BIOCELL
2004, 28(2): 171-199

ISSN 0327 - 9545
PRINTED IN ARGENTINA

Cordoba Biology Society

(Sociedad de Biología de Córdoba)

Abstracts from the
XIV Annual Scientific Meeting

Edited by Lic. Ana Laura Correa & Dra. Graciela Racagni

August, 7-9, 2003
Villa General Belgrano, Córdoba, Argentina

Board of the Society 2001-2003

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ACKNOWLEDGMENTS

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1.

PHOTODYNAMIC STUDIES OF CATIONIC PORPHYRIN 5-(4-TRIMETIHYL AMMONIUM PHENYL) - 10, 15, 20 - TRIS (2, 4, 6 – TRIMETHOXY PHENYL) PORPHYRIN: BIOLOGICAL CONSEQUENCES IN A HUMAN CARCINOMA CELL LINE

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Photodynamic therapy (PDT) is a promising treatment for a photosensitizing of solid malignancies involving the administration of a photosensitizing compound and the accumulation of the sensitizer in the target cells, followed by selective irradiation of the lesion with visible light. Intervention of the excited photosensitizer with molecular oxygen results in the formation of singlet oxygen, which are believed to be responsible for cell destruction. The aim of the present study is to evaluate the photodynamic effects of 5-(4-trimethylammonium phenyl) - 10, 15, 20 - tris (2, 4, 6 - trimethoxy phenyl) porphyrin (CP) on Hep-2 cells. The uptake is not an energy dependent process until evaluated concentration (10 μM). CP does not affect the cell viability in the absences of light (0.1; 1 and 10 μM). In contrast, the combination of CP with visible light induces a decrease in cell survival that depend of the porphyrin concentration (1 and 5 μM) and light dose (27 and 54 Jcm^{-2}). Irradiation for 7.5 min produced chromatin fragmentation characteristic of apoptosis, while the cells showed typical morphological changes of necrosis after 15 min irradiation. CP is localizated in the mitochondria. These results suggest that the porphyrin CP has interesting photobiological properties.

2.

STUDIES PHARMACOKINETIC AND PHOTOTHERAPEUTIC OF CP AND CF3 PORPHYRINS *IN VIVO*: APPLICATION OF PHOTODYNAMIC THERAPY

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Photodynamic therapy (PDT) is emerging as an alternative modality for cancer therapy. It induces neoplastic cell death for photoachievable sensitizers. It offers improved selectivity for targeting tumor compared with conventional chemotherapy and radiotherapy. We have analyzed the photosensitizing properties of two porphyrin: 5-(4-N-(2′,6′-dinitro-4'-trifluorophenyl) phenyl)-10,15,20–tris(4-trimethoxyphenyl) porphyrin (CF3) and 5-(4-trimethylammoniumphenyl)-10-15,20-tris (2, 4, 6-tri-methoxy-phenyl) porphyrin (CP) on cell cultures. In darkness, CF3 and CP did not alter the survival of cells cultures, but produced mortality cell when irradiated. The aims of this work were evaluate the photodynamic effects of CF3 and CP on Balb-c rats. The photosensitizers are accumulated in liver and duodenum. They are eliminated from the body, via bile-gut. They do not cross the blood-brain barrier and do not produce toxicity in the skin. In phototherapeutic studies, it was observed almost complete tumor ablation (90%) for tumor injected with CP after 7-15 days post-irradiarion, while CF3 was not observed significant regression. The results show that CP could be adequate sensitizer for PDT.

3.

STUDY OF HUMAN CHONDROCYTE FROM OSTEARTHRITIC CARTILAGE

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The normal cartilage is characterised morphologically by a small number of chondrocytes which are resposible for the production and organization of an exracellular matrix. Ostheoarthritis (OA) is a degenerative cronic disease of the cartilage. It´s still poorly understtod. Chondrocyte heterogeneity within normal and OA cartilage is important to understand the pathogenesis of this disease. The structural characteristic of the chondrocytes from the three main zones. It was demostrated thet the chondrocyte´s phenotype varied (clonal, normal, secretory and degerating cell) acording to the location within the tissue. Objetive: to use of light microscopy in morphological characterization of chondrocyte population within human ostheoarthritic cartilage. Cartilage samples were removed during arthroscopy from the knee cartilage from an OA patient. Samples were carefully oriented in order to obtain thick and thin sections parallel and perpendicular to the surface of the cartilage. Two different types of cell aggregates were found. The typical "clonal" chondrocytes were large cells with an euchromotatic nucleus inside of a lacunar with a special peipheral matrix. It is of particular interst that the "clonal" chondrocytes synthetize and secrete increased concentrations of metallo-proteinases in OA cartilage.

4.

DIFFERENTIAL INFECTIVITY OF HUMAN PLACENTAL CHORIONIC VILLI AND ISOLATED TROPHOBLAST BY *Trypanosoma cruzi*

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The first barrier to produce congenital Chagas disease by *Trypanosoma cruzi* is the placental trophoblast.. Objectives: **a)** to quantify *T. cruzi* infection in chorionic villi explants and isolated trophoblast from human normal placentas *in vitro*, **b)** to analyze the effect of placental soluble factors on *T. cruzi*, **c)** to correlate viability of *T. cruzi* with levels of nitric oxide (NO). Chorionic villi explants, isolated trophoblast (24 h), myocardium and VERO cells were co-cultured with trypomastigotes Tulahuen strain of *T. cruzi* for 3 h, 24 h and 72 h. It was quantified: 1) *T. cruzi* in tissue and cells by PCR, PAS/H and immuno-histochemistry, 2) viability of *T. cruzi* and no levels in the supernatant culture media. 0.013% (3 h), 0.022% (24 h) and 0.057% of the areas of chorionic villi were infected, with 4.5 ± 2 parasites per nest and PCR +, whilst 52% of VERO cells were infected with 75 ± 20 parasites per nest and similar results for isolated trophoblast ($p < 0.05$). None o few live parasites were found in supernatant culture media of explants-*T. cruzi* co-cultures (24 and 72 h) and NO showed trypanocidal levels. Parasite dead increased ($p < 0.05$) when *T. cruzi* was incubated with control supernatant culture media and the phenomenon was not observed when media were preheated at 65ºC. Conclusion: low productive infection in chorionic villi explants with no or scarce viability of *T. cruzi* in supernatant culture medium, but high infection in isolated trophoblast and VERO cells (as control). These results could be due to placental no and thermolabile agents. Human normal placenta may play a crucial role in the congenital Chagas disease.

5.

VIABILITY, PROLIFERATION AND APOPTOSIS IN ETHANOL/TROPHIC FACTORS-EXPOSED NEURAL CREST CELLS

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The embryonic neural crest cells (NCC) segregate from neural tube anlage, distribute to the embryo producing many derivatives, and are sensitive to prenatal ethanol exposure. In this work, we study alterations of trophic parameters of *in vivo* and *in vitro* NCC treated with teratogenic ethanol doses, and the possible prevention by trophic factor treatment. On *in vivo* experiments we shown anomalies of neural tube closure and increased apoptosis (whole-mount acridine orange affinity) in NCC after direct exposure to 150 mM ethanol for 10 h (sufficient dose to induce *Fetal Alcohol Syndrome* in mammals) The observed changes were prevented by simultaneous addition of *Neurotrophin-3* (NT-3) (40 ng/ml) with the ethanol treatment. On *in vitro* cephalic NCC, high cell mortality was observed (Calcein AM-Ethidiun H1 test), as well as diminution of cell proliferation (BrdU labeling), after 3 h of ethanol treatment. Ethanol plus NT-3, but not with *Ciliary Neurotrophic Factor* (CNTF) prevented the drop of cell proliferation. Considering the expression of NT-3 (but not CNTF) in the vecinity of NCC and the early expression of TrkC receptors on these cells, the results open new perspectives to evaluate the potential use of trophic factors to prevent alterations induced by prenatal ethanol exposure. Moreover, trophic alterations induced after a short exposure to ethanol add evidences on the contribution of cephalic NCC to the phenotype of the *Fetal Alcohol Syndrome*, and support the present view about the real potential risk of alcohol ingestion (even at low doses and/or for a short time), during any stage of the pregnancy or lactation.

6.

CHEMOTACTIC MIGRATION INDUCED BY TROPHIC FACTORS ON NEURAL CREST CELLS

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Neural crest cells (NCC) migrate through the embryo body and distribute with precision in "target" fields, where they differentiate in many derivatives. The known factors modulating the dispersion of NCC are not sufficient to full explain the oriented migration of this cell population. In our laboratory, we showed that soluble factor(s) from embryo skin induce chemotactic response in a (melanocytic?) sub-population of NCC. In this work, we evaluate the directional response of NCC exposed to gradients of *Stem Cell Factor* (SCF) and *Neurotrophin-3* (NT-3). Using real time videomicroscopy and strict directional-based quantitative criteria, we showed a chemotactic behavior of NCC, with a typical dose-response curve and maximal effect with 25-50 ng/ml SCF or 40-80 ng/ml NT-3 in the source of the gradient. The angular analysis of the cell tracks also showed that, besides the NCC oriented towards the trophic factors, another population is repeled by a high concentration of the same factors in the source of the gradient, suggesting a dose-dependent response. Considering that SCF is expressed in the "target" region (skin) of pre-melanocytic NCC and the c-kit receptors are present in this cell population during their migration towards the skin, as well as that NT-3 exists during the embryonic neurogenesis and their receptor TrkC is expressed in the early stages of NCC, these results indicate that —besides the classical trophic functions— the SCF and NT-3 could to participate in the spatial orientation of NCC towards their "target" fields. These data amplify the functional scope of trophic factors integrating them into new directional mechanisms of migratory embryo cells.

7.

PHOTOINACTIVATION INDUCED BY ZnPCOCH3 OF CARCINOMA CELL (HEP-2)

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Photodynamic therapy is an alternative therapy for treatment of solid and superficial tumors and is being investigated for the treatment of arteriosclerosis, restenosis, and psoriasis. This therapy is based on the administration of a photosensitizer, which is selective retained by neoplastic tissues, and then subsequently activated with visible light of an appropriate wavelength. This new therapy results in a sequence of photochemical and photobiologic processes that cause irreversible photodamage to neoplastic tissues. This work describes the photobiologic properties of tetramethoxy Zn (II) phthalocyanine (ZnPcOCH3) on larynges carcinoma human cell line (Hep-2). The uptake of ZnPcOCH3 was linearly increasing with time for the first 1-2 h of incubation and tends to a saturation value after long incubation ≥ 3 h. Cell toxicology induced by phthalocyanine was first analyzed in dark condition and no toxicity in terms of cell survival was detected at 5×10^{-7} M. In contrast the combination of photosensitizer with visible light induced a decrease in cell survival of 20%. The morphologic analysis of the cell preformed with Hoechst-33258 revealed that the cell death after irradiation for 15 min produced chromatin fragmentation, characteristic of apoptosis.

8.

EFFECT OF CYCLOHEXIMIDE ON THE ACTIVATION OF ENDOPROTEASE IN *Amaranthus* GERMINATION

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The development of endopeptidase activity during imbibition of amaranth seeds has been studied. Proteolytic activities are essential for the mobilization and reutilization of protein reserves during seed germination. Azocaseinolytic activity can be detected in dry seeds with optimum pH of 5.5 and 6.5. An increase in this activity is observed afterward. The development of endopeptidase activity in cereals and legume seeds, the most studied in this respect, is due to the *de novo* synthesis of enzyme protein. In amaranth seeds a different mechanism, on the basis of cycloheximide (CH) effects, is suggested. The increment in the endopeptidase activity was not inhibited by CH suggesting that *de novo* synthesis of enzyme was not involved. Indeed, this increment is greater in the presence of CH. The inhibition of synthesis of an inhibitor of endopeptidase is a possible explanation. PAGE on polyacrylamide-gelatin showed an activity band in dry seeds. Other three bands were added by imbibition for three days. By DEAE-Sepharose chromatography of a protein extract of seeds, three peaks were observed in embebed seeds, and two peaks in dry seeds. The endopeptidases were inhibited by PCMB suggesting that SH groups are important for their activity. Our results suggest that the endopeptidases of amaranth seeds can be classified as cysteine-proteinases involved in protein reserve degradation. These enzymes, apparently, were activated at imbibition through a mechanism different to *de novo* synthesis.

This work was supported by grants from SECYT (UNRC).

9.

ACTIVATION OF Na^+/H^+ EXCHANGER IN RESPONSE TO MANITOL IN *Trypanosoma cruzi* EPIMASTIGOTE FORMS

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Trypanosoma cruzi has a complex life cycle that adapts to the variety of conditions imposed by the environment, for example changes in osmolarity occur in the insect vector rectum, where metacyclogenesis is induced. Our laboratory demonstrated that 0,5 M manitol induced an increase in the numbers of parasite intermediate forms and changes in growing parameters. The aim of this work was to determinate the early biochemical events triggered by manitol in epimastigote forms. Manitol induced the acidocalcisome alkalinization, demonstrated by an increase in the orange acridine absorbance and by fluorescence microscopy. Moreover, manitol evoked an IPs/IP_3 accumulation increase in with [^{3}H]myo-inositol labeled parasites. This effect was inhibited by U73122, a specific PLC inhibitor, and by BAPTA-AM, a calcium intracellular chelator. On the other hand, manitol induced an increase in Ca^{2+} signaling in Fura-2AM loaded cells. The IPs/IP_3 accumulation and the Ca^{2+} signaling were inhibited by EIPA, an Na^+/H^+ exchanger inhibitor. This fact suggest Na^+/H^+ exchanger activation was involved in both processes. Finally, acidocalcisome Na^+/H^+ exchanger together with the Ca^{2+}/nH^+ exchanger, may be responsible of Ca^{2+} signaling and the subsequent PLC activation.

10.

DIETARY FATS AND SUPRAMOLECULAR ORGANIZATION OF UROTHELIAL PLASMA MEMBRANE

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Experimental and epidemiological evidences support the hypothesis that dietary fatty acids play a role in urinary tract tumorigenesis altering the plasma membrane structure. In this context, we determined the changes of the lipid composition and of the hexagonal array of uroplakins in the urothelial luminal surface of rats receiving different dietary fats (corn, fish. olein oil rich diets and comercial diet). Lipids from purified urothelial luminal surface were analized by HPTLC. The ultrastructural organization was studied by EM and image processing of negatively stained urothelial plaques. Hetrodimers and homodimers resulting from chemical "cross-linking" were analized by SDS-PAGE as a complementary protein topology study. There was a direct correlation between the contents of phosphatidylethanolamine (PE) and docosahexanoic acid. Contrary, sphyngomyelin, cholesterol and phosphatidylcholine contents were in inverse relationship with respect to that of the PE. Major changes of the lipid composition and heterodimers formed by "cross-linking" were observed in the olein oil membrane. The properties of the lipids found in this membrane suggest the tendency to microdomains formation while the heterodimers found in the olein membrane were indicative of a longer particle center-to-center distance than that of the control.

11.

EXPERIMENTAL CONDITIONS TO STUDY CYTOTOXIC EFFECTS OF ARSENIC IN CHO CELLS

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In the southeastern region of the Province of Córdoba elevated arsenic levels in drinking water averaged 178 ppb. It was observed a dose-response relation between ingestion of arsenic from drinking water and HACRE ("hidroarsenicismo crónico regional endémico"), highly related to bladder, skin and lung cancers. In mammals, arsenic induces stress response, cytoskeletal alterations and MAP Kinase activation. However, lack of an animal model has limited progress on the understanding of the mechanism of arsenic-linked carcinogenesis. *Objective*: To develop a model in cultured cell to study the molecular events which may mimic arsenic carcinogenesis. Cells culture: the non-tumoral cell line CHO K1 were incubated through different times in DME medium containing 10% fetal bovine serum, with the addition of different amounts of sodium arsenite. Western blot and immunocytochemistry were used to determine the Hsp70 (heat shock protein 70 kDa) induction and to visualize the actin filaments, respectively. Cells grown for $1^1/_2$ hours in the presence of the sodium 200 µM arsenite followed by 3 hours of recovery, showed the Hsp70 induction. If the cells were cultured for $2^1/_2$ hours in the presence of sodium arsenite (200 µM), they responded with striking changes in their cell shape and remodeling of the actin filament network since actin acquires perinuclear localization. We observed that CHO cells will undergo stress response after acute arsenic exposure and that this response is associated with expression of Hsp, morphologic changes and cytoskeletal alterations. So, 200 µM Sodium arsenite and $2^1/_2$ hours will be useful conditions to future experiments.

12.

INFLUENCE OF ACETYLATED TUBULIN ON THE REGULATION OF H^+-ATPASE ACTIVITY OF *S. cerevisiae*

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Brain membrane tubulin can be isolated associated to the α subunit of Na^+,K^+-ATPase. This tubulin corresponds mainly to the acetylated isotype. The activity of Na^+,K^+-ATPase is inhibited by its association with acetylated tubulin (AT), *in vitro* and *in vivo*, showing a direct correlation between the inhibition grade and the amount of associated AT. Recently, we found that AT is also able to inhibit, *in vitro*, the plasma membrane H^+-ATPase of *S. cerevisiae*. By studying plasma membrane of *S. cerevisiae*, in this communication we show that: a) the inhibition produced by brain AT on H^+-ATPase, measured by its capacity to hydrolyze ^{32}P-ATP, correlates with an increase in the amount of AT bound to the membrane and b) the effect of AT on the enzyme is inhibited by ATP concentrations between 0.1 and 0.5 mM. When *S. cerevisiae* cells were incubated in presence of 50 mM Mes-Tris, pH 6.8, containing glucose, an increment of H^+-ATPase activity was observed, which was accompanied by a decrease in the amount of AT bound to membrane. However, when the cells were incubated in physiological solution containing 10 mM glucose, a pH decrease of the medium was observed with correlated with a decrease in the amount of AT bound to the membrane. These results indicate that the H^+-ATPase activity of *S. cerevisiae* is modulated by the association/dissosiation of AT/ H^+-ATPase complex. This type of regulation could play an important role in the pH homeostasis.

13.
MOLECULAR CHARACTERIZATION OF CALTRIN-LIKE PROTEINS FROM HUMAN SEMINAL PLASMA

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In the previous meeting we reported the presence of two protein fractions of 15 and 34 kDa in human seminal plasma that reacted with caltrin antibodies and were designated caltrin-like proteins. A procedure for purification and the sequence of the first 35 amino acid residues of the 15 kDa protein (p15) were also reported. This fragment showed homology with the sequences of three proteins previously isolated from human prostate and seminal vesicles but their functions are still unknown. In this communication a comparative study on the molecular characteristics and structural differences between p15 and p34 is presented. The purification procedure for p15 was modified changing the steps of saline fractionation and using Sephadex G-70 for exclusion chromatography to purify p34. The pIs estimated by isoelectrofocusing were 5.8 and 7.0 for p15 and p34 respectively. When both proteins were submitted to SDS-PAGE under no-reducing conditions, p15 showed a mixture of monomer and dimmer molecules in the native state while p34 moved as a 27 kDa protein suggesting the presence of disulfide bridges between Cys residues of the same polypeptide chain. The presences of sugars was assessed by periodic acid staining (PAS) or by specific interaction with Con A and WGA lectins conjugated with alkaline phosphatase (AP). Positive PAS and Con A assay obtained with p34 suggest the presence of carbohydrates likely bound by N-glycosidic bonds to the polypeptide chain. The structural differences between p15 and p34 may be associated to specific functions as it was determined with caltrin proteins from other species.

14.
IDENTIFICATION OF GLYCONJUGATES IN THE PORCINE PLACENTA

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The lectins have contributed important information for the identification of different glycoconjugates in diverse structures normal or pathologic. The aim of this study was to identify the presence of glycoconjugates in porcine placenta during pregnancy using lectins. Histologic sections of the porcine placenta of +/- 28, 55, 70 days and a term, fixed in formaldehyde 10% buffer phosphate and embedded or not in paraffin, were used. The following lectins conjugated with FITC, were used as markers: PNA (Arachis hipogaea: β-gal (1-3) gal Nac); Con A (Concanavalin A: α-man, α-glc), PSA (*Pisum sativum*: α-man) and VFA (Vicia faba: man, glc). Through direct immunofluorescence it was detected the presence of glycoconjugates with residues mannose and glucose in trophoblastic epithelial cells in contact with uterine lumen in all gestational periods, but not in chorion and amnion, except for placental tissues at 55 days of gestation. Working with Con A was more positive in all gestational periods, especially at 55 days of pregnancy.

15.
EFFECTS OF EICOSATRIENOIC ACID (20:3N-9) ON SOME PROMALIGNANT-RELATED PROPERTIES OF THREE HUMAN CANCER CELLS LINES

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Dietary fatty acids modulate the biological behaviour of certain tumours. Eicosatrienoic acid (ETA, 20:3 n-9) an unusual metabolite of oleic acid, counteract the beneficial parameters showed by human squamous cell carcinoma culture induced by essential fatty acids, such gammalinolenic and eicosapentaenoic acids (EPA 20:5 n-3). This may be linked to the essential fatty acid deficiency (EFAD), a metabolic condition in which ETA arise in tissues. Thus we comparatively studied the effect of EPA and ETA, on certain tumour cell parameters linked to tumour progression and metastases, such as E-cadherin expression, Diene conjugation (DC), a measure of lipid peroxidation and cell proliferation. Human tumour cell lines (T-24 from urothelium, MCF-7 breast and HRT-18 colon) were used an EPA-added cultures an anti-proliferative effect was observed in all parameters of the three lines. ETA showed a differential effect depending on the cell line. Urothelium line did not express E-cadherin, a fact which was not modified for added fatty acids. DC production was decreased by ETA. In mammary MCF-7 cells ETA increased E-cadherin expression, enhanced the lipid peroxidation and decreased the cell proliferation. On colon HRT-18 cells ETA diminished the E-cadherin expression, as well did lipid peroxidation whereas cell proliferation was increased. Unusual ETA behaves as pro-tumorigenic lipid on colon and urothelial line, whereas showed opposite effect on some tumour parameters of breast cancer cells. These results partially expand the pro-tumorigenic effect of EFA on colon and urothelial lines, besides epidermis. They are potentially relevant considering that eicosatrienoic acid is abnormally increased by EFAD a condition linked to a pro-tumorigenic proneness, which may sub lying in certain alimentary or metabolic disbalances of food lipid sources.

16.
DIFFERENT DOSES OF SALBUTAMOL MODIFIES LUNG LIPIDS OF MALE RAT

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We have demonstrated that therapeutic doses of inhaled Salbutamol (a β-adrenergic bronchi dilator agonist) increase phospholipid (PL) contents on lung surfactant. We evaluated lipidic profile changes in total lung when different doses of Salbutamol were inhaled in these concentrations: 1.3 μmol/ml (low dose) (LD), 13 μmol/ml (therapeutic dose) (TD) and 130 μmol/ml (high dose) (HD). Male Wistar rats were separated in four groups: LD group, TD group, HD group and Co group, which inhaled saline solution (Control). We determined: total lipids (TL), triglycerides (TG), total (TC) and free cholesterol (FC) and PL (See table). We conclude that TD favors a better formation of phospholipids, main component of lung surfactant, which improves respiratory function. We can see that there is not a lineal dose-effect behaviour.

Lung lipids	Co	LD	TD	HD
LT (mg)	27.8±4.7	24.33±0.3	21.0±0.	20,6±0.3
TG(μg/mg lip)	40.85±1.8	33.95±2.2	14.8±1.5	27.17±2.7
CT(μg/mg lip)	20.75±1.2	19.1±3.0	45.24±5.0	64.08±7.2
CL(μg/mg lip)	15.51±2.5	14.2±1.0	21.94±1.4	45.93±1
PL(μmol/mg lip)	0.060±0.006	0.105±0.007	0.205±0.05	0.132±0.01

17.
DEVELOP OF EXPERIMENTAL MODEL IN *Bufo arenarum* LARVAE FOR THE EVALUATION THE PHOTO-SENSITIZERS TOXICITY

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Current studies in photodynamic therapy (PDT) focus on the characterization of new photosensitizers. In our laboratory we are heading to the search of new more effective and less toxic photosensitizers. It is of great importance to investigate the toxic activity of these therapeutic compound potentials to know their reaches and limitations. These properties can be determined using *in vivo* toxicity test. The objective was to develop an experimental model of embryotoxicity test in *Bufo arenarum* larvae. A substance of activity photosensitivity, the methylene blue (MB) was used. The values of the LC50 (median lethal concentration) and body growth development for this molecule under conditions of darkness and illuminated were obtained. Larvae of *Bufo arenarum* in stadium I, obtained from a fecundation *in vitro* were used, maintained in glass Petri dishes in the proportion of 10 larvae (for dish) per 40 ml Ringer diluted solution. To obtain the LC50 in darkness the larvae were exposed to different concentrations of MB. Using the maximum non-toxic dose in darkness (373 μM) was carried of the illumination. The results show that 706 μM concentration corresponds the LC50 in darkness and 373 μM with illumination of 30 min was able to kill to 98.3% of the larvae. The 20.5 μM concentration with 30 min of illumination corresponded of the LC50. In conclusion, combination of MB and light are the responsible for the larval death. The analysis of the corporal dry weight didn't show differences among the different treatments. Experimental model adapts for the obtaining of the LC50 and embryotoxicity tests with photosensitizers drugs and they can be used with new photosensitizers studied in PDT.

18.
GLUTAMINE SYNTHETASE FROM NODULATED PEANUT ROOTS: EFFECT OF INHIBITORS AND ACTIVATORS. SEPARATION OF ISOENZYMES

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Glutamine synthetase (GS) catalyzes the ATP dependent assimilation of ammonium into glutamine, using glutamate as a substrate. Plant GS and glutamate synthase (GOGAT) are responsible for the initial assimilation of ammonia into organic compounds. In nodulated legumes ammonium proceed from rhizobia. The aim of this work was to purify and characterize GS from nodulated roots of peanut. The separation of iso-enzymes also was attempted. Germinated seeds were inoculated with *Bradyrhizobium* sp SEMIA 6144. Plants were grown in fixed conditions of temperature and light. A protein extract of roots was purified by chromatography on DEAE-Sepharose eluted with a 0.5-1.5 M NaCl gradient. The purified enzyme was not affected by p-CMB or NEM. However, optimal activity of enzyme was observed in the presence of reductors such as 2-mercaptoethanol, reduced glutathione and cysteine. Ca^{2+}, Hg^{2+} and Mn^{2+} were inhibitory, while EDTA, Co^{2+} and Cu^{2+} increased the activity. Inhibition of enzyme was caused by 5'-mononucleotides whereas a stimulation was observed in the presence of NAD^{+}, NADPH or 3'-mononucleotides. By native PAGE 2-3 isoenzymes can be detected. When the revelation of isoenzymes was accomplished in the presence of 5' or 3' mono-nucleotides the effects over each isoenzyme were similar to those observed *in vitro*. We may conclude that GS from nodulated peanut roots shows compatible properties with GS of other plants.

This research was supported by grants from SECYT (UNRC).

19.
PHOTODYNAMIC THERAPY. INDUCTION OF MALIGNANT CELL DEATH MEDIATED BY PHTHALOCYANINE WATER-SOLUBLE

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Photodynamic therapy (PDT) is a bimodal therapy for certain types of cancer that involves the use of a photosensitizing chemical following by illumination with suitable wavelength of light. Only the combination of both result cytotoxic for biological systems. The photoactivation generates reactive oxygen species leading to an oxidative damage and subsequent cell death. The effect *in vitro* induced by a hydrophilic new phthalocyanine on larynx human cells named Hep-2 are studied. Parameters such as dark toxicity and effects on PDT-mediated cell viability are evaluated, as well as the PDT treatment leading to characteristic type of cell death induced by photosensitizer water-soluble. No loss of cell viability occurred when the treatment with Pc (0.1, 0.3 and 0.5 μM) occurred in the dark for 12 h (without irradiation). On the contrary, 12 h incubation with Pc followed by visible light irradiation (15 minutes) induced a significant decrease of cell viability. A lethal photodynamic effect with 0.5 μM Pc followed by 15 minutes of irradiation resulted in complete loss of cell viability (1% living cells). Sublethal effect (50% living cells) was found using experimental conditions such as 0.1 μM Pc followed by 15 minutes of irradiation and 0.5 μM Pc followed by 1 minute of irradiation. Our results indicate that the ability to induce cell death was dependent on the photosensitizer and irradiation dose. The cell death could contribute to the development of experimental protocols with potential for clinical applications in PDT of cancer.

20.
PHOSPHORYCHOLINE PHOSPHATASE: ONE ENZYME WITH TWO CATALYTIC SITES

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Different species of *Pseudomonas* genus induce a phosphorylcholine phosphatase (FCPase) activity in presence of choline as the sole carbon and nitrogen source. This activity can be assayed using p-nitrophenyl-phosphate (p-NPP) or phospho-rylcholine (PC) as substrates and their kinetic properties suggest the existence of independent catalytic sites. The aim of this study is to demonstrate the presence of these sites. Seven species of *Pseudomonas* were grown in a basal salt medium supplemented with 20 mM choline chloride. The enzymatic activities were purified by precipitation with ammonium sulfate and separation on a Sephacryl S-200 HR. The molecular weight and isoelectric points were calculated. Kinetic studies included pH effect, Mg^{2+} activation, Al^{3+} inhibition, Km apparent determination and enzymatic activity in presence of p-NPP and PC, simultaneously. Five species of *Pseudomonas* showed FCPasa activity, similar MW (≈ 55 kDa) and kinetic properties. The enzymatic activities of the two remaining species were ineffec-tive with PC as substrate and their MW were similar (≈ 27 kDa), but minor than the others. Using p-NPP the kinetic properties were identical to the obtained with FCPase. *P. fragui* FCPase showed an important reduction of the activity with PC, but not with p-NPP, in presence of both substrates and Al^{3+} ion simultaneously. These results support the existence of different catalytic sites for each substrate, because the decrease of the molecular weight coincides with loss activity on PC, main-taining identical kinetic properties with p-NPP. It could be involved a unique gene with different open reading frame or two independent genes.

21.
PROSTATIC BINDING PROTEIN (PBP) EXPRESSION IN ACUTE BACTERIAL PROSTATITIS

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Inflammation of the prostate gland constitutes an important problem in urology. The bacterial infection is the principal etiology of acute prostatitis. PBP is the main epithelial secretory protein of the rat ventral prostate; it is a glycoprotein of 50 KDa that belongs to the Secretoglobin family, whose founder member, Uteroglobin, is an antiinflammatory molecule that inhibits quimiotaxis and Fosfolipasa A_2 activity. The physiological roll of PBP is not known completely yet. The objective of this work was to determine the participation of the PBP in acute prostatitis in an experimental model induced by bacteria. Wistar rats 3 months old were inoculated with a suspension of *E. coli* (10^9 UFC) beneath the capsule of the ventral prostate, and control rats were injected with sterile PBS. Animals were sacrificed 24, 48 and 72 hours after inoculation. Samples of ventral prostate were fixed and processed for inmunocitochemistry and morphologic analysis by photon and electron microscopy. A semiquantitative analysis of PBP expression was done by Dot Blot in tissue homogenate. A great neutrophil interstitial infiltrate was observed 24 hours after inoculation, and epithelial cells exhibited a marked hypertrophy with significant increase ($p < 0.05$) in PBP expression. In 24 hours-treated animals, specific label for PBP spread all over the cytoplasm, while in control it was restricted to the apical portion. Protein increase stayed 48 hours after inoculation and inflammatory cells diminished, whereas 72 hours after, acini exhibited a noticeable disorganization with loss of the alveolar lumen and diminution of PBP levels in homogenate. Results obtained demonstrate an early response to inflammation that includes PBP expression increase that contributes to control inflammatory infiltration. Decrease of the protein at 72 hours would allow inflammatory cells to invade the acini lumen. These results indicate that PBP would play a key role in acute inflammation *in vivo*.

23.
ASCORBIC ACID EFFECTS ON THE OXIDATION OF CONIFERYL ALCOHOL CATALYZED BY A BASIC PEROXIDASE FROM TOMATO HAIRY ROOTS

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Tomato hairy roots treated with 100 mM NaCl secrete to the culture medium a basic peroxidase isoenzyme, which has high affinity and catalytic efficiency with coniferyl alcohol (CA), a substrate marker of peroxidases involved in lignification. Ascorbic acid (AA) has been reported as an inhibitor of CA oxidation by peroxidases, but with different effects on apoplastic or cell wall-bound isoenzymes. The aim of this work was to analyze the effect of AA on the oxidation of CA catalyzed by the partially purified basic peroxidase above mentioned. AA was added in the range 1 to 40 µM after 1 minute of the beginning of the reaction. Its effect on CA oxidation was determined by following the decrease in $A_{212\,nm}$ produced by CA oxidation at the optima conditions previously determined. A 20% inhibition of CA oxidation was observed after the addition of 1, 5 or 10 µM AA. Higher concentrations: 20, 30 and 40 µM AA, produced a lag phase, a period during CA was not oxidized at all. Fot 20 and 30 µM AA, CA oxidation was reestablished after this lag phase of 3 minutes, but at a lower rate, aproximately 35% of the initial velocity (v_i). When 40 µM AA was added, only 10% of v_i was obtained after the lag period. Similar results were reported for wheat apoplastic Px. The results suggests that apoplastic AA could regulate the activity of this peroxidase involved in lignification, and hence the participation of this enzyme in the response to the salt stress.

Grants of CONICET, Agencia Córdoba Ciencia-FONCYT, SECYT-UNRC.

22.
CORRELATION BETWEEN GLUCEMIC LEVELS, AGE AND SEX OF UNSELECTED DIABETIC PATIENTS

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Diabetes mellitus is a metabolic disease characterized by high levels of blood glucose. We studied a population of diabetic men and women who came to the Hospital of San Luis. Diabetes type and treatment were not established. We tried to determine if sex and age influence blood glucose levels of diabetic patients. 320 diabetic patients were studied (157 men and 163 women) in an age range of 40 – 70 years old. We determined blood glucose by enzymatic method in fasted patients, and glycosilated hemoglobin A_{1c} by chromatography-espectrophotometry. Comparisons where made using tertiles by age and sex and t-Student test. Results are shown in the table. We can infer that blood glucose level in women under 50 years old is significantly lower than in men of the same age; this could be due to the presence of female sex hormones. We can also say that glycosilated hemoglobin in women decreases significantly with the age when compared to men.

Men			Women		
Age	Glucose (g/l)	HbA_1 (%)	Age	Glucose (g/l)	HbA_1 (%)
46±5.5	**1.58 ±0.2	10.65±1.8	45±6.4	**2.85±0.4	9.78±1.5
54±6.5	*2.19±0.3	9.86±1.3	54±7.0	*1.75±0.3	9.21±1.4
65±4.9	1.95±0.3	**7.3±1.2	65±4.7	1.86±0.4	**10.7±1.5

24.
PLC ACTIVATION BY G PROTEIN IN BARLEY ALEURONE STIMULATED WITH GIBERELLIN

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Aleurone layer is considered a useful model to study hormonal signal triggered by GA and ABA. Synthesis and secretion of hydrolytic enzymes are stimulated by GA and antagonized by ABA. Our laboratory demonstrated that 5 µM GA increases the lipid kinase activities, IP_3/IPs levels and polyphosphoinositides turnover. These results showed PLC is involved in GA response. Furthermore, α-amylase secretion was inhibited by U73122, a PLC inhibitor. The goal of this study was to determinate the mechanism by which PLC is stimulated during secretion process. Aleurones were stimulated with 5 µM mas7, a mastoparan analogue, G protein activator. Mas7 increased PI-k and PIP-k activities at 5 min and 20 min, while that DAG-k and PA-k did at 20 min and 60 min. Similarly, mas7 evoked IP_3/IPs accumulation in [^{3}H]*myo*-inositol radiolabelled aleurone, this effect was inhibited by U73122 treatment. In addition, mas7 triggered α-amylase secretion which was reduced to 50% by PLC-inhibidor. Secretion stimulated by GA was potentiated by mas7. In this work we demonstrated, by using mas7, that PLC activity in barley aleurone is regulated by G protein.

25.

RESPONSE OF THE TOMATO PEROXIDASE GENE *tpx1* TO BIOTIC LICITORS

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Peroxidases (Px) have been involved in several physiological and biochemical processes, such as abiotic and biotic stress responses and lignosuberization. This is a defense mechanism against pathogens that limits their spread at the infection site. In tomato, *tpx1* gene encodes an isoperoxidase with a pI 9.6 which has been related to lignification. Furthermore, *tpx1* expression is induced by salicylic acid that is an endogenuos elicitor for the activation of defense responses. To study the effect of biotic elicitation on *tpx1* gene expression, we used tomato (*Lycopersicon esculentum* Mill. cv. Pera) hairy roots as a model system. We used chitosan as purified biotic elicitor (200 mg/l) and the specific phytopathogenic fungus *Fusarium oxysporum f .sp. lycopersici* as complex elicitor (10^5 y 10^6 conidia/ml). Soluble (SP) and ionically-bound to cell wall (IBP) proteins were extracted. Px activity was measured and isoenzymes were separated by IEF and cationic PAGE. Total RNA was isolated and transcript accumulation was tested by Northern blot. Px activity of tomato hairy roots treated with chitosan increased about 50% at 2.5 h post-elicitation. It was coincident with an increased intensity of the band with a pI 9.6 in the IEF and accumulation of *tpx1* transcripts. In the SP fraction chitosan stimulated Px activity about 76% without alteration of isoenzyme patterns or *tpx1* transcription. Treatment with *Fusarium* conidia didn´t altere isoenzyme patterns or *tpx1* transcription, but enhanced Px activity in the SP fraction with both concentrations, and in the IBP only with 10^5conidia/ml at 48 h post-elicitation. In conclusion, *tpx1* expression was induced by chitosano at 2.5 h, but not by *Fusarium* conidia at longer times.

27.

CORTICOTROPHS AND MELANOTROPHS CELLS IN ADENOHYPOPHYSIS OF VIZCACHA: IMMUNO-CYTOCHEMICAL STUDY

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Cells in the pars distalis (PD) and pars intermedia (PI) of adenohypophysis secrete ACTH and MSH, respectively. These hormones arise from a common precursor, proopiomelanocortin (POMC), through proteolytic cleavages in corticotrophs and melanotrophs. The aim of study was to analyse the morphology and distribution of these cells in adenohypophysis from adult male vizcacha (*Lagostomus maximus maximus*). The immuno-cytochemical study showed ACTH-immunoreactive cells in the cephalic and dorsal regions of PD. They were found in small clusters or isolated, and they were elongated, spherical and stellate in shape, with an eccentric nucleus. They often had cytoplasmatic processes enveloping neighboring cells and near to sinusoids. The cytoplasmatic immunostaining was heterogeneous. MSH-immunoreactive cells were confined to the PI. They were elongated or spherical in shape, whit eccentric nucleus and cytoplasmatic immunostaining homogeneous. The most ACTH cells were also MSH positive. ACTH or MSH immunoreactive cells were not observed in the pars tuberalis. This study permitted to distinguish the localization of both cell types in the adenohypophysis. In PI the cells melanotrophs were immunostaining with the serum anti-ACTH due to that this hormone is forerunner of MSH. Nevertheless, themselves not the possibility is ruleed out that these cells synthesize ACTH.

26.

ULTRAESTRUCTURAL STUDY OF THE EPIDIDYMAL CORPUS OF VIZCACHA (*Lagostomus maximus maximus*)

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Vizcacha, seasonal rodent, revealed morphological changes in prior studies realized to light microscopy. The objective of this work was to study to electron microscopy (EM) different cell types in the pseudostratified epithelium of the epididymal corpus during seasonal reproductive cycle of vizcacha that understands an active period (summer-autumn) and a period of regression gonadal (winter). In the epididymal corpus were observed the following cell types: principals, basal, clear and halo cells. Active Period: principal cells revealed irregular nucleus and cytoplasm with RER radially arranged, numerous dense bodies and vesicles of apical position. The basal cells presented elongate nucleus with poor content of organelles. The clear cells showed oval nucleus and pale cytoplasm with poor development of organelles. The halo cells was located in the base of epithelia. They showed oval nucleus with chromatin placed to the nuclear wrapping and pale cytoplasm with scarce developed of organelles, dense bodies of forms and size variables. The lumen showed abundant residual material. Regression period: principal cells with cytoplasmic vacuoles, dense bodies and RER of radial disposition with glycogen were observed. The clear cells were more numerous in this period and presented a greater development of all their organelles. The basal and halo cells did not show morphologic variations important. The increase of dense bodies and vacuoles in principal and clear cells would involve mechanism of phagocytosis and endocytosis more activated of the epididymis in regression.

28.

ALTERNATIVE FORMULAS DESTINED TO COW MILK ALLERGIC CHILDREN

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Alternative formulas could change growth and/or immunologic parameters in cow milk allergic children. The most used are soybean (So), formula without lactose (SL). Equine milk (LY) would be an advantageous nutrient. The objectives of this worw are to study anti-LV, anti-LY and anti-So antibodies and quantify LB, LT and LTCD8(+) in LV allergic patients, and to compare the main proteins in each formula. 62 allergic children (ages 1 to 3) grouped depending on the main food ingested in the latest 6 months were studied: 9 having mother's milk (LM), 16 having So, 10 having SL, 9 having casein (Cas), 8 having LY and 10 control ones. Anti-LV antibodies were determined by double immunodiffusion (IDD) and passive hemaglutination (HAP). LT were quantified by spontaneous rosettes (RE), LB and LTCD8(+) by IFD. Proteins were determined by poliacrilamide electrophoresis (SDS-PAGE). Anti-LV antibodies were found by IDD in allergic patients, 1/8 having LY and 3/10 control ones. Two patients showed anti-LY antibodies. Using HAP, 2/8 having LY and 10/10 controls, showed anti-LV antibodies. Total LTs in the So group as well as in the SL group were lower than controls ($p < 0.02$). The So group showed diminished LTCD8(+) values compared with the ones showed by LM or LY groups ($p < 0.02$). Proteins of LV, So and native LY were identified as α-lactalbumines. In LY boiled for 10 minutes that band was absent. LY would be an adequate alternative food to replace and complete children's diet, specially when boiled for 10 minutes to modify the potentially allergenic protein fraction.

29.

STUDY OF MORPHOMETRICS PARAMETERS OF THE PORCINE PLACENTA BY IMAGE DIGITAL ANALIZER

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The porcine placenta is epitheliochorial, non-invasive, diffuse and folded, so that the maintenance of the pregnancy supports in the interdigitations of the epithelial trophoblastic cells with the epithelial uterine cells during pregnancy. The objective of this work was to study the tissue placental through an analysing digitalis of images that allow measuring the total area and the epithelial area of the placental tissue from 28, 55 days of pregnancy and at term. The macro is conformed by five main sections: imaginig, processing, segmentation, parameter measurements and finally the obtained results in pixels are transformed in micrometers. Result: in placentas of ± 28 days of gestation, total villi area was: x = 108.99 ± 48.76 µm and epithelial area: x = 51.58 ± 12.04 µm. In placentas of 55 days of gestation, total area was: x = 148.04 ± 17.93 µm and epithelial area was: x= 42.20 ± 17.43 µm. In placentas at term total area was: x = 201.99 ± 40.23 µm and epithelial area was: x= 66.99 ± 11.76 µm. These results suggest that the area of epithelial trophoblastic cells are more developed at the beginning of porcine placentation.

30.

MORPHOLOGY OF FABACEAE ROOT SYSTEMS

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Root architecture determines the efficiency of soil nutrient adcquisition and internal structure shows adaptation to the environment. The objective is to study root systems of *Fabaceae* species from central Argentina and to relate them to their habitat. Species of the following genera were analyzed: *Adesmia*, *Acacia*, *Caesalpinia*, *Coursetia*, *Desmanthus*, *Desmodium*, *Galactia*, *Geoffroea*, *Hoffmanseggia*, *Prosopis*, *Rhynchosia*, *Robinia*, *Senna*, *Stylosanthes*, and *Zornia*. Root system development was studied in the lab during the first year. Arboreal species were analyzed with exposed profiles. Transversal cuts and histochemical analysis were carried out according to D'Ambrogio de Argüeso (1986). The studied species show alorrhyzous systems with variants related to soil profile. Plagiotropic roots are observed in highland grass steppes (*Senna birostris* var. *hookerina, Senna subulata*) and in soils with CO_3Ca accumulation (*Prosopis caldenia*). Bud roots were found in *Acacia caven, Caesalpinia gilliesi, Senna aphylla, Geoffroea decorticans, Robinia pseudoacacia, Adesmia cordobensis*, and *Hoffmanseggia glauca*. In anatomical studies, the following variants are observed: 1) lignified, woody roots with predominance of xylematic area, 2) formation of big parenchymatic rays, 3) high parenchyma production in phloem and phelodermis, 4) *cambium* differential activity, 5) formation of accesory *cambia*. The last four structures produce an increase of parenchyma with water and starch accumulation. These structures are found in areas with hydric stress where the abundant starch, besides acting as reserve, when it hydrolyzes, produces an increase of water retention in the parenchyma.

31.

IMMUNOMODULATING PROPERTIES OF *Minthostachys verticillata (M.v.)* ON HUMAN LYMPHOCYTES AND BASOPHILS

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Minthostachys. *verticillata* is a traditional medical herb that shows antimicrobial activity against *Staphylococcus aureus*, antiviral properties against HVS-1 and bronchodilating effect in asthma. The objectives of this work are to investigate the stimulating ability over lymphocyte expansion of *M.v*. derivates compared with PHA-M, PWM and BCG; to characterize the proliferated cell populations and determine the ability of different doses of *M.v*. derivates to inhibit basophil degranulation. 16 patients presenting cow milk allergy and 12 non allergic ones were evaluated. In all cases the lymphocytarian transformation test was performed. Leukocyte cell cultures were prepared and stimulated with each of the mitogens or *M.v.* derivates. The LT population was charac-terized with murine anti human CD8 monoclonal antibodies by IFD. To compare the response against the specific antigen and *M.v*. derivates the basophil degranulation test was performed *in vitro*. *M.v.* derivates had mitogenic effects similar to the ones shown by PHA-M, PWM or BCG. The proliferation rates reached by *M.v*. derivates were higher than those shown by controls ($p < 0.001$). Over 40% of proliferated cells showed LT CD8(+). Doses of 100 µg/ml of decoction and 1.2 mg/ml of essential oil did not reach the minimums degranulation rate. Derivates of *M.v*. resulted to have mitogenic and immunomodulating activity on LT CD8(+) and did not cause basophil degranulation. As proinflammatory characteristics were not developed they could have enhanced the Th1 deviation on allergic patient's cell cultures.

32.

A COMPARATIVE ULTRAESTRUCTURAL STUDY OF THE PARS TUBERALIS OF VIZCACHA *(Lagostomus maximus maximus)*

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The pituitary pars tuberalis (PT) mammalian species extends from the median eminence and surrounds the infundibular stalk to the anterolateral zone of the pars distalis (PD). The PT is composed of specific granulated cells and folliculo-stellate cells. The mean diameter of the secretory granules and the presence of variable amounts of glycogen particles varies in a species-specific way. The purpose of this work was to compare the PT of the vizcacha *(Lagostomus maximus maximus)* with the PT of the other species. Adult male vizcachas were captured in their habitat, the pituitary gland was rapidly dissected and procesed by electronic microscopy. In PT of vizcacha two **specific granulated cell types** could be distinguished, mostly based on the diameter of the secretory granules: **type I** or the small granules: their diameter ranging between 60 and 200 nm. **type II** or the large granules: their diameter of about 150 - 500 nm. Both cell types exhibit well-developed endoplasmic reticulum, golgi, abundant cytoplasmic glycogen and numerous nerve endings on the plasmatic membrane. Therefore, this rodent represent an alternative experimental model for histophysiological studies of the PT.

33.

INCIDENCE OF HUMORAL IMMUNE RESPONSE DURING THE PORCINE GESTATION

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The placenta is the principal immunoendocrine organ during pregnancy. The molecular and cellular mechanism of the feto-maternal responses in the porcine placenta in connection with abortion remains unclear. The close similarity between swine and humans exists in the organization of their MHC locus, in the sequence of their MHC antigens and immunoglobulins and their κ/λ light chain ratio. The aim of this study was to determine the presence of IgG total in placental tissue in different gestational periods. By radial immunodiffusion, using human IgG, the average value in swine serum (n = 5) was: non-pregnant 26.88 mg/ml; pregnant 30 days 29.88 mg/ml; 91 days of gestation 19.24 mg/ml of IgG. Through direct immunofluorescence it was detected the presence of total immunoglobulin on fetal placental tissues at 28, 55, 70 days of gestation and at term. For this we used one antibody human anti-IgG conjugated with FITC. Fluorescence (++) on fetal placental chorionic villi in all the gestational periods was observed. This result is detected in fetal trophoblastic epithelial cells in contact with uterine lumen. Taking into account these results the antibodies detected in fetal swine placenta would pertain to the type of asymmetric protector antibodies. These results suggest that the humoral immune response has a very important function in the immunomodulation of swine pregnancy.

34.

NEW CONTRIBUTIONS ON THE METABOLISM OF GIBBERELLINS BY *Azospirillum* SPP

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Several mechanisms have been proposed to explain how the inoculation with *Azospirillum* spp. improves growth and development of many important crops. Phytohormone production, including gibberellins (GAs), is one of them. The objective of this work was to obtain an *in vitro* model for GA biosynthesis in *Azospirillum* spp., by using the bacterial ability to transform GA precursors in chemically defined medium. The bacteria were growth in selective NFb (nitrogen free broth) medium with addition of deuterated ([17,17,$^2H_2$]-GA) precursors. To identify and quantify metabolites of [17,17,$^2H_2$]-GA precursors from bacterial metabolism, capillary gas cromatography coupled to mass spectrometry (GC-MS) was carried out. The results obtained suggest the existence of at least two pathways for GA biosynthesis in *Azospirillum* spp. One, resembling the early 13α-hydroxylative pathway found in plants, with the main product being GA_1, and other like the non hydroxilative pathway of plants, which product is GA_3. The sequential production of these compounds may be divided in two phases: the first one involves the conversion of GA_{12} to intermediate precursors, GA_9 and GA_{20}, by P450DM; the second one involves transformation of the intermediate precursors to the biologically active GA_3 and GA_1 by 2OGDD.

35.

NOTE ON ARGENTINIAN SPECIES OF *HYPOLEPIS* BERNHARDI (DENNSTAEDTIACEAE: PTERIDOPHYTA)

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Hypolepis is a genus distributed in tropical and south temperate regions of the world. The number of species is estimated in 40, about 15 of them in America. It is characterized for being medium-sized to large, terrestrial, epiphytic or epilithic ferns, the stems long creeping and branched, bearing pluricellular hairs. Lamina remote, often large, 2-4 pinnate, surface often pubescent with pluricellular, sometimes glandular trichomes. Veins pinnate in the segments, free, ending behind the margin. Sori marginal, protected by a reflexed, more or less modified lobe (pseudoindusium). Spores monolete, ellipsoidal, with a reticulate or usually coarsely echinate, thin perisporium. Two species are found inhabiting Argentina: *Hypolepis repens* (L.) C. Presl and *Hypolepis poeppigii* (Kunze) Maxon. Until the moment, there is no enough information about this genus in Argentina, due to that, diagnostic features, illustrations, ecological characteristics and the differences between the species are included in this work. *Hypolepis poeppigii* is found inhabiting the south of Argentina and the south and central part of Chile. *H. poeppigii* and *Hypolepis repens* are the unique species of the genus in the austral part of South America. The main features that allow the identification of those species are the kind, proportion and position of the hairs on the lamina. *Hypolepis repens* has capitate, pluricellular hairs on the rachis, costae and costules, being glabrate between veins. *Hypolepis poeppigii* has glandular pluricellular trichomes and simple pluricellular hairs with acute apical cell, the second ones are found on the edges of the lamina, pseudoindusium and the complete surface of the blade. The last species is found in the forests with high humidity, inhabiting the banks of lakes and streams, often epiphytic on trees, sometimes forming large clones. *H. repens* is a rare species of local occurrence inhabiting the mountain regions up to 800 m a.s.l. which is found in the banks of the creeks.

36.

JASMONATES INVOLVED IN SALT RESPONSES IN TOMATO CV. *MONEYMAKER* AND ITS MUTANTS *TSS1* Y *TOS1*

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Jasmonates are a known group of phytohormones involved in biotic and abiotic stress. In this study we compared the jasmonate basal levels between the wild cv. *Moneymaker* and its mutants, *tos1,* hypersensitive to osmotic stress and *tss1,* hypersensitive to ionic stress. Changes in these compounds were then analyzed in response to salt stress. Seedlings with expanded cotyledons were treated with 100 mM NaCl for 11 days. Jasmonate(s) evaluation was performed by HPLC-GC-MS. NaCl treated-seedlings of *tos1* decreased the basal level of the jasmonic acid precursor, the 12-oxo-phytodienoic acid (OPDA) and its derivatives 11-OH-JA and 12-OH-JA; in *tss1* OPDA increased. In the cv. *Moneymaker* the only increase registered was in 12-OH-JA. Other jasmonates such as jasmonic acid and methyl-jasmonate did not modify by effect of the saline stress. These results suggest that NaCl preferentially affect the jasmonate biosynthesis pathway in the mutant *tos1*, hypersensitive to osmotic stress, while in *tss1*, hypersensitive to ionic stress, OPDA increased although no change in jasmonic acid was observed. The precursor OPDA is considered as a signal compound *per se*. Both mutants differed from the wild cultivar in their response to salinity.

37.

APPROACH OF PROBLEMATIC SITUATIONS AS A CENTRAL AXIS IN THE LEARNING PROCESS OF BIOLOGICAL CHEMISTRY FOR VETERINARY MEDICINE

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It is known that students do not enjoy learning Science and they neither get an intellectual satisfaction with courses given in the scientific area. This situation is present in Biological Chemistry and students usually comment about how bored and difficult are the subjects. Furthermore the performances are low, specially in the first years at the University careers. To understand the importance of the teaching-learning of Natural Science is necessary to consider an appropriate motivation, find a sense to the proposed contents and be able to learn them. Problem based learning is a "learning which results from the process of working towards the understanding of, or resolution of, a problem" (Barrows and Tambling, 1980). We outline here an alternative way of teaching Biological Chemistry based in the approach of Problematic Situations related with Veterinary Medicine. An specific problematic situation was proposed to the students related to the application of nucleic acids structure, replication, transcription and translation in Biotechnology and its implicancies in animal production. Different instructions of working were proposed with which students had to do a variety of activities including different degrees of difficulty. The students not only showed a great motivation for the study of the thematic but they also presented anxiety to try new problems of Biotechnology and its advantages in the animal production. We may conclude that through the approach of real situations related with the career and the knowledge construction by their own we can collaborate in the generation of the better and lasting knowledge.

38.

THE SIGNIFICANCE TO JOINT PRACTICE/THEORY IN CURRICULA DESING TO TECHNICIAN CAREERS

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The aim of this study was to analyze the practice/theory relationship such as an articulator nucleus of the curriculum of the Laboratory Technician career of the UNRC from pedagogical practice. From theory and curricular dimension we try to see the relationship between curriculum and teaching with special emphasis on the desing of career and subjet curriculum. We focus the attention on the university classroom and the learning/teaching process and consequenthly the significat apprenticeship using qualitative analyses methodology. This study provides analysis data from career curriculum, subjet curriculum, graduates, students, teachers surveys and class observations. To analize and valid data we applied triangular methodology between sources and methods. The results showed no coincidence between prescript curriculum and class activity. The increase of the number practice hours does not guarantee a significat apprenticeship. The results showed that to produce curriculum changes is necessary to take into account the scientific knowledge advance as well as the teaching perspective.

39.

HOME-RANGE OF COPPER-COLOURED LIZARD FROM PAMPA DE ACHALA: *Pristidactylus achalensis* (SAURIA: POLYCHROTIDAE)

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The animals choose, inside the habitat of the population, a specific place where they live. It is called home range and it is the place or specific area in which they develop their biological activities, like sunbathing, feeding and /or reproduction. *Pristidactylus achalensis* is one of the 20 endemics species from Pampa de Achala and it is protected inside the Parque Nacional Quebrada del Condorito (Córdoba). The aims of this work were to estimate the average size of home range of males, females and immature lizards and to determinate the sites of preference of this species. The data were obtained during five months, between November 2002 to March 2003. Nine set of data were taken in two sites (Z1= 13500 m^2 with 11 specimens and Z2= 4400 m^2 with 21 specimens). The lizards were captured and signed with beads placed round their necks with a copper wire. The distances between lizards were taken with a tape measure and laser binoculars (Bushnell Corp.) and transferred to an orthogonal system. The angles were measured with a Brumton compass. The data were analyzed with the software VISIO 3.0 and NIH IMAGE 1.7 and the size (perimeter and area) of the home range were calculated with the minimum convex polygon method. The average distance among lizards was 59.44 m in Z1 and 30.63 m in Z2. The average of the home range was 63.06 m^2 in Z1 and 13.94 m^2 in Z2. For females in Z1 and Z2 the average of home range was 45.84 m^2 and 15.62 m^2 for males. The Z1 area presents the 80 % of its area covered by grass and big granitic outcrops, just the 20% of the area is appropriate to lizard shelters. Z2 presents rocks of medium size (suitable for caves) covering the 80% of the area. This difference between the structure of the areas could explain the low number of lizards in the first area compared with the second one. The bigger size of the home range in Z1 could to be due to the low number of lizards in this area and a bigger distance among them which decrease the competition for space. The lower size of the home range of males could to be due to their territorial behavior.

40.

STRESS RESPONSE CHARACTERIZATION OF MICROBACTERIUM SP., A GRASS ENDOPHYTE

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One strategy to avoid negative interactions between native and introduced microorganism in the rizosphere is to employ endophytic microorganisms adapted to their plant host with an ecological advantage compared with indigenous rizosphere microflora. Therefore it is necessary to understand how those endophytic bacteria behave and respond under different conditions. In this sense the aim of this work is to characterizate the different stress responses of an endophyte of grasses, *Microbacterium sp* that is able to promote wheat growth under saline stress on greenhouse trials. Growth curves were made in Nutrient Broth supplemented with 5 mM; 10 mM and 20 mM peroxide hydrogen for oxidative stress; 250 mM and 1M NaCl for saline stress and growth at 42ºC for thermic stress. Comparing growth profiles and the viability between control and stress conditions, i.e. saline, thermic, and oxidative we can conclude that the strain is highly tolerant to the stresses tested. This resistance could indicate a broad range of adaptation that the microorganism could display in order to face environmental changes.

41.

POACEAE NOT PREVIOUSLY CITED FOR THE PROVINCE OF SAN LUIS

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San Luis includes two well delimited physiographic provinces: the "Pampeanas" hills and the "Chaco- pampeana" plain, broadly formed under temperate climatic conditions (12-18°C). The former comprises two mountains chains: the "Sierras de San Luis" and the "Sierras de los Comechingones", both of them being continuations of the great central massif of Argentina. The latter includes the quaternary sediments which have filled the spaces between the hills and have extended towards the south of the province. The soils have a sandy texture and poorly structured, deficient in organic matter, and low in fertility. As regards San Luis flora, it has been studied by different authors; at present, we are conducting flora surveys with special emphasis on the Poaceae, which make up the natural grasslands and are important fodder resources. Specimens were collected from different places and at different times of the year, and the herbaria of the (Facultad de Ingeniería y Ciencias Económico Sociales, UNSL) and VMSL (Estación Experimental Agropecuaria INTA San Luis) were consulted. As a result, 57 new Poaceae taxons-not previously cited for San Luis-were found: 12 species have a high value as fodder, 7 a medium one, 10 a low one, 4 none and 24 are of unknown value.

42.

SCREENING METHODS FOR POTENTIAL MYCOTOXIN PRODUCERS IN HARVESTED BLACK PEPPER FROM DIFFERENT REGIONS OF BRAZIL

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A mycological survey was carried out on 115 samples of whole dried black pepper seeds, from two main production regions of Brazil. A high incidence of contamination was verified on both regions when 99% of the samples showed filamentous fungi contamination. A total of 497 species of 9 different genera was isolated (*Aspergillus*, *Eurotium, Rhizopus, Penicillium*, *Curvularia*, *Cladosporium*, *Absidia, Emericella* and *Paecilomyces)*. The genus *Aspergillus* was the predominant (53.5%) followed by species from *Eurotium* genus (24.5%). *Eurotium chevalieri* (16.4%) was the most predominant species followed by *Aspergillus flavus* (14.6%) present on 55 samples of black pepper (47.8%) analyzed. Twenty five samples (21.7%) were contaminated with aflatoxigenic strains of *A. flavus* and *A. parasiticus*. In relation to the types of aflatoxins produced by mycotoxigenic strains, it was observed that twenty five strains (44.6%), out of 56 isolated of *A. flavus* produced aflatoxins. Among these, 28% (7/25) of the strains produced aflatoxin B_1 (AFB_1), and 72% (18/25) produced AFB_1 and AFB_2. One out four (25%) isolates of *A. parasiticus* produced AFB_1, AFB_2, AFG_1 and AFG_2. From 12 samples, *A. ochraceus* species were isolated in low frequency (3.5%). Two strains of *A. ochraceus* from a total of 16 isolated were producers of ochratoxin A (OA). With respect to the aflatoxins and OA natural contamination, none of the samples presented detectable levels of these mycotoxins using thin-layer chromatographic analysis.

43.

CHANGES IN TEACHING STRATEGIES TO IMPROVE STUDENTS' ACADEMIC PERFORMANCE

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In 1992, the Lab Technician career curricula was modified to improve the graduates' working possibilities. A new subject, Laboratory III, was included in the last year of the career. This subject deals with those techniques graduates need to handle for diagnosing infectious illnesses and food poisoning. It was originally designed in 4 modules, fragmenting in this way the students' knowledge and the acquisition of practical abilities. Surveys that were carried out among students showed that most of them had difficulties to obtain passing marks; making it clear a strong need for changes in learning strategies. First, we worked on content articulation; then, students practical abilities were fostered through practical real situations; finally, group work was promoted to allow then discuss and analyze problems inherent to their own professional practice. These changes were implemented in 2000. Three years later, the effect of these changes on students' performance was evaluated. This evaluation showed that 70% of the students passed the subject with high marks. To conclude, we believe the methodological changes implemented since 2000 have clearly contributed to the students' performance improvement.

44.

OCCURRENCE OF OCHRATOXIN-A IN WINE AND GRAPE JUICE FROM RÍO DE JANEIRO, BRAZIL

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Ochratoxin A (OA) is receiving attention worldwide because of the hazard it poses to human health. The aim of the present study was to test the distribution of OA in grape-juice, pulps of frozen grapes national and imported table wine obtained from markets in Río de Janeiro, Brazil. Analytic methodology using on immunoaffinity column with a final separation using on reverse phase (C_{18}) and fluorescence detection in HPLC showed a limit of detection of 21 ng L^{-1} The mean recovery was 91% for red wines and 82% for white wines. While the mean recovery for juices and wine grapes was 81 and 79% respectively. Out of 64 samples of grape juice and frozen pulps, 25% were positive for OA, the mean content being 37 ng L^{-1} with a maximum concentration of 98.8 ng L^{-1}. In wines, 28.7% of 80 samples were positive with levels ranged from 21 to 71 ng L^{-1} (mean concentration 36.6 ng L^{-1}). OA was detected in red, white and rose table wines samples at median concentrations of 36.6, 26 and 35.4 ng L^{-1}, respectively. The examined red wine presented the highest percentage of contaminated samples (38%) and the highest levels (37 ng L^{-1}). The white wine presented levels above 26 ng L^{-1} in 17.7% of the analyzed samples. Risk assessments analysis showed that levels of contamination detected in red wine sold in Río de Janeiro, Brazil, was not enough to surpass the secure virtual dose, established in 5 ng Kg^{-1} of body weight day by intake.

45.

EFFECT OF ANTIOXIDANTS MIXTURE ON BACTERIAL POBLATIONAL SUCCESION ON MAIZE STORED

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This study was carried out to determine the effectiveness of butylated hydroxyanisole (BHA) and propyl paraben (PP) combination on bacterial population succession on stored maize. Maize kernels were treated with an antioxidant mixture. The kernels were stored in an australian type silo (100 kg). Five maize samples at each sampling period were collected. Sub-samples of each sample were obtained, and a total of six sampling period were done (period June/ November 2002). Enumeration, isolations and identification of bacterial propagules were done. Bacillus Gram (+) with spores population were maintained until fourth sampling period in maize treated with antioxidants, while a 100% of contamination were found in fifth sampling period in maize stored without antioxidants. Cocobacillus Gram (+) were maintained until third sampling period at both maize stored. And bacillus Gram (+) were found at first sampling period in control silo. The fact that bacillus Gram (+) with spores were maintained in the majority of sampling period may be the result of endospores in these microorganisms. Generally, endospores from *Bacillus spp.* are resistant to dry and destruction by antimicrobial chemical. These resistance mechanism could explain the presence of sporulate bacillus during storage period.

46.

ACADEMIC PERFORMANCE IN FINAL EXAMS FROM AN INNOVATOR CURRICULAR PROJECT IN THE TEACHING OF BIOCHEMISTRY IN VETERINARY MEDICINE

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Biochemistry is a discipline that study the biological structures and their functioning in the organisms with the basis of the chemistry. The students arrived to the university with a natural apprehension to exact science. The present curricular project was stated in the motivation, the integrated curricular theory, the dynamic theory and the interdisciplinary work. It was specially considered the thematic sequence based in the use of atomic models for biomolecules, their structure- function relationship and subcellular, cellular and tissue localization in the normal and pathologic physiology of the animal. The methods used were theory moments with color multimedia support, interactive workshops, an integrative activities in laboratories and with animated CD-Rom. In the academic performance evaluation the students opinion revealed: the learning of biochemistry was good (27%), very good (12%), even though difficult. The students preferred the CD- Rom method (18%) and the workshops (16%), that is, the innovative activities. From 234 students, 60% obtained regular condition and 88% passed final exams. It seems an innovative and successful project, because of the comprehension of the reasons and the purpose of the discipline. Moreover, the final evaluation, last instance of the learning, has been congruent with the method used in the construction of the knowledge.

47.

NEW DATA ON THE INHERITANCE OF THE ¨XY FEMALE¨ CONDITION IN *Akodon azarae* AND THE PROBLEM OF FERTILE XY FEMALES OF *AKODON* (RODENTIA, SIGMODONTINAE)

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In nine species of *Akodon*, the presence of an important proportion of XY fertile females has been observed. This fact results of great interest for the analysis of the mechanism of sex determination. Up to now, we have studied 195 animals of one of these species, *Akodon azarae*, comprising specimens trapped at Chucul (province of Córdoba) and their descendants. The correlation of the sexual phenotype of XY individuals with the X chromosome C-banding pattern is confirmed: males present an X of type 1 (X_1) and XY females have either a type 2 or a type 3 X (X_2, X_3). All XX females carry an X_1 chromosome, while its homologue can correspond to any of the three types. The analysis of the offspring of 21 matings let us corroborate the transmission of X_2 and X_3 chromosomes to the progeny, and that the reception of an X_2 or X_3 chromosome by an XY individual, determines the fertile female phenotype. Six of the XY females produced came from X_1X_3 mothers and carried the X_3 as expected. The occurrence of XY females in the offspring of XX mothers has not been reported in other authors studies. These data, together with those obtained from *in situ* hybridization (FISH) experiments by other investigators in *Akodon montensis* from Brazil, indicate that sexual reversion is probably due to a mutation of a gene of the X chromosome that as its result does not respond to *Sry*, a mutation probably originated by a chromosomal rearrangement.

48.

ATACK OF THE PHYTOPHAGOUS NEMATODE *Globodera tabacum* (LOWNSBERY, 1954) BEHRENS, 1975 ON TOBACCO CULTIVAR IN THE PROVINCE OF JUJUY, ARGENTINA

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Tobacco (*Nicotiana tabacum* L.) is the most widely grown commercial non-food crop in the world and plant-parasitic nematodes can cause serious damage to them. In the north of Argentina, the presence of the nematode *G. tabacum* has been detected in tobacco *Virginia "cv. 493"* roots. Different studies have been carried out to evaluate the nematode-host relationship through the analysis of histogical alterations induced in the tissues attacked by the pathogen. Root sections, healthy and attacked by the nematode, were fixed in FAA and processed according to conventional techniques for optical microscopy. The cortex and the vascular cylinder showed modifications that derived in the formation of syncytia and in an important reduction of vascular tissues. The plant response corresponds to hosts susceptible to the pathogen. This completes its life cycle forming cysts filled with eggs on the roots. The nematode population and the cultivar studied have a close relationship between each other. The incidence of the pathogen on the production of the cultivar has not been evaluated yet.

49.

EFFECT OF STRUCTURAL HETEROZYGOSITY ON THE SPERMATOGENESIS OF *Graomys centralis* AND *G. Griseoflavus* (RODENTIA, MURIDAE) HYBRIDS

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In the center of Argentina, *Graomys* is represented by two species: *G. griseoflavus* (2n = 36, 37 or 38) and *G. centralis* (2n = 42). Since the reproductive isolation between these species is recent they are very similar at morphological and structural gene levels. The males of interspecific hybrids are sterile. In this study, we analyzed the effects of the Robertsonian heterozygosity on the gametogenesis in *G. centralis* and hybrids mice. The hybrid animals were obtained by crossbreeding among male *G. centralis* and female *G. griseoflavus*. Testis were fixed with Bouin reagent, sectioned at 5 µm thick and stained with PAS-hematoxilin. Spermiogenesis in *G. centralis* was divided into 12 stages. Each of these stages was found to have a well-defined cell association. The spermatids and the spermatozoa of hybrid animals were not observed, while the spermatogonia and the Sertoli cells were identified. The more differentiated germinal cells were primary spermatocytes in pachytene phase of meiosis. These results can be due to the incompatibility of haploid complements of the parents for the normal mating and disjunction of the meiotic chromosomes in the hybrid nuclei. Sterility of hybrid males is a mechanism of post-zygotic reproductive isolation and might have promoted an isolation reinforcement by pre-zygotic and premating mechanisms described in previous studies. The existence of these reproductive barriers explains the absence of hybrids in regions where the two species superpose their distribution areas.

51.

GEOGRAPHICAL VARIATION IN THE ADVER-TISEMENT CALL OF *Physalaemus biligonigerus* (ANURA: LEPTODACTYLIDAE) IN THE PROVINCIE OF CÓRDOBA

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Advertisement call of anuran amphibians constitutes an effective mechanism of reproductive isolation, it is used in taxonomic studies and contributes information that allows clarifying relationship among populations. The objective of this work is to compare calls among isolated populations of *Physalaemus biligonigerus* in the province of Córdoba under the supposition of the existence of cline variation. Acoustic records corresponding to 6 localities of Córdoba were analyzed (San Clemente, Cañada del Sauce, Río Cuarto, Alejandro, San Marcos and La Carlota). The total size of the sample was 120 calls (14 males). The temperature in the moment of the signal record was in a range of 18 to 22ºC. The signals were analyzed through oscilograms and sonograms (software Canary V.1.2.4). The variable considered in the oscilograms were: duration of the sectors 1 and 2 (DS1 and DS2), duration of 10 pulses of each sector (DPS1*10 and DPS2*10), measured in ms; in the sonogram: higher frequency (FA) and lower (FA), measures in Hertz; the latitude of each locality was included as an independent geographical variable. We used descriptive statistic to analyze the variations of each variable; and multivariate discriminant analysis, with highly significant difference among variables (Wilks' = 0.0397; $p < 0.0000$) and cluster analysis for classification. We conclude that differences exist among the call of the studied populations, putting on in evidence the tendency to a gradient cline, that it could be awarded to the geomorphologic, fitogeographic and latitudinal characteristics of the localization of the six studied populations.

50.

GASTROINTESTINAL PARASITES OF GUANACOS (Lama guanicoe) OF MIDWEST ARGENTINA (MENDOZA AND SAN JUAN)

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Since there is a growing interest in the conservation and the use of the guanaco in farming systems, its necessary to know more about its parasites. The objective of this work is to gain knowledge about the parasites in captive and wild guanacos of midwest Argentina. 35 fecal samples were collected, 19 during May 2002 (6 in Reserva Don Carmelo [Puna] and 13 from Reserva La Payunia [Patagonia, Mendoza]). During 2003, 16 captive animals were sampled. Fecal samples were examined by modified Teuscher technique, double sedimentation and sporulated with potassium bichromate. The following parasites were found: ova of *Nematodirus* spp. and *Trichuris* spp., *Eimeria macusaniensis* and *Eimeria* spp. Significant differences were not observed for the prevalence of *Nematodirus* spp and *E. macusaniensis* between wild and captive populations ($p > 0,05$). *Trichuris* spp. was found in less than half of the animals. *Eimeria* spp. had a greater incedence amongst captive animals and significant differences were not found between the two wild populations. *Nematodirus* spp, *Trichuris* spp. and *E. macusaniensis* are the parasites most frequently found in these guanacos. Finding *Nematodirus* and *Trichuris* in captive and wild (from different zones) suggest an ample distribution and capacity to adapt to different ecological conditions.

52.

HEMATOLOGY OF CAPTIVE GUANACOS (Lama guanicoe) OF MENDOZA PROVINCE

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Hematological parameters are critical to assess animal health. There is limited information with respect of the guanaco (*Lama guanicoe*). The objective of this work is to describe hematological parameters in captive guanacos. During May 2003 blood was collected from the yugular vein of 12 captive guanacos, 6 male and 6 female, from Malargue and Mendoza Zoo. The animals were in corrals and fed alfalfa hay and supplemented with feed. Thin blood films were fixed with methanol and Giemsa stained for white blood cell (WBC) differentials, WBC counts performed on Neubauer haemocytometer. Packed cell volume was determined by the microhematocrit.
Results:

	Mean	(SD)	Range
Hematocrit (%)	37	(5.26)	28-44
WBC µl	8418	(3690)	3200-16250
Neutrophilsµl	4301	(3026)	1760-13243
Bands µl	80	(135)	0-487
Lymphocytes µl	2973	(1696)	943-5450
Monocytes µl	137	(87)	0-298
Eosinophils µl	1081	(854)	160-2504
Basophils µl	2	(8)	0-27

The number of leukocytes were lower than those reported in wild caught guanacos of Patagonia. Eosinophils counts were higher than those reported in captive guanacos in Chile.

53.

ADVERTISEMENT CALLS IN THE GENUS *MELANOPHRYNISCUS*: POBLATIONAL STUDIES IN CORDOBA AND BUENOS AIRES

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The advertisement call is an important element in the acoustic communication of anurans. It could be utilized to attract conspecific females and to delimitate a territory, furthermore it is a powerful mechanism of reproductive isolation. The acoustic signals are used in amphibian systematic and are useful in elucidating phylogenetic relationships. *Melanophryniscus* is a monophyletic genus. Taxonomist recognized 13 species segregated in 3 groups. The *stelzneri* group contains *M. stelzneri* that inhabits Córdoba Mountains. The taxonomic position of the populations of austral hills of Buenos Aires is under study at present. It were analyzed the advertisement calls of two populations of *Melanophryniscus stelzneri* inhabiting the Córdoba mountains (Atos Pampa (APA) and Río de los Sauces (RSA)) and one population inhabiting Sierra de la Ventana, Buenos Aires (SVE) which until the moment has no nomenclatural position. Samples were: APA (n = 17), RSA (n = 31) and SVE (n = 29). The acoustic signals were obtained during 1999 in Córdoba and 2000 and 2001 in SVE using a digital recorder. Temperatures of air and water were also taken. The software Canary 1.2.4 was used for the digital sound analysis. Each emission was characterized by call length, first segment length, second segment length, number of pulses of the first and second segments, dominant frequency, number of harmonics and modulations. The comparison between populations was carried out using analysis of Principal Components. Temperature range was 20-23ºC. Significant differences among calls of the three populations were found and the two first factors taken explained the 87.1% of accumulated variance. The calls analyzed from APA and RSA had harmonics (between 0- 2), in SVE were between 0 and 4. There were no modulations in calls of APA while in RSA and SVE the modulations were slight. The results show the existence of significant differences in the advertisement calls at regional and sub-specific level of entities of the genus *Melanophryniscus*.

54.

DETECTION OF *Pratylenchus alleni* FERRIS, 1961 (NEMATODA: TYLENCHIDA) IN RASPBERRY ROOTS IN THE SOUTH OF ARGENTINA

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The analysis of raspberry roots (*Rubus idaeus* L.) var. Heritage from Neuquén, allowed to observe the presence of specimens of the lesion nematode *P. alleni*. Studies on the damage caused by *Pratylenchus* spp. on *Rubus* have shown that the nematode was found in the radical system, affectting the roots mainly in their absoption function. To evaluate nematode–host relationships through the histological analysis the infected roots were cut in small pieces, fixed in FAA and processed until their inclusion in histowax. Histological sections of 9 µm of width, stained with hematoxylin-safranin-fast green, were analysed with optical microscope. In the parenchyma tissue of cortex, larvae, adults and eggs could be observed. The adult nematodes appeared related to the development of alleys delimited by functional parenchyma. Empty cells were also dectected. Very developed vesicular-arbuscular mycorrhizae were also observed. The incidence of the nematode on the production of raspberry cultivar in the south of Argentina is not known yet.

55.

EFFECT OF CALTRIN I ON THE PROGESTERONE-INDUCED ACROSOME REACTION IN MOUSE EPIDIDYMAL AND EJACULATED SPERM

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It is known that caltrin I (calcium transport inhibitor) binds to the acrosomal region on the head of epididymal sperm and inhibits acrosomal proteases release and/or activity by blockage of extracellular Ca^{2+} uptake and spontaneous acrosome reaction (AR) To analyze if caltrin affects the activity of progesterone, a physiologic inducer of the acrosomal exocytosis, the functional state of epididymal and ejaculated sperm submitted to *in vitro* capacitation and induction of AR by addition of 15 µM progesterone was evaluated using the chlortetracycline fluorescence assay (CTC). Epididymal spermatozoa were capacitated in the absence or presence of 0.01 mg/ml caltrin while ejaculated sperm recovered from the female reproductive tract 2-4 h *post-coitus* were maintained in capacitation medium in the absence of caltrin. In the last cells, the presence of bound caltrin was confirmed by indirect immunofluorescence. Epididymal spermatozoa showed 15.1% spontaneous AR in absence of caltrin while 8.5% was recorded in presence of the protein. Ejaculated spermatozoa showed 7.2% spontaneous AR. By addition of progesterone the spontaneous AR of epididymal sperm increased to 41.1 and 49.7% in absence and presence of caltrin respectively and to 42% in ejaculated spermatozoa. Results indicate that caltrin inhibition on extracellular Ca^{2+} uptake reduces premature exocytosis to guarantee acrosomal integrity required for sperm-zona pellucida primary binding but does not interfere with the AR promoter action of progesterone.

56.

ECOPHYSIOLOGY OF FLUORESCENT 51B *PSEUDOMONAS*

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Bacteria exerting beneficial effects on plant development are termed PGPR. The gratest number of strains are members of the fluorescent *Pseudomonas*. With the purpose of studying effects of fluorescentes 51B *Pseudomonas* on biological parameters of *Capsicum annum*, two lots were inoculated: (A) before sowing, (B) was germinated in germination chamber, inoculated and maintained at 4ºC 14 hours before sowing. In each lot two treatments (T1 and T2) and a control (T) were established. T1 received 10^7 ufc /ml and T2 10^6 ufc/ml. The experiment was conducted in pots under natural conditions. Plant height, flowering, fresh and dry weight from shoots and roots were evaluated at 80 days for (A) and 60 days for (B). No significant differences were found in each lot; however, significant differences ($p \leq 0,05$) between lots were observed for all biological parameters. Fluorescentes 51B *Pseudomonas* revealed beneficial effects on plants in (A) and inhibitory effects in (B). The initial inoculum size was the same for both, but (B) was submitted to cold. This allows to conclude that ecophysiological behaviour of 51B can be regulated by environmental factors; in this case, by cold, which determined the form of interaction with the host, on whose growth it produced an inhibitory effect.

57.

PRELIMINARY *T. cruzi* SEROLOGICAL STUDY AND ELECTROCARDIGRAPHIC EVALUATION OF CANINES FROM BARRIO LA FAVORITA, MENDOZA

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Dogs are the main domestic reservoir of *T. cruzi*. They have shown to serve as sentinels of vector transmission in control programs. Even though Mendoza is an endemic region, studies regarding reservoirs is scarce. The objective of this work is to evaluate the prevalence of *T. cruzi* infection in dogs that are taken to the neutering center of the Mendoza City Municipality at Barrio La Favorita, a geographic zone considered to be of high economic, social and sanitary risk, actually under a program of vector control. All dogs that were taken to the center on Tuesdays during October-December 2002 were sampled. Diagnosis of *T. cruzi* was made by Indirect Hemo Aglutination Test, sera with a titer equal or greater than 1/16 would be confirmed by ELISA. Electrocardiograms (standard leads) were obtained from all dogs. 35 dogs were studied (16 male, 19 female) with an age from 4 months to 10 years (mean 1.5 years). All 35 of them were seronegative for *T. cruzi*. Only one dog showed an abnormal EKG. If the results of this preliminary study are confirmed, they would indirectly suggest that an effective control of vector transmission has been undertaken.

58.

SEASONS EFFECT ON THE PROGESTERONE LEVELS IN THE FIRST THIRD OF THE PREGNANCY IN SOWS RAISES IN INTENSIVE OUTDOORS SYSTEMS

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The fertility is an important parameter in the pig farms rentability. Subfertility problems was reported in summer, the nutrition and the environmental temperature could be involved. The objetive of the study was to determine the plasmatic levels of progesterone (P), LH, PRL, glucose (GLUC) and free fatid acids (NEFA) comparing the periods summer-autumn (S-A) and winter (W) in intensive breeding outdoor sows. The blood samples were obtained by the cranial cava vein puncture in oestrus sows and with 20 (P20) and 40 (P40) days of pregnancy, during 3 months of S-A and 3 months of W. NEFA, GLUC, LH, PRL and P were quantified. The maximum and minimum temperature was registered daily. The results showed lower P levels in the S-A than in W in P20 sows, without any difference into de NEFA and GLUC levels; although the sows were exposed to high temperatures (higher than 25ºC) around 8 hours in the day during the summer. Thus, the changes in the P are not consequence of a low voluntary feed in take produced by the high temperatures. Also, the low P levels seem to be independent of the LH and PRL levels, since both hormones don't show differences among the seasons. In conclusions, the P20 sows have low levels of P in the S-A, which is not a consequence of a lower feeding in take neither a low LH concentration. This smaller P concentration would make that sows mated in the summer are more sensitives to the environmental factors what would put in risk the pregnancy mainteinance.

59.

GENE HOMOLOGUES OF *Pseudomonas aeruginosa* PHOSPHORYLCHOLINE PHOSPHATASE IN OTHERS SPECIES OF PSEUDOMONAS

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Phosphorylcholine phosphatase (PCP) and phospholipase C (PLC) are produced by *Pseudomonas aeruginosa* grown with choline, betaine or dimethylglycine. Choline and both enzymes have been involved in the bacteria pathogenesis. Different tissue membranes of the host organism are degraded as follow: PLC hydrolyses phosphatidylcholine to DAG and phosphorylcholine. The last compound, by PCP action, produces choline and Pi. Recently, the responsible gene of PCP activity, *P. aeruginosa* PA5292, was identified. The aim of this work was to know if the proposal mechanism was specific for *P. aeruginosa*. PA5292 gene was amplified by PCR and used as a probe in Southern blot experiments. Homology was found in the plant pathogenic species: *P. cichorii, P. syringae, P. agarici, P. tolaasii, P. aspleni* and in the non-pathogenic species *P. fluorescens, P. putida, P. aureofaciens* and *P. fragi.* Parallelly, PCP but not PLC activity was detected. Bioinformatic analysis revealed the presence of *pcP* homologues in the *P. putida*, *P. fluorescens, P. syringae* pv syringae and pv tomato genomes. The identified genes are: PP5130, Pflu3115, Psyr3119 and PSPTO0436, respectively. Homologue genes for PLC were not found. Taking together, our results indicate that the studied species do not share a similar infection mechanism with *P. aeruginosa*.

60.

CIRCADIAN PARAMETERS MODIFICATION IN RAT PUPS BORN FROM MOTHERS WITH SUPRA-CHIASMATIC NUCLEI LESION

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In mammals, the circadian clock of fetuses and neonates is synchronized during the development to environmental cycles of 24 hours for circadian signals transferred by the mother. The circadian rhythms of individual rats, maintained under constant conditions, show rhythmic behavior synchronized among them and with those of the mother. In our laboratory we demonstrated that the extirpation of the maternal superior cervical ganglia (SCG) or the pineal gland during the early gestation interrupts the synchronization of the enzymatic and behavioral rhythms in rat pups. The effects of SCG extirpation on the maternal synchronization would be mediated by the pineal gland. Here, we studied if the lesion of the suprachiasmatic nuclei (SCNx) in day 7 of gestation affects the period, phase, amplitude and alpha of drinking rhythm. In rat pups born from mothers with SCNx, drinking behavior were monitored in constant darkness for 24 days beginning at weaning. The period, alpha, amplitude and phase values were tightly clustered in pups from each control group, while in pups from each SCNx group a significant dispersion was observed. The distribution of rhythmic parameters between litters in the SCNx group was significantly different to that of the control group ($P < 0.01$). In conclusion, the modification of the rhythmic parameters suggests that the maternal SCN lesion would disrupt the pups circadian clock synchronization.

61.

CHARACTERIZATION AND PGPR ACTIVITY OF PEANUT NATIVE MICROSIMBIONTS

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The increase in the yield of some leguminous plants inoculated with selected rhizobia strains has been associated with the rhizobia ability to act as plant growth promoting rhizobacteria (PGPR). Several researches show that rhizobacteria may also exhibit growth-promoting effects on not leguminous plants. Like other PGPR, rhizobia produce siderophores, cyanides and show antagonistic activity against phytopathogen fungi. They are also able to solubilize organic and inorganic phosphate. Because of their adaptation to the ecological conditions from a local soil, the native strains are preferentially used to inoculate a determinate area. Thus, the genotypic characterization, the symbiotic performance determination as well as the identification of strains with other PGPR properties is recommended. Methods: tolerance to adverse conditions, phosphate solubilization, siderophores production and antifungical activity, ERIC-PCR. Three of the six isolates studied were fast growers. No relationship was found between growth rate and tolerance to adverse conditions. Fifty percent of the isolates was able to solubilize inorganic phosphate or produce siderophores and 67% showed antagonism against plant pathogenic fungi. Isolate NLH 32 showed all the PGPR activities tested. The fingerprint profiles obtained with primers E1/E2 showed a great heterogeneity between the isolates.

62.

SYNCHRONIZING EFFECTS OF MELATONIN AND DIAZEPAM DURING THE RAT DEVELOPMENT

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During gestation or neonatal period the mammalian circadian pacemaker can be synchronized by several rhythmic cues transduced from the mother. We have previously shown that maternal pineal denervation during early pregnancy, disrupts maternal synchronization of testis malate dehydrogenase (MDH) activity and drinking behavioural rhythms in rat pups. On the other hand, a correlation between brain GABAergic activity and melatonin effects on adult rodent behavioural parameters has been demonstrated. Moreover, melatonin or diazepam injections to denervated mother during the last week of gestation, synchronized pups MDH activity In this study melatonin and diazepam synchronizing effect on drinking behaviour rhythm in pups born from pineal denervated mothers was analysed. Removal of the maternal superior cervical ganglia was performed on day 7 of pregnancy. Each drug was injected to pregnant mother from day 16 to 20 of gestation (18:00 h). Mothers delivered and reared their pups in constant darkness and circadian drinking behaviour was registered in pups during three weeks after weaning. Pups born from melatonin or diazepam treated denervated mother and control group show behavioural parameters highly clustered but significantly different from pups born from denervated mothers group. These results support the hypothesis that melatonin share with diazepam a synchronizing GABAergic pathway.

63.

ISOLATION, IDENTIFICATION AND CHARACTERIZATION OF *Pseudomonas syringae* PV. *coronafaciens* IN A*vena sativa* PLANTS

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Avena sativa is a plant of agricultural and economical interest valuable for human and animal consumption. In our country, this crop is affected by fungal and bacterial diseases that decrease its yield. Our laboratory has been studying the choline influence (or any of its metabolites) in enzymes induction involved with *P. aeruginosa* pathogenesis for a long time. So, it was found interesting to begin the study of phytopathogens *Pseudomonas* and the possible relationship between choline and the production and maintenance of infection. Bacterial colonies isolated from Suco rural zone oat samples with bacteriosis symptoms were pathogenic for healthy oat plants and the subsequent re-isolation confirmed Koch postulates. Taking into account biochemical and physiological tests, the isolation was identified as *P. syringae,* belonging to phytopathogen fluorescent *Pseudomonas.* It was proved through studies of virulence and pathogenicity factors that the microorganism produced siderophores, levansucrase, lipases, proteases, lecitinase, ice nucleation activity and tabtoxin. Plants, bacterial and fungal glutamine synthetase is inhibited by this toxin. Tabtoxin production and phosphoryl choline phosphatase (PCP) activity were favored by choline when it was used as carbon or nitrogen source. Our results support the importance of choline and its metabolites for this bacterium in phosphorous and nitrogen metabolism. It is concluded that the isolated and characterized bacterium is the oat halo blight causal agent, *P. syringae* pv. *coronafaciens*.

64.

DEVELOPMENT AND MATURATION OF THE PITUITARY-GONADAL AXIS IN THE *Lagostomus maximus maximus*. MORPHOLOGICAL AND BIOCHEMICAL STUDY

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The puberty in mammals is not a single event occurring at one time, but is an acceleration of developmental processes that have being under way since the start of sexual differentiation, and that no one so far has been able to define this sequence of events in a way that accounts for differences between the sexes and mammalian species. The stimulation of increased circulating levels of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) is a good index of the change in responsiveness of the hyphophysial-gonadal axis from the time of birth to sexual maturity. In the present study the growth and the maturation of the pituitary-testicular axis were examined by morphological and biochemical studies in adult (A) and immature (I) male vizcacha. Body (BW), pituitary (PW) and testicular (TW) weight were measured. FSH and LH were determined by IRMA and double-antibody heterologous RIA. Plasma Testosterone and Estradiol were measured by RIA. Results are showed in the table. A close relationship between endocrine system and maturation processes was observed in this roedor.

	PC (kg)	PH (mg)	PT (g)	FSH (mUI/ml)	LH (ng/mgj)	T (ng/ml)	E (ng/ml)
A	5.5± 0.35	49.81±2.20	5.98± 0.30	8.48 ± 0.89	6.30± 0.58	2.12± 0.17	2.59±0.6
I	2.3±0.50	20.21±1.61	0.28± 0.08	18.28±2.79	1.35± 0.13	0.80± 0.12	ND
	p<0.001	p<0.001	p<0.001	p<0.0001	p<0.01	p<0.005	

65.

LEGUME GENES INVOLVED IN THE NODULE AND LATERAL ROOT FORMATION

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During the symbiotic interaction of bacteria of the family Rhizobiaceae with legume plants, a specialized organ is formed from this interaction, the root nodule, in which the bacteria reduce atmospheric nitrogen to ammonia, The plant then uses this ammonia for amino acid and protein synthesis, in turn supplying the rhizobia with carbon. The induction of several plant genes accompanies nodule organogenesis and functioning. There is considerable evidence demonstrating that nodule and lateral root formation in legume plants are closely related, in particular, the similar patterns of gene expression. We investigated the differences in the gene expression of an early nodulin gene (*ENOD40*) and an expansin gene (*MaExp1*) in *Melilotus alba* (white sweetclover) roots, comparing uninoculated roots and roots with *Sinorhizobium meliloti*. We utilized RT-PCR, northern and *in situ* hybridization analysis on the roots to determine the transcript levels and localization. Our results showed that *ENOD40* and *MaExp1* were expressed in both nodules and the lateral roots, and that the transcripts were localized to comparable tissues. Although the role of these genes in the nitrogen-fixing system in legume plants is still under investigation, *ENOD40* and *MaExp1* from *M. alba* can be added to the growing list of genes with similar spatial patterns of expression in both organs.

66.

PRENATAL ALCOHOL ADMINISTRATION AND OLFACTORY STIMULI EFFECT ON GLUTAMIC ACID DECARBOXYLASE ACTIVITY OF THE RAT OLFACTORY SYSTEM

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Previous works have demonstrated that alcohol prenatally treated rats showed an abnormal preference to olfactory stimuli from a male or a female. In the present work, we have studied the glutamic acid decarboxylase (GAD) activity in olfactory bulbs, the accessory (BOA) principal (BOP) and amygdaloid area that contains the medial and central nuclei, for female and male rats prenatally treated with an alcohol dose of 1.5 g/k (from days 14 to 17).Males and proestrous females, were killed at 01:00 h. Female alcohol prenatally treated rats showed an increase in GAD activity of BOA ($p < 0.05$) and BOP ($p < 0.01$) compared to rats prenatally treated with saline solution (controls). However, in males, this alcohol dose induced a decrease of enzymatic activity only in BOA ($p < 0.05$). No changes were observed in the other cerebral areas of females and males. The olfactory stimuli from an intact male, over 1 h, decreased both GAD activity of BOA ($p < 0.01$) BOP ($p < 0.01$) and the amygdaloid area ($p < 0.01$) of females prenatally treated with alcohol. In males, alcohol prenatally treated, olfactory stimuli from an estrous female promoted an increase of GAD activity of BOA ($p < 0.01$) and BOP ($p < 0.05$). These results indicate that depending of the area studied, an alcohol dose of 1.5 g/k affects GAD activity of females and males differently. Alcohol plus olfactory stimuli reverted BOA enzymatic responses in females as well as in males.

67.

***IN VITRO* FERTILIZATION AND INTRACYTOPLASMIC SPERM INJECTION IN THE MONGOLIAN GERBIL (*Meriones unguiculatus*)**

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The Mongolian gerbil is a rodent in which the copulation rate varies among individual breeding pairs. No reliable *in vitro* fertilization (IVF) techniques have established so far. In this study, we compared the efficiencies of IVF and intracytoplasmic sperm injection (ICSI) techniques in producing fertilized oocytes of gerbil. Oocytes were recovered from the oviducts of superovulated gerbil and hamster females. Gerbil spermatozoa were collected from the cauda epididymis. Zona-free hamster and gerbil oocytes were inseminated by gerbil spermatozoa. IVF: zona-intact gerbil oocytes were inseminated in HTF medium supplemented with or without hypotaurine (Hyp) and L-epinephrine (Epin). ICSI: sperm heads were injected into the ooplasm and cultured *in vitro*, or were transferred into the oviducts of pseudopregnant recipient females. After 5 h of incubation, gerbil spermatozoa penetrated into the ooplasm of 58% and 43% of zona-free hamster and gerbil oocytes. However, they penetrated only 3%, 16% and 6% of zona-intact gerbil oocytes in HTF, HTF+Hyp y HTF+Hyp+Epin. Male pronucleus formation was observed in 2%, 11% y 4% of oocytes, respectively. By ICSI, 63% of oocytes formed a male pronucleus and arrested their development at the 2-cell stage. 57 oocytes were transferred into oviducts and 7 (12%) morulae/blastocysts were recovered on day 6. These findings indicate that gerbil oocytes can be fertilized *in vitro* more efficiently by ICSI than by conventional IVF.
Supported by Science and Technology Agency, Gov. of Japan.

68.

ADAPTATIVE MECHANISMS OF TWO *BRADY-RHIZOBIUM* STRAINS UNDER HEAT STRESS AND TREHALOSE METABOLISM

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Environmental stress, as hight temperatures of soil, affect crops and rhizobial growth, nodulation and nitrogen fixation. It decreases crops improvements, peanut among them. Our aim was to analize the effects of heat stress on some biochemical mechanisms of peanut rhizobia: *Bradyrhizobium* USDA 3187 and *Bradyrhizobium* TAL 1371. These were grown at 28ºC and/or 37ºC on YEM. Bacterial growth, viability, proteins, cellular trehalose and cellular potassium were measured. The trehalose metabolism was analized by trehalos 6-P synthase and trehalase activities, after trehalose cuantification. Viability increased at 24 hs of growth, when temperature of incubation was increased at 37ºC, in both strains. The potassium content decreased in exponential and stationary phases of growth in USDA 3187 and TAL 1371. Trehalose was detected in USDA 3187 and TAL 1371, at control conditions, and increased in stationary phase of TAL 1371 at 37ºC. Trehalose 6-P synthase activity was not modified under heat stress in USDA 3187, but trehalase activity decreased. Adaptative mechanism of this strain, is probably due to another compatible solute accumulation different of trehalose. On the other hand, in TAL 1371, the synthetic activity did not change, but trehalase activity raised under heat stress. These results do not agree with trehalose accumulation detected. This fact might involve another possible synthesis mechanism of trehalose. In this strain trehalose would behave as a compatible solute in heat stress as occur in other rhizobial strains.

69.
EFFECT OF THE PRENATAL STRESS ON SOME IMMUNE PARAMETERS

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Different types of gestational stress in rodents and non-humans primates produce an abnormal regulation of the hypothalamic-pituitary-adrenal axis (HPA) in the adult offspring that could induce a long-term alteration of the immune function. The number and the percentages of blood leukocytes provide an important indication of the immune system activation and the leukocytes distribution in the body. The objective of this work was to investigate the effect of chronic prenatal stress in the leukocytes subpopulations distribution in response to acute stress in rats. Three months old males offspring, whose mothers were chronically stressed by immobilization (IMO) during the pregnancy (EP) and non-stressed controls (C) were used. Blood samples were collected immediately for baseline measures (0 min). After the acute IMO (20 min) and after 60, 90, 120, 150 and 330 min. later, blood samples were collected. Corticosterone (COR) plasmatic profile, totals white globules, and leukocyte subpopulations were measured. The spleen of the EP and C animal were extracted under basal conditions and acute IMO and spleen somatic indexes were calculated. In EP and C offspring the leukocytes, lymphocytes and neutrophils profile are similar, however depressed responses in this profiles in the EP and postnatal acute IMO were observed. The spleen somatic index is smaller in EP. The basal plasmatic COR levels are bigger in EP, but they increase less than in C after postnatal stress. A correlation between COR and lymphocytes and COR and neutrophils exists. In conclusion the prenatal stress produces an alteration in the leukocytes distribution in response to postnatal stress, possibly mediated by COR. This redistribution could affect the immune response in these animals.

71.
ROLE OF THE ALDOSTERONE IN THE ANTINATRIURETIC RESPONSE TO ACUTE AND CHRONIC STRESS

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The stress response appears front of stimuli that threaten the integrity of the organism. Due to the central role of sodium in the maintenance of the corporal liquid volume, exist mechanisms that control their excretion, among those the aldosterone (A). The levels of this hormone increase with the stress, in accord with a decrease in the sodium renal excretion and in consequence, it could influence the development of hypertension. The objective of this work was to evaluate the A participation in the antinatriuretic effect to the acute and chronic immobilization (IMO) stress. Male Wistar rats under standard conditions, with access to NaCl 1.5% (drink) 3 previous days to the beginning of the experiment was used. Four groups was considered: control (CC), control stress (CE), control + drug (CD) and stress + drug (ED). The blocker of A receptors, spironolactone (6 mg/kg/día i.p.) was used. In the 1 (acute stress) and 7 (chronic stress) days, 6 hs post-IMO, urine for the volume, [Na+] and osmolarity determinations was colected. The CE group excreted less [Na+] than the DC ($p = 0.0003$). This excretion was also smaller in the ED group that in the CD ($p = 0.0024$), while the ED animals excreted more sodium than the CE ($p = 0.0097$). The renal effect was similar at the 1 and 7 days. Not differences neither in the osmolarity or urine volume were observed. The IMO-induced antinatiuresis was partially reverted with spironolactone in the acute and chronic stress. These results would indicate the participation of the aldosterone in the stress-induced antinatriuresis, although other mechanisms would be involved in this renal response.

70.
COMPLETE MINERALIZATION OF TETRADECYL TRIMETHYLAMMONIUM USING *Pseudomonas putida A*.

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Quaternary ammonium compounds (QAC's) are commercial chemicals commonly used in a variety of products. Considering the resistance to biological degradation and the complete mineralization of QAC's achieves with consortia of at least two microorganisms; the purpose of this study was to evaluate the pure culture potential for the complete degradation of a QAC's, tetradecyltrimethylammonium (TDTMA). *P. putida A* grew in liquid HPi-BSM medium containing 50 mg/l of TDTMA as the sole C, N or C and N source. The growth occurred concomitantly with TDTMA disappearance and the level consumed at 48 h was 28 mg/l. At 72 h the TDTMA consumption increased a 10% but the growth was not significant. The increase in cell protein when the culture reached the stationary phase was 11.4 mg/l. This value represented about 86% the initial N-TDTMA incorporated into the cell biomass. When cells grew on glucose plus TDTMA, analysis of supernatants showed that it was fully consumed in 96 h. The increase in cell protein was 18.8 mg/l and 97% the N-TDTMA was incorporated into cell biomass. A monooxygenase activity was measured and implicated in the TDTMA degradation initial steps. This enzyme activity was present in cell-free extracts of cells grown on TDTMA as the C, N or C and N source but not in cells grown on glucose and NH_4Cl showing that the TDTMA oxidation is dependent on inducible systems. Taking into account that others bacteria can't grow on different QAC's as a sole C and N source, this strain is fairly suitable for biodegradation studies.

72.
PREVALENCE OF *Clostridium botulinum* IN SOIL OF ARGENTINA

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Botulism continues to worry the authorities in Public Health, and in food and agricultural industries. The knowledge of the geographical distribution of botulinum toxin-producing clostridia (BTPC: *C. botulinum, C. baratii, C. butyricum* y *C. argentinense*) is necessary to evaluate the incidence of this disease. We examined 2007 soil samples from five politic-administrative regions of Argentina. Aliquots of suspensions were inoculated in chopped-meat medium broth after heat shock (80ºC, 10 min) and incubated for five days at 34ºC. Supernatants were tested by bioassay. BTPC were isolated onto solid media. Toxin was obtained in high concentration by dialysis and its LD_{50} was calculated and identified by quantitative neutralization (1000 LD_{50}/mouse). Prevalence of BTPC in soils of Argentina was 23.4%. We found significative differences ($p < 0.01$) amongst the regions: Cuyo, 38.0% (183/481); Noroeste, 29.4% (83/282), Pampeana, 24.8% (120/484), Patagónica, 14.7% (72/490) and Nordeste, 4.1% (11/270). There were different serotypes: A 56.7%, B 15.3%, F 3.8%, G 0.4%, Af 3.6%, A+B 3.0%, A+F 0.2%, B+F 0.2%, not identified 16.6%. BTPC distribution in soils of Argentina is not homogenous. These results could explain the different incidences of botulism in the studied regions. However, other factors would be implicated.

73.

BACTEREMIA AND SOLUBLE MEDIATORS IN EXPERIMENTAL BACTERIAL INFECTIONS

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The innate immune response is one of the first host defensive barriers against infections. The events triggered during the earlier period of infections could be responsible of the disease´s physiophatogeny. The aim of this work was to study the serum level of IL-6, TNF-α, NO and the bacteremia in mice infected with different bacterial species, at different post-infection times, and to analyzed the influence of the age of mice in the evolution of the disease. 60^{th} to 90^{th} days old BALB/c mice were intraperitonally infected with 9×10^8 CFU/ml (66.6% mortality at 8 hs p.i) of *E. coli* ATCC 35218 or *Streptococcus agalactiae*. At 0, 3 and 6 hs p.i mice were bleeded for colony counting and serum determination of TNF-α, IL-6 (ELISA) and NO (Griess). 60 days old mice infected with *E. coli* showed at 6 hs 2690 CFU/ml and with *S. agalactiae* (GBS) > 100.000 CFU/ml; while 90 days mice showed 343 and 0 CFU/ml respectively. The levels in controls (uninfected mice) were IL-6: 0 pg/ml, TNF–α: 11.7 ± 10.6 pg/ml and NO 13.3 ± 2.29 μM. 60 days mice infected with *E. coli* had the maximum levels at 3 hs p.i. IL-6: 2951 ± 373.4 pg/ml ($p < 0.01$), TNF –α: 83.4 ± 9.5 pg/ml ($p < 0.01$) and NO 19.9 ± 4.3 μM. In 90 days mice, the levels were: IL-6: 2271 ± 152 pg/ml ($p < 0.001$), TNF-α: 50.6 ± 10.8 pg/ml and NO: 17.23 ± 4.0 μM. In 60 days animals infected with GBS, the values of the concentrations were: IL-6: 267.2 ± 139 pg/ml ($p < 0.05$) TNF-α: 28.6 ± 8.0 pg/ml and NO: 18.8 ± 2.83 μM; in the 90 days mice: IL-6: 517 ± 26 pg/ml ($p < 0.02$), TNF-α: 22.6 pg/ml y NO: 9.6 ± 0.4 μM. In conclusion younger animals were more susceptible to infection with both *E. coli* and GBS. There was a close relation among sepsis and soluble mediators; the increase of IL-6 and TNF-α serum levels preceded the bacteremia peak.

74.

EFFECT OF CHANGING THE PERIOD OF EXPOSURE TO DAILY STRESS ON THE SODIUM AND WATER RENAL EXCRETION

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Chronic immobilization stress induces antinatriuresis and antidiuresis in rats with option to drink NaCl 1.5% solution. When the influence of a particular stressor on the organisms response is evaluated, the nature of agents, the severity and the duration of the daily exposition should be considered. The objetive of this work was determine the effect of changing the period of exposure to daily stress on the renal response in rats with saline overload. Male Wistar rats, 250-300 g of body weight, maintained under standard conditions was used. Three days before to the experimental day, NaCl 1.5% solution were offered to drink. Daily immobilization (IMO) among 10-12 AM for 7 days was used. Initial and final body weight and daily food intake were registered. Four groups were considered according to the duration of the stressor: control (C); IMO 15 min. (IMO 15); IMO 60 min. (IMO 60) and IMO 120 min. (IMO 120). The days 1 and 7, after the IMO, urine was collected for the volume and Na^+ concentration determination. The day 7 adrenal glands were extracted. The stress caused antinatriuresis, increment of the relative weight of adrenal glands and smaller food intake, independently of the duration of the IMO. Not differences neither in salt intake or diuresis was observed. Stress reduced the gain of body weight, except in IMO 15 animals. In conclusion, the effects of the stress on these variables are independent of the duration of the stimulus.

This work was supported by grants from SECYT (UNRC).

75.

ENTEROTOXIN D EXPRESSION IN *Staphylococcus aureus* REGULATORY MUTANTS

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Staphylococcus aureus is an important pathogene found in human and animals. It is responsible for the synthesis of several virulence factors. Staphylococcal enterotoxins are an important risk factor for human health. Several global regulatory genes such as *agr*, *sar* and *sae* control the production of most of *S. aureus* virulence factors. The aim of this work was to evaluate the influence of genetic environment on the expression of enterotoxin D (*sed*) gene and analyze *agr* and *sae* genes regulatory effects on that exoprotein expression. In this work, double agr^- and sae^- mutants were obtained after the transfer by transduction with phage 80 α, the mutation of *agr:tetM* to different sae^- mutants. *sed* carrying plasmid was transferred by transduction to the different wild type *S. aureus* strains and to its isogenic mutants sae^-, agr^- and sae^-agr^-. After the separation of the proteins of the culture supernatants by SDS-PAGE, enterotoxin D (SED) was detected by Western-blot. The results obtained showed an increase in the production of enterotoxin D in the mutants agr^- and a significant decreased in sae^- mutants while double mutants showed intermediate levels in the production of the analyzed exoprotein respecting to the levels observed in the unique mutants. These studies show that *sae* would act as a positive regulator and *agr* as a negative regulator of SED synthesis. *agr* regulatory role on the strains analyzed in this study was different to the one described in bibliography for another mutant agr^-, *S. aureus* ISP546. The genetic background might be the cause of the observed differences.

76.

ANTIBIOTYPE AND HEMAGGLUTININ DETECTION IN *Clostridium botulinum* STRAINS ISOLATED FROM INFANT BOTULISM CASES

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Intestinal colonization of *Clostridium botulinum* (*Cb*) and later absorption of its toxin cause infant botulism (IB). It affects infants under one year of age. Specific physiological characteristics of these strains are unknown. Our aim was to determine in Cb strains from Mendoza IB cases: antimicrobial resistance pattern and hemagglutinin production. We studied 16 serotype A strains isolated from IB cases and a control strain (A Hall). Minimal Inhibitorial Concentration (MIC) was determinated by agar dilution (NCCLS) and broth elution methods for ampicillin (AMC), amoxicillin (AMX) and ampicillin-sulbactam (AMC-S). Twofold dilutions of toxin were mixed with 2% human red blood cells suspension to determinate hemaggltunin titer. AMC, AMX and AMC-S MIC by agar dilution method were between 0.125 and 1 μg/ml. By the other method, MIC were between 0.5 and 2 μg/ml. Control strain MIC was similar. All strains from IB cases were hemagglutinin negative, but A Hall strain showed a titer of 64. So far, these results suggest that antimicrobial susceptibility is not an important factor to characterize these strains. For that purpose, hemaglutinin production could be used.

77.

IDENTIFICATION AND DETERMINATION OF THE PROFILE OF RESISTANCE TO ANTIBIOTICS OF *STREPTOCOCCUS* AND *ENTEROCOCCUS* STRAINS ISOLATED FROM BOVINE MASTITIS

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The infectious pathogen *S. agalactiae* and environmental streptococcus *S. dysgalactiae* and *S. uberis* are known as bovine mastitis etiological agents. The aim of this work was to identify the species of 80 streptococcus and enterococcus strains isolated from intramammary infections (IMI) coming from dairies in the provinces of Córdoba, Santa Fe and Buenos Aires. These strains were analyzed by bacteriological tests and API20 Strep system and the resistance to antibiotics (penicillin, cefalotine, erythromycin, oxaciline, neomycin) generally used in therapy was determined. Seventy four of the strains (92.5%) were identified by conventional methodology while 98.7% was confirmed by the fast system. Total isolation of *S. agalactiae* and the 98.1% of *S. uberis*, classified by traditional methodology, were confirmed by commercial system. Five of the six strains of both *S. dysgalactiae* and *E. faecalis* were identified by bacteriological method. The calculus of k = 0.8 (0.7 - 0.9) showed an agreement between both methods. The classification based on bacteriological tests is useful for the identification of *S. uberis* and *S. agalactiae* strains isolated from IMI. The highest percentages of resistance and intermediate susceptibility in the analyzed strains were found for neomycin. In five strains, 1 *S. agalactiae*, 1 *S. uberis* and 3 *E. faecalis*, resistance to four and five of the studied antibiotics was detected. The identification of the microorganisms isolated from IMI from dairies in the central argentine dairy region and the knowledge of profile of susceptibility to antibiotics will help to select the most effective therapeutic agents for the treatment and control against mastitis.

78.

EFFECTS OF DIFFERENT STRESS CONDITIONS ON AN *Azospirillum brasilense* EPS^{++} MUTANT

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One of the current problems in our country, mainly in the south of Córdoba province, is soil salinity. An alternative to revert its negative effect on the crops, is to use PGPR. In this sense, we have studied the effect of saline stress on *Azospirillum brasilense* Cd - cereal interaction, demonstrating impairment in the colonization of maize and wheat roots by the bacteria. In order to investigate the mechanism of this alteration, we have obtained different mutants, one of them being EPS hyperproducer. It has been shown that this mutant has a synthesis deficiency of the alanine-alanine ligase and it is unable to colonize maize roots, both under normal and saline stress conditions. In the present work, we analyze the tolerance of the mutant under different stress conditions. *A. brasilense* Cd (ATCC29710) (wild type) and *A. brasilense* Cd PG1 (isogenic mutant with Tn-5 insertion) were grown under hypoosmothic (distilled water), hyperosmothic (NaCl 500 mM) and thermic stress (42º C). Colonies forming units were measured at different times. Results indicate that both, the wild type and the mutant utilized different mechanisms to counteract the effects of the stress. The mutant also showed to be more sensible than the wild type, revealing that the mutation turn the bacteria more vulnerable to adverse conditions.

79.

BACTERICIDAL ACTIVITY AND SYNERGISM AMONG STRAINS OF LACTOBACILLIS ON UROGENITAL PATHOGENS

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The lactic acid bacteria's capable of producing bacteriocins are considered a potential factor of control to improve the microbiological stability of different vaginal ecosystem. The objective of this work is to studying the MIC and the synergism among produced by lactobacillis strain with inhibitory activity on urogenital pathogens. 100 lactobacillis strains were isolated from vaginal exudates and were identified like: *L. fermentum*, *L. gasseri* and *L. acidophilus* by biochemistry tests. a) The detection of the bactericidal activity, was carried out by the crossed fluted technique; b) For the synergism study the well in the agar technique was used; c) The purification of the bacteriocins was carried out by precipitation with $SO_4(NH4)_2$ and passage by the column of Sephadex G60; d) The minimum inhibitory concentration was carried out by the technique of serial dilutions of the bacteriocins; e) Character of the inhibitory substance. From the 100 lactobacillis strains isolated, 12 strains producers of bacteriocins were detected and 2 strains were selected, *L. fermentum* and *L. acidophilus* for being the best producers of inhibitory activity. The synergic effect was observed among the strains of *L. fermentum* and *L. acidophilus*, the zone of inhibition of growth were bigger than the zone of inhibition of each strain separately. The substance activity was kept for the increase of the temperature to 100ºC and 121ºC. These substances presented a PM minor to 10.000 D. The activity of the substances was reflexed with the same activity to pH 4 and 5, but there was no activity to pH 9 and 11. It presented a homologous and heterologous spectrum of inhibitory activity. The MICs of the bacteriocins were of 10-320 AU/ml for *L. fermentum* and 320-640 AU/ml for *L. acidophilus.* The production of bacteriocin with synergic activity and that expressed their action even diluted, would permit the use of these strains in the probiotic design.

80.

PGPR CAPACITY OF SALINITY TOLERANT NATIVE STRAINS

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For the last ten years, we have being studying the effect of saline stress on the interaction of *Azospirillum brasilense* Cd, (a PGPR) an cereals. Having demonstrated that saline stress impairs the bacterium colonization of maize and wheat roots, our actual objective is to obtain native strains salinity tolerants. Surface sterilized agropyro and maize seds were germinated and transferred to pots with sterile sand: perlite added with NaCl. After one week, plants were inoculated with native strains, isolated from saline soils and previously demonstrated to be PGPR. Shoot and root fresh and dry biomass were measured three weeks later. Results were analysed by ANOVA at $p < 0.05$. Plants inoculated with native strains presented a greater root biomass than the no inoculated (either under stress or under control conditions), and one of them, MEP218, increased the hydric status of the plant. Tests for antibiosis between the selected PGPR (or their supernatant) and fungal pathogens *Sclerotinia sclerotiorum* and *Sclerotinia minor* were conducted in potato dextrose agar (PDA). Plates were examined for signs of clear zones indicating growth inhibition. Supernatants were analysed by SDS-PAGE. Some of the strains exhibited antibiosis against the pathogens tested. Most of the isolates released antimicrobial compounds to the medium heat resistants.

81.

DETERMINATION OF BIOCHEMICAL AND PHYSIOLOGICAL GROWTH PARAMETERS IN PEANUT MICROSIMBIONTS UNDER HYPERSALINE AND HYPERTHERMIC STRESS

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To expand peanut production into new regions it is necessary to know the adaptive response of the nitrogen fixer micro-organisms that interact with cultivations. The object of the present work was to contribute with the knowledge of physiological and biochemical response (growth, viability, biomass, protein content, trehalose content; lipids and periplasmic glucans respectively) of a peanut microsimbiont under different types of stress (hyperthermia and hyperosmolarity). This response will be compared with the one of other rhizobia that have been already studied, and it probably contributes in the selection of a great tolerant strain for future inoculation of cultivations essays. The strain used was *Rhizobium* TAL 1000, and the culture medium was YEM in four different conditions: control, hyperthermic, hypersaline, hypersaline and hyperthermic. For the parameters determination we employed OD, CFU recount, wet weight, protein quantification, trehalose quantification, periplasmic glucans profile and lipid determination. Results indicates no differences in bacterian growth and protein content for the different stress conditions. On the other hand viability, biomass and trehalose content suffered an increase in the hypersaline and hyperthermic condition. The polymerization degree of periplasmic glucans was affected under the stress conditions applicated, this was proved in its MW profile. Lipidic pattern did not change but phospholipids increase a 20 % in the hypersaline and hyperthermic condition. Results suggest that periplasmic glucans and phospholipids may be implicated in the adaptive response of this strain. Furthermore they indicate a response that locates the strain used into the group of tolerant organisms.

82.

RENAL RESPONSE ADAPTATION TO THE CHRONIC STRESS IN RATS WITH SALT OVERLOAD

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The stress exposition has a dependent and specific effect of the intensity, duration and stress type. Moreover, exist differences in the individuals response to the stress. These factors are determinant to induce adaptation of this response. The objective of this work was to investigate if strong immobilization (IMO) stress of different duration, induces renal adaptation in rats with salt overload. Male Wistar Rats of ± 300 gr body weight (BW) under standard conditions was used. 3 groups of rats were considered: control (C), 30 min. (E30) and 60 min. (E60) IMO daily. All groups had access to NaCl 1.5% 3 days before the beginning of the experiment. Blood and urine samples were obtained the days 1, 7 and 10 post IMO. BW, Packed cell volume (PCV), glucemia, and urinary [Na^+],volume and osmolarity was determined. The results show high glucose and PCV values in E30 and E60, in the 3 days considered. IMO reduced the BW gain in the 10 days of treatment, originating this difference in the first 7 days. Sodium excretion was lower in the E30 and E60 rats than C, but no differences between both IMO was observed. The osmolarity was smaller in the E60 and a tendency to an antidiuretic effect was observed in this group. In conclusion, not differences in glucose, PCV and [Na+] among the IMO animals through the time were found. This would indicate that it was not stress adaptation. However, the E60 showed a significantly bigger PCV after the 10 days of IMO, a decrease urinary osmolarity and a tendency to the antidiuresis what would evidence a marked effect of stress when it is more prolonged.

83.

EFFECTS OF STRESS ON LIPID METABOLISM IN RATS

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Frequent association of hypertension with risk factors as mellitus diabetes, dislipidaemia and obesity increase cardiovascular risk. This should be understood as the possibility to suffer a cardiovascular disease during a time, generally 5 or 10 years. Thus, it is important to remark the hypercholesterolaemic effect of catecolamines and psychosocial factors accompanying stress in several species. Stressed Wistar male rats were used to verify the effects of intermittent chronic stress on blood lipids and plasmatic lipoproteins. This was done experimentally, comparing stressed Wistar male rats (S), with non stressed control animals (C) of the same group. A group of 16 rats under biothery standard conditions were divided in two groups. Each group had the same number of individuals. One group was exposed to stress by immobiliy on a (IMO) plaque for 2 hours a day. The rest of the rats were non stressed controls. All the animals had their body weight meassured. Total cholesterol concentration (Col), triglicerids (TG), HDL, LDL and glucemia (stress marker) were also measured by enzymatic methods on the 1st, 7th, 14th days immediately post IMO. The values of the meassured parameters were greater in S animals with respect to C animals for every considered period of time. The major increase was evident at the 14th day. Plasmatic TG increased more than 200% and plasmatic Col showed an increment of 62%. Among plasmatic lipoproteins, LDL was the one which showed the greatest increment this day (40% S vs C). Besides, it was observed that stressed animals had gained less body weight. The conclusion may be that stress by IMO, either chronic or acute, produces lipidemic changes. This could induce an increase of aterogenic risk.
This work was supported by grants from SECYT (UNRC).

84.

DIRECTED WORKSHOP AS A PEDAGOGIC PROPOSAL IN THE GENERAL BIOLOGY COURSE

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General biology is a regular biology course dictated in the 1st year in the veterinary carreer or the UNLPam.The new students come from different regions of the country carrying heterogenous previous knowledge and lacking integration in the contents of this discipline. The aim of this work is to report the implementation of a new pedagogic strategy named directed workshop (DW). Each content of the General Biology program is offered throngh theoretical and practical classes, being all the teachers of the subject present. Students suggest the themes, which are then selected according to the difficulty they found in their learning process. Students group themselves according to the selected themes and solve the given problematic monitored by a teacher. This experience that has been put into practice since the year 2002, allowed the knowledge socialization generated by the interrelation among students and also among students and teachers, it favored the integration of the disciplinar contents reducing the inequalities between the previous knowledge detected at the beginning of the course. Besides the implementation of this new pedagogical experience the teaching-learning system increased the percentage of matriculation retention.

85.
INTERACTION BETWEEN BACTERIOCINS FROM *LACTOBACILLUS* ON *E. coli* STRAINS ISOLATED FROM URINARY INFECTIONS

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Escherichia coli is the most frequently pathogen isolated from urinary infection in women. *Lactobacillus* has the capacity to produce bacteriocins that can inhibit the other microorganisms growth and so, they can be employed therapeutically. The objective of this work was: to analyze sinergism, antagonism and indifference interactions between bacteriocins produced by *Lactobacillus acidophilus* and *Lactobacillus fermentum* on different *E. coli* strains. Sixty-four *E. coli* strains recovered from women with urinary infections were identified by conventional tests. Their sensibility to bacteriocins produced by *L. acidophilus* (23 strain) and *L. fermentum* (60 strain), isolated from vagina, were analyzed by crossed streak technique. It was employed Rogosa broth plus 1.2% agar. Interaction between bacteriocins from both *Lactobacillus* on each *E. coli* strain was made by agar well diffusion. *E. coli* was inoculated on medium agar, following 100 µl of free cellular supernatants containing bacteriocins of each producer strain was placed in wells of 7 mm; incubated at 37ºC by 16 h, and then interaction (sinergism, antagonism or indifference) was analyzed. Crossed streak technique revealed that the 64 *E. coli* strains (100%) were sensitive to both bacteriocins with significative inhibition ratios (10 to 22 mm). The agar wells diffusion assay permits demonstrate that 26 *E. coli* strains exhibit sinergic action (40.6%) while 38 strains (59.4%) were indifferent. There was not evidence of antagonic effect. Results indicates that *Lactobacillus* strains used in the assay can be used like as a probiotic with the proposal to control urinary infections.

86.
***IN VITRO Trypanosoma cruzi* INFECTION IN HUMAN PLACENTA: PHOSPHOLIPASES AND NEURAMINIDASE AFFECT THE ENZYME ACTIVITY OR PLACENTAL ALKALINE PHOSPHATASE AND ITS RECEPTIVITY TO IGG**

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Trypanosoma cruzi is the parasite responsible of Chagas' disease. Placental Alkaline Phosphatase (PLAP, EC 3.1.3.1), is a surface GPI anchored protein from human placenta. Structure studies on PLAP showed that it contains many sialic acid residues, which would be important for its active site. *T. cruzi* expresses a stage-specific transialidase-neuraminidase which could desialyte PLAP's sialic acid residues and cleave PLAP from the cell surface with its phospholipases C and A_2. Since *T. cruzi* seems to somehow affect PLAP's activity, we studied the effect of phopholipase A_2, phospholipase C and neuraminidase on PLAP's enzyme activity and IgG receptivity in cultures of placental villi and direct interactions with purified PLAP. Enzyme activity, zymmogrames for PLAP, and immunocitochemistry for PLAP were determined as well as its receptivity to human IgG. Results were compared with measured enzyme activity and IgG receptivity on placentas from chagasic women and cocultures of normal placenta with trypomastigotes. As conclusion we suggest that the considered enzymes can not only affect directly the IgG receptivity of PLAP and its enzyme activity but also favor the release of PLAP into the plasma of chagasic women. The released molecules of PLAP would have lower enzyme activity probably due to the direct interactions with the parasite´s molecules and IgG.

87.
EFFECTS OF INOCULATING WITH FLUORESCENT *PSEUDOMONAS* 51B ON GERMINATION, EMERGEN CY AND GROWTH OF TWO BEANS CULTIVARS

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After physiological maturity, deterioration promotes dropings in seeds quality. This is related with degradation of cellular membranes, as well as degraded growth rate, emergency of plants, and germinative power. These variables can be improved by means of inoculations with rhizobacteria which stimulate growth of plants. Objective of this study was to determine the effect of inoculating flourescent *Pseudomonas* 51B, on germination and emergence percentages in the field, as well as on growth (fresh and dry weight, total length of plants) of two cultivars of beans. With this purpose, groups of a) vigorous seeds (control), b) with natural deterioration and c) with artificial damage, were divided in two subgroups each: without and with inoculation (density of rhizobacteria: 10^8 cfu/ml) Although inoculation of seeds in the three groups did not modify germination of either cultivar as compared to those not inoculated, in the control group of white bean it only increased the percentage of emerged plants in 45% at the 8° day after seeding. On the other hand, *Pseudomonas* 51B only promoted growth of the control of black bean, increasing significantly total fresh and dry weight of emerged plants in the field as compared with material which was not treated with inoculant; whilst white bean has shown the same behaviour in the group with natural deterioration. However, in the case of black bean, this material has shown an antagonic effect as compared with bacterian inoculation. These results make evident the promoting and/or deleterious performance of fluorescent *Pseudomonas* 51 B on the growth of white and black bean plants according to vigour level of their seeds.

88.
ROLE OF GLUTATHIONE IN GROWTH AND SYMBIOSIS IN *BRADYRHIZOBIUM* SP-PEANUT

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The glutathione (GSH) plays an important role in the mechanism of defense of the microorganisms and the plants against different environmental stresses. Previous studies with *Bradyrhizobium* sp SEMIA 6144 (*Arachis hypogaea* L. symbiont) showed an increase in GSH content under acid stress. In order to know the role of GSH, a GSH-deficient mutant (*Bradyrhizobium* sp 6144-ΔS7Z) was obtained by disruption of *gshA* gene, which encodes the enzyme γ-glutamylcysteine synthetase. The objective of the present work was to determine the GSH-deficient mutant strain growth and symbiosis with peanut. The mutant strain growth was significantly reduced in MSM medium at different pHs (7 and 5.5) and the GSH content was very low, about 4% of the wild-type level. There was no reversion in the mutant strain growth in the MSM medium supplemented with 100 µM GSH and the endogenous GSH level was approximately the same that observed without the addition of GSH, whereas, the wild-type strain increased the GSH content in this condition. Possibly, the mutation caused an alteration in the transport of this molecule The symbiotic properties of the mutant was similar to that found in the wild-type strain indicating that the mutation does not affect the symbiotic ability of the mutant to form effective nodules. The coinoculation experiment of peanut with a combination of wild-type and mutant cells 1:1 ratio showed that the mutant strain only occupied a 25% of total nodules. Thus, the *gsh*A mutation appears to affect the ability to compete during the process of peanut nodulation.

89.

EFFECTS OF THE INTERMITTENT CHRONIC STRESS ON THE SODIUM RENAL HANDLING

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One of the most remarkable effects in the stress situations is the development of hypertension, which is increased when high salt was intaked. The objective of the present work was to investigate the effect of the short-term, chronic, intermittent IMO, on the renal functionality in salt overload rats. Male Wistar rats under standard conditions was used. Three days before to the experimental day, NaCl 1.5% solution were offered to drink. In the experimental day, the rats were divided into two groups: (C) control and (E) immobilization stress (IMO), 30 minutes daily, during 14 days. In the 1, 7 and 14 days of stress, after IMO, urine was collected and volume, sodium and potassium concentration were determined. Initial al final body weght and daily food and saline solution intake were registered. The day 14 the adrenals were extracted. The acute and chronic stress caused antinatriuresis, ($p = 0.007$), and not differences in the urine volume was observed. The salt intake was different among days ($p = 0.01$) and marginally significant among groups, being smaller in the stressed rats. Stress reduced the body weight gain, being smaller in the first 7 days coincidently with a reduced food intake and a increase in the adrenal weight. In conclusion, chronic stress, although of short duration, produces anorexy, loss of body weight and increase of the size of the adrenal. Also, the possibility to excrete a salt load is modified and could impact in the genesis of the hypertension.

90.

IDENTIFICATION BY RFLP, AT A LEVEL OF SPECIES, OF *Streptococcus uberis* STRAINS ISOLATED FROM BOVINE MASTITIS

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Streptococcus uberis is known as the most important environmental pathogen involved in bovine mastitis that causes huge economical losses in milk production, in its biological value and in its industrial sub-products quality. The aim of this work was to identify *S. uberis* strains by the Restriction Fragment Length Polymorphism (RFLP) analysis of 16S ribosomal DNA. These strains were isolated from dairies of the milk producer area in the central region of Argentina and previously characterized bacteriologically. Complementary 16S ribosomal DNA primers of *Streptococcus uberis* were used to amplify genomic DNA fragments by polymerase chain reaction. PCR products were digested with three restriction enzymes HhaI, RsaI and MspI. The 54 *S. uberis* strains that were identified by the traditional method presented different results in one to three bacteriological tests respecting the reference strain *S. uberis* ATCC 19436. The analysis of restriction profiles showed that 43 of the 54 strains (79.6%) identified as *S. uberis* by the conventional method, presented a restriction profile coincident with the one of the reference strain. Thirteen of the 17 strains (76.5%) from clinical origin and 30 of the 37 (81.1%) isolated from subclinical mastitis were confirmed by RFLP as belonging to *S. uberis*. The results of this study showed the variability of the biochemical profiles of the strains characterized as *S. uberis* based on its fenotype. The Restriction Fragment Length Polymorfism (RFLP) analysis of 16S ribosomal DNA technique appears as a useful tool for the fast identification of *S. uberis* strains isolated from bovine mastitis.

91.

A GROUPAL TECNIQUE "PUZZLE IN GROUP" FOR THE APPROACH OF THE COMPREHENSION DIFFICULTIES AND COMUNICATION OF THE INFORMATION IN TEACHING BIOLOGY

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The aim of this work is to present experiencies carried out in the educational centres from General Pico, La Pampa, Argentina, in different teaching levels (Polimodal and Superior). The pupils population is, heterogeneous in terms of interests and capacities. However, there are shared factors like difficulties in reading – writing strategies necessary for the comprehension and comunication of the information that is alloted. The information that is distributed agrees with the present Biology programs of Polimodal, Natural Science and Biochemistry. A didactic strategy, groupal technique, named " Puzzle of Groups" is selected. It is based in the shaping of basic, heterogeneous groups which are given information about the same topic and each pupil is given a different section of it; in this way a temporary experts group is formed, receiving the teacher's role in later presentations of discussions. The flexibility capacity of the technique allowed group transformations and adaptations according to the teaching level and the difficulties observed. The study was centered in the classroom and permitted to observe the pupils strategies and study habits. Learning was transformed in a shared activity because pupils learn expressing ideas, discussing and confronting opinions. It moved the teacher from his/her place giving him/her the part of guide who helps the internal processes of development centering the activity in the student, thus letting him/her perform his/her individual tasks. Besides, it provided the scope for processes of evaluation.

92.

WATER QUALITY VARIATIONS IN PIEDRAS MORAS DAM (CORDOBA, ARGENTINA)

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Lakes and dams are exposed to environmental degradation, and their low renovation tax makes them vulnerable to pollution as a result of human activities. The task of this work was the prediction of water quality for non-sampled sites, by means of spatial interpolation technique (krigging) and the semivariogram model. Piedras Moras Dam (32º 12'S, 64º 19'W) is the last dam in the Ctalomochita or Tercero River basin (832 ha.). It is used as a reservoir to supply drinking water, recreational and watersport activities. Seven sampling stations were selected. Samples were collected seasonally, with twin repetitions during three consecutive years. Physico-chemical and biological variables were evaluated, according to standard methods. The applications of Surfer for Windows, Idrisi and Variowin were used. Both experimental information, and the one obtained by a digital processing of the images Landsat TMV, were integrated in a GIS. Dispersion of total nitrogen was different for each season. Total phosphorous in Spring showed its higher levels at Rio Soconcho and the Beach. Highest levels of chlorophyll-a occurred in Spring at the Porks effluent, Soconcho River and the Beach. Dissolved oxygen showed no differences in its dispersion pattern, and temperature had no specific annual tendency. Found variation has a structurated variability part, meaning that next located observations will have similar values, and a random variability part, meaning that the value of any sample can not be deterministically assesed. According to our results, water quality was between admisible limits. Risks of algal blooms could happen all along the year, diminishing its occurrence during Fall.

93.

THE EXPERIMENTAL AUTOIMMUNE OOPHORITIS (EAO) MODIFY SERUM LEVELS OF LH AND PROGESTERONE. EFFECT OF ICV INJECTIONS OF ALPHA-MSH

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Infertility and other ovarian disorders are due to changes in the immune system and reproductive hormones. There is a strong correlation between EAO in rats and the human illness with one of the mediators of the inflammation being peptide α-MSH. The current work was carried out with the idea of obtaining more information about the way this peptide acts in an inflammatory process such as EAO. The illness was induced in female rats by means of an injection of ovarian homogenate with a complete Freud adjuvant. An analysis was performed to see if intracerebro-ventricular (icv) injection of α-MSH was able to block or modify the hormones related to the reproductive process. Daily injections of α-MSH did not block the development of the illness. However when the serum profile of the LH and P hormones was studied, it was observed that during the proestrus the rats with EAO had higher LH values than those found in the controls, or in control rats treated with the peptide. The icv injection of α-MSH in rats with EAO produced a decrease in LH values. P values increased during the development of the illness during the proestrus day between 17.00 and 20.00 h. In the continuous diestrus (D_2), in rats with EAO high P serum levels were observed, although when they were treated with the peptide these levels decreased to similar levels of those found in D_2 of control rats. LH serum values were only modified in D_2 control rats treated with α-MSH. It can therefore be concluded that the peptide reduces both hormones values in the proestrus day in rats which have the induced illness. However, this hormonal decrease is insufficient to block development of the EAO.

94.

CELLULAR DAMAGE INDUCED BY TOXINS FROM *Helicobacter pylori* STRAINS ISOLATED FROM CLINICAL CASES

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Helicobacter pylori infection leads to different clinical and pathological outcomes in humans including chronic gastritis, peptic ulcer disease and gastric neoplasia. A major virulence factor produced by *H. pylori* is a cytotoxin, termed VacA, which causes vacuolar degeneration of cells *in vitro* but only about 50% of *H. pylori* isolate can produce the vacuolating toxin. This consideration led us to analize the *H. pylori* toxins activity from 18 strains recovered from patients with gastric simptomatology. Culture supernatants were collected, concentrated 30X and sterilized by filtration. Vero cells were inoculated with two fold toxin dilutions in Minimum Essential Medium containing or not 10 mM $ClNH_4$, and incubated at 37ºC, 5 days. The effects of the toxin were analized by light microscopy and documented by photographs. Exposure of culture cells to the toxins led to structural alterations with marked vacuolation and peripherical nuclear desplacement. Data indicated a 72% of positivity (toxin titers range from 5 to ≥ 640). These findings demonstrate the incidence of *H. pylori* strains with capacity to produce vacuolating toxin associated to severe gastric damages.

95.

RECOLECTION PLACE AND ITS INFLUENCE ON THE MICROBIAL ACTIVITY OF THE ESSENTIAL OIL OF *Psila spartoides*

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Essential oils (EO) are complex mixtures of terpenes; it has been probed their effectiveness against harmful microorganisms, as well as their potential biotechnological uses. For these reasons the antimicrobial activity of the EO should be studied. The aim of this work is to study the antimicrobial activity of *P. spartoides* collected in La Rioja, Córdoba and Río Negro. The EO were obtained by hidrodistyllation and analized by gas cromatography. The antimicrobial activity was probed using the Disc Difusion Technic (DDT) against *S. aureus, S. epidermidis, B. cereus, M. luteus, E. faecalis, E. coli, Klebsiella sp, P. mirabilis, P. aeruginosa* and *C. albicans*. 200 µl of each inoculum were spread over plates containing Mueller-Hinton Agar; paper filter discs impregnated with 10 µl of each EO, were placed on the surface of the media. The plates were left 30 minutes at room temperature, then they were incubated at 37ºC during 24 h. Then, the inhibition zone around the disc was measured. *P. spartoides* from Córdoba inhibited 80% of $Gram^+$ bacteria, 50% of $Gram^-$ and *C. albicans*. The specie obtained from Río Negro was active against $Gram^+$ and *C. albicans* but not against $Gram^-$ ones and *P. spartoides* from La Rioja was active against $Gram^+$ only. The differences in the antimicrobial activity could be attributed to the amounts of terpenes in the EO. The most abundant terpene of *P. spartoides* from La Rioja was limonene (35.8%), from Río Negro was camphor (26.5%) and from Córdoba camphor (50.5%) and limonene (4.3%). The latest specie demonstrated the best antimicrobial activity and inhibition spectrum probably due to the differences in the chemical composition of the EO what could be attributed to the recollection places.

96.

ACTION OF MAJOR COMPONENTS OF *Minthostachys mollis´*s ESSENTIAL OIL ON EUCARIOTIC CELLS

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The essential oil (EO) of *Mynthostachys mollis* revealed antiherpetic action *in vitro*. Their major componentes could be also responsible of the toxic action on eucariotic cells, so it was necessary investigate this capacity. The EO of *M. mollis* and its major components toxicity was analysed on eucariotic cells by different methologies. Different concentrations of EO (obtained by hidrodistillation and chromatografied assayed) and its major components: pulegone (P), mentone (M) and limonene (L), pure or in similar concentrations as the vegetal presents, were employed in the following assays. 1) Maximal non-cytotoxic concentration (MNCC): each sample was seeded on cell cultures (Hep-2) and incubated to 37ºC, 5 days. Cell alterations were observed by microscopy. 2) CC 50%: in identical conditions and by tripan blue exclusion values from survivor cell curve were extrapolated. 3) LD 50% was calculated counting dead *A. salina* vs. total ones, after 24 h using Reed-Muench method. The MNCC values (mg/ml) were, EO: 0.87; P: 0.132; M: 0.370 and L: 0.450, showing pure terpenes more toxicity with respect to EO.

97.

DIETARY LIPIDS MODULATE EICOSANOIDS RELEASE AND APOPTOSIS OF A MICE LUNG ALVEOLAR CARCINOMA

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Certain polyunsaturated fatty acids (PUFAs) as arachidonic acid (AA) and its metabolites, the eicosanoids, influence diverse cellular signalling pathways which regulate normal and tumour cellular growth, differentiation and apoptosis. However, the molecular mechanisms in by which PUFAs and derivatives play these roles is unknown. The objectives of this work are to investigate the effect of dietary PUFAs on the ciclooxygenase (COX) and lipoxygenase (COX) derivative eicosanoids and their influence in the neoplasic cells (NC) apoptosis. Lung alveolar carcinoma cell suspensions (1 X 10^6 /ml) were obtained by transplants of tumor in syngeneic BALB/c mice fed on basic diet plus 6% corn oil rich in essential fatty acids n-6; a non-essential fatty acid, as oleic oil (n-9), and commercial diet (control). AA metabolites produced by (COX) as 12(S)-HHT, and (LOX) as 12(S)-HETE and 15(S)-HETE were detected by HPLC. Apoptosis was evaluated by flow cytometry with Annexin V Fitc kit and by inverse-light (phase-contrast) microscopy. NC isolated from tumor of corn oil fed mice showed a significant increased number of apoptotic cells. The LOX:COX ratio was higher (5.25) in corn oil NC diets than in oleic (3.54) and in control (3.76) respectively. Dietary lipids modulate cancer cells apoptosis modifying the AA eicosanoids (LOX) 12(S)-HETE and 15(S)-HETE and (COX) 12(S)-HHT ratio. These results may be implicated in the dietary prevention of neoplastic process.

99.

ETHANOLIC EXTRACT OF *Minthostachys mollis*. ACTION AGAINST DIFFERENT VIRUSES

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Considerating the essential oil of *Minthostachys mollis* is active against *Herpes simplex* virus, the study of its antiviral capacity against other viruses is important. The aim of this work was to determinate the antiviral activity of ethanolic extract (EE) obtained form *M. mollis* against *Poliovirus* (RNA) and, *Herpes suis* and *Adenovirus* (DNA) in Hep-2 cells. Cytotoxic capacity of EE was evaluated on cells incubated with different concentrations of the vegetal at 37ºC (72 h). So, Maximal Non-Cytotoxic Concentration (MNCC) was calculated in 2.60 mg/ml and it was employed in antiviral assay. Cell monolayers were infected with viral dilutions and incubated with EE. Cythopathic effect reduction by Reed-Muench method (RMM) and number plaque reduction by Dulbecco method (DM) of the treated virus *vs* control were 90% and 99.9% for *Herpes suis* virus respectively, 32.25% for *Poliovirus* (DM) and 72% for *Adenovirus* (RMM). The EE of *M. mollis* was only considered active against *Herpes* virus because it inhibited more than 99% virus production, suggesting its potential application in the treatment of herpetic infections.

98.

***Minthostachys mollis:* DIFERENT METHODS TO DETERMINE THE ANTIMICROBIAL ACTIVITY OF ITS ESSENTIAL OIL**

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Minthostachys mollis is a native aromatic plant usually found in the province of Córdoba. Its antibacterial and antiviral activities have been extensivelly studied, both of them caused by the essential oil (EO). Sesquiterpenes, monoterpenes, carbonilated products and polienes made a complex mixture, which are probably the substances responsible of the antimicrobial activity. The objetives of this work is to study the antimicrobial activity of the EO and the pure components of *M. mollis* using bateria and the yeast *C. albicans*. The microorganisms used were *S. aureus, S. epidermidis, B. cereus, E. coli, Klebsiella sp, P. mirabilis, P. aeruginosa* and *C. albicans.* The EO, pulegone (P), mentone (M), limonene (L) and α-pinene (P) were probed against them using the Disc Difussion Method (DDM). Minimun Inhibitory Concentration was assayed only with samples that showed probed antimicrobial activity using the DDM and the microdilution technic (MT). The Minimun Bactericidal Concentration(CBM) was also realised. The EO was active against *B. cereus, S. aureus, E. coli,* and *Klebsiella sp.* The CIM for *B. cereus* was 421 mg/ml with the DDT and 52.62 mg/ml with the MT. The CIM using the MT was 52.62 mg/ml for *S. aureus* and 105.24 mg/ml for *E. coli*. With the DDM no CIM was detected. P and L showed activity against all microorganisms, except with *P. aeruginosa*. P was active against *B. cereus, S. aureus* and *Klebsiella sp,* while M did not show any antimicrobial activity. The cromatographic analisys of the EO revealed: 62.97% of P, 16.40% of M, 1.87% of L and 0.16% of P. The MT was more sensitive than the DDT. P and L could be the responsibles of the antimicrobial activity.

100.

DIACYLGLYCEROL METABOLISM IN MIDGUT CELLS OF *Panstrongylus megistus* (HEMIPTERA: REDUVIIDAE)

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In insects, the metabolism of dietary lipids allows the storage of reserves, which will be used as fuel. We previously shown that in the enterocytes of *P. megistus*, absorbed fatty acids from lumenal lipolysis were used in synthesis of neutral lipids, through the phosphatidic acid pathway. In this work, we focus on biochemical events that regulate the synthesis and release of diacylglycerol from midgut of *P. megistus*. It was analyzed: **a-** the kinetic of blood and lipid digestion, **b-** the activity of phosphatidate phosphohydrolase (PAP) and triacylglycerol hydrolase (lipase) in midguts on basal condition (without feeding) and at different times post-feeding (pf). In addition, a histological study to evaluate the pattern of lipid storage in this tissue was performed. Midguts were homogenized and supernatants at 10,000 x *g* and cytosol were employed for the enzyme assays. For microscopic study, tissues from different conditions were fixed, processed by high-resolution light microscopy and stained with Sudan. The results showed that between days 3-5 pf, the lumenal content represented ~ 50% of the initial blood meal whereas at day 15 pf, it represented about 6%. The activity of PAP increased from 24 h pf and was maximum 4-8 days pf. Lipase activity was maximum 7-12 days pf and decreased to basal levels from day 14 pf. In basal condition, the tissue showed small lipid droplets. In contrast, at days 10-15 pf the enterocytes were full of large lipid droplets, in position close to the lumen. [1]- The changes PAP and lipase suggest the importance of both enzymes in the regulation of intracellular metabolism of intestinal diacylglycerol, [2]- The pattern of lipid storage in midgut cells shows an active processes of lipid uptake and trafficking, and confirm the transient nature of the midgut triacylglycerol pool.