>U</b>	
Pereira C	61	Rosales E	62	Uribe M	94
Pereira S	123	Rossi D	24	<b>V</b>	
Pérez Chaca V	105	Rosso NE	9, 44, 142, 143	Vaca L	161
Pérez Chaca MV	91, 124, 125, 126	Rossomando P	154	Valek J	69, 132
Pérez Padilla A	12	Rotelli AE	144	Vallcaneras S	162
Pérez Romera E	139	Rozzen E	145	Vallés P	100, 146
Perinetti C	115	Ruete C	100, 146	Valsecia M	49
Petenatti EM	63, 64, 127, 176, 177	Ruiz A	2	Varas SM	34
Petenatti ME	127, 176, 177	Ruiz O	134	Vargas E	95
Petryna A	24	Rüttler M	115	Vega AE	29
Pieri E	113, 117, 128	Rüttler ME	129, 171	Vega IA	74, 75, 163
Piezzi RS	73	Saad JR	7, 86	Vega OA	164
Pizarro M	115	Saad R	2	Veiga V	32
Pizarro MA	129	Sagua MD	124, 126	Velasco M	97
Poey V	24	Salas M	98	Velásquez E	169
Polo M	130	Salceda S	15	Velázquez L	96, 165
Ponce C	2, 3	Saldeña T	40	Veneciano J	19
Ponce Zumino A	131	Saldeña TA	147, 148, 151	Verdaguer MN	131
Portiansky EL	65	Salerno J	23	Verdes P	14, 60, 166, 167
Prieto G	56, 68, 69, 97, 98, 132	San Martin-B A	63, 64	Viera C	3
Puebla DA	79	Sánchez CO	149	Viera TB	147, 168
<b>Q</b>		Sánchez J	24	Villa MC	10
Quargnolo J	17	Sanchez M	150	Villagra SE	169
Quattrin F	16	Santandrea MJ	83	Villegas NL	70
<b>R</b>		Saraví FD	17, 40, 148, 151, 168	Villegas Gabutti C	122, 170
Raba J	102, 103	Sartor T	13, 32, 150, 178	Vincenti A	8, 21
Ramos G	129	Scappini E	19, 174	Vincenti AE	107
Rapisarda M	49	Scardapane L	73, 138, 139	<b>W</b>	
Rastrilla A	164	Seltzer A	153	Wendel G	67, 101, 123
Rastrilla AM	121, 162	Siewert S	77	Wittouck P	41, 72, 160
Rege C	133	Silvestri F	171	<b>Y</b>	
Reginato M	134	Sisso J	53	Yanzón CS	171
Renna N	135	Sombra L	89, 90, 152	Yelós M	115
Renna NF	171	Soria M	153	Yunes R	33
Restelli M	15	Sosa A	63, 64, 123, 154	<b>Z</b>	
Ribes A	139	Sosa MA	13, 32, 150, 178	Zanin L	172
Rigatuso R	16	Sosa ME	86, 155	Zirulnik F	91, 105, 125
Rigo H	28	Sosa Z	121		
Rinaldi M	136	Sosa ZY	164		
Rinaudi L	22, 137	Stati A	81		
		Stefanini de Guzmán A	54		
		Stege PW	29, 103		
		Stevani E	155		