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### Abstracts from the

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### PARADOXICAL EFFECT OF COLCHICINE IN CARRAGEEN ACUTE PERITONITIS

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Colchicine (Co), in leucopenic (LD) and non-leucopenic doses (NLD), in rats with carrageneen (Ca) induced pleuritis produces an increase in pleural exudate at 3 and 24hr, and a decrease in neutrophils (N) and monocytes (M). Objective: to study the similarity of this findings in comparison with Ca peritonitis. Wistar rats (n=7), 200g, 24h fasting, water ad lib., avoiding coprophagy, were submitted to the following experiments: 1. 1ml IP saline; 2. 1ml 1% IP Ca, at 3 and 24h; 3. 0.5mg/kg OG Co 24 h. 4. 2mg/kg, OG Co, 24h, 0.5mg/kg OG Co 60min and Ca 3h; 6. 0.5mg/kg OG Co 60min, Ca 24h; 7. 2 mg/kg OG Co 60min and 3h; and 8. 2 mg/kg OG Co 60min, Ca 24h. N and M counts performed in blood obtained by cardiac puncture. N and M counts in P exudates. P samples: mieloperoxydase (MPO) reaction. Ca yielded: P exudates at 3 and 24hr (1.5-2ml), N=4500±200 and M=4100±150, 24h; 245±20 U/ 100mg/protein MOP, 3 and 24h. Blood: N=4000±100 and M=300±50, 3 and 24hr. Co (NLD) in P, 24h: N=25000±400 and M=12000±300. In blood: N=1900±150 and M=100±90. Co LD vielded in P N=3000  $\pm$ 70 and M=5400 $\pm$ 400; in blood N=5400 $\pm$ 120 and M=140  $\pm$ 20. Co (DNL) and Ca 3 hr yielded in P N=8500 $\pm$ 200 (p<0.01), same at 24h, M=2500  $\pm$ 200 (p<0.05), MPO 380  $\pm$ 30 (p<0.05), 3hr, and 470±25 (p<0.05), 24h. DL Co in P, 3h:  $N=5500\pm150$  and  $M=5000\pm100$ , 24h  $N=30000\pm500$  (p<0.01), M=27000±400 (p<0.01). Conclusion: NLD Co produced increase in Ns and Ms in P, while LD showed decrease in both. Co previous to Ca, 3 and 24h, with both doses, yielded a minimal peritoneal exudate with a remarkable increase in Ns and Ms.

## 3. BIOFLORA PREVENTS INTESTINE ULCERS PRODUCED BY DICLOFENAC IN RAT

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Bioflora, composed of four human live enterobacteria is administered in acute bacterial diarrhoea since it inhibits the development of intestinal anaerobe. Objective: to study Bioflora as a probiotics that might prevent the occurrence of ileum ulcers produced by Diclofenac through the same mechanism above mentioned. Wistar rats (n=7 in each group), 24 hr fasting, water ad lib., avoiding coprophagia were submitted to the following experiments: 1. 1 ml saline by orogastric (OG) gavage every 12 hr during 2 days. 2. 1 ml Bioflora every 12 hr, 2 days. 3. 10mg/k Diclofenac, OG, each 12hr, 2 days. 4. Bioflora after Diclofenac every 12hr, 2 ds. 5. Bioflora culture flask. Rats were sacrificed by ether overdose, and sterile laparatomy to remove 20 cm terminal ileum were performed. The intestine was opened along the antimesenteric border edge, taking pictures of ulcers to quantified by computerised planimetry. Three hundred grams were weighted, and placed in a flask containing PBS, pH 7.5, to be macerated and cultured in blood-agar plates during 4 days. Anaerobic colonies development was confirmed (U.F.C.) performing bacteria count. Ulcer area in 20 cm terminal ileum yielded: 1.0 mm<sup>2</sup>, 2.0 mm<sup>2</sup>, 3. 107±12, <u>4.</u> 7±3 (P<0.01). Gram positive germ U.F.C. yielded: <u>1</u>. 1.6 x  $10^5$  /g tissue, **2.** 3.2 x  $10^4$  (<0.05). **3.** 4.16 x  $10^6$  (n.s.). **4.** 8.53 x 10<sup>4</sup> (<0.01). U.F.C. (live bacteria) developed in Bioflora flasks. Conclusion: Bioflora inhibit development of ileum ulcers by Diclofenac through an intestinal anaerobic bacteria-blocking mechanism.

### 2.

## INFLUENCE OF PLASMA MACROMOLECULES ON ERYTHROCYTE AGGREGATION IN HORSES

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Plasma proteins have multiple effects on blood rheology, mainly on erythrocyte (E) aggregation-disaggregation process. Fibrinogen is responsible of 25% plasma viscosity (PV), though its role on E aggregation is not yet clear. Ceruloplasmine is also high in equine blood, therefore explaining the high aggregability in this species. IgM contributes to increase aggregation in human blood. Objective: to study the influence of some plasma molecules on equine E aggregation (EA). Blood samples of 8 healthy thoroughbred horses (aged 2-5 yr) by jugular venipuncture anticoagulated by EDTA were utilised. Centrifuged plasma was divided in: whole plasma (P) and defibrinated plasma (DP), by means of Cl<sub>2</sub>Ca recalcification and clot removal. Assays: fibrinogeneno (Fb mg/dl) by gravimetry; IgM (mg/dl) and ceruloplasmine (Cp mg/dl) both by immunoturbidimetry. EA: registering variation of transmitted light through automated shaked blood sample every 2 min. Parameters obtained at 40% hematocrit: 1) aggregate size (S) and 2) aggregation rate (R). Es were suspended in P and DP. Fb absence confirmed by high resolution electrophoresis. Results (x±SD): 1) R in P vs DP [30±5, p<,01] but not in S[.05±.09]. No correlation between R and Fb or IgM. There was n.s. correlation between S vs IgM [r= .84, p<.05]. There was decrease in DP, 68%, regarding P. Conclusion: Fb decreases R aggregates, while their S is associated with IgM leve. Ceruloplasmine showed no modification upon aggregation process.

## STUDY OF THE DECISION MAKING PROCESS FOR THE MEDICAL CAREER

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Medicine still is an attractive career for a lot of applicants. As its decision making process is related, among others, with their social origin and the instructional level of their parents, these variables were mainly studied in our School of Medicine. An ad-hoc questionnaire was applied to a random sample of 129 female and 74 male admitted students. 54% of their parents had complete or incomplete tertiary instructional level. 40% of women's fathers and mothers had complete or incomplete university studies as occurred in 48% of men's fathers and 35% of their mothers. Analyzing the possible influences received when making the decision, 61% of women and 48% of men referred none at the same time as the rest stated the influence of a close relative (15% of women and 19% of men reported one of their parents as being a physician). The main reason for choosing Medicine was interest for Biology in 51% of women. Its social role was the second option for them (43%). This option was the first one in men (34.60%) whilst: the second was Medicine as an independent profession (30%). The social prestige of medical profession was mentioned by almost 20% of men and less than 5% of women. Exceeding the social role of Medicine, differences were significant in every case (p<0.008) when statistically analyzed through X2's or Fisher's tests. Reported data would indicate the influence of parent's instructional level in the decision for attending to the university The influence of relatives for Medicine was not relevant. By contrast, the reasons leading to it were striking between genders. Complementary qualitative studies could be advisable.

### VARIABLES RELATED WITH THE REPRODUCTIVE SYSTEM IN DIABETIC MALE ONE YEAR-OLD RATS

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Murine diabetes induced by streptozotocin reveals diminution of plasmatic testosterone and spermatogenetic alterations. Variables linked to male reproductive system were analyzed in one year-old spontaneous diabetic eSMT rats. Blood for basal glycemia and for plasmatic testosterone was obtained by tail and heart punctures, respectively, from 10 rats (5 eSMT and 5 Wistar -controls-). Testicles and epididymes were dissected and weighed. One of the epididymes was minced, suspended in 5% formalin and spermatozoa counted using a Neubauer's chamber. Data, expressed as mean ± SE, were statistically analyzed. Relative plasmatic testosterone (ng/ml/100g bio-mass): eSMT:  $0.14 \pm 0.11$  vs. W:  $0.59 \pm 0.03$ , p< 0.01; *Basal glycemia* (mg/dl): eSMT: 297.00 ± 34.00 vs. W: 93.00  $\pm$  5.80, p< 0.001; **Body weight** (g): eSMT: 314.40  $\pm$  33.27 vs. W:  $450.00 \pm 18.81$ , p < 0.05; **Testicular weight** (g): eSMT:  $1.98 \pm 0.37$ vs. W:  $3.13 \pm 0.18$ , p< 0.05; *Epididymal weight* (g): eSMT:  $0.85 \pm$ 0.14 vs. W: 1.24  $\pm$  0.18, p<0.05; *Number of spermatozoa/mg of* epididymal tissue: eSMT: 379.080  $\pm$  60.104 vs. W: 190.406  $\pm$ 35.579 (p<0.05). Relative testicular and epididymal weights were no significant. When comparing the spermatozoa number at this age with that registered at 7 months of age (eSMT: 530.950  $\pm$ 66.711- not reported yet-), we assume that these rats are undergoing aging processes in congruence with occurred in the abovementioned murine models.

## 7. EPIDIDYMES IN DIABETIC RATS EXPOSED TO DIFFERENT DIETS

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Considering that nutritional and metabolic factors can modify reproductive functions, this communication refers to epididymal data obtained from eSMT rats, a synthetic type 2 diabetic line, exposed to different diets. Weanling male animals receiving standard diet were divided in (1) ad libitum diet -ALD-(n = 7) and (2) restricted diet -RD- (24 hours fasting each 48) (n = 9). At 12 months of age, rats were sacrificed with ether overdose. Epididymes were dissected and weighed. One of them was minced, suspended in 5% formalin and spermatozoa counted in a Neubauer's chamber. The other was fixed in 10% neutral formalin and underwent a routine histological process being sections stained with Giemsa, Periodic Acid Schiff (PAS) and Alcian Blue. Data, expressed as  $x \pm SE$ , were statistically analyzed (ALD vs. RD). Body weight (g)  $(297.85 \pm 32.73 \text{ vs.})$  $436.55 \pm 14.34$ , p<0.01). Epididymal weight (mg) (727.43 ± 100.49) vs.  $1041.55 \pm 37.808$ , p< 0.01). The number of spermatozoa/mg. of epididymal tissue (306325  $\pm$  14532 vs. 364221  $\pm$  28950) appeared high but no significant. The histological study of the epididymal tail was congruent with the spermatozoa count revealing high number of luminal located sperm cells. As seen, DR rats not only showed an increased absolute body and epididymal weights but a high number of spermatozoa from a quantitative and qualitative standpoint. When considered together an opportunely reported improved biochemical profile, these results add reproductive evidences to a delay of the ageing process occurring in RD eSMT diabetic rats.

6.

## STUDY OF GLYCOCONJUGATES IN THE KIDNEY OF 1 YEAR –OLD DIABETIC RATS BY USE OF LECTINS

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Among distinct histochemical methods, diabetic nephropathy may be more specifically studied by use of lectins. This paper analyzes glycoconjugates in nephronic zones of 1 year-old spontaneous diabetic rats (eSS and eSMT) through this method. Kidneys of male eSS, eSMT and Wistar rats (controls) (n = 5 in each line) were fixed in Bouin's fluid and 10% neutral formalin and underwent paraffin embedding. Endogenous peroxidase was inactivated by a 3% solution of H2O2 (100V) in methanol. Then, specimens were treated with bovine serum albumin and incubated at 4°C during all night long with Arachis hypogaea (PNA), Ricinus communis (RCA), Triticum vulgare (WGA) and Urex europaeus (UEA-I). The day after, sections were exposed to Avidin-Biotin and Diaminobencidine. Subsequently, they were stained with hematoxylin and visualized under a Zeiss microscope. Both diabetic lines appeared weakly stained with WGA in superficial (S) and juxtamedullary (JM) glomeruli and resulted negative for PNA, RCA and UEA-1 in the flattened epithelium and the basement membrane (BM) of Bowman's Capsule (BC) S and JM corpuscles appeared negative to RCA and UEA 1 in the flattened epithelium and BM of BC. A weak reaction for WGA was registered in the BM of S and JM proximal convoluted tubules. These results may be attributed not only to the dismetabolim of eSS and eSMT but could be specifically related with their respective evolving nephropathies at the studied age.

### A STUDY OF BLOOD VISCOSITY IN PATIENTS AFFECTED BY DIABETIC MICROANGIOPATHY

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Hemorheological factors, as blood viscosity, participate in the pathogenesis of varied angiopathies. Diabetes is one of the most risky factors for developing circulatory complications and appears linked to many dermatologic microcirculatory lesions: erysipelas -like erythema, rubeosis, perionychial telangiecta- ses and pigmentary purpura. Thus, the study of whole blood viscosity, at low speed, may provide information indirectly related with erythrocyte aggregation whilst, at high speed (230 sec.-1), may do it with erythrocyte deformability. Otherwise, the study of plasmatic viscosity may give data related with non cellular factors. This report intends to establish possible relations between the changes produced in plasmatic and whole blood viscosities of diabetic patients and the appearance of dermatologic lesions related with diabetic microangiopathy. Then, 18 diabetic patients with skin lesions and 18 healthy ones were analyzed. The aforesaid viscosities were determined at different speeds (2.30; 4.60; 11.50; 23; 46; 115; 230 sec..-1) using a cone-plate viscometer, Data were compared through the Mann Whitney's Test. Significant differences for all values of plasmatic viscosity were registered between sick and normal patients (p< 0.0001). For whole blood viscosity, this difference was only significant at lower speed than 46 sec.-1 (p<0.001). These results reveal that blood viscosity could participate in the development of diabetic microangiopathy and, through it, in the appearance of the abovementioned dermatologic lesions.

### THE HUMAN STOMACH DURING ITS EARLY FETAL DEVELOPMENT

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Considering the relevance of the pre and postnatal development of digestive system, this paper reports the differentiation of the stomach in 9th and 10th week-old six human fetuses obtained from spontaneous abortions in public hospitals. Serial 7 µm thick sections from the abdominal zone were sequentially exposed to varied histological and histochemical procedures. Then, they were planimetrically processed and superposed for spatial reconstruction. At these ages, the stomach appeared as the most dilated portion of the digestive tube showing fornix, body and antrum when longitudinally cut and a slight tubular aspect dorsally and ventrally flattened when transversally sectioned. Its location and general shape resembled those of postnatal life. From the inner to the outer surface, the histolo-gical study revealed: (a) a high single columnar epithelium with nuclei at different heights as well as scattered small pits, cover-ed by round cells and simulating intraepithelial glands; (b) a tunica propria with loose connective tissue, rich in reticular fibers; (c) lack of muscularis mucosae; (d) the inner layer of the muscularis externa; (e) a continuous myenteric plexus and (f) a noteworthy cellular density in the submesothelium. These data suggested: (1) an epithelial proliferation pattern; (2) the intraepithelial-like glands as the origin of future gastric fovea; (3) a possible primitive propul-sion movement based on the incomplete muscularis externa; (4) an ended migration process from neural crests to myenteric plexus and (5) the probable differentiation of the outer layer of the *muscularis externa* in the submesothelial cellular density.

### 11.

## IN VITRO EFFECT OF INSULIN ON RED BLOOD CELL RHEOLOGICAL BEHAVIOUR IN HYPERTRIGLYCERIDEMIC OBESE AND DIABETIC RATS

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Previous studies in our laboratory in line- $\beta$  rats (hypertriglyceridemic obesity and diabetes model) have shown hemorheological alterations associated with triglyceridemic and glycemic levels and these alterations were more pronounced in aging animals. In the present work, the in vitro effect of two physiological insulin concentrations (120 and 480 pmol/l) upon erythrocyte deformability and osmotic fragility of β-line rats aged 200 days were studied (N=12). Blood was obtained by cardiac puncture. RBCs were separated by centrifugation, washed in saline, divided in three aliquots and incubated during 15 minutes: Aliquot A: PBS containing 100 mg/dl glucose without insulin (control); B: PBS plus 120 pmol/l insulin; C: PBS plus 480 pmol/l insulin. The supernatant was removed and RBCs were suspended: a) in saline for erythrocyte deformability estimation (Tk), and b) in 0 to 0,145 M NaCl for the study of osmotic fragility. Statistical analysis: Student's t test for paired data. We were observed a significant decrease in the Tk of B and C aliquot (regarding control) but not in the hemolysis curves (hemolysis percentage vs NaCl concentration). These results suggest that insulin interacting with RBCs increases erythrocyte deformability, but does not produce changes in the erythrocyte membrane associated with osmotic fragility.

#### 10.

## PRACTICAL APPLICATION OF GRAPHICS RELATED WITH POSTSTENOTIC ARTERIAL FLOW AS A COMPLEMENTARY DIAGNOSTIC TOOL

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Altered blood flow resulting from arterial stenoses produces distal turbulences and tensions affecting blood cells. When enduring after surgical treatment, the zones reached by that altered flow could evidence the appearance of relapses. Taking advantage of an opportunely reported original software based on the method of the finite elements, adapted to the physical characteristics of blood and apt to simulate different blood flows, we performed and analyzed graphics of flow functions. Moreover, we could determine the distance requested to normalize the poststenotic flow, which appeared longer to that considered during the surgical replacement. This led to reveal the possible endurance of still damaged endothelium and the probability of sequels forcing new interventions. From the simulations performed on real models.  $170 \pm 5$  graphics with maximal curves for each diameter of the affected vessel and for each percentage of obstruction were obtained. To simplify the interpretations, these graphics were summarized in one graphic resulting from the linear approximation applied to the graphic values of all the studied cases. Then, using the diameter of the affected vessel and the percentage of obstruction as input data the referred requested distance could be achieved. In sum, this complementary tool may improve usual diagnostic methods when combined with them.

## 12. IONIC STRENGTH INFLUENCE OF THE MEDIUM ON ERITHROCYTE AGGREGATION

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Erythrocyte aggregation (EA) is a process influenced, among other factors, by erythrocytes surface charge. In this work, the effect of medium ionic strength  $(\mu)$  upon EA was studied, considering the glycocalyx of the red blood cells (RBC) as a polyelectrolyte fixed on an electrically neutral surface, whose surface charge will be modified when the medium ionic composition is altered. Washed RBCs, were incubated at 10% Hct during 30 min at 37°C, in media with different  $\mu$ : 0.516; 0.20 (normal) and 0.07 moles/1 respectively. RBCs were resuspended in 2% dextran solution (PM: 500.000) at 40% Hct. EA was measured by transmitted light, obtaining two parameters: sono, which estimates the size of the aggregates and 2k<sub>2</sub>n<sub>0</sub>, which characterizes the kinetic of the process. Statistical analysis: ANOVA A significant decrease of both parameters was observed both at high and low i as compared with the normal  $\mu$ (p<0.01). The results of this work are similar to those obtained by some of the authors in a previous work, where they found a shape index variation of the RBC both at high and at low  $\mu$  in the same way (stomatocytosis). Conclusion: the influence of the  $\mu$  in both cases, aggregation and erythrocyte shape, is related to the effect produced by the medium on the RBC glycocalyx ionic atmosphere altering the electric potential of the membrane surface  $(\psi_0)$ . This effect on the  $\psi_0$  might be mediated by two factors: the masking effect of the medium ions, and the dissociation grade of the surface charged groups.

## EFFECT OF ALIMENTARY RESTRICTION UPON ERYTHROCYTE AGGREGATION IN A MODEL OF HYPERTRIGLYCERIDEMIC OBESITY

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Studies conducted in our laboratory, on different age groups of the β (beta) rat genotype (with moderate hypertriglyceridemic, normocolesterolemic obesity and glucidic intolerance), inbred in the Fac. de Cs. Médicas in Rosario, showed a lower tendency for red blood cells (RBCs) to form aggregates on the  $\beta$  line (obese) with respect to the  $\alpha$  line (eumetabolic). In this work the effect of alimentary restrictions over determining factors in the erythrocyte aggregation process in obese rats was studied. At weaning the animals were divided into two groups: A: (control; n=10) fed ad libitum, and B: (restriction; n=12): alimentary supply 30% lower than that consumed by group A. After 200 days Hematocrit (Htc), erythrocyte deformability (ED), triglicerydemia (TG), fibrinogen (Fb), albumin (Al), globulins (Gl) and erythrocyte aggregation (EA) of RBC suspensions in its own plasma and in Dextran 500 were determined. Statistics: Student's t test for unpaired data. It was noted that the group subjected to alimentary restriction did not show a significant difference in ED, Fb, Al or EA in its own plasma, a difference in Htc, TG and Gl which decreased and EA in Dextran 500 which increased. Our results would be yielding evidence of the presence of changes in the membrane of red blood cells unrelated to lipid or protein plasma levels because the RBCs of both groups do not differ in the tendency to aggregate when they are in their own plasma.

### 15.

## GINGER IN PROTECTION AND AGGRAVIATION OF GASTRIC MUCOSA LESIONS INDUCED BY ETHANOL. ROL OF SENSORY NEURONS

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Objetive: to study the dose-dependent ginger (GIN) effect on the gastric mucosa aginst ethanol injury and its probable mechanism of action. Wistar rats (n=7 in each group), 200 g, 24h fasting, water ad lib. were submitted to the following experiments: 1. 1ml saline by orogastric gavage, 60 min wait (control); 2. 100-500 mg/k IG GIN 60 min (control); 3. 1 ml 96% ethanol (ETOH), 1ml, IG, 20 min (control); 4. 2% lidocaine chlorhydrate (LIDO) (blocking local anesthetic of sensory neurons), IG, 30 min (control); 5. 20mg/ k Indomethacin (INDO) (1-2 cyclooxygenase inhibitor) SC, 60 min (control); 6. 100 mg/k GIN, then ETOH; 7. 500mg/k GIN, then ETOH; 8. LIDO, then 500 mg/k GIN, finally ETOH; 9. INDO, then 500 mg/k GIN and then ETOH. The rats were sacrificed by ether overdose. Laparatomy, gastrectomy and aperture along great curvature were performed. Gastric macroscopic lesional area (GMLA) percentage was assessed by computerised planimetry. Histological samples were obtained (HE). Results evaluated with Student t test. GMLA %: **1.**  $1.0 \pm 0.1$ ; **2.**  $1.0 \pm 0.1$ ; **3.**  $35.5 \pm 5.5$ ; **4.**  $1.0 \pm 0.1$ ; **5.**  $1.0 \pm 0.1$ ; **6.**  $5.1 \pm 6.0$  (p<0.01); **7.**  $45.6 \pm 5.1$  (n.s); **8.**  $1.0 \pm 0.1$ ; **9.**  $30.5 \pm 5.8$  (n.s). Histological examination showed normal gastric mucosa in groups 1, 2, 4, 5 y 8. It can be concluded that GIN at low doses (100 mg/k) protects the gastric mucosa against ethanol injury; in contrast al higher doses (500 mg/k) the lesions are aggravated; and in the protecting mechanism take part the sensory neurons.

#### 14.

### **BLOOD RHEOLOGY IN NORMAL PREGNANCY**

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An efficient placenta flow is vital for normal fetal development and growth. Blood flow is determined by a driven flow, blood vessels diameter and flow intrinsic properties. The objective of the present work was to analyze the hemorheological profile in normal pregnancy second trimester. One hundred and two non-medicated non-smoking normal pregnant women, with neither fetal growth retardation nor hypertension were compared with an age-matched control group. Assays: hematocrit (Htc), hemoglobin (Hb), total protein (TP), albumin (Al), globulin (G) and fibrinogen (Fb), erythrocyte aggregation (EA) by photometry, blood viscosity (BV) and plasma viscosity (PV) with a cone-in-plate viscometer and erythrocyte deformability (ED) estimated by filtration index (FI). Statistics: Student's t Test for unpaired data. The results showed a change in blood rheology in the second gestation trimester due to plasma and cell alterations: a) EA, globulin levels and PV increase in pregnant women in comparison with controls; b) Al decrease, and c) no statistical differences in BV at native Htc. The modified protein profile, namely Al -considered as an antiaggregant agentand G and Fb (plasma physiological aggregant agents) might be the cause of a higher EA in pregnant women. Besides, higher Fb levels could be responsible of increased PV in pregnancy. On the other hand, lower ED (greater FI) in pregnancy points out the existence of possible cell membrane alterations. Lack of difference between BV at native Htc could be due to physiological hemodilution during pregnancy evidenced through Htc lower value, that remarkably counterbalances the above-mentioned effects (ED and PV), given rise to an adaptive change in order to preserve an efficient placenta flow.

### 16.

### EFFECTS OF A PHYSICAL TRAINING PLAN IN PUBESCENT OBESE $\,\beta$ RATS

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Rats of  $\beta$  line develop obesity and hipertriglyceridemia around puberty and become diabetic at adult age.

We have already shown that in adult  ${\mathfrak B}$  rats a moderate physical training plan did not make modifications in body weight nor in plasmatic parameters.

The aim of the present work was to evaluate in  $\beta$  rats the effects on obesity and diabetes of implementing the plan since puberty.

From 70 to 130 days old, eight  $\beta$  male rats were trained three times a week, 15 minutes a day, employing an ECO 3/6 rodent treading machine at 30  $\pm$  1 m/seg. Control (C) and trained (T) rats were fed ad libitum.

At 130 days old, there were not significant differences between groups (Mean  $\pm$  S.E.M):

- Body weight (g): C:  $412.2 \pm 5.14$ ; T:  $434.7 \pm 11.23$
- Tail length (cm): C:  $18.11 \pm 0.05$ ; T:  $18.09 \pm 0.22$
- Retroperitoneal fatty panicles weight (g/100g of body weight): C:  $5.280 \pm 0.251$ ; T:  $5.640 \pm 0.299$
- Triglyceridemia (g/l): C:  $2.41 \pm 0.04$ ; T:  $2.55 \pm 0.27$
- Fasting glycemia (g/l): C: 1.48  $\pm$  0.04 ; T: 1.66  $\pm$  0.10
- Glycemia after 120 minutes of an oral glucose overload (g/l):
   C: 1.69 ± 0.06; T: 1.85 ± 0.09

This physical training plan, even implemented since puberty, did not avoid obesity and diabetes development in  $\beta$  rats. Probably it should be combined with caloric restriction.

## IN VITRO EFFECT OF DIFFERENT COMPOUNDS OF ALUMINIUM ON INTESTINAL CALCIUM ABSORPTION

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Aluminium (Al) is a toxic metal for both human and animal beings. The main route of access to body in healthy individuals is gastrointestinal tract. In the intestinal lumen Al can reacts with several dietary substances producing a wide range of chemical species. The goal of present work was to analyze whether Al chemical speciation has influence in its biological effect. 45Ca transport (JCa) was measured in a model of in vitro chicken everted small intestine, in presence of different chemical forms of Al, inorganic ones (I): AlF<sub>3</sub>, AlCl<sub>3</sub>, Al(OH)<sub>3</sub>, and organic ones (O): Al citrate (CAl), Al maltol (MAI), Al lactate (LAI), at nominal concentrations varying from 2 to 200  $\mu$ M (pH 7.3). Al-free controls were incubated with respective ligands. For each compound and concentration the mean of n = 4 measurements were used. JCa (% control) was plotted against log [Al], then  $\rm I_{\rm max}$  (maximal inhibition) and  $IC_{50}$  ([Al] produces half of  $I_{max}$ ) parameters were calculated. Curves were compared by analysis of covariance. To characterize Al chemical species, FT-IR (Fourier transformed infrared) spectra of compounds lyophilized in absence and presence of 1 mM Ca were obtained. Furthermore, Al speciation in incubation solution was simulated by mean of computer software. All the essayed Al compounds diminished JCa displaying a downhill sigmoid dose-response curves, excepting CAI that resulted be linear and Al(OH)<sub>3</sub> that did not show no defined trend. I<sub>max</sub> values were similar (~40%), but there were differences in IC<sub>50</sub> (in  $\mu$ M): AlCl<sub>3</sub>=99.8  $\pm$  0.4, AlF<sub>3</sub>=59.4  $\pm$  0.3\*, MAl=94.2  $\pm$  0.5, LAl=86.6  $\pm$  0.6\*; \*P<0.05 vs. AlCl<sub>3</sub>. I forms and LAI showed a pronounced shifts of FT-IR peaks in presence of Ca, in wavelength range 450 to 3500 1/cm, denoting a strong interaction between Al and Ca. These results suggest in vitro Al action on JCa would be dependent upon chemical form of metal (inhibitory effect: AlF, > LAl > AlCl<sub>3</sub> = MAl > CAl > Al(OH)<sub>3</sub>), which would determine its biodisponibility and thereby its biological action mechanism.

### 19. CHANGE OF ENVIRONMENT. ITS EFFECT ON WEIGHT, GLYCEMIA, PANCREAS AND KIDNEYS IN eSS' RATS

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eSS diabetic rats, bred in the Rosario School of Medicine, were for some time maintained in the Instituto de Investigaciones Médicas (IIM), being designated eSS'. Since genotype-environment interactions were found in eSS, we decided to study the effect of food supply and habitat changes in eSS'. 10 males and 9 females were transported from IIM - where they had been bred until 3 months with a diet prepared with natural ingredients - to our bioterium. Body weight (BW), fasting glycemia and after 120 min of glucidic overload (G0 and G120) were registered. From then on, they received the usual balanced commercial diet. At the age of 6 months, BW, G0 and G120 were registered and the animals were sacrificed with ether overdose; pancreas and kidneys were weighed and examined histologically. Data obtained before and after the environmental change were compared and in all variables, differences between sexes were assessed; G0 and G120 were higher in males and BW increased in both sexes. Pancreas weight did not differ between males and females, the latter showing higher number of large islets. In both genders islets were round or policyclic. Kidneys in males were heavier, not differing with females when considering their relative weight. Less smaller glomeruli were found in females. The stress caused by the change of habitat and nutrition might, in part, be responsible for the levels of glycemia in eSS'males; females seem to resist much better the effects of environmental changes.

#### 18.

## ALUMINIUM EFFECTS ON CALCIUM UPTAKE IN CHICKEN ISOLATED DUODENAL CELLS

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The objective of this work was to study in vitro actions of aluminium (Al) on kinetics of radiocalcium uptake (45Ca<sub>UPT</sub>) in chicken isolated duodenal cells. Epithelial cells, obtained according to method described by Liang et al., were incubated in aerobiosis, during 30 min. with 0 and 100 µM Al lactate, at [Ca<sup>2+</sup>] varying from 0.01 to 5 mM (pH 7.4). Apparent constants  $V_{max}$  and  $K_{m}$  were calculated from  $^{45}Ca_{\mathit{UPT}}$  vs.  $[Ca^{2+}]$  plots by a non-linear fitting method using the Marquardt-Levenburg algorithm. Curves were compared by analysis of covariance. In other series of experiments, enterocytes were treated with Al in presence of: i) diltiazem (D) or nifedipine (N) (blockers of L-type voltage-dependent Ca channels, VOCC): 1, 10 and 100 μM; ii) A23187 (Ca ionophore): 1 μM; iii) Bay K8644 (Ca channel activator, acting on dihydroxipiridine site): 1 µM; iv) capsaicin (activator of Epithelial Calcium Channel, ECaC): 2 and 20 μM; v) U73122 (activator of store-operated Ca channels): 1 nM. Controls were incubated with respective vehicles (C). Results were expressed as % of respective Al-free control. Al significantly reduced both V<sub>max</sub> (15.3  $\pm$  1.9 vs. 22.1  $\pm$  3.4 nmol Ca/mg prot.) and K<sub>m</sub> (1.4  $\pm$  0.2 vs. 2.1  $\pm$  0.4 mM) as compared to Al-free control (P< 0.05). D from 1  $\mu$ M (101  $\pm$  2 %) and N from 10  $\mu$ M (100  $\pm$  3%), completely annulled Al inhibition as compared to C (56  $\pm$  3%). Bay K8644 (82  $\pm$  4%), U73122 (84  $\pm$  3%) and capsaicin (2  $\mu$ M=  $97 \pm 2\%$ ,  $20 \mu M = 89 \pm 3\%$ ) reduced Al inhibition. A23187 increased Al inhibition (45  $\pm$  3%, n = 4, P< 0.05, ANOVA). In summary, Al could reduce Ca entry in chicken enterocytes acting directly on *VOCC*, preferably in the open state of channels.

### 20.

## GLUCIDIC PROFILE AND MORPHOLOGY OF PANCREAS AND KIDNEYS IN RATS DERIVED FROM e AND eSS LINES

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The aim of this paper is to study the glucidic features and histopathology of pancreas and kidneys of the two lines of rats that shared the same habitat, designated e'and eSS', after years of isolation from eSS. 17 eSS' (9 males and 8 females) and 16 e' (7 males and 9 females), aged 9 months, were fed with a diet based on natural ingredients. They were weighed; basal glycemia and after 30, 60 and 120 min of glucose oral overload, were measured. Taken all the variables together, line, sex and interaction effects were verified. G0 showed line effect; G30, G60 and G120 sex effect and weight showed differences in line and sex. Optic microscopy revealed round, policyclic and star-shaped pancreatic islets in eSS'males, smaller than in e'. Only round and starfish-like islets were found in females. eSS'males had a higher proportion of renal glomeruli smaller in diameter and more marked lesions in the tubular epithelium. Results revealed sexual dimorphism for weight and glucose intolerance, features repeatedly found in eSS. e' males present a notoriously altered glucidic profile and eSS', with lower weight and normal fasting glycemias, displays in that environment a marked glucose intolerance.

### HEPATIC ESTEATOSIS IN AGED SPONTANEOUSLY DIABETIC RATS FROM eSS AND eSMT LINES

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Hepatic esteatosis is frequently found in patients with type 2 diabetes. eSS and eSMT lines of rats display this syndrome, more severe in males. We investigated the presence of hepatic pathology in animals of both lines and genders and Wistar eumetabolic males, aged 14 months and more. Livers were weighed, some parts were embedded in paraffin and sections were stained with H-E and PAS; others were frozen and stained with Oil red to identify lipids. When diabetic lines were analyzed, ANOVA showed line-sex significant interaction for body weight and relative liver weight, probably due to the larger biomass of eSMT males and the lower relative liver weight of eSS females. When comparing males, Wistars had higher total weight, followed by eSMT and smaller total and relative liver weight. Coarse cytoplasmic granularity in hepatocytes were more evident in eSMT males. Eosin and PAS negative vacuoles were seen in the central and middle lobular areas, more abundant in eSMT males and smaller in females of both lines; their distribution corresponded with Oil red stain. The pattern of lipid deposits in dysmetabolic lines is similar to that of hepatic esteatosis observed in diabetic patients. Diabetes per se, without obesity, may be the cause of hepatic esteatosis in eSS. In eSMT this lesion is more marked, probably due to its larger biomass. The histopathological features and the expression of the diabetic syndrome are less conspicuous in females of both lines.

### 23.

# DIRECT EFFECT OF THE METHANOLIC FRACTION OF LIGARIA cuneifolia (ARGENTINE MISTLETOE) EXTRACT ON RHEOLOGICAL PROPERTIES AND ERYTHROCYTE SHAPE

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The aim of this study was to investigate the direct effect of the methanolic fraction of Lc extract on murine erythrocyte shape and deformability. Solution was prepared by mixing methanolic fraction of Lc extract (25 mg%) and polyvinyl-pyrrolidone carrier (PVP: 12,5mg%) (FMLc). Blood samples obtained by cardiac puncture from inbred line of male Wistar rats were divided into three equal aliquots; one was used for control (C) (n=5), the second was added PVP (n=5), and the third FMLc (n=5). They were incubated 30 minutes at 37 °C; the rigidity index (RI) was determined by the filtration method through nucleopore membranes and the cellular shape by direct microcospic observation. The Morphological Index was calculated (MI=  $\Sigma$ [ shape index multiplied by cell number/ whole number of cells]). Statistics: ANOVA and Dunnet's test were used. Results: RI: C:  $14,40 \pm 0,23$ ; PVP:  $24,50 \pm 0,29*$  and FMLc:  $34,33 \pm 1,92*\#$ . MI : C:  $-0,80 \pm 0,05$ , PVP:  $-1,53 \pm 0,07*$ , FMLc:  $-1.97 \pm 0.19 * #, (*p<0.05 vs control, # p<0.05 vs PVP).$ Both PVP and FMLc have shown a direct on the erythrocyte membrane producing a change of shape from biconcave disc to spherostomatocyte (MI more negative). A decrease in erythrocyte deformability (RI increased) showed an interaction of PVP and FMLc with the erythrocyte membrane.

#### 22.

### TESTICULAR AND EPIDIDYMAL HISTOLOGY FROM DIABETIC RATS FED WITH DIFFERENT DIETS

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Nutritional factors modify the functions of male genital systems. We studied histologically the testis and epididymis of  $(\beta)$  rats (obese, late diabetic), which were fed with different diets after weaning: 1.- standard commercial food (Cargill) ad libitum (DAL). 2.- standard food restricted to the 70% of the control (R). 3.- Hyperglycemic diet: DAL + saccharose (HG). 4.- Hyperlipidic diet: DAL + bovine fat (HL). At 250 days of age the animals were sacrificed with ether overdose and weighed. The dissected testis and epididymis were weighed. Testis were fixed in Bouin's liquid and postfixed in 10% formalin in PBS. Epidydimis were fixed in 10% formalin in PBS. Specimens were submitted to histologically proceedings. Stained with PAS-H, Giemsa, Masson-trichrome. Testicular histology from restricted fed rats, a tubular structure are similar to those described in control animals. Conversely, in DAL, HG, HL rats, different types of abnormal tubular structures could be observed: nucleated cells in the luminal content, and/or interspace between germ cells. Epididymis in R luminal tubules were plenty of spermatozoa in comparison with DAL, HG,HL. Tubular epithelia were similar to other control rats (Wistar) in R. Conversely, in DAL, HG, HL rats epithelial border were irregular. So, in restricted fed rats testicular and epididymal histology seemed simlar to other control animals, otherwise, in diabetic rats (DAL, HG, HL) differential lesional degree of testis and epididymis were detected.

### 24.

## INVITRO EFFECT OF HIALURONIC ACID ON ERYTHROCYTE RHEOLOGY

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We have demonstrated that Rheumatoid Arthritis diffuse aggression to cell membranes has an expression in red blood cells making erythrocyte deformability a reliable indicator of the disease activity. Besides, it was recently reported that arthritic patient blood serum has a hialuronic acid (HA) increase associated with the disease activity. Our objective is to relate serum HA concentration ([HA]) with erythrocyte deformability impairment.

HA was purified by chromatography. Blood samples from normal donors were divided into two aliquots. Purified HA was added to one of them (HA group, n=5); while to the other one it was added the same volume of neutralized elution (control group, n=5). Both groups were incubated for 30 minutes at 37°C, and serum ([HA] $_{\rm s}$ ) and erythrocyte rigidity index (RI) were determined by filtration through nucleopore membrane. Statistics: Wilcoxon test for paired data, results are expressed as median and confidence interval (CI 95%). Results: Group HA: [HA] $_{\rm s}$  = 103 (100-105) µg/ml, RI = 16.55% (15.09-19.75). Control group: RI = 8.79% (5.59-10.21), p<0.01.

Conclusions: These results demonstrate that hialuronic acid impairs erythrocyte deformability, supporting the hypothesis that serum HA can damage Rheumatoid Arthritis patient erythrocytes.

## COLONIC HISTOMORPHOMETRY AND AUERBACH'S PLEXUS NEURONAL COUNT IN *Trypanosoma cruzi* (Te) INFECTED RATS

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Male "I" line rats were separated after weaning in the following experimental groups: 1.- control (C); 2.- infected with 10<sup>6</sup> Tc (Tc); 3.- Tc + benznidazole (Tc+B); 4.- Tc+ interferon-gamma (Tc+I); 5.- Tc+ benznidazole + interferon-gamma (Tc+B+I). Autopsy was performed at 6 months after infection. Proximal and distal colon were dissected out and fixed in Carnoy's liquid, histologically processed and semi-serially cut at 6 µ. They were stained with Giemsa, PAS, and Alcian blue. Histomorphometry was performed with a lineal device and graticulated grid attached to eye objective. Data are expressed as: mean ± SEM. Neuronal counts (neurons/ mm<sup>2</sup>) in proximal colon: 1.-C:  $29.31 \pm 3.30$  (n=7); 2.-Tc:  $40.71 \pm$ 4.82 (n=11); 3.- Tc+B:  $40.73 \pm 4.98$  (n=5); 4.- Tc+I:  $40.66 \pm 4.63$ (n=5); 5.- Tc+B+I:  $31.47 \pm 3.23$  (n=6). Neuronal counts in distal colon: 1.-C: 17.43  $\pm$  1.97 (n=8); 2.- Tc: 16.81  $\pm$  1.43 (n=11); 3.-Tc+B:  $23.58 \pm 3.06$  (n=5); 4.- Tc+I:  $19.75 \pm 2.80$  (n=4); 5.- Tc + B + I:  $26.61 \pm 1.82$  (n=4). Corporal body weight, colon weight, colon length, mucosal thickness, muscular thickness, goblet cells/crypt count and neuronal count showed no differences between infected non-treated and, infected and benznidazole or interferon-gamma treated rats.

### 27.

## INTESTINAL HISTOMORPHOMETRY AND AUER-BACH PLEXUS STRUCTURES IN DIABETIC RATS FED WITH DIFFERENT DIETS

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A diabetic line of rats  $(\beta)$  was fed with different diets after weaning: 1. - standard commercial food (Cargill) ad libitum (nr). 2.standard food <u>restricted</u> to the 70% of the control (r). At 250 days of age the animals were sacrificed with ether overdose and weighed. Gut was dissected out and was flushed with PBS at 4°C. The length and weight of the gut were then measured. Samples of small intestine, proximal colon and distal colon were fixed in Carnoy's fluid., embedded in paraffin wax, semiserially cut at 6 μ. Tissues were stained with PAS-hematoxilyn, Giemsa and Masson-trichrome. For histomorphometry a calibrated micrometer attached to the ocular was used. Samples were processed with NADH histochemistry. 1-Small intestine (g): nr:  $8.76 \pm 0.35$ ; r:  $7.67 \pm 0.27$  (P<0.05). 2-Colon (g): nr:  $2.22 \pm 0.06$ ; r:  $1.85 \pm 0.06$  (P<0.001). 3- 5-Small intestine histomorfometry (nr=8; r=9): a-Wall thickness ( $\mu$ ): nr:  $872.12 \pm 39.46$ ; **r**:  $619.40 \pm 29.06$  (P<0.01). **b- Mucous thickness** ( $\mu$ ): nr: 720.15  $\pm$  19.20; r: 570.23  $\pm$  28.66 (P<0.01). c- Muscular thickness ( $\mu$ ): nr: 151.96  $\pm$  30.93; r: 49.17  $\pm$  3.73 (P<0.01). 4-**Distal colon** (n=5): a- Wall thickness ( $\mu$ ): nr: 428.69  $\pm$  11.90; r:  $359.64 \pm 24.46$  (P<0.05). Other determinations: (n.s.). **5-Proximal** colon (n=6): (n.s.). 6-NADH histochemistry: in ad libitum fed rats, intact zones to disarmament of the plexus could be seen, more in small intestine, less in colon.

Intestinal histomorphometry and Auerbach plexus structures depend upon diets in an animal model of diabetes type 2.

### 26.

## EFFECTS OF ALLOXAN DIABETES ON THE DEVELOPMENT OF SPONTANEOUS PERIODONTAL DISEASE IN RATS

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This study was done in IIM/Fm "m" male rats. Diabetes was induced by an intraperitoneal injection of alloxan at weaning. Only animals with glycemias above 300 mg/dl were used (n=13) and studied between the 6<sup>th</sup> and 11<sup>th</sup> week of age.The control group (n=43) was studied between the 3<sup>th</sup> and 15<sup>th</sup> week. The left lower jaw was resected and the development of periodontitis was evaluated by measurements done on a digital radiograph or at the microscope.

The diabetic animals showed reduced resorption areas (C: 0.59± 0.05 mm², D: 0.34±0.03, P<0.0001). The mineral density of the periodontal bone was significantly lower in the diabetic state (C: 3.26±0.42 mg/mm², D: 2.48±0.3, P<0.001). The resorption foci had similar bone density in both groups (C: 1.35±0.19 mg/mm², D: 1.16±0.15). The average radicular length inserted in the alveolar bone was not affected during the experiment. This variable was greater in diabetics than in control animals (1st. molar, C: 51.61±2.96%, D: 72.35±1.57; 2nd. molar C: 49.02±3.30, D: 69.8±1.63, 3rd. molar C: 52.5±3.38, D: 73.8±1.98). In both experimental groups, the distance between the cemento-enamel junction and the alveolar crest (CEJ-AC) was higher in the lingual than in the buccal view. The diabetic state produced a significant increased in the slope of the regression of this variable as a function of age.

The effects a) on mineral periodontal density, b) on the size of the resorption areas and c) the increased bone support of molar roots, may be explained by reduced bone turnover. The effects on the distance CEJ-AC require adicional investigations.

### 28.

### ROLE OF PARACETAMOL ASSOCIATED WITH AINES IN-HIBITORS OF COX-1 AND COX-2 IN THE PROTECTION OF GASTRIC MUCOSA IN RATS

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We study Paracetamol (P) at different doses (100 and 500 mg/kg) as-sociated with none steroids antiinflamatories inhibitors of the ciclo-oxigenasa 1 and 2 (COX-1 and COX-2) to evaluate if they produce ci-toprotection or protection of gastric mucosa before been attacked by ethanol 96% (E), and verify if it depends on Prostaglandins (PGs) or by another mechanism. Wistar rats (n=7 per group) were submitted to the following experiments: I: 1:1ml saline, Orogastric OG and 60min. expected (control)(C); 2:E 1 ml OG, 20min C; 3: P 100 mg/kg OG, 60 min. C; 4: P 500mg/kg OG, 60 min C; 5: P 100mg/kg OG then E; 6: P 500 mg/kg OG then E; 7:Rofecoxib (R) (COX-2 inhib) 50mg/kg OG, 60 min C; 8:R, then P 100mg/kg OG then E; 9: R then P 500mg/kg OG then E; 10: Ketorolac (K) (COX-1 inhib.) subcutaneous (SC) 5 mg/kg 30min C; 11: K then P 100 mg/kg OG then E; 12: K then P 500 mg/kg OG then, E; 13: Indometacina (IN) (COX-1 and COX-2 inhibitor) SC 10mg/kg, 30min C; 14: IN then P 100mg/kg OG then E; 15: IN then P 500mg/kg OG then E. II: 1: Mercury Bichloride (MB) 1% SC 30 min then P 100 mg/kg OG and then E; 2: MB then P 500 mg/kg OG then E; III: 1: Lidocaina 1% OG 1ml, 60 min then P 500 mg/kg OG then E. Rats sacrificed by ether over-dose. Laparotomy and gas-trectomy. Macrospoy (%) computerized planimetry. Histology (H-E). Student's t and ANOVA. Macroscopy: **I:** 1: 0%; 2: 35,5± 5.5%; 3: 0%; 4: 0%; 5: 0% (p<0.001); 6: 25± 7% (p<0.05); 7: 0%; 8: 45± 5% (ns); 9: 5%; 10: 0%; 11: 50± 5% (ns); 12: 15± 6% (p<0.03); 13: 0%; 14: 40± 6% (ns); 15: 15± 2% (p<0.03); II: 1: 0% (p<0.001); 2: 15% (p<0.03); III: 1: 0%. Conclusion: 1- the P at low doses (100mg/kg) produced gastric citoprotection mediated by PGs and in 500mg/kg doses gave partial protection none PGs dependent; 2- P did not use Tioles in its mechanism citoprotector in both doses and 3- there was not participation of the senso-neuronal mechanism.

## 29. DEXAMETASONE AND SELECTIVE AINES INHIBITORS OF THE CICLOOXIGENASA I AND II IN THE DAMAGE OF THE GASTRIC AND INTESTINAL MUCOSA

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Rofecoxib (R) is a none esteroid antiinflamatorie selective in-hibitor of the ciclooxigenasa 2 (COX-2); Ketorolac (K) is an AINE that in low doses has a selective effect to inhibit the ciclo-oxigenasa 1 (COX-1) and in greater doses (10 mg/kg or more) in-hibits both COXs. The Indometacina (IN) is a powerful inhibitor of both COXs. The aim of this job was study if Dexametasone (D), a glucocorticoid inhibitor of COX-2, with antiinflamatory and inmunosupresive actions would be able to increase the wounds produced by AINEs COX-1 or COX-2 in gastric and intestinal mucosa. Wistar rats (n=7 per group) were submitted to the following experiments: 1. Saline 1 ml orogastric (OG), 24hs expected (Control)(C); 2. D, 1 mg/kg, subcuta-neous (SC), 24hs (C); 3. K, 5mg/kg OG, 24hs (C); 4. R 50mg/kg OG 24hs (C); 5. IN 10mg/kg SC, 24hs (C); 6. K 5mg/kg OG then D 1mg/kg SC, 24hs; 7. R 50mg/kg OG, then D 1mg/kg SC, 24hs; 8. IN 10 mg/kg SC, then D 1mg/kg SC, 24 hs. Rats sacrificed by ether overdose. Laparotomy, gastrectomy and enterectomy. Macrospoy (%) com-puterized planimetry of the gastric mucosa (GM) lesional area and the small intestinal (SI) erosions (mm<sup>2</sup>). Histology (H-E). Stu-dent's t and ANOVA. Macroscopy: 1. GM  $0.1\pm0.1\%$ , SI  $0.1\pm0.1$  (ns); 2. GM  $0.1\pm0.1\%$ , SI  $0.1\pm0.1$  (ns); 3. GM  $0.1\pm0.1\%$ , SI  $0.1\pm0.1$ (ns); 4. GM  $0.1\pm0.1\%$ , SI  $0.1\pm0.1$  (ns); 5. GM  $66\pm0.8\%$ , SI  $180\pm25$  (p<0.001); 6. GM  $0.1\pm0.1$ , SI  $0.1\pm0.1$ (ns); 7. GM 0.1± 0.1, SI 0.1±0.1(ns); 8. GM 87±7%, SI 280±35 (p<0.01). Conclusion: the association of D with AINEs (COX-1 or COX-2) did not aggravate the wounds of GM and the ones of SI, on the other hand associated with AINEs (COX-1 and COX-2) produced aggrava-tion of the gastrointestinal wounds.

## 31. IDENTIFICATION OF BONE \( \alpha \), MACROGLOBULIN (\( \alpha \), M) RELATED-PROTEINS IN RATS TREATED WITH MFP. ITS ROLE IN FLUORINE (F) BIOAVAILABILITY

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Sodium Fluoride (NaF) and sodium monofluorophosphate (MFP) are both used in osteoporosis treatment, and produce the same increase on bone mass. However, they have significant differences. When NaF is administered, fluoride is the plasmatic leading specie. After a dose of MFP, fluoride and F bound to  $\alpha_2$ M are detected, bone F content is higher and unknown bone proteins with F have been found. The objectives of this work were to verify whether proteins with F are products of  $\alpha_2$ M-MFP complex metabolism and to investigate their role in F bioavailability from MFP.

Plasmatic α<sub>2</sub>M was purified from IIM/FcM rats, then it was used to produce antibodies in Ginea Pig. Twenty day old rats of the same strain recibed 1 ml/day of MFP 80 mM (n=8) or NaF 80 mM (n=8). After 30 days, treatments were stopped. Four rats of each group were killed, the others were kept without treatment for 30 days. Femurs and plasma were obtained from each animal at 30 and 60 days. Plasma F levels were measured, noncollagenous bone proteins were obtained and analyzed by Western Blotting.

Bone from 30-day MFP-treated rats contained proteins reactive to anti- $\alpha_2$ M. After stopping treatment, low molecular weight proteins increased and those of high molecular weight decreased. Plasma F increased in animals treated with NaF after stopping treatment, this phenomenon was not observed in MFP treatment. In conclusion, F containing proteins come from  $\alpha_2$ M and their metabolism produce lower molecular weight compounds. The event would cause effective fluoride levels in the osteoblastic environment and would explain the steady plasmatic F levels through MFP treatment.

## 30. BREAST CANCER MORTALITY RATES IN ROSARIO IN THREE DECADES

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In Argentina, as in several countries, breast cancer is the most frequent cause of cancer death among women. This report is based on data from death certificates of all women residents in Rosario whose cause of death was breast cancer, during three 5-year-periods: 1977-81, 1988-92, and 1996-2000, in order to know these rates evolution. Deaths, which were allocated to the last residence of the decedents, were aggregated into the 22 census areas of the city. Age-standardized mortality rates (SMR) by area were calculated. They were correlated with some socio-demographic variables. The SMR for the whole city was not different in the three studied periods: 35.6%, 38.2%, and 33.9%, respectively. However, while SMR remained low in some areas (i.e. area 20: 1977-81: 26%,000); 1988-92: 27%,000; 1996-2000: 26%,000) and high in other areas (i.e. area 1: 1977-81: 33%,000; 1988-92: 38%,000; 1996-2000: 36%,000) in the three decades, there were great variations in the remaining areas (i.e. area 17: 1977-81: 22%,000; 1988-92: 56%,000; 1996-2000: 25%, area 3: 1977-81: 33%, 1988-92: 58%, 1996-2000: 50% In the 1996-2000 period, negative correlation coefficients were found between SMR and variables considered as socio-demographic pointers, such as natality, fertility, infant mortality rates, and chronic malnutrition (proportion of six years old boys with grown retardation).

### 32. SPEECH AND AUDITION DISORDERS IN UNIVERSITY YOUNG PEOPLE

Drogo GA, Bloj L, Casaprima V, Dulout A, Fissore L, Jannelli A, Marcozzi N, Perfumo S, Soler E, Chinelatto M, Ingaramo L. Esc. Fonoaudiología. F. Cs. Médicas (UNR). Rosario.

Objective: to detect disorders in audition and language phonological aspects. Sample: 120 adolescents, Biophysics, School of Medicine (UNR). The speech area was studied through a survey asking about manners and behaviors young people recognize in relation to their verbal productions. It was also evaluated voice quality, prosody, articulatory function, respiratory tract, phono-respiratory coordination and obstruction. Anamnesis in audition and tonal audiometry were performed; a survey was done to investigate the knowledge young people have about the harmful effects of noise, their audition habits and the presence of further symptoms after exposure to loud music. Results (%). Speech area disorders: 56 prosody, 42 obstruction, 22 phono-respiratory coordination, 21 voice quality, 20 respiratory tract and 10% articulatory function. Surveys about pronunciation and speech speed: 37 of the young people referred problems in their pronunciation, only 9 had actually articulatory mistakes. Speech speed: 69 showed a disorder; a high percentage said that they spoke fast. Audition area, 100 knew the harmful effects of noise, 37 listened to music at a high/maximum volume, 92 went to places with amplified music and 87 had further symptoms after exposure; 24 had perceptive hearing loss. Conclusion: high number of young people with a difficulty in rythmicalprosodical aspects, in agreement to the answers from the surveys were observed. Pronunciation problems are due, in a high percentage, to linguistic difficulties. While in audition area, though participants knew about noise harmful effects, they persisted in their harmful habits, which could be related to the decrease in the auditory threshold found.

### ESTERASE POSITIVE MACROPHAGES IN RECTUM FROM OVALBUMIN SENSITIZED RABBITS

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Mononuclear phagocyte system consists of cells that have a common lineage which primary function is phagocytosis. Macrophages (MΦ) are the main cell of this system. In rectal mucosa, MΦ are present within the epithelium, in the subepithelial zone and in chorion, diffusely distributed in each area. Mø precursors originate in the bone marrow, circulate in bloodstream and enter various tissues where mature and become activated. Cytochemical analysis revealed high activity of alpha-naphtyl acetate esterase in their lysosomes. M $\phi$  are involved at all stages of the immune response, either innate or adaptative. The aim of the study was to analyze modifications in number of esterase positive M\$\phi\$ in rectal lamina propria from ovalbumin (OVA) sensitized rabbits. New Zealand rabbits (n=20) were divided in to 2 groups (G). G1: normal control. G2 twice OVA subcutaneously sensitized. Specific anti-OVA-IgE levels were determinated by Passive Cutaneous Anaphylaxis test (PCA). Samples from rectum were snap frozen and stained for non-specific esterase using naphtyl acetate as substrate. Ten high microscopical power fields (HPF-400X) in ten slides were counted in each animal. Results were expressed as the arithmetic mean and SD of esterase positive Mφ/HPF. Statistical analysis was made using t test. PCA was positive at 160 fold dilutions. In G1 6,45  $\pm$ 1,94 esterase positive cells per field were found. In G2: 12,12 ±2,52 M\$\phi\$ (p <0,0001). The Mφ increase in sensitized animals may represent a recently recruited population of monocytes.

### 35

### N-ACETYLCISTEIN (NAC) AND GASTRIC STRESS

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Thee aim of the present work was to study the effect of the antioxidant agent NAC in the gastric stress. Randomized groups of Wistar rats (n=7 each group), 200g weight, 24hr fasting, and water ad lib. And avoiding coprophagy were submitted to the following experiments: 1. 1 ml saline in bolus by orogastric (OG) gavage, and gastric stress (S) provided by immobilization and immersion in 18°C water during 3 hr.(control); 2. 20mg/kg NAC, intraperitoneal (IP), 30 min, and then S. The rats were sacrificed by ether overdose. Laparatomy, gastrectomy and aperture along the great curvature were performed, and percentage of gastric macroscopic lesion area was tabulated by computerized planimetry. Tissue samples for histological examination were obtained (H.E. stained). Data evaluated by ANOVA and Student's t Test. 1. 75±7%; 2. 15.5±2.1% (p<0.01) in the gastric antrum and  $1.0\pm0.1\%$  (p<0.001) in the gastric body. The microscopical examination showed: normal gastric mucosa in the gastric body and necrotic lesions in the antrum. It is concluded that NAC is a gastric mucosa protector in stress.

### 34.

## MAST CELLS IN SACCULUS ROTUNDUM FROM RABBIT IN LOCAL ACUTE ANAPHYLACTIC INMMUNE RESPONSE

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Sacculus rotundum is located close to ileo-cecal valve and contains mainly lymphatic tissue. Owed to its digestive physiology, rabbit is specially interesting as a model to study mucosal immunity. Mast cells belong to an heterogenous family of cells that come from stem cells in bone marrow. After circulating in blood they colonize different tissues where develop into mature mast cells. The aim of the study was to evaluate Alcian Blue positive mucosal mast cells in sacculus rotundum from rabbit in sensitized and sensitized plus orally challenged rabbit. Thirty New Zealand rabbits were divided into three groups: G1: normal, G2: sensitized, G3: sensitized, orally challenged and sampled 4 hours after challenge. Sensitization was performed subcutaneously with ovalbumin (OVA). Specific anti-OVA IgE levels were evaluated by positive passive cutaneous anaphylaxis test (PCA). Samples from sacculus rotundum were fixed in Carnoy and stained with Alcian Blue (AB) ph<1. 200 high microscopical power fields (HPF) were counted in each group. Results were expressed as an arithmetic mean and SD. Statistical analysis was made using Student t test. PCA was positive at 160 fold dilutions. In G1=  $3.4 \pm 0.84$  mast cells/ HPF, in G2=  $7.04 \pm$ 2,16 mast cells/ HPF (p<0,001), in G3= 5,28  $\pm$  1,43 mast cells/ HPF (p<0,05). Results showed that sensitization induces the production of cytokines that stimulate mast cell proliferation. The oral challenge, with the passage of the antigen through the mucosal barrier, induces the mast cell degranulation, with the consecuent decrease in AB-positive cells visualization.

### 36.

## DESCRIPTIVE STUDY OF KNOWLEDGE AND PRACTICES IN DRUG CONSUMPTION IN FIRST YEAR STUDENTS OF MEDICINE FROM A PRIVATE INSTITUTE

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Objective: to find out the knowledge and practice of drug consumption in 1st year students of Medicine from a private institute. The 1st day of the beginning of the Level Course, 33 students were anonymously surveyed with their consent. The questionnaire consisted of 52 questions (open and closed) gathering social, economical and cultural identification data related to the knowledge about and consumption of drugs. Results (%): (sample aged x=18yrs old: 58% male, 42% female): 79% take drugs, 63 by medical prescription, or 9 on their own. 21 of the students started at a very young age and 63 just take them occasionally. Drugs most commonly used: analgesics and antiinflammatories (70), and vitamins (9). Drugs bought in pharmacies (72) and in medical products laboratories (9). Drugs which they have knowledge and information about right from their doctors (49), from their relatives ((33), from medical brochures and advertising (12). Regarding to references they have about: drugs not needing medical prescription, analgesics (55) are in the 1st place; drugs considered dangerous: anorexigens (40), psychodrugs (30) and anabolics (6); drugs considered harmless or innocuous, analgesics are 67; drug contraindications: pregnancy (35), arterial hypertension (10); most common side effects in drugs: digestive disorders (64), drowsiness (15), dizziness (9), allergies (3). Conclusion: Since it is a young population, a high % of students take drugs, on their own, although most of them are sold with a medical prescription. While drugs are mainly bought in pharmacies, a great % of the students knew through other means, different from medical prescription. Students information students about danger, contraindications and side effects was impressive, due to the very little seriousness they give to the subject.

### ADAPTIVE GASTRIC STRESS

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It is already demonstrated in rats that after 4th day chronic stress the necrotic area induced by acute stress decreases. The objective was to study prostaglandins, sensitive neurons and heat stress proteins (HSP) role in gastric adaptation to stress. Randomized Wistar rats groups (n=7 each group), weighting 200-230g, 24 hr fasting, water ad libitum, avoiding coprophagy were submitted to the following experiments: 1. 1ml subcutaneous (SC) saline (S). 2. Stress by immobilization and 23°C water immersion, during 3.5 hr for 4 consecutive days, (S) (control) 3. Stress, 30 mg/kg SC Indomethacine (In), 5 days previous (pretreatment: Pt) and 3.5 hr stress. 4. At day 5, 500mg/kg ginger (Pt), ablation of sensitive neurons and 3.5hr stress. 5. Stress, at day 5, 10mg/kg Prazosin (P) (Pt) (HSP blocker) and 3.5hr stress. Laparatomy, gastrectomy, aperture along great curvature and tabulation of gastric mucosa necrotic area (%) by computerized planimetry and histological examination (H-E), were performed. Evaluation by Student's t Test and ANOVA. Results: 1. 80±5%, 2. 40±6% (p<0.01), 3.80±6% (p<0.01), 4.82±5% (p<0.01), 5.85±7% (p<0.01).

Conclusion: The adaptation of the gastric mucosa was confirmed at day 4 of gastric stress. Endogen prostaglandins, heat shock proteins and gastric sensitive neurons take part in its mechanism.

### 39.

### RELATIONS BETWEEN PLASMA VISCOSITY AND LIPO-PROTEINS OF HIGH AND LOW DENSITY IN MENO-PAUSAL WOMEN

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It is known that cardiovascular disease risk is high in menopause. It has also been proved the inverse association of the lipoproteins with atherosclerosis risk. The aim of the present work was to investigate the extent of the effect of lipoproteins on plasma viscosity (n<sub>p</sub>) in menopausal women. The n<sub>p</sub> of 41 menopausal women was determined with a cone-in-plate viscometer, the serum of HDL-C and LDL-C with the enzymatic colorimetric method and data were correlated. The results demonstrated that the LDL-C is positively associated with the  $n_p$  (r = 0.34, p < 0.02) and the HDL-C does this negatively ( $r = -0.39^{r}$ , p < 0.01). A multiple regression model revealed that 25% of the n<sub>n</sub> variation can be attributed to these lipoproteins (F = 6.36, p = 0.008, p-LDL = 0.01, p-HDL = 0.02). With the coefficients obtained from the statistical study the following equation it could be deduced:  $n_p = 1.69 - 0.00439$ . HDL-C + 0.00133. LDL-C. These results demonstrate that these lipoproteins have a significant and opposite effect on the  $n_p$ , which correlates with its association with atherosclerosis risk. The present work shows in which way a parameter of the blood rheology as it is the n can represent a link between the metabolic alterations and the circulatory modifications in menopause.

#### 38.

### VARIATION OF MEMBRANE FLUIDITY WITH TEMPERATURE

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In this work membrane fluidity at different temperatures was studied. For this the coefficient of fluorescence (r) anisotropy of the hydrophobic fluorescent tracer trimethylammonium, 1,6 diphenyl-1,3,5-hexatriene (TMA- DPH) bound in the lipid bilayers of the membrane of human red blood cells (RBCs), at temperatures of 26°C, 32°C, 37°C and 42°C was measured. This r coefficient refers to the restriction of the tracer movement in the lipid bilayers and it gives information of their dynamics indicating the so-called membrane fluidity. The higher the value of r the lower the level of fluidity. Anticoagulated blood was obtained with heparin of 15 healthy volunteers; RBCs were washed twice and resuspended at a concentration of 4 x 10<sup>6</sup> cells/ ml in phosphate buffer. TMA-DPH was added to obtain a final concentration of 2 x 10<sup>-6</sup> M and incubated shaking during 10 minutes. The anisotropy was calculated by the following equation:  $r = (I_{\parallel} - I_{\perp}) / (I_{\parallel} + 2 I_{\perp})$ , where  $I_{\parallel}$  and  $I_{\perp}$ correspond to the fluorescence measured with the polarized parallel emission and perpendicularly to the excitation. Results of r measurements made at different temperatures were as follows: at 26°C:  $0.225\pm0.010$ ; at 32°C:  $0.223\pm0.011$ ; at 37°C:  $0.216\pm0.009$ ; at 42°C: 0.207±0.010. The difference between the r values at different temperatures had no statistical significance. In the data statistical analysis, Anova and Newman-Keuls test were applied. We conclude that, within the temperature range studied, r is kept constant.

### 40.

## THE RELATIONSHIP BETWEEN HEMATOLOGICAL VARIABLES AND ERYTHROCYTE DEFORMABILITY IN NORMAL INDIVIDUALS

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The deformability of red blood cells (RBC) plays an important role in circulation to allow erythrocytes penetrate small capillaries. Any changes in it might reflect alteration in the RBC membrane, in its geometry or in the cell internal viscosity. The object of the work was to study the relationship between RBC deformability and a) the mean corpuscular volume (MCV) related to the cell geometry and b) the mean corpuscular hemoglobin concentration (MCHC) related to cytoplasmatic viscosity. In blood anticoagulated with EDTA (1mg/ml) of 17 healthy individuals, the hematocrit (Htc), the hemoglobin concentration and the RBC number were determined to calculate the MCV and the MCHC. To measure erythrocyte deformability, a 10% RBC suspension buffered saline (PBS) was filtered through Nucleopore membranes with 5 µm diameter pores and the results were expressed as rigidity index (RI), which is the inverse of deformability. RI =  $(T_s - T_b) / (T_b) \times 100/Htc$ , where  $T_s$  is the RBC suspension time of passage and T<sub>b</sub> is the medium suspension time of passage. RI figures co-related positively and significantly with MCV (r= 0.59, p< 0.01) but no relation was found between the RI and the MCHC. These results might indicate that macrocytes find it difficult to filtrate through 5 µm-diameter pores. Some pathologies associated with macrocytes might be related with a reduced erythrocyte deformability.

### 41. LOVASTATIN ENHANCES *IN VITRO* RADIATION-IN-DUCED APOPTOSIS, IN CELLS OF A RAT LYMPHOMA

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We previously demonstrated that Lovastatin (LOV), a hypocholesterolemic drug, has an antimetastatic effect in vivo, and, in vitro, increases radiation-mediated cytotoxicity on cells of lymphoma TACB (L-TACB). Both LOV and radiation are known to induce apoptosis after arresting the cell cycle on G<sub>1</sub> and G<sub>2</sub>, respectively. Our aim was to determine whether the effect observed was the consequence of LOV acting synergically or additively with radiation to enhance the apoptotic process. Flasks with L-TACB cell suspension (2.5 x 106 cells) in RPMI with 10% FBS were treated as follows: I) no treatment (control); II) LOV, 20 µM; III) radiation, 9 Gy; IV) LOV, 20 μM and radiation, 9 Gy. After incubating at 37°C, in 5% CO<sub>2</sub>, for 72 hours, cell viability was estimated with the MTS/ PMS test (4 repeats). Early apoptosis was detected with Anexin V, after 12 hours of incubation (2 repeats). Cell viability in group IV  $(\text{mean} \pm \text{SE}) (140\pm25.7)$  was lower than in group I (318 $\pm$ 9.8), group II (235±14.8) or group III (287±23.0) (p<0.01). The proportion of cells in early apoptosis were highest in group IV (2.3%) as compared with groups I (0.3%), II (0.7%) or III (1.2%) (p<0.01). It can be concluded that cytotoxicity in this *in vitro* model is due, in part, to the induction of the apoptotic process. The results with the combined treatment indicate that LOV acts synergically with radiation, enhancing the apoptotic process induced by the latter. These conclusions could be relevant in cancer therapy in humans, because they may allow to improve the therapeutic index or decrease the adverse effects by decreasing the therapeutic dose.

### 43.

## MODULATION OF IL-10R EXPRESSION IN PRIMARY TUMOR AND METASTATIC CELLS OF A RAT B CELL LYMPHOMA (L-TACB)

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We have previously demonstrated that IL-10 increase the *in vitro* proliferation rate of L-TACB metastatic cells, without affecting primary tumor cells growth. Our aim was to investigate the expression of IL-10 receptor (IL-10R) at protein and mRNA level, in L-TACB primary tumor (I) and metastatic (II) cells. Inbred e rats were challenged with L-TACB s.c. After  $\cong 20$  days, tumor and lymph node metastasis were excised. Total RNA from tumor and metastasis, spleen of tumor bearing rats (positive control) and normal kidney (negative control) was extracted. Semiquantitative RT-PCRs showed no differences in IL-10R mRNA between I and II cells. Also, protein homogenates from total cells (a), membrane (b) and citosolic (c) fractions were prepared. The presence of IL-10R was determined by Western blot. The quantity of IL-10R in II(a) was  $\cong$ 2 times higher than that detected in I(a), and fractions II(b) and (c) were higher than I(b) and (c). Conclusion: 1) Regulation of IL10-R expression would be post-transcriptional; 2) IL-10R translation is higher in metastatic cells; 3) The positive modulation of IL-10R in L-TACB would be part of the transition from the tumoral to the metastatic phenotype.

## 42. SPONTANEOUS ENTEROPARASITOSIS IN MICE OF THE CBi STOCK. <sup>2</sup>M.D.

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Parasitic infections are chronic processes difficult to erradicate. Nevertheless, resistant individuals have been detected in population studies; this resistance could be under genetic control. To determine if disparate genotypes show cuali and/or quantitative differences in the host-parasite relationship, the spontaneous enteroparasitosis in four lines of the CBi stock was examined. Eight males and 8 females, 80 to 100 days old, from lines CBi+ (+), CBi- (-), CBi/L (L) and CBi/C (C) were sacrificed to remove the intestine. An aliquot of the intestinal content was analyzed by direct microscopy; the parasitic burden was quantified with crosses (0 to 5+). The remaining content was fixed with SAF to count helminths, and expressed as total number of parasites (nP). The effect of genotype and sex on the proportion of positive mice was evaluated with  $\chi^2$ ; the quantitative variables were analysed with the Kruskal-Wallis non-parametric test. Trichomona muris was found in all (C) males and females but not in the other genotypes (p<0.01). All (-) mice were strongly positive for Spironucleus muris, at variance with (+), (C), and (L), that showed lower parasitic burden and proportion of positive animals (p<0.01). Syphacia obvelata was observed in all genotypes while Aspiculuris tetraptera, though positive in all the lines, showed a significant lower proportion of parasitized mice in (+) (p<0.05). (-) males exhibited the highest **nP** p<0.05). The results evince differences attributed to genotype in the host-parasite relationship, since all the lines shared the same environment from birth. A sex effect in the number of helminths was observed in genotypes (-) and (C).

#### 44. BENZNIDAZOL (BZ) EFFECT IN RAT SARCOMA DEVEL-OPMENT

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We have demonstrated high amounts of NO2 in spontaneous rat Sarcoma E100 (SE100) primary cultures. Association between serum and tumour size has been confirmed. There are few NO controlled biological reactions, and involvement in carcinogenesis and tumour cells destruction in tumours has been demonstrated. BZ, in vitro trypanosomicide drug, inhibits stimulated macrophage NO synthesis, due to transcriptional inhibition in inducing enzyme. Objective: to study BZ effect on SE100 growth in vivo and in vitro, and its toxicity. In vivo experiments: m adult rats groups received 100 mg/kg/d or 200 mg/kg/d orally (vehicle carboxy methyl cellulose, CMC), Control (CG): CMC, starting 24h previous SC tumour challenge (5x10<sup>5</sup> cells) during 14ds. BZ-treated rats, without tumour challenge were sacrificed to assess: oxalacetic (GOT) and piruvic glutamic transaminase (GPT), cholinesterase, alkaline phosphatase and lactic dehydrogenase levels, coagulation and haemogram. In vitro: replications from primary SE100 cultures (105) cells/well) were incubated (24hr) and 0.1, 0.5 and 1 mM BZ in dimethyl-sulfoxide (DMSO) was added. Tumour proliferation assessed by 3Htymidine incorporation (cpm). NO2 in each culture assessed by Griess reaction. Haematological parameters: no differences with CG. DMSO alters SE100 growth in vitro, at the highest concentration. Experimental example: CG (cpm) 1695±355, n=6 vs 0.5mM BZ, 2599±310, n=3, p<.01 and vs 0.1mM BZ, 2543±244, n=3, p<.01. NO2 (μM), pooled treatments: BZ: 21.8±2.7, n=9 vs CG: 27.6±6.9, n=6, p<.04. There was no BZ dose- or time-dependent experimental toxicity in rats m, though SE100 growth is enhanced both in vivo and in vitro, which could be due to NO synthesis alterations.

### MODIFICATIONS PRODUCED IN A SPONTANEOUS (Lbe56) LYNPHOMA DEVELOPMENT IN Mycobacterium vaccae (Mv) OR Rhodococcus rhodochrous (Rr) TREATED RATS

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We have demonstrated that Mv modulates the host response against tumour challenge. The Actinomycetal Rr, an environmental agent, could also modify Lbe56 growth, previous- and post inoculation of tumour cells. Heat-killed Rr or Mv 10<sup>8</sup> and 10<sup>7</sup> cells/dose saline suspension was sc injected to e rats. Experimental groups: A- I) Mv 10<sup>8</sup> -30 day, Mv 10<sup>8</sup> -15 d, Lbe56 (300 cells) 0 d, Mv  $10^8 + 15$ d, Mv  $10^8 + 30$ d; II) Mv  $10^7 - 30$ d, Mv  $10^7 - 15$ d, Lbe56 0d, Mv 10<sup>7</sup>+15d, Mv 10<sup>7</sup>+30d; III) Rr 10<sup>8</sup>-30d, Rr 10<sup>8</sup>-15d, Lbe56 0d, Rr 10<sup>8</sup> +15d, Rr 10<sup>8</sup> +30d; IV) Rr 10<sup>7</sup> -30d, Rr 10<sup>7</sup> -15d, Lbe56 0d,  $Rr10^7 + 15d$ ,  $Rr10^7 + 30d$ ; V) S -30d and 15d. Lbe56 0d, S +15d and 30d. B- parallel experiment: same schedule inoculating suboptimal Lbe56 (100 cells/rat) dosis. No tumour growth observed, hence all the rats were reinoculated after 60ds (500 cells). Results: A- no differences in take % and tumour growth [mm<sup>3</sup>=(a<sup>2</sup>xb)/2, a=minor diameter]. B- take % frequency at d 12, I (5/12), II (8/12), III (8/12), IV (8/12) and V (9/12).

Tumour Volume (mean ± SE mm<sup>3</sup>), days 14, 19 and 24

I	520±257 (n=9)	6641±1757 (9)	22173±3220*
II	1171±289	18260±3951	31171±4680
III	996±240	17300±3441	31731±4390
IV	877±244	14786±2807	36398±4727
V	892±260	12005±2247	35705±5100*
			p<0.03

Two Rr or Mv sensitising doses did not modify tumour growth. However, the Lbe56 non-tumourigenic inoculum emphasises the immunomodulator Mv effect, pointing out the probable utility of anti-tumour Mv vaccines.

### RENAL FUNCTION IN PARTIALLY HEPATECTOMIZED RATS AT 48 HS

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Physiopathological observations suggest the existence of a link between liver and kidney, may be involving neuronal and humoral factors. The present study was undertaken to evaluate the tubular and hemodynamic renal parameters during a period of 48 h after a partial hepatectomy (PH) performed on adult male Wistar rats. Water and sodium balance, and systemic parameters related to liver and kidney functions, were measured. Besides, hemodynamic and tubular renal functions were evaluated by mean of conventional clearance techniques. A significant decreasing in both glomerular filtration rate (in ml/min.100 g b.w:  $C=0.83\pm0.03$ ; PH= 0.71  $\pm$ 0.02) and urine excretion of creatinine, along with a slight reduction of renal plasma flow without any change in filtration ratio, were observed. PH rats presented an increasing of FE% H<sub>2</sub>O, agreeing with the significant reduction of water balance (reduced ingestion without change in urine volume). Urine osmolarity was also diminished (in mOsm/l:  $C = 2374 \pm 59$ ; PH = 1881 ± 186). Althoug the PH there were a reduction of sodium balance due to reduced Na ingestion, FE% Na was augmented (C=  $0.20 \pm 0.01$ ; PH =  $0.60 \pm$ 0.02). Changes in Na metabolism were not accompanied of modifications in the renin-aldosterone system. These preliminary findings would indicate that some renal functions are modified at early stages of liver regeneration. Renal tubular alterations could be attributed to both changes in hemodynamic parameters and systemic factors associated with changes in liver function.

#### 46.

### SODIUM LITHIUM EXCHANGE IN ERYTHROCYTES FROM PRENATAL HEMOPOIESIS

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Disorders in the sodium-lithium countertransport (SLC) on the cell membrane have been referred. The risk of developing hypertension in new borns with fetal stress (programmed hypertension) has also been included in such disorders. Thyocianate anion induces in prenatal erythrocytes (umbilical cord blood) an increase in the value of Vm (SLC) but not in the value of Km in this mechanism of transport. Previously reported results suggest the blockade of sulfhydryl groups of membrane with N-ethylmaleimide do not modify the asymmetry (re-orientation of the free transport) of the system. This article analize SLC when cells are treated with N-ethylmaleimide (into a solution with thyocianate anion). Eflux of lithium (extracellular sodium dependent) was measured in prenatal erythrocytes from umbilical cord (normal new borns). Cells treated with this inhibitor and different rates of intracellular sodium/potassium were used.

A significant difference among the values of Vmax with the replacement of Cl- by SNC- was seen, but the presence of Nethylmaleimide (concentration that saturates sulfhydryl groups) did not affect the action of SNC- upon the Vmax.

Better understanding of the kinetics characteristics of the SLC (involved in the pathogenesis of some clinical entities) will give valuable information not only to interpret the mechanism that could explain the origin but also in the possible clinical and therapeutic research in the future.

### IRON (Fe) AND STRESS OXIDATIVE IN INTESTINE OF PARTIALLY HEPATECTOMIZED ALUMINUM (AI) TREATED RATS

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The aim of the study was to investigate the content of iron (Fe) and its relationship with the level of stress oxidative in small intestine of partial hepatectomized (PH) aluminium (Al) - treated rats. Adult male Wistar rats, weight average 300g, were divided in to 4 groups (n= 5 each one): Sham (SH) rats with simulated surgery, hepatectomized (PH) removal of 65% of the liver; SH and PH treated with Al (OH), 80mg/kg body weight injected intraperitoneally 3 times a week during 3 months. Animals were sacrificed 48 hs after the surgery, and the mucose of the small intestine was obtained. Intestinal Fe content was determined by espectrofotometry of atomic absorption. Intestinal antioxidant capacity was evaluated with: Catalase activity (method of Beers and Sizer), Glutation (GSH) content by Ellman's method. Lipoperoxidation level (LPO) was determined as indirect measurement of reactive O, substances (ROS, stress oxidative) (Ohkawa's method). Results: the intestinal content of Fe was increased in all the experimental groups compared with SH (34% in PH, 25% in SH+ Al and 100% in PH + Al). LPO (nmoles de MDA/g intestinal tissue) SH: 50.8±5.2, PH: 135.3± 23.3\*, SH+Al: 95.7±11.9\*, PH+Al: 95.1±16.6\*; Catalase activity (U/mg protein) SH: 1.1±0.2, PH: 0.3±0.05\*, SH+Al: 0.4± 0.1\*, PH +Al:  $0.2 \pm 0.01*$ ; GSH(µmoles/g tissue) SH 2.6 ± 0.3, HP 2.7 ± 0.2, SH+AL 3.1  $\pm$  0.5, PH +Al: 2.2  $\pm$  0.4. (\*p<0.05). Intestinal Fe augmented in all the experimental groups, probably increases reactive O<sub>2</sub> substances producing the increase of LPO observed. The diminution of the activity of Catalase could be explained by an interaction of the Fe with the enzyme.

### EFFECTS OF ALUMINUM ON THE BILIARY SECRETION IN PARTIALLY HEPATECTOMIZED RATS

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The relationship between the biliary secretion and the initial phase of hepatic regeneration was studied in rats partially hepatectomized rats exposed chronically to aluminium (Al). Male Wistar rats were used, weight average 300g divided in 4 experimental groups (n=8 each): A: controls, B: treated with Al (OH), 80 mg/Kg B.W administered i.p. 3 times per week during 3 months, C: studied 48 hs after a hepatectomy of 65% (HP), D: treated with Al + HP. The bile flow (FB), rate of biliary excretion (VE) of: biliary acids (AB) measured by the technique of Talalay modified by Berthelot, cholesterol (Col) by enzymatic method, proteins (Prot) by method of Lowry and phospholipids (Fl) by technique of Duck-Chong were determined. Bile flow independent AB fraction rate (FIAB) was considered by extrapolation of the straight line regression obtained after correlating the biliary flow and the rate of biliary excretion of AB. The data were analyzed by ANOVA or Kruskall-Wallis The results were expressed as mean value±SEM. FB (µl/min.100 g de rata), A: 6.6±0.2, B:4.3±0.2\*,C:4.4±0.3\*, D:2.9±0.1\*#; VEAB (nmol/ min.100grata) A:176±9, B:122±6\*, C:113±6\*, D:75±10\*#; VECol(nmol/min.100g rata) A:4.1±0.1, B:2.2±0.1\*, C:1.8±0.1\*, D:1.3±0.1\*#; VEFL(nmol/ min.100g rata) A:35.0 ±1.4, B:32.0 ±0.8, C:29.0 ±3.3, D:22.0 ± 0.5\*; VEProt(µg/min.100g rata) A:21.0 ±0.7, B:14 ±0.8\*, C:12 ±1.3\*, D:8.0 ± 0.7\*#; FIAB(nmol/ml A:4.6 ± 0.8, B:2.7 ±0.3\*, C:2.7 ±0.4\*, D:1.6 ± 0.3\*; \* and # p < 0.05 with respect to A and C, respectively. The aluminum produced deleterious additive effects on the installed impairment of biliary secretion in hepatectomized rats. These alterations are compatible with an arrest of the vesicular transcitosis which mediates the transport to the apical pole of lipids and proteins. The fact that this route permits the insertion of canalicular transports responsible for the transference of bile compounds which are involved not only with the dependent but also with the independent bile flow would explain the diminution of both fractions of bile flow.

### 51.

### SERUM LEVELS OF ANTI HSP 70 KD ANTIBODIES AND AUTOANTIBODIES IN PATIENTS WITH PULMONARY TUBERCULOSIS

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Heat shock proteins (hsp) from mycobacteria are knwon to play a role both in normal and pathological immuno regulatory situations. As such we attempted to analyse the serum levels of anti Hsp 70kd and 65kd (from M. bovis) in tuberculosis patients (TB) with different degree of pulmonary affectation and the eventual relationship with the presence of autoantibodies like ANA and dsDNA. The sample comprised 33 TB patients (mild 13, moderate-advanced -Mo-Ad-19, and HIV-coinfected 11) mean general age  $39.24 \pm 18.24$  years (sd) and 7 healthy controls (51.12  $\pm$  13.88). Antibodies to hsp 65 and 70 kd were measured by ELISA whereas presence of anti ANA and dsDNA were assayed by IF. Results were as follows: hsp 65 kD (optical density, mean  $\pm$  sd) HCo=165.4  $\pm$  38.9, Mild= 138.2  $\pm$ 33.3, Mo-Ad=  $186.4 \pm 71.7$ , HIV+= $299\pm 251$  (HIV vs other groups p<0.025). Hsp 70 kD HCo=177.5  $\pm$  33.9, Mild=155  $\pm$  20, Mo-Ad=189.5  $\pm$  60.7, HIV+=237  $\pm$  96 (HIV vs other groups p<0.02). Presence of both autoantibodies was only detected in 4 patients at low titers (1/20, 1/40) resulting in no significant association with anti-hsp antibodies. Anti-hsp antibodies in TB patients appeared

### **50.**

### STUDIES ON THE SIALIDASE ACTIVITY IN WOMEN WITH BACTERIAL VAGINOSIS

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Bacterial Vaginosis (BV), is a syndrome due to aerobic and non aerobic microbial overgrowth whose pathogenesis is not fully understood, although Gardnerella vaginalis sialidase is thought to play an important role. In this first step we have proceeded to analyze sialidase activity in vaginal washings from two groups of young and sexually active women undergoing no treatment, one of them with bacteriological evidence of BV (n=29) and the other one with normal flora (n=23). Patient samples consisted of vaginal fluid for microbiological diagnosis and vaginal lavage (in 5ml of physiologic saline) to assess sialidase activity. One hundred  $\mu l$  of vaginal washings were incubated with 100  $\mu l$  of sustrate (2-(3'-metoxyphenil)-N-acetil-Dneuraminic acid) in microplate at room temperature during 1 h. Metoxyphenol was detected with 50 µl of L-4-aminoantipirina 4 mM and 50 µl of potassium ferricyanyde 6 mM, with optical density being measured at 492 nm. Absorbance values were compared the data yielded by pure metoxyphenol and sialidase activity was expressed in nanomoles of formed metoxyphenol.

Control group:  $3.052 \pm 0.222$ 

BV group:  $5.293 \pm 0.956$  (p < 0.03)

Washings from women with BV had increased values sialidase activity, lo cual evidencia una asociación con este syndrome.

### 52.

# NUTRITIONAL LEVEL AND INTERNAL AND EXTERNAL SUBCUTANEOUS BODY FAT LAYERS THICKNESS IN RESTRICTED AND AD LIBITUM OUTDOOR REARED PIGS

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Fat chemical composition is an important determinant of pork meat quality. Saturated and unsaturated fatty acids are differentially distributed between the internal and the external layers in the subcutaneous fat depot, saturated fatty acids been preferentially deposited in the inner backfat layer. To study feed restriction effect on backfat thickness, the depth of the external (E) and the internal (I) subcutaneous layers were measured in the carcass of 83 pigs (44 castrated males and 39 females) under ad libitum or restricted (25%) feeding levels, in two seasons (winter and summer). In summer, I/E ratios were lower in restricted than in ad libitum pigs and in females than in castrated. (ad libitum - M:  $1.52 \pm 0.20$ ; F:  $1.28 \pm 0.14$  / restricted - M:  $1.30 \pm 0.09$ ; F:  $1.03 \pm 0.10$ ). A similar result was observed in winter (ad libitum - M:  $1.78 \pm 0.13$ ; H: 1.65 $\pm 0.21$  / restricted - M: 1.59  $\pm 0.17$ ; F: 1.10  $\pm 0.06$ ). It is concluded that, in relation with human health, feed restriction, particularly in winter, could be a profitable practice in terms of internal backfat layer depth reduction because of the enhanced quality of pork meat with a decreased porcentual content of saturated fatty acids, besides its obvious impact on carcass leanness.

## HEMATOCRIT VALUES IN TWO GENOTYPES OF CAMPERO INTA BROILERS WITH DIFFERENT BODY WEIGHT GAIN

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Meat-type chickens have been intensely selected for rapid growth, high meat yield and superior feed conversion, causing changes in other physiological parameters. Often, the high yielding meat lines experience metabolic stress in the form of hypoxemia, resulting from high demands for blood oxygen by a rapidily developing tissue mass. In response to such metabolic demands, broilers often have higher values for packed cell volume or hematocrits (PCV). With the aim to characterize the PCV behaviour in 12 males (M) and 12 females (F) of two genotypes of Campero INTA broilers (D: DonBo and T: Traditional) with different body weight gain, triplicate blood samples were collected from the left brachial vein into heparinized microhematocrit tubes. Traditional birds showed a higher body weight gain than DonBo in the age interval (49-83 days) under study (M: T 43.5±0.63, D 39.2±0.74, p<0.05; F: T  $33.3 \pm 0.91$ , D  $26.8 \pm 0.67$ , p<0.05). Genotypes also differed in PCV mean values (M: T 34.9±0.79, D 32.6±0.60, p<0.05; F: T 35.7± 0.87, D 33.2±0.43, p<0.05). Although Campero birds were developed for range breeding systems and show lower weight gain rates than commercial broilers, in these genotypes PCV values also paralleled body weight gain figures. In consequence, higher growth rates would need PCV levels at a higher concentration to accommodate oxygen demands notwithstanding the particular growth pattern of these meat-type chickens.

### 55

## IN VIVO BREAST MEASUREMENTS AS ESTIMATORS OF BREAST WEIGHT IN DIFFERENT GENOTYPES OF CAMPERO INTA BROILERS

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To assess the predictive value of different in vivo measurements on carcass breast weight, length, width and surface at 49, 63 and 77 days of age were measured in three genotypes of Campero INTA broilers (NaTA, DonBo and Traditional). One hundred and twenty birds (20 males and 20 females of each genotype) were used. NaTA and DonBo showed shorter breasts than Campero Traditional without significant differences between them. DonBo and Traditional birds did not differ in breast length but both of them showed higher mean values for the trait than NaTe. Breast surface showed the following increasing order (NaTA-DonBo-Traditional). In vivo measurements, except breast surface at 49 days in males (p<0.05), were not correlated with carcass breast weight in NaTe birds. In DonBo broilers the six length and surface measurements and breast width at 49 days in males and the nine of the them in females, correlated well with the trait weighed at slaughter. Significant correlations with breast length at 77 days, breast width at 49 days and breast surface at 49, 63 and 77 days were observed in Campero Traditional males. Althouh easily measured the assayed in vivo predictors showed a non uniform behaviour. Breast surface, the new estimator proposed, was the best one but genotype differences precludes a generalized advice.

#### 54.

## FEED INTAKE, DAILY BODY WEIGHT GAIN AND FEED EFFICIENCY IN CAMPERO INTA BROILERS WITH DIFFERENT MATERNAL GENOTYPE

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Live body weight (g); daily body weight gain (g/day), daily feed intake (g/day) and feed efficiency (weight gain/feed intake) between 62 and 70 days of age were measured in 15 males of three hybrid combinations of Campero INTA broilers with AS paternal genotype and Rubia INTA (Campero NaTA), Plymouth Rock Barrada (Campero DonBo) and E strain (Campero Traditional). Traditional broilers, with higher body weight than the other two combinations showed the highest weight gain and the highest feed intake in the period studied. Differences in these traits were not significant between NaTA and DonBo genotypes. As feed efficiency reflects the relationship between body weight gain and feed intake, non significant differences between these two traits could finally result in a significant difference in the ratio between them. That was the case for the comparison between Traditional and DonBo genotypes, the former being more efficient than the latter (0.4290  $\pm$  0.0312 vs.  $0.3476 \pm 0.0089$ , p < 0.05). NaTA birds showed a greater weight gain with a similar feed intake and consequently a more favorable conversion ratio than DonBo  $(0.3882 \pm 0.0202 \text{ vs } 0.3476 \pm 0.0089,$ p < 0.05). The lower feed consumption showed by NaTA birds in comparison to Traditional hybrids resulted in a concomitant lower body weight gain, being both genotypes equally efficient. Economic considerations about genotype differences in a biological trait as average feed efficiency must be accompanied by an explicit mention of both body weight gain and feed intake figures.

### 56.

### PLOIDY ANALYSIS OF THE GERMINAL EPITHELIUM OF Genypterus blacodes (PINK CUSKEEL)

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Polyploidy indicates the quantity of repetitions in the fundamental number of chromosomes. It is a process observed in different species and tissues but its biological significance is unclear. The ovary of Genypterus blacodes is a typical cystovary of teleosts. Dividing septa extend from the internal part of the ovarian wall toward the central lumen of the organ forming the crests. These septa are covered by single epithelium: the germinal epithelium. In the present work we used computer-assisted microscopy to analyse the morphology of G. blacodes ovary germinal epithelium. Specimen samples in spawning stage were stained using the Feulgen technique to determine the amount of DNA and the presence of polyploid cells during the proliferating phase using a digital image analyser. The germinal epithelium of the ovary of G. blacodes showed important metaplasic transformations along the reproductive cycle, passing from plane to ciliated cubic or cylindrical during the spawning stage and evidencing glycoproteins secretion that will probably form the mucous coat that covers the eggs during spawning. Epithelial cells with 2 or 4 nuclei were also observed during this stage. They were 2N cells (diploid, 60%); 4N (tetraploid, 19%); 2Nx2 (two diploid nuclei, 17%); 4Nx2 (two tetraploid nuclei, 1%) and 2Nx4 (four diploid nuclei, 3%). Polyploidy development in vertebrates has been associated to hypertrophic stimuli i.e. hypertension (myocardial cells), nursing (mammary glands), gestation (endometrial cells), microbial infection (lymphocytes) or oncogenesis (tumour cells). During the spawning, duplication of genes in G. blacodes could be related with the increase of the glycoproteins synthesis from the germinal epithelium.

### 57. GLYCOCONJUGATES IN THE ESOPHAGOUS OF

Odontesthes bonariensis (ATHERINOPSIDAE)

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Glycoconjugates (GCs) are the mayor constituents of mucous secretion in fish digestive tracts. Mucous cells and their mucus secretion are closely related to both endogenous and exogenous agents. The "silverside" Odontesthes bonariensis is one of the most important commercial and sport freshwater fishing resources in the Pampean Region of Argentina. The "silverside" has been recently used for stocking and intensive cultivation in different countries. The objective of this study was to continue with the histochemical characterization of the mucous cells (MC) in the esophagous of O. bonariensis collected from Lake Los Padres (Gral. Pueyrredón, Bs.As.). Samples were routinely processed. Sections were subjected to histochemical techniques for the identification and visualization of GCs. With orcein technique the mucus secretion of MC revealed the existence of sulphated mucins. A sequence of procedures utilizing Alcian Blue at different pH values and molarities showed the presence of strong and weakly sulphating GCs and with carboxyl groups. Metachromatic reactions at pH 4.2 and pH 5.6 reflected the presence of GCs with O-sulphate esters and/or carboxyl groups. GCs synthesized by MC are asssociated with different aspects, in particular GCs sulphated are related to lubrication functions. Mucus activity suggests that, as it has been found in other freshwater fish, the esophagous is mainly involved in the physical transport of the food bolus to the stomach with the aid of abundant secretion of mucus.

### 59.

### ULTRAESTRUCTURAL STUDY OF MARGINAL SPHINC-TER OF THE SEA ANEMONE *Phymactis clematis* (ANTHOZOA)

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The marginal sphincter of the sea anemone Phymactis clematis is endomesogloeal type with histological and physiological characteristic of smooth muscle. The study by transmission electron microscope showed that the sphincter is constituted by a mesogloeal axis with lateral branches and myocytary bundles. The myocytes show centrally an electrodense region corresponding to the nucleus and peripherically the cytoplasm. As in a vertebrate smooth muscle, in a myocyte longitudinal section a filament free region of sarcoplasm containing isolated mithocondria, rough endoplasmic reticulum and smooth endoplasmic reticulum, may be seen. In the peripheral sarcoplasm grouped myofilaments may be observed stretching in longitudinal direction that connect to dense bodies wich are cytoplasmic condensation scattered at regular intervals. They may have an actin-fixing protein and would have the same function as the Z line of the skeletal muscle. Areas of higher condensation in the site of contact of two adjacent plasmatic membranes may be seen among myocytes. These connections might act as links allowing the simoultaneous contraction of the smooth muscle. Myocyte ultraestructure of *Phymactis clematis* marginal sphincter is similar to smooth muscle fibres of higher vertebrates.

#### 58.

## HISTOLOGICAL ANALYSIS OF THE LIVER OF Oligosarcus jenynsi (PISCES, CHARACIDAE) FROM LOS PADRES LAKE (PROVINCE OF BUENOS AIRES)

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The histological structure of the liver provides data on zoological, phylogenetic and pragmatic aspects of teleost fish. Liver lesions in fish are often associated to environmental pollution. Los Padres lake represents a very attractive place for tourism located in Buenos Aires province. The evaluation of its trophic status involves the estimation of limnological as well as biological parameters. Among the latter, fishes play different roles in food webs. The characid Oligosarcus jenynsi is one of the most common species in this waterbody, inhabiting vegetated waters. The aim of the present work is the characterisation of histological structures of *O. jenynsi* liver, within the frame of a wider investigation carried out to estimate the trophic status of Los Padres lake. The material used was processed according to classical methodology and techniques. The liver is covered by a thin capsule of connective tissue. The hepatic parenchyma is divided into irregular lobules, separated by the hepatopancreas associated with connective tissue. Even though we can not speak of true hepatic triads, we often find ducts associated to blood vessels, to which we give that name. The hepatocytes contains a central nucleus and the chief stored substances are glycogen and lipid. The hepatopancreas is evident because of its marked basophilia, surrounding a branch of the portal vein. Encircling the hepatopancreas cells, a basal membrane and a connective tissue layer are observed, both with abundant reticular fibers. The biliary ducts, scattered within the hepatic tissue, are covered with cuboidal epithelium. Melano-macrophages centers are not observed in the hepatic parenchyma. This investigation shows that the hepatic organization of Oligosarcus jenynsi is similar to that of other teleosts.

### 60.

### MUSCULAR ACTIVITY OF MARGINAL SPHINCTER OF CNIDARIAN OF DIFFERENT ENVIRONMENTS

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The muscular activity of marginal sphincter of Oulactis muscosa, Aulactinia marplatensis and Aulactinia reynaudi from intertidal of Santa Clara del Mar were compared with the same species from Punta Cantera. The marginal sphincter isolated was stimulated at increasing KCl concentrations and analyzed in the form of a doseresponse curve expressed in tension in grams (g = grams force) vs concentration. Statistical analysis was performed using the Student's test. P values < 0.05 were considered statistically different. Maximal tension developed in species from Punta Cantera were O. muscosa: 1.50 g; A. marplatensis: 1.33 g y A. reynaudi: 1.29 g. while species from Santa Clara del Mar developed maximal tension significant lower: O. muscosa: 0.68 g; A. marplatensis: 0.51 g and A. reynaudi: 0.59 g. However we found no significant differences between the species from Punta Cantera. The same result was observed for the species from Santa Clara del Mar. The specimens from Santa Clara del Mar also presented lower velocity of response and slow relax period. These results are agree with the study zone wich present a slow pendent to the sea, so that the specimens are unaffected for the dashing of waves. This response is different to the observed one in the same species from Punta Cantera. This work apport knowledge to an adaptative behaviour in lower methazoos on different substrates.

## CHLOROPLASTS DEVELOPMENT IS STIMULATED BY NITRIC OXIDE TREATMENT IN SEEDLINGS OF LACTUCA SATIVA L. CV. GRAND RAPIDS

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Certain types of germination and chloroplast differentiation are light-dependent processes. In this work, we demonstrate that NO is a bio-active molecule which promotes the cotyledon development and the chloroplast differentiation -- even in absence of light-- in *Lactuca sativa* L. cv. Grand Rapids. UV-B excited chlorophyll can be used to show the chloroplast distribution in a section of fresh material (Elkin & Park, 1975, Planta 127: 243-250).

When the material is appropriate, grana can be observed because of their intense bright-red autofluorescence.

In the present work, we analyzed changes in seedlings' lettuce cotyledons kept for 1 to 10 days under light or darkness, in water or treated with the NO donor, sodium nitroprussiate (SNP) 100  $\mu M$ . The cotyledons were explanted and the sections hand-cut. Afterwards, sections were immersed into an alkaline medium. Then, sections were observed under optic microscope with white and UV light. The analysis of results indicates that: \* Seedling's chloroplasts grown in the presence of light showed autofluorescence during the second day of culture. \* For the same time, the seedling's chloroplasts grown in water at darkness did not show autofluorescence, whereas the seedling grown in NO and darkness showed autofluorescence. These results represent a further evidence that NO is a molecule that promotes chloroplast development and chlorophyll biosynthesis in the absence of light.

### 63.

### SALINITY SENSITIVE ALKALINE PHOSPHATASE ACTIVITY OF MUSCLE OF *Cyrtograpsus angulatus* FROM MAR CHIQUITA LAGOON: RESPONSE TO DOPAMINE

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The role of alkaline phosphatases (AP) in osmoregulatory process in hyperrregulating crabs is still controversial. Biogenic amines have been involved in the regulation of several physiological process although their transduction mechanisms and tissue targets are still poorly understood. The aim of this work was to determine the occurrence, response to salinity (S) and possible regulation by dopamine (DA) of AP activity of muscle of Cyrtograpsus angulatus. Male adults crabs were maintained a) for 33 days in 35%S (t0) b) transfered at t0 from 35 to 10%S. To study the effect of DA, crabs acclimated to 10%S were administered with saline (control) or saline+10-4M DA solutions at the base of chelipeds. AP activity was determined in chela muscle homogenates (0.25M Sucrose/ 0.5mM EGTA-Tris pH 7.4) by measuring pNPP hydrolisis (9.5mM) in the presence of 100mM Tris (pH 9.0) with or without MgSO<sub>4</sub>. Three independent experiments were carried out. ANOVA was used as statistical analysis (p<0.05).

At 35%S AP activity was detected both with (+) and without (-) Mg<sup>++</sup>. Activity (nmoles pNPxmin<sup>-1</sup>xmg prot<sup>-1</sup>) +Mg<sup>++</sup> (535±10) was higher than -Mg<sup>++</sup> (237±5). After 4 hours from the transfer to 10%S both activities (-Mg<sup>++</sup>=182±5; +Mg<sup>++</sup>= 388±12) were lower than at t0. 10<sup>-4</sup>M DA decreased 45±4% +Mg<sup>++</sup> activity (control=621±18; +DA=343±23) but not -Mg<sup>++</sup> activity. The results suggest a possible differential involvement of AP activities and DA in ionoregulation of *C. angulatus*.

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## Na<sup>+</sup>-K<sup>+</sup> ATPase ACTIVITY OF MUSCLE OF *Cyrtograpsus* angulatus AND *Chasmagnathus granulata* FROM MAR CHIQUITA LAGOON (BS. AS. PROVINCE)

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The aim of this work was to study biochemichal characteristics and the response to salinity (S) of Na+-K+ ATPase activity of muscle of Cyrtograpsus angulatus and Chasmagnathus granulata. Male adults crabs were maintained a) for, at least, 10 days in 10%S for biochemical studies; b) for 33 days in 35 or 10%S for long-term acclimation studies; c) transferred at t0 from 35 to 10%S. Na+-K+ ATPase activity was determined in chela muscle homogenates (0.25M Sucrose/0.5mM EGTA-Tris pH 7.4). The activity was determined by measuring ATP hydrolisis (0.1-16mM) in the presence of (mM): 20 Imidazole (pH 6.2-7.8)/ 100 NaCl/ 30KCl/ 0.5 EGTA (Control: without KCl, with 1mM ouabain). Three independent experiments were carried out. ANOVA was used as statistical analysis (p<0.05). In C. angulatus the highest activity (nmoles Pi xmin<sup>-1</sup>xmg prot<sup>-1</sup>) occurred at 1mM ATP (62±8). At higher concentrations than 3mM ATP, activity was 40% of that with 1mM ATP. In C. granulata the highest activity was obtained with 13mM ATP (127±23), being inhibiting by 80% with 16mM ATP. Maximal activity occurred at pH 7.4. Upon long-term acclimation muscle Na<sup>+</sup>-K<sup>+</sup> ATPase activity of *C. angulatus* was higher in 10%S (65±4) (35%S 28±2). After 4 hours from the transfer to 10%S it was similar to that at t0. The results show the occurrence of Na+K+ ATPase activity in muscle of C. angulatus and C. granulata with differential sensitivity to ATP, pH and salinity. The possible existence of either different isoenzymes or mechanisms of regulation of Na<sup>+</sup>-K<sup>+</sup> ATPase activity in muscle of these crabs remains to be investigated.

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## BRANCHIAL Na<sup>+</sup>,K<sup>+</sup>-ATPase ACTIVITY OF *Cyrtograpsus* angulatus AT DIFFERENT SALINITIES. EFFECT OF SEROTONIN

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We have shown previously that C. angulatus exhibits a differential short-term response to salinity of Na+,K+-ATPase activity in individual gills. The aim of this work was to study the long-term response to salinity (S) of Na+,K+-ATPase activity in anterior and posterior gills of C. angulatus and the effect of serotonin on this activity under differential environmental salinities. Male adult crabs were maintained for 33 days in 35% or 10% S. To study the effect of serotonin, crabs maintained for 12 days in 35 or 10% S were injected with saline (control) or saline + 10<sup>-4</sup>M serotonin. Na<sup>+</sup>,K<sup>+</sup>-ATPase activity was determined in 10000xg supernatant from gill homogenates (0.25 M sucrose/0.5 mM EGTA-Tris pH 7.4) by measuring ATP hydrolysis in the presence of (mM): 20 imidazol (pH 7.4)/100 NaCl/30 KCl/0.5 EGTA (control: without KCl, with 1 mM ouabain). Three independent experiments were carried out. ANOVA was used as statistical analysis (p<0.05). At 35 or 10% S the activity (nmoles Pixmin<sup>-1</sup>xmg prot<sup>-1</sup>) was higher in posterior gill (g) 6 compared to anterior gill 4 (35% S: g4 =230±28; g6=683±9; 10% S:  $g4=236\pm26$ ;  $g6=966\pm72$ ). In 10% S the activity in g6 was higher than in 35% S (41 $\pm$ 7%). No differences occurred in g4. At 30 min, 10<sup>-4</sup> M serotonin inhibited the activity in g4 in 10% S (control: 70±8; +serotonin: 32±4) but no effect occurred at 35% S. In g6 serotonin had no effect. The results suggest a different role of anterior and posterior gills in long-term ionorregulation in C. angulatus and the possible occurrence of mechanisms of regulation via serotonin in individual gills.

# ANATOMICAL DIFFERENCES BETWEEN MATURE AND YOUNG STEMS IN PLANTS OF *CUPHEA GLUTINOSA* CHAM ET SCHLECHT. (LYTHRACEAE) FROM DRY AND WET PLACES

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*Cuphea glutinosa* is a native specie from the bonaerenses southeast sierras of industrial and medicinal importance.

The objective of this work was to determine the anatomical characteristics of mature and young stem of plants growing at different conditions of water and light, in order to determine the possible sites of active substances storage.

Mature and young stems from plants of Sierra de los Padres were used. Some plants grow in a dry sector with important amount of light available all day, and other in a flooded sector where they receive direct sunlight only in the afternoon. Samples were collected and fixed in FAA, then sectioned and stained with traditional methods.

Some differences can be observed among young stems of different growth conditions areas, which were represented by: the epidermis; the chlorenchyma; the pith; the development of the phloem tissue, and the amount of fibres. At the same time, mature stems differed mainly by the reddish-orange coloured substances present in the cells of cortical parenchyma and from the phloem tissue.

These structural differences observed in young and mature stems, may be attributed to the different environmental conditions that affect the sites of individuals growth.

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### 67.

## SEASONAL PREVALENCE OF INTESTINAL CANINE PARASITES FROM PUBLIC SQUARES OF MAR DEL PLATA CITY, ARGENTINA

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Intestinal canine parasites could be transmitted from dogs to other animals and either to humans throughout faeces. Success in the development of infectious stages depend of specific climatic conditions. The aim of this work was to know the parasitic prevalence by taxa and by season in canine's faeces collected from public squares of Mar del Plata city, in order to estimate if seasonality exerts a relevant influence on the sanitary risk to infectious to humans. Canine faeces were collected from 21 public squares in Mar del Plata city (Argentina), between june 2001 and april 2002. The parasitic analysis was made by the Willis method. In order to compare prevalences among seasons  $\chi 2$  test was used. All the squares were found contaminated with canine's faeces and in 19 (90.48%) of them were found parasites. The parasitic species were: Trichuris vulpis, Toxocara canis, Ancylostomids, Amoebae, Coccidians, Pseudophyllidea, Taenia sp. and Capillaria sp. Total prevalence comparison by seasons was found significantly higher in winter than in summer ( $\chi 2=18.74$ ; p<0.05), mainly for *T. canis* ( $\chi$ 2= 7.63; p< 0.05). For *T. vulpis*, prevalence was higher in winter  $(\chi 2= 16.06; p < 0.05)$  and higher in spring and summer  $(\chi 2= 10.52;$ p<0.05 and  $\chi$ 2=5.14; p<0.05, respectively) compared with autumm. For Ancylostomids, a greater prevalence in autumm than winter  $(\chi 2=5.53; p<0.05)$  was registered. These results suggest that winter is the season with higher risk for T. canis transmission, winter, summer and spring for *T. vulpis* and autumm for Ancylostomids.

### 66.

### DESCRIPTION OF THE REPRODUCTIVE SYSTEM OF *Uca uruguayensis* (CRUSTACEA:DECAPODA)

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*Uca uruguayensis* is a brachyuran crab which inhabits the supralitoral zone of the Mar Chiquita Lagoon (Buenos Aires Province, Argentina). It is found on the external margins of a crab community, occupying galleries in the mud. The goal of the present study is to describe the reproductive system of *U. Uruguayensis*. 30 individuals collected by hand over the supralitoral zone were analyzed. Gonads were dissected for a routine histological study; processed and stained with H&E and Toluidin Blue. Testicle is sac shaped with a grey granular surface. It is small in size, and located in the dorsal region of the cephalothorax. The vas deferens is white, with a rounded end contacting the testis. The histology of the testis shows numerous cysts that surround the collector duct. Each cyst is enclosed by a thin connective tissue layer, whose interior shows germinative cells. The three regions of the vas deferens are formed by columnar or cubic epithelial cells. The lumen lacked of spermatophores, and only some of the individuals showed a hyaline secretion in the posterior portion of the lumen. Ovaries are sacular; their position is dorsal to the digestive system and next to the hepatopancreas. Some females showed grayish ovaries, meanwhile in other ones they were of an intense red color. The connective tissue that surrounds the ovary, subdivides it internally. The subunits are formed by germinative cells in different developmental stages (oogonia or oocites in early previtelogenesis). The present analysis allows us to conclude that the reproductive system of *U. uruguayensis* is similar to that found in other Brachyura and presents a rest phase in winter.

### 68.

## ELIMINATION OF ORGANIC ANIONS MAINLY EXCRETED BY KIDNEYS IN RATS WITH EXTRAHEPATIC CHOLESTASIS (EC) OF 72 HOURS

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Modifications in para-aminohippurate (PAH) systemic depuration were observed in rats with EC of 21 hours. PAH is an organic anion mainly excreted by kidneys. The aim of this study was to evaluate the depuration of PAH in rats with EC of higher evolution. Adult male Wistar rats were employed. The common bile duct was ligated during 72 h in a group of rats (L, n = 8). A parallel group of Sham rats was processed (S, n = 7). After 72 h, Total and Direct Bilirubin (BT, BD, mg/l) were determined as parameters indicative of hepatic function. A pharmacokinetic study was done by administration of a single dose of PAH (30 mg/kg b.wt). The data were fitted to a biexponential curve with PKCALC computer program. The following parameters were determined: systemic depuration of PAH (ClPAH, ml/min/100 g), elimination rate constant from the central compartment (K1-0, min<sup>-1</sup>), Total, Central and Peripheral Volumes of Distribution (VdT, VdC, VdP, ml/min/100 g). At the end of the experiments kidneys were used for histological studies. Results: (\*P<0.05): BT:  $S = 2.51 \pm 0.36$ ,  $L = 71.34 \pm 4.31$ \*; BD:  $S = 1.30 \pm 0.36$ 0.24, L =  $64.85 \pm 4.16$ \*; CIPAH: S =  $3.46 \pm 0.21$ , L =  $2.45 \pm 0.25$ \*;  $K_{10}$ : S = 0.34 ± 0.02, L = 0.23 ± 0.02\*; VdT: S = 28.43 ± 2.79, L =  $31.12 \pm 4.37$ ; VdC: S =  $10.11 \pm 0.55$ , L =  $10.51 \pm 0.59$ ; VdP: S =  $18.32 \pm 2.52$ , L =  $20.61 \pm 4.16$ . No morphological changes were observed in L rats. Cholestatic rats showed a significative diminution in K1-0 with the consequent diminution in the ClPAH. These results suggest renal impairment in L groups produced by the original hepatic damage.

## DETERMINING FACTORS OF SULFANILAMIDE (SA) RENAL ELIMINATION IN RATS WITH DYSTROPHIC CALCIFICATION

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The aim of this study was to evaluate the renal elimination of SA and the contribution of some related parameters in rats with dystrophic calcification. Adult male Wistar rats treated with an overdose of vitamin D3 (300000 UI/kg b.w., i.m., 10 days before) (T) and control rats (C) were used. Systolic arterial pressure (PAS), aortic (Caao) and plasma calcium (Cap) levels were evaluated. Renal Clearance of SA (ClrSA) and glomerular filtration rate (GFR) were assayed by conventional clearance techniques. Renal Blood Flow (RBF) was determined using fluorescence microspheres. Excreted (CE), filtrated (CF) and secreted (CS) loads of SA were calculated. The abundance of the renal Organic Anion Transporter 1 (OAT1) was determined by Western Blot. Results (\*P<0.05): Cap (mg%):  $C=9.4\pm0.1~(n=6), T=9.3\pm0.1~(n=4);$  Caao (umol/g dry wt):  $C=22\pm2$ (n=9), T=50±5\* (n=7); PAS(mmHg): C=96±3 (n=9), T=136±8\* (n=8); ClrSA (ml/min/100g): C=0.227±0.023 (n=6), T=0.158 ± 0.003\* (n=6); RBF (ml/min/100g): C=5.3 ± 0.7 (n=7), T=1.6±0.4\* (n=6); GFR(ml/min/100g): C=0.782±0.080 (n=8), T=0.471±0.039\* (n=5); CE (ug/min/ 100g): C=3.16±0.46 (n=6); T=2.18±0.11\* (n=6), CF(ug/min/100g): C=0.86 ±0.10 (n=6), T=0.52±0.35\* (n=6); CS(ug/min/100g): C=2.28 ±0.35 (n=6), T=1.66±0.08 (n=6). OAT1 (%): C=100±6 (n=6), T=173±26\* (n=8). Optical microscopy studies revealed a reduction in glomerular size and dilatation of Henle's loops. The diminution of RBF justifies the lower GFR and the consequent lower CF in T rats. The increment in OAT1 abundance might compensate the influence of the lower RBF on the drug secretion.

### 71. SERO – EPIDEMIOLOGICAI STUDY OF CHAGAS' DIS-EASE AND SYPHILIS IN INPATIENTS

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Syphilis and Chagas are two diseases transmisible through blood transfusion or from the mother to the foetus. Our aims were: a) to get to know the prevalence of both diseases in all the analysed sera; b) to evaluate the possible association between sera of hospital patients and sera obtained from blood donors as regards possitivity of Syphilis and Chagas; c) to evaluate the possible association between sera of controlled pregnant women(CPW) and non controlled pregnant women (NCPW) as regards possitivity. During the year 2001, 9,472 Syphilis samples and 9,108 Chagas samples were applyed. Serological methods: Chagas: HAL and

samples were analysed. Serological methods: Chagas: HAI and ELISA. Syphilis: VDRL and TPPA. Statistical tests: comparison of ratios based on a normal distribution. Chi square with Yates corrections. The decision rules were made taking into account a p< 0.05. The prevalence of Syphilis was 1.72% and the prevalence of Chagas was 6.75%. the prevalence of Chagas is significantly higher than that of Syphilis (p< 0.00001). A significant relationship was found between the samples of hospital patients and the samples of blood donors as regards prevalence (p< 0.00001). There was no significant association between CPW (n = 545) and NCPW (n= 1,390) concerning the seroprevalence of Syphilis. A significant relationship between CPW (n = 550) and NCPW (n = 1,128) was not found either with respect to prevalence of Chagas. The significant prevalence of Chagas over Syphilis shows a marked migration from endemic regions and a greater control over Syphilis transmission. In pregnant women seroprevalence of Chagas and Syphilis is similar in CPW and NCPW. The lack of statistical significance when evaluationg this association has, however, clinical significance which is even higher if we consider that the ratio of NCPW widely exceeds that of CPW.

#### 70.

### RENAL HEMODYNAMIC AND TUBULAR ALTERATIONS AFTER ACUTE URETERAL OBSTRUCTION IN RATS

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Bilateral Ureteral Obstruction (OUB) may cause acute renal insuficiency. The physiophatologic mechanisms involved in short time OUB have not been completely elucidated. The aim of this study was to evaluate some parameters of renal function after acute OUB. Adult male Wistar rats were employed. Both ureters were ligated during 24 h, then were released and the studies were done after 24 h (n = 5). A parallel group of Sham rats (Sh, n = 6) was employed. Plasma urea levels were determined before the obstruction (t=0 h), the release (t=24 h) and the experiments (t=48) by spectrophotometric techniques (Ur, g/l). P-aminohippurate renal clearance (Cl PAH, ml/min/100 g), glomerular filtration rate (GFR, ml/min/100 g), fractional excretion of water (FE% H2O), sodium (FE% Na), potassium (FE% K) and the ratio urine to plasma osmolality (U/P) were determined employing conventional clearance techniques. Data were analysed with unpaired "t" Student Test or ANOVA, and p < 0.05 (\*) were considered statistically significative. Results: Ur Sh= (t=0 h):  $0.54 \pm 0.04$ , (t=24 h):  $0.51 \pm 0.03$ , (t=48 h):  $0.72 \pm 0.06$ , Ur OUB= (t=0 h):  $0.56 \pm 0.03$ , (t=24 h):  $3.32 \pm 0.14$  \*, (t=48 h): 2.91  $\pm$  0.30\*(\*p<0.05 vs t=0); Cl PAH: Sh = 5.26  $\pm$  0.78; OUB =  $0.49 \pm 0.18*$ ; GFR: Sh =  $0.582 \pm 0.053$ , OUB =  $0.127 \pm 0.053$ 0.020\*; FE H2O%: Sh =  $0.876 \pm 0.089$ , OUB =  $9.86 \pm 1.57*$ ; FE%Na:  $Sh = 1.28 \pm 0.19$ ,  $OUB = 50 \pm 11*$ ; FE%K:  $Sh = 32 \pm 4$ ; OUB = 88 $\pm$  5\*; U/P: Sh = 3.71  $\pm$  0.44; OUB = 1.56  $\pm$ 0.06\*. Marked renal hemodynamic alterations and modifications in the renal handling of water and electrolytes are observed in this experimental model of short time OUB, which persists after release of obstruction.

### 72. LIPID PARAMETERS AS CARDIOVASCULAR RISK FACTOR IN CHILDREN AND ADOLESCENTS

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Objetive: to compare distribution and prevalence of lipid parameters cutt-off in children and adolescents, both sexes in Rosario public schools. Cardiovascular (CV) disease is a frequent Morbidity and mortality cause in Western Countries. It is already Demonstrated that altered lipid metabolism is a CV risk factor. The gradual and progressive atherosclerotic process stars in chilhood. A school population of 538 children and adolescents, both sexesmales=138; females=400), age range: 9 to 19 years old was studied evaluating triglycerides (TG), total cholesterol (TC), (HDL-C) y (LDL-C) by enzymatic methods (Wiener Lab). Lipid parameters prevalence in participants at risk were characterised according to sex and age. Normal distribution of studied variables was rejected by Shapiro Wilk'test. Comparison of female vs male values showed stadistical significant differences only for HDL-C in females above 15yr (Man-Whitney's U Test). Results: population at risk, consedering International percentile cut-off values (%):

•	Females (n=400)		Males (n= 138)	
Percentile	9-14 yr	15-19 yr	9-14 yr	15-19 yr
TC 95	0	4	2	8
LDL-C 75	4	25	9	23
TG 75	19	16	14	3
HDL-C 25	0	1	0	1

Conclusions: a) values are in accordance with other groups in Argentina; b) prevalence for lipoprotein risk factor increases with age, except to TG. Our results emphasize the importance of detection and identification or risk factors since chilhood in order to modify them preventive rules.

## TWO CASES OF CHRONIC MYELOGENOUS LEUKEMIA (CML) TREATED WITH IMINITAB (GLIVEC) IN DIFFERENT PHASES

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IMINITAB, inhibitor of cytoplasmic transduction signs, and hindering neoplastic cells growth, is a new therapeutic agent for chronic myelogenous leukemia (CML). It is a tyrosine kinase berabl inhibitor, inhibiting also the c-kit receptor protein in gastrointestinal neoplasia and small cells lung cancer.

The aim of the present work was to evaluate the effect of this agent in CML patients in two different time-periods, namely the chronic phase and the acute one. We hereby present two patients: 1) a 48 yr old patient with radioactive contamination history, and 2) a 19 yr old patient. In both cases diagnosis was confirmed by BM and BM biopsy, neutrophile alkaline phosphatase, and Ph chromosome t(9;22) (q34;q11). There were non-compatible BM donors available. Both patients were treated with hydroxyurea, hydroxyurea plus interferon, and one of them adding ARAC. Since there was no favorable response an Iminitab course was started. Patient (2) with blastic crisis remitted for 12 month until subsequent relapse and death. Patient (1) treated during chronic phase is still in remission. Neither of them attained negative Ph chromosome. Up to now, current reports show a high percentage of relapse in patients treated during the acute phase, while the chronic ones present a smaller number of relapses. It is to be noted the importance of the follow up during the chronic phase, due to the short time drug utilization in our country (May 2001). Good tolerance and sustained remission in CML patients allow us to be optimistic regarding this therapeutic agent.

### 75.

### PARAQUAT INDUCED EMBRYOTOXICITY ON Bufo arenarum $Rucci\ AN$ .

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Paraquat, PQ, is a nonselective herbicide widely uses in Argentina. The evaluation of PQ induced embryotoxicity on Bufo arenarum by using the Frog Embryo Teratogenesis Assay-Xenopus was the main purpose of this work taking into account that this anura type is broadly distributed in agricultural region. Methods: when the embryos reached the stage 8 (mid blastulae) they were randomly assigned to eight different experimental groups: the first group remained free of PQ treatment and constituted the control; the seven remaining groups were treated with different concentrations of PQ ranging from 0.80 to 16.00 mg/l. All experimental units were conducted according to the FETA-X ASTM Designation. The treatment was interrupted 120 hours post-fertilization when the larvae reached the developmental stage 27. Results: all PQ concentrations used increased the percentage of mortality (p<0.01). A PQ concentration of 5.473 mg/l was the estimated dose that produced a 50% of mortality (LC<sub>50</sub>). The PQ concentration of 8.705 was the estimated dose that produced a 50% of malformations (TC<sub>50</sub>). A teratogenic index (TI) of 0.63 was derived All the PQ concentrations assayed produced a strong growth retardation evaluated as the head-tail lengths (p<0.01). **Conclusions:** Keeping in mind that PQ application rate for aquatic weed control ranges from 0.1 to 2 mg/l, ours results showed that, although the PQ TI<sub>50</sub> of 0.63 would show that this herbicide is a non-teratogenic compound, increased the percentage of mortality and strong growth retardation at all PQ concentrations assayed were observed.

### 74.

## ERYTHROCYTE VISCOELASTIC RECOVERY: COULD BE A HEMORHEOLOGICAL MARKER OF HETEROZYGOUS $\beta$ THALASSEMIA?

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The  $\beta$  thalassemia is a hereditary anemia and an heterogeneous genetic disease associated with defective expression of the  $\beta$  chain of human hemoglobin. The main objetive of this work was to investigate any hemorheological abnormality in  $\beta^0$  and  $\beta^+$  carriers and if this abnormality was the same according to the different types of carriers  $\beta^0/\beta$  or  $\beta^+/\beta$ .

A thalassemic group was compared hemorheologically with both, an iron deficiency anemia group charaterized by the same red cell morphology (hipochromia and microcytosis) and a normal group, Hemorheological parameters (red blood cell deformability, membrane elastic modulus and surface viscosity) were determined applying a laser diffractometric method (ektacytometry). Creep and recovery curves are recorded using this method. The recovery curve can be fitted by an exponential decay of first order, being the fitting degree expressed by the  $\chi^2$  parameter. Hemorheological parameters of  $\beta$  thalassemia group showed no significant differences when compared with the other two groups (iron deficient and normal group). The shape of recovery curves recorded from  $\beta$  thalassemic patients ( $\beta^0/\beta$  or  $\beta^+/\beta$ ) was significantly different from ( $\chi^2$  =  $172,27 \pm 92,57$ ) when compared with the other two groups ( $\chi^2$  =  $50.97 \pm 16.8$ ) (p<0.001). On the basis of such observations it can be concluded that the shape of the recovery curve might be considered as a marker of  $\beta$  thalassemia.

### 76.

### Toxocara canis LARVA MIGRATION IN MICE

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Toxocara canis is a nematode found in the small intestine in dog. The eggs are eliminate in the faeces and become infected in two or three weeks' time, if moisture, temperature and soil conditions are favourable. The puppy becomes infected when eating the larval eggs; the released larvae go through the wall and reaches the liver and lungs via the blood streams. The larvae then cross the lung alveolus, go up the trachea to the esophagus and reach the small intestine where they become adult. In adult dogs the larvae reach the striated muscles, the liver, kidneys and brain. They are immobilized and wrapped in the inflammatory granuloma. Adult dogs can also acquire T. canis via placenta and / or breast feedeing from their mother. The aim of this paper was to study male Rockland mice susceptibility to T. canis infection through second-stage larva migration and also the migration time in tissues and their histologic patterns. The eggs were obtained from *T. canis* female uterus. Male Rockland mice were infected with eggs having second-stage larvae, grouped into three lots of five mice each according to the number of inoculated eggs and slaughtered. The liver, lungs, small and large intestine, kidneys, spleen and brain were studied. When inoculating 500 to 1000 larvae, 500 were observed in the liver, 200 in the lungs and only 50 in the brain in four weeks time. Eosinophilic granulomas and necrosis centers were observed in the liver while migration larvae with necrotic areas and granulomas were detected in the lungs. Larvae in the brain were also found. When inoculating 500 to 1000 larvae, a greater response was obtained. The Rockland mice model showed the same susceptibility as the studies carried out by other authors with BAL/c, C57BL/10, etc.

### STUDY OF EMERGENT PARASITES IN +HIV PATIENTS

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Parasitic Infections in immunocompromised people are highly susceptible to non-virulent or little pathogenic organisms. Immunosuppression by HIV and other causes are risk factors for parasitosis. It is necessary to develop and regulate the diagnosis of intestinal parasites by finding the etiological agent with new techniques and cultures to identify strains. Cryptosporidium parvum, Isospora belli, Ciclospora cayetanensis, Microsporidium spp., Sarcocystis spp. are AIDS markers while Giardia lamblia, Blastocystis hominis, Entamoeba histolytica, Dientamoeba fragilis are potential markers. The aim of this work was to study emergent parasites located in the digestive tractus of + HIV patients and to use the appropiate methodology for its diagnosis. Thirty + HIV patients from Rosario were studied. Their ages ranged from 20 to 50 years old. Their faeces were collected with SAF. The detection of Cryptosporidium parvum, Isospora belli, Microsporidia and Dientamoeba fragilis was performed using 1% safranine, Weber-Green modified trichromic, Ryan Blue, Alcohol-resistant acid trichromic and diluted Giemsa. The parasites found were Blastocystis hominis 8 (26%), Giardia lamblia 4(13.3%), Microsporidium spp. 1 (3.3%), Blastocystis hominis y Giardia lamblia 3 (10%). At least one parasite was found in 55% of the patients. The entire protozoa associated to the HIV patient produce different diarrheic states.

## 79. TAXONOMIC STUDY OF THE *OXALIDACEAE* OF THE SANTA FE PROVINCE

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The family *Oxalidaceae* comprises 6 genera and circa 900 species from tropical and subtropical to cold regions of both hemispheres. In Argentina the family *Oxalidaceae* includes two genera, but only one of them is present in the Santa Fe province: *Oxalis* L. The present work contributes to the knowledge of this family providing keys to the identification of different taxa based on morphological characters and a map of their ecological and geographical distribution. The methodology consisted of a deep bibliographical review, consultation of national herbaria with important collections of the province (SF, SI, UNR), field work with some of the species in their communities and lab work to confirm their identity. As a preliminary result 10 species of the genera *Oxalis* L. were assessed. Their geographical distribution is concentrated mainly in the centre and northern departments of the province. It is here provided taxonomic information, illustrations and a distribution map.

#### 78.

### EVALUATION OF HUMAN SPERM DNA ALTERATIONS: COMET ASSAY

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Reactive oxygen species would be able to generate base oxidation and strand breaks at the sperm DNA. These alterations could impair the embryo development or the differentiation of any of the embryonic cellular progenies if the fertilization takes place. The aim of the study was to develop the method of single cell gel electrophoresis or comet assay, with slight modifications, in order to investigate the effects on human sperm DNA caused by the oxidative stress induced by H<sub>2</sub>O<sub>2</sub> or the exposure to ionizing radiation. Motile spermatozoa from samples of normozoospermic donors were exposed to increasing concentrations of  $H_2O_2$  (17,6  $\mu$ M to 140,8  $\mu$ M) or UV radiation (15 W for 1 h). Then, the sperm cells, included in 1% agarose gels, were electrophoresed under alkaline conditions (20 V for 5 min). The sperm DNA was stained with the silver method. The total length of sperm DNA migration for each treatment group was assessed using a microscope. The statistical analysis of the mean results among the different treatments was performed by the ANOVA test followed by the Dunn' test or by the Student t-test when only one treatment was applied. The results of the comet assays showed significant dose-dependent increases in sperm DNA migration for spermatozoa treated with H<sub>2</sub>O<sub>2</sub> respect to controls (p < 0.001). A significant decrease in the sperm DNA migration was observed in spermatozoa treated with UV radiation respect to controls (p < 0.001). In contrast to the H<sub>2</sub>O<sub>2</sub> treatment, the UV radiation would cause the cross-linking of the nucleotides, which could explain the observed results. The comet assay appears to be a sensitive method to assess potential damages in human sperm DNA.

### 80.

### ADHERENCE OF Candida dubliniensis, IN VITRO

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Candida albicans and Candida dubliniensis are yeasts that share phenotypic characteristics and are phylogenetically closed related; they are frequently isolated from HIV-infected individuals. Candida krusei is an emergent opportunistic yeast, which has been isolated in increased numbers and is intrinsically resistant to fluconazole. Our aim, in this study, was to compare adherence capacity, as a potential virulence factor, among C. dubliniensis, C. albicans and C. krusei strains. The experiment was performed with six strains of C. dubliniensis, isolated from the oral mucous of VIH (+) patients, one of C. albicans and one of C. krusei, both isolated from stools. Each of them was grown in Sabouraud broth during 24 hours at 37°C. They were washed twice with PBS and taken to a final concentration of 10<sup>7</sup> yeasts/ml. Buccal epithelial cells (BEC) were used which were washed twice with PBS and taken to a final concentration of 5x10<sup>5</sup> cells/ml. Adherence assays were performed according to Kimura et al technique and the number of yeasts attached to 100 BEC were counted. We repeated the same technique for three times, in duplicate, on different days. The data collected from the adherence assays were subjected to analysis of variance. C. dubliniensis strains showed adherence values between those of C. albicans and C. krusei, and these differences were statistically significant (p<0,0001). The strain of C. dubliniensis, which showed the highest adherence value, was isolated from a patient with extensive oral lesions. The fact that *C. albicans* is more adherent than C. dubliniensis and the later more than C. krusei agrees with the isolation frequency of these species in the buccal cavity of VIH (+) infected patients, according to previous results.

### PHENOTYPIC IDENTIFICATION OF STRAINS BELONG TO Trichophyton GENUS

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The aim of this study was to compare different phenotypic methods for *Trichopyton* strain identification, so as to distinguish among their species and to select the most useful techniques to apply in the mycological laboratory. Fifty-one strains isolated of clinical samples of the CEREMIC laboratory and 8 strains belonging to our collection were studied. The macroscopic and microscopic characteristics were examined in different culture media. Physiological tests proven were: urease activity, hair perforation in vitro and alkalinization of bromocresol-purple casein glucose agar (BCPC-G-A). In order to establish the association among *T.rubrum* (T.r), T.mentagrophytes (T.m) and 5 variables (the three physiological tests above mentioned, red pigment production and microconidia shape) the ratio of Odds were calculated. Thirty two percent of the strains were isolated from tinea pedis, 30% from tinea unguium, 24% from tinea corporis, 4% from tinea capitis, 4% from tinea cruris and 4% from tinea manuum. Microconidia shape not always showed the typical morphology expected for these species. According to physiological test the strains were classified as: 32 T.r, 14 Tm, 1 T. soudanense and 4 strains remained yet without identification. The close associations were obtained among the species and the variables BCPC-G-A, hair perforation and red pigment production, being the ratio of Odds 735, 496, and 40.5 respectively. Our results suggest that the alkalinization of BCPC-G-A should be used to carry out a correct distinction between T.r and T.m in the mycological laboratory.

### 83.

### ABH ANTIGENS IN HUMAN DENTAL PULP

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As a consequence of the caries or trauma there are changes in the dental pulp tissue that give origin to inflammatory processes which coexist next to a wide variety of soluble and membranous immunologycal indicators that have been described so far. These indicators are: Pg E2, Interleukin 1β, CD44, and others. The antigens of Histo-blood group ABH and their associates are present in a large number of cells and tissues. This study aims at analyzing the ABH antigen presence in human dental pulp cells in healthy teeth, using as reference cells of the urinary deposits of the same subject. Materials and methods: Antiserums: Monoclonal Anti-A, Anti-B (Wiener Lab) Lectine: Anti-H, Cell panel: ABH. Healthy teeth obtained from patients under orthodontic treatment with A, B and O blood groups were processed. The pulp tissue was extracted from them. It was placed in 100 µl of buffer phosphate pH 7.2. It was frozen and defrosted from 3 to 5 times so as to produce a thermal shock. 3 batteries of geometric dilutions of the material studied in PBS were done with the floating material. Each battery was faced with equal volume of antiserum of a different specificity. It was incubated for 10 minutes and it was detected with a red corpuscle isogroup solution at 5%. It was centrifuged at 1000 rpm for a minute and macroscopic agglutination was observed. The absence of agglutination indicates the presence of soluble antigen. Results: The presence of antigens A, B and H in pulp tissues of the teeth of the patients under study was proved. This detection coincides with the presence of antigen in the urinary deposit cells together with the patient blood group. Conclusion: The presence of ABH antigens has been proved in the pulp tissue of healthy teeth.

#### 82.

### QUALITY OF FORAGE CROPS FOR ANIMAL FEEDS

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Preservation by fermentation or dehydration of forage crops for animal feeding, is an usual method for their use in those seasons when fresh forage is not available and for obtain higher yields of nutritive value per hectare.

Presence of undesirable microrganisms such as moulds and bacteria, undesirable chemicals such as mycotoxins, and metabolic disorders can damage the animal health.

147 samples of forage crops (55 of sorghum, 49 of lucerne and 43 of maize) for feed ration of cattle, were analyzed. For determining final acceptability of the samples, chemical-fermentative characteristics (Mc Donald, 1991) and myco-toxicological characteristics (Pitt & Hocking, 1999; determination of aflatoxins -AF- and deoxynivalenol -DON- by ELISA) were evaluated.

Animal feeds of lucerne and maize were the most contaminated samples. Aflatoxins was the most frequent contamination (45.58% of all analyzed samples, 55.81% of the lucerne samples and 22.45% of the maize samples). The sorghum silage weren't successfully stored by "bridge" system (all the samples were unaceptables for consumption).

We studied the statistical association between variates using Fischer's exacted Test, to select the parameter as a marker of forage quality. We were able to determinane that both AF and DON, can be employed as indicators of lucerme and maize contamination.

### 84.

## ROLE OF SALICIC ACID IN DISEASE RESISTANCE TO PVX IN $SOLANUM\ TUBEROSUM$

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In plants, the control of a pathogen is often determined by simple gene-for-gene interactions, in which a resistance response is induced only if the pathogen encodes a strain-specific avirulence (avr) gene and the plant carries a resistance (R) gene. Resistance is typically manifested as a hypersensitive response (HR), leading to the the production of signal molecules such as salicylic acid (SA). Previously studies in tobacco have shown that SA induces the expression of the alternative oxidase (AOX), a mitochondrial membrane enzyme that catalyses the alternative respiratory pathway. The goal of this work is to study the role of SA and its effect on AOX during the plant defence response again pathogen attack. Our research model is Potato virus X (PVX) and Solanum tuberosum cv Pentland Ivory (genotype Nb nb nb nb). Nb is a single dominant gene from Solanum tuberosum that confers hypersensitive resistance to PVX strains carrying the avirulent 25 kDa movement protein. Transgenic Nb plants expressing the salicylic acid hydroxylase (nahG) showed that SA is required for the induction of local and systemic acquired resistance induced by the avirulent PVX strain ROTH1. Furthermore, we have characterized the potato AOX gene in order to study the importance of the AOX in the expression of HR-associated programmed cell death in plants.

### RELATIONSHIP OF SPERM DNA WITH MOPHOLOGY AND ANTISPERM ANTIBODIES

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The examination of human semen according to WHO criteria is essential to male infertility evaluation. Sperm morphology abnormal is an indicator of poor fertilizing potential. Disorders of chromatin structure can cause unexplaine infertility. The antisperm antibodies (ASA) presence in ejaculate, not only change the motility but also decrease the fertile capacity.

Our objetive was to study in infertile men the relationship between sperm DNA denaturation with mophlogy and presence of ASA. Human sperm samples from 31 men were studied. All samples were evaluated for standars semen parameters according to WHO criteria (WHO 1999), research ASA and integrity of DNA.

In the morphlogical evaluation Papanicolaou stain was applied. Acridine orange stains green with normal double-stranded DNA and red with single-stranded DNA. The presence of ASA was carried out with TAC II (mixed agglutination test) obtained for our work team and validate with a MAR-screen (Gatti V, Act Urol. Ital: 1998, 12(6), 321).

From 31 semen samples that were studied, 16 (52%) presented denatured DNA and abnormal morphology, the association in these parameters were statistically significant. ( $\chi^2 = 11.50$ , p<0.05) When the presence of ASA was evaluated, we found that 16 samples with denatured DNA, 14 (87.5%) with presence of ASA in the spermpatozoa. The presence of spermatozoa with denatured DNA

is related with the presence of ASA ( $\chi^2 = 29.74$ , p<0.05) To conclude it is very important to use a sequential diagnosis way that allows to evaluated structural, dynamic and funcionale characteristics of spermatozoa. The diagnosis basis should include a semen basic analysis, a funtionale study and the search of ASA in spermatozoa.

### 87

### NON RADIOACTIVE PRECURSOR IMPORT INTO CHLOROPLASTS

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Eukaryotic cells have a subcellular organization based on organelles. Protein transport to these organelles is cuantitatevely important because the majority of cellular proteins are codified in nuclear genes and then delivered to their final destination. Most of the chloroplast proteins are translated on citoplasmic ribosomes as larger precursors with an amino terminal transite peptide, that is neccesary and sufficient to direct the precursor to the chloroplast. Once inside the organelle the transit peptide is cleaved and the mature protein adopts its folded form.

In this work we developed a system for the expression and purification of the pea ferredoxin-NADP<sup>+</sup> reductase precursor (preFNR) for its import into chloroplasts in non radioactive conditions. We constructed a preFNR fused in its carboxy terminus to a 6 histidines peptide (preFNR-6xHis) that allows its identification using a comercial especific antibody. The construction was expressed, purified, processed and precipitated, rendering a soluble and active preFNR-6xHis that was used in binding and import into chloroplasts experiments. The reisolated chloroplasts were analyzed by SDS-PAGE, electroblotting and revealed by immunodetection using either colorimetric or chemioluminiscent reactives. We performed also import experiments labeling preFNR and preFNR-6xHis with radioactive methionine as controls. We conclude that preFNR-6xHis is bound and imported into chloroplasts as the wild type preFNR and that both colorimetric or chemiolumiscent detection methods are useful to avoid the manipulation of radioactive material.

#### 86.

### MODIFIED SPERM STRESS TEST (MOST) AND THIS RELATION WITH IMMUNE RESPONSE

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Reactive oxygen species attack cell membrane phospholipids, generating fatty acid peroxides and other degradation products, inducing immovilization, senescence and death of spermatozoa and can be measured in an indirect form through the M.O.S.T. (Calamera JC, Hum. Reprod. 1998; 13: 2484-88). The integrity and the funcionality of the spermatic membrane are essential for the viability and phisiological changes necesaries for the process of fertility, the antiespermatic antibodies (ASA) alter your permeability affecting the fertile capacity and motility. The macrophage participate in immune response how show cell the antigen and cytotoxic cell, through the liberation of cytoquines. Ours objetive was evaluate the relation beetwen the M.O.S.T. and the mechanism of immune response. Human sperm samples from 25 men were studied. All samples were evaluated for standard semen parmeters according to WHO criteria (WHO 1999). The presence of ASA was determinated with TAC II (mixed agglutination test) obtained for our work team and validate with a MAR-screen (Gatti V, Act Urol. Ital: 1998, 12(6), 321). The concentration of macrophags with neutral red stain. MOST ratios were calculated by dividing final motility over initial motility (cutt of= 0.39). The 25 samples sperm studied, there was 18(12%) with unnormal MOST, and 14(77.7%) presented ASA, and 10(55%) high concentration the macrophage (>0.6.106 /ml). The statistic analysis proved significant association beetwen positve AAE and altered MOST ( $\chi^2 = 12.44 \text{ p} < 0.05$ ), too meeted association beetwen high macrophage and unnormal MOST ( $\chi^2 = 6.48 \text{ p} < 0.05$ ). All the samples with ASA contained unnormal values of the MOST. To give that immunologic respect of the semen contribute beetwen in 5 15% in the male infertility we do considerer important incorporate to human sperm assay.

### 88

### PRESENCE OF ABH ANTIGENS IN PERIAPICAL TISSUES IN HEALTHY HUMAN TEETH

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The ABH antigens are glycolipids of membranes present in the glycocalyx of the cells in charge of the adhesion, cell recombination and contact inhibition processes. The purpose of this study is to propose an in vitro experimental model to show the presence of ABH antigens in periapical tissues in healthy human teeth. Three teeth from healthy individuals were extracted owing to orthodontia instructions. They were worn with the turbine and with saline solution irrigation. The suspension was collected with Eppendorf tubes and it was frozen at −30°C. They were submitted to consecutive processes of freezing and defrosting to release the membranous antigens. We worked with the inhibition haemoagglutination technique using anti – A and anti- B monoclonal antiserums and anti-H lectine. They were faced with the A, B or O corpuscle controls. They were centrifuged at 1000 r.p.m. and agglutination was registered. The lack of agglutination revealed the presence of antigens and positive agglutination showed their absence. The findings demonstrate the presence of A antigens and the lack of B and of H antigens in teeth of A individuals, the presence of B antigens and the lack of A and H antigens in teeth of B individuals and the presence of H antigens and the lack of A and B antigens in teeth of O individuals. This finding, which is not described in the literature, may provide a methodology for the study of teeth in healthy patients and patients with various pathologies. No presence might be related to immune phenomena altered by adhesion, which could be the cause of chronic damage.

#### 20

## PRELIMINARY STUDIES ABOUT THE INTERACTION OF COBALT WITH SULFANILAMIDES. OBTAINMENT OF Co(II) AND Co(III) COMPLEXES WITH SULFATHIAZOLE, PHTHALYLSULFATHIAZOLE AND SULFANILAMIDE

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There are many areas of interest for coordination chemistry of sulfa drugs. As part of a research program devoted to the study of the interaction metal ions-sulfa drug, we began Co studies. Complexes were obtained by mixed of aqueous solutions of CoCl, and the ligand, L (L = sulfathiazole, ST; phthalylsulfathiazole, FST or sulfanilamide, SA). Co(III) complexes were obtained similarly, plus H<sub>2</sub>O<sub>2</sub>. The obtained precipitates (Co(II)-ST: blue developing pink; Co(II)-FST: gray; Co(II)-SA: green; Co(III)-ST: brown; Co(III)-FST and Co(III)-SA: both black) were analyzed. *Electronic spec*tra: (registered in a Jasco V-530, 25°C, solvents: H<sub>2</sub>O, DMSO, methanol; for CoCl<sub>2</sub>, the ligands and the complexes): although there were observed differences in all of the solvents, the major ones were in methanol, shifted to lower wavelength. For d-d transitions, the major shifted respect to CoCl, were observed with Co(II)-FST, suggesting an enhance of the energy in the bonding metalligand. Contents of Co in Co(II)-ST: 11%, so, it is possible to thing in an stoichiometry L/Co = 2. IR spectra of Co(II)-ST: changes were observed in the signals of the thiazole ring (1540 cm<sup>-1</sup> en HST, 1510 cm<sup>-1</sup> en Co(II)-ST). It was not possible to obtain <sup>1</sup>H RMN spectra, due probably to the paramagnetism of Co(II). Bacteriological studies: it was determined the Minimum Inhibitory Concentration (MIC) as norms of the National Committee for Clinical Laboratory Standards, testing with E. coli (ATCC 25922 and a Hospitaler strain). E. coli ATCC 25922 was resistant to the complex (CIM: > 128 µg/mL), but the Hospitaler one was sensitive (CIM: 32 µg/mL), given the same value than the ligand (NaST).

### 91.

## CONSTRUCTION OF A BACTERIOPHAGE QB DERIVATIVE TO BE APPLIED AS A CONTROL IN RNA VIRUSES DIAGNOSES

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The use of RT-PCR for the detection of RNA viruses is an important tool in clinical and research laboratories. Due to RNA lability, it is necessary to have a control to verify the efficiency of both, sample processing and amplification reaction. We present the development of an internal control derived from Qß phage to be spiked into the specimen, processed and co-amplified with the same target primers. Qß is a coliphage with single strand RNA genome. This genome was cloned as a cDNA in an expression vector under the control of the T7 promoter (pBRT7QB). The system was used for the production of phage particles and its infectivity and stability was checked along time during a year. The primers KY78/KY80, which are commonly used in the detection of HCV, were inserted in the phage genome. They were cloned into the open reading frame of A1 coat protein. Oligonucleotides were designed for the introduction of KY78/KY80 in the phage genome by PCR. This amplicon was cloned into pBT7Qß to replace a wild type region with a fragment flanked by KY78 and KY80. The recombinant phage (QB78-80) was tested in infection experiments with the same strategy used with wild type phages. QB78-80 phage particles viability was demostrated, however tittles obtained with the recombinant phages were lower than those obtained with Oß. To confirm the in vivo results, RT-PCR was performed with the QB78-80 and HCV RNAs as templates and the KY78 and KY80 primers. Products obtained were the expected ones and they were confirmed by hibridization with specific probes and detection by colorimetric method. The success in the development of an internal control for the HCV molecular diagnoses suggests that this strategy is an useful tool for the design of internal controls for the molecular diagnosis of others RNA viruses.

#### 90.

### CAPTURE OF MERCURY BY SULPHATIAZOLE

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Mercury is a heavy metal, toxic in anyforms. Its influence is accumulative and produces important effects on the neurological behavior. Mercury was an important constituent of diuretics, antibacterials, antiseptic, skin unguents and laxatives. More effective therapeutic forms had been replaced mercury compounds in last decades. Actually, it is a problem the environmental contamination of mercury as toxic waste. Epidemic of poisoning by mercury were produced both in human poblation and wild animals in many countries. Hg(II) salts have industrial use and mercury wastes can be contaminate rivers and another water bodies, where bacterials might form methyl mercury, one of the most dangerous compounds of the metal.

Sulfathiazole (sodium salt), NaST, antibacterial from the sulfonamide group, forms with Hg(II) a white precipitate: Hg[(sulfathiazolato), (OH),] when aqueous solutions of sulfathiazole and Hg(II) are mixed in molar ratio ≥2. The very slight solubility of this compound in water (0.69 mg/100 mL) let us think sulfathiazole us a "catcher" of Hg(II) inorganic salts there is an accidentally spreading in the environment. We designed an experience in which an aqueous solution of NaST (0.22 mmol/5 mL) were added to 0.1 mmol HgCl<sub>2</sub> in 5 mL H<sub>2</sub>O, and a white precipitate was inmediately obtained. Later, it was taken aliquots of 1 mL at different times (0.5; 1; 1.5; 4; 24; 48 and 72 hs) from the floating. Contents of Hg was determined in each sample by atomic absortion in a perkin Elmer 3110. At 4 hs the contents of Hg was < 5ppm. It was not later dissolution of the precipitate, so, these results let us propose NaST us a possible "catcher" of inorganic Hg(II) if it is accidentally spreading in the environment.

## 92. OPTICAL PROPERTIES OF PROTEIN SOLUTIONS USED AS BUFFERS IN OPTICAL BIOSENSORS

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Optical biosensors, based on null ellipsometry technique, can be used for detection and quantification of protein in solutions. Polarized light goes through the sample and is reflected at the biosensor surface of the biosensor (biochip). The reflected light intensity is related with the protein concentration adhered on surface. So its measurement allows the desired detection and quantification. During the measurement, some non-specific proteins present in the solution may be adsorbed on the biochip interfering with the results. These non-specific unions may be reduced by the use of buffer solutions. Also, the light absorption and scattering, produced by the particles of the buffer solution, interfere the results too. These phenomena must be evaluated for the employed buffers.

In the present work, the optical extinction, the change in the refractive index and the kinetic adsorption are studied for several protein solutions like albumin, low fat milk and gelatin in PBS and PBS-tween.

The results show that the extinction grows as the concentration does. For albumin solutions exceeding 0,005% with or without Tween, it was not detected any increase of the light intensity reflected on the biosensor surface. In fact, the final intensity is lower than the incident, indicating that the high value of optical extinction interfere with the observation of the albumin adsorption process. The obtained results may also be applied for the selection of the adequate composition in buffers of other optical instruments.

### BETA THALASSEMIA AND ALPHA GENE TRIPLICATION

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When heterozygous beta thalassemia coexists with alpha genes numerical alteration, the clinical and haematological phenotype is affected. The clinical and haematological alterations in beta thalassemia carriers in association with alpha gene triplication were evaluated; 109 unrelated patients were studied. Alpha gene triplication  $(\alpha\alpha\alpha^{\text{anti3,7}})$  was studied in all patients with Hb under 11 g/dl (males and females over 50 years of age ) 10,5 g/dl (females under 50 years of age and children). So, 48 patients were studied. Molecular characterization of beta thalassemia was performed by PCR-ARMS. The alpha gene triplication was investigated by means of PCR allele specific. The association of heterozygous β-thalassemia with alpha gene triplication ( $\alpha\alpha\alpha^{anti3,7}$ ) was found in two patients out of the 48 studied, i.e., a 10 year old child (a) and a 36-year-old woman (b), both of Italian ancestry. The  $\beta$ -thalassemia mutation in (a) was  $\beta^+$  IVS II-745, and in (b)  $\beta^0$ 39 mutation. Haematimetric parameters in beta thalassemia carriers in association with alpha gene triplication were lower than in controls,  $\beta^+$  thalassemia carrier children, and β° thalassemia carrier women respectively. Degree of anaemia was in correspondence with thalassemia intermedia. Haematimetric parameters are in accordance with literature reports.

#### 94.

### ALTERATIONS OF THE FLT3 GENE IN ACUTE PROMYELOCYTIC LEUKEMIA

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Alterations in the FLT3 gene including internal tandem duplications (ITDs) and D835 mutations occur frequently in acute myelogenous leukemia. We investigated the prevalence and clinico-biological correlations of FLT3 ITDs and D835 mutations in 90 patients with acute promyelocytic leukemia (APL) receiving the AIDA protocol. Twenty patients in which both presentation and relapse material was available were analysed sequentially. Thirty-three patients (37%) harbored the ITD, and 7 (7.7%) the D835 mutation in blasts obtained at diagnosis. Presence of ITDs was strongly associated with high WBC count ( $P \le 0.0001$ ), M3 variant ( $P \le 0.0004$ ), and the short (BCR3) PML/RAR $\alpha$  isoform (P $\leq$  0.003). There was no difference in response to induction in the two ITD+ve and ITD-ve groups, while a trend towards inferior outcome was observed for ITD+ve cases when analysing disease-free survival (DFS) and relapse risk (RR). These differences, however, did not reach statistical significance.

### 95.

## ALTERATIONS OF SPERM MEMBRANE AND OXIDATIVE STRESSS IN INFERTILE PATIENTS

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Fertilization process involves complex changes of plasmatic sperm membrane. Evaluation of its integrity trough functional tests ins an important parameter to study male infertility. There is increasing evidence that oxidative stress (OE) is a significant factor in the pathogenia of male infertility. The determination of indicators of oxidant injury provides useful information for the study of fertilizing capacity. Our objective was to evaluate the alterations of the sperm membrane against OE. 43 semen samples were studied from patients that consulted for infertility at Reproduction Laboratory of "Eva Perón" School Hospital. Sperm was study according WHO. 17 samples of free leukocytes (<1 x 10 6/ml) and antisperm antibodies (<25% mixed agglutination) were chosen. Structural and functional integrity of membrane was evaluated trough eosina test (ET) and the hiposmotic test (HT). To evaluate the oxidant injury the modified sperm stress test (MOST) was applied. The analysis of association between variables was carried out as a partial correlation study. The MOST partial correlation coefficients with ET and HT were 0,00646 and 0,3969 respectively, from which only the last one is significantly different from zero (p=0,022). Results of study were expressed in qualitative way taking into account the reference ranks for each variable, applying the exact probabilities of Fisher test. According to results we conclude that there is a statistically significant association between the sperm percentage affected by the OE and with functional changes (HT). The study of parameters related to sperm structure and function allows to improve knowledge of ethiology of their alterations.

### 96.

### ERYTHROCYTE MORPHOLOGY IN BLOOD STORED UNDER BANK CONDITIONS

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The purpose of the present work was to study the morphology of erythrocyte from whole blood during 23 days storage at a blood bank. Whole blood stored in CPD-adenine were studied from 17 healthy adult volunteers. Red Bood cells morphology was evaluated with the Zipursky-Forconi method at two times (0 and 23 days) during the storage interval. In each aliquote, 100 erythrocytes were observed and classified being the Bessis. Percentages of more relevant morphology classes of RBC are summarised in Tabla 1 (average  $\pm$  standad deviation)

Morfology	Day 1	Day 23	p
Normal	84,06±9.37	13,47±9.22	< 0.001
Echinocytes	5.94±8.84	86.41±9.15	< 0.001

Statistical differences were determinated by using Student "t" test. The results demostrate that the mean percentage of normal morphology (the most deformable class, is significantly increased in the samples at the first day (P<0.001). Whereas the altered erythrocytes (echinocytes) were significantly greater in the samples on day 23 (p<0.001). The cell morphology is influenced both by extracelllular factors and by coccneted with the structural conformation of the cell membrane Transformation discoyte-echinocyte has been explained by many factors. Transformation of ATP, increase of intracellular calcium, changes in pH, alteration in the composition of the cell membrane). The study of morphology is an useful parameter which can be used to evaluate the alteratio of RBC during storage.

#### 07

### CONTRIBUTION OF ANATOMIC FEATURES TO RECOGNIZE BUDDLEJA GENDER (BUDDLEJACEAE)

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Buddlejaceae has been considered a subfamily, Bubbleioideae, in the Loganiaceae, today considered a new family in the Escrofulariales, (Cronquist, 1981). Three species from Santa Fe province were considered: Buddleja tubiflora Benth., Buddleja grandiflora Cham. et Schlecht., Buddleja Brasiliensis Jacq. ex Spreng. Objective: to supply anatomic features to recognize *Buddleja* L gender. The material was from herbariums and FAA fresh fixed, embedded in parafin. Transversal and longitudinal cuts were manual and with Minot microtome performed. Leaves were made diaphanous and stems macerated according to conventional techniques. Stains: Safranin and Safranin-Fast Green. LM and SEM observations. Anatomic features: in superficial view, epidermic cells show wavy anticlinal walls and striate cuticle, stomata are only found in the abaxial epidermis, being the ranunculaceae and rubiaceae types predominant; star-shaped hair, in candelabrum and glandular with bicellular head (more abundant abaxially), can be seen in both epidermis. Leaf architecture: pinnal, camptodromus, brochidodromus, the last marginal veining is buttonhole, areolae are square or polygonal. Transversal cut: bifacial petiole, concave-convex, with a central vascular beam. Leaf lamina shows dorsoventral, hypostomatic structure, thin mesophile, with a round to polygonal cells palisade layer on the surface. The stem is square section, sometimes with four wings, laminar, discontinuous pericycle, made up of sclereid fibers; lack of intra-axillary phloem in the vascular system, continuous secondary log, crossed by uniseriated medullar radii; vessels with variable diameter 20-60µm; generally 200µm long, paratracheal parenchyma. Polygonal crystals with various shapes, abundant in medullar parenchyma.

### 99.

### ERYTHROPHAGOCYTOSIS IN YOUNG AND OLD INDIVIDUALS

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As the donor age increases there is a concomitant decrease in the percentile contribution of whole blood by a heavy density fraction of red blood cells (RBC). The aim of this work is to investigate in young and old individuals, the interaction between monocytes and different erythrocytes suspensions: a) senescent and young RBC (SeRBC, YRBC) obtained by differential centrifugation; b) RBC stored with its own serum (RBCS) and without serum (RBCwS) and c) RBC desialyzated with neuraminidase (Ne) and trypsine (T). Healthy individuals blood samples (20-40 years old, n= 50 and > 70 years old, n=49) were studied The suspensions were subjected to the erythrophagocytosis assay and the % of active monocytes (AM) were determined. Non sensitized RBC (NRBC) and ex vivo sensitized RBC (SRBC) were used as negative and positive controls. Values of AM obtained with old individuals were: SeRBC: 21.9 ± 1.0, YRBC:  $2.9\pm1.2$ , RBCS:  $16.2\pm1.4$ , RBCwS:  $3.1\pm0.8$ , NeRBC:  $11.1 \pm 1.3$ , TRBC:  $3.2 \pm 1.1$ , NRBC:  $3.0 \pm 1.2$ ; SRBC:  $31.2 \pm 1.8$ and with young individuals: SeRBC:  $17.1 \pm 1.5$ , YRBC:  $3.1 \pm 0.9$ , RBCS:  $11.1 \pm 1.1$ , RBCwS:  $2.8 \pm 1.2$ , NeRBC:  $10.8 \pm 1.4$ , TRBC:  $3.5 \pm 1.0$ . NRBC:  $2.9 \pm 1.3$ ; SRBC:  $30.5 \pm 1.7$ . The results obtained with SeRBC and RBCS show the involvement of autologous IgG in the selective removal of RBC. These values were higher in old individuals indicating that this process would increase in aged donors. Our observations suggest that the desialylation would not participate in the increased removal of erythrocytes observed in old individuals.

#### 98.

### PRENATAL DETERMINATION OF FETAL *RHD* USING DNA FROM MATERNAL PLASMA

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Prenatal determination of fetal *RHD* is required when the fetus is at risk of haemolytic disease of the newborn due to maternal alloimmunisation. Invasive methods, such as amniocentesis, to obtain fetal DNA for genetic analysis pose a risk to the fetus. Fetal DNA has been recently found in maternal plasma providing new possibilities for prenatal RHD determination by non-invasive PCR based procedures. The aim of this work was to assess the feasibility of RHD genotyping of the fetus by PCR amplification of fetal DNA obtained from maternal plasma. We analyzed plasmas from 6 RhD negative pregnant women with male fetuses. Fetal DNA was extracted from maternal plasma through absorption and elution in silica gel membranes. Plasma from RhD negative individuals who were not pregnant were used as control. RHD genotype was determined by a multiplex PCR strategy that analyses intron 4 of the RHCE (1238 bp) and RHD (587 bp) genes and the 3' unstranslated region of the RHD gene (250 bp). The fetal origin of the DNA was confirmed by amplification of the SRY gene. Four fetuses were RHD positive and 2 were RHD negative. None of the control plasma samples were RHD positive. The SRY gene was amplified in all plasma samples from the pregnant women. For all cases, 100% correlation was observed with RhD phenotype performed at birth. Fetal RHD status may be determined by PCR in DNA extracted from maternal plasma of pregnant women. These preliminary results suggest that this noninvasive procedure may be useful for the management of RhD negative mothers. A large scale evaluation is needed before considering application in clinical situations.

### 100.

### SPECIFIC ERYTHROCYTE ADHESION ENERGY DETER-MINATION THROUGH ROTATIONAL VISCOMETRY

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Erythrocyte adhesion provides an experimental model for the study of intercellular interactions. We present a simple reo-mechanic technique to determine the specific adhesion energy between adjacent cells consisting in plotting curves of viscosity vs. fluid shear stress obtained for red cells suspensions using a rotational cone-plate viscometer Brookfield LVDIII+ and different shear rates at 23°C. Such curve, plotted in a semi-logarithmic scale, exhibits a linear segment with negative slope, followed by a segment that converges asymptotically to the minimum apparent viscosity of the suspension. Extrapolating the linear section of the curve to intercept the value of minimum viscosity, a critical value of shear stress ( $\tau$  N/ m<sup>2</sup>) is obtained, at which total dissociation of the cellular doublets is achieved. Specific energy ( $\Gamma$  N/cm) is calculated using the Derjaguin relation for rotational flow:  $\Gamma = (0.1 \text{ xA}_0 \text{xt})/(2\pi \text{xR}_0)$ , where  $A_0 = 50.10^{-8} \text{cm}^2$  is the erythocyte's exposed area, and  $R = 10^{-8} \text{cm}^2$ <sup>4</sup> cm is the radius of curvature of the last membrane adhesion point. In each determination, 1 cm<sup>3</sup> of normal red cells suspension (40%) hematocrit) in autologous plasma or 4.1% Dextrán 70 (MW 70 kDa) solution in PBS was employed. Results were compared to those obtained with a validated method:

	Reo-mechanic method		Erythroagregameter	
	RC in plasma	RC in Dx 70	RC in plasma	RC in Dx 70
$\tau_{c} (N/m^2)$	0.365±0.04	0.535±0.07	0.411±0.05	0.490±0.06
Γ (N/cm)	2.85±0.29	4.25±0.54	3.27±0.35	3.90±±0.60

Specific energy quantification allows to estimate even a weak intercellular adhesion strength, and also to analyze the fluctuations of this parameter in circulatory pathologies.

## METHANE PRODUCTION BY ALFALFA PASTURE IN *IN VITRO* RUMEN: PROCESS EFFECT AND RELATION WITH DEGRADABILITY

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The aim of this work was study the methane production (CH $_4$ ) in *in vitro* rumen by alfalfa pasture. Three samples of the same alfalfa pasture were collected every 15 days (A, B and C). The samples were chopped (1 cm) and froze at -20°C (F, fresh) or dried at 60°C, milled and shifted (2 mm mesh) (D, dried), and incubated in *in vitro* rumen systems at 39°C during 24 h. pH and redox potential (ORP, mV) were determined at 0 and 24 h; gas production (ml), CO $_2$ , CH $_4$  (mmol/l) and *in sacco* rumen degradability (DDM %) at 24 h.

EFFECT		DDM	pН		ORP		Gas	CI	H4
		%	0h	24h	0h	24h	ml	mmol/l	l/kgMS
Sample	Α	79.2c	6.30	5.42	-295	-289	41.0	33.2 a	33.2 a
	В	75.9b	6.30	5.51	-302	-286	48.2	50.0 b	50.0 b
	C	70.0a	6.31	5.47	-295	-284	49.2	51.4 b	51.4 b
Significati	on	*	ns	ns	Ns	Ns	ns	ns	*
Process	D	73.7a	6.4b	5.49	-300	-295	51.0b	17.54	42.4
	F	76.3b	6.2a	5.44	-295	-277	41.2a	17.47	47.3
Significati	on	*	**	ns	Ns	***	**	ns	ns

 $\underline{Note} : *p \leq 0.05; **p \leq 0.01; **** p \leq 0.001. \ a, b, c \ works \ different \ in \ the \ row \ indicate \ differences.$ 

At 0 and 24h the control systems pH were  $6.6\pm0.0$  and  $5.72\pm0.33$ ; ORP were  $-320\pm0.5$  and  $-320\pm0.0$ . At 24 h, gas production was  $25.0\pm0.37$  and CH<sub>4</sub> production (mmol/l) was  $12.64\pm0.74$ . The process effect was significative for gas  $_{24h}$ , pH $_{0h}$  and ORP $_{24h}$ ; and the sample effect for DDM and CH<sub>4</sub> ( $_{1/kgMS}$  and  $_{1/kg}$  MSD). F samples presented higher DDM, lower gas production and pH higher regarding S. pH $_{0h}$  was related with ORP $_{24h}$  (r: -0.95, p≤ 0.01) and ORP $_{0h}$  with pH $_{24h}$  (r -0.95 p≤ 0.05). CH $_{4}$  production evidenced an inverse tendence regarding DDM.

### 103.

## DICHOTOMIC KEY FOR EGGS MORPHOLOGIC DIFERENCIATION OF COOPERIA SPP, PARASITE NEMATO DE OF CATTLE

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Cooperia spp is a endemic nematode resistant to ivermectin in Argentina. Sommer (1996, J of Helm, 70:143-151) by means of images it was able to discriminate against in 85% the different trichostrongyle eggs. The objective of the present work was to prove dichotomic keys that allow to differ by means of characteristic quantitative to the eggs of *Cooperia spp* of the other trichostrongyle. By means of the optic microscope and ocular micrometer the long one and the high one were measured from 25 to 50 eggs of obtained trichostrongyle for flotation (Solution of sugar) of the faecal matter of 10 bovine Angus between 8 and 10 months of age with natural infestation of Cooperia spp and Haemonchus placei. The obtained patterns of the bibliography for these two variables were used alone and cocktails for the formulation of 6 dichotomic keys that they allowed to differentiate the goods Cooperia and Haemonchus. The obtained proportions with the key were compared (Schwarz, 1981; Rolan, 1973) with the obtained ones for the cultivation of larvas, these like indicators of maximum capacity discriminate. The width of the results oscillated from a maximum of error from 80% to a minimum that allowed to discriminate against 100% of Cooperia spp. Of the six evaluated keys, the high of the eggs showed the biggest discrimination capacity: 60% (p < 0,05) the 5 remaining keys varied among any capacity to a maximum of 40% (p < 0,05) of differentiation. For that evaluated you can conclude that it is necessary to continue with the adjustment of new keys.

#### 102.

### KINETIC OF DRIED MATTER RUMINAL DEGRADATION OF WHEAT AND CORN STRAWS

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The aim of this work was to study the kinetics of dried matter ruminal degradation of wheat and corn straws in rumen of sheep feed with alfalfa hay. The sample, wheat (WS) and corn straws (CS), were dried at 60°C, milled and shifted with 2 mm mesh. Three g dried matter (DM) of sample in nylon bags (17mgDM/cm²) were incubated within the rumen of two sheep during 0, 2, 4, 8, 12, 18, 24, 48, 72 and 96h, in three different periods. The observed results in ruminal dried matter degradation (RDMD) were fitted at (1) Orskov and McDonald (1979): RDMD = a + b (1 - e^-ct') and (2) Danhoa (1988) models: RDMD = a + b (1 - e^-ct'); where a soluble fraction, b slowly degradable fraction, c degradation rate of b, a + b potential degradability and lag latent phase. The best fit was determined with f test (P > 0.05).

	Orskov and McDonald,				Danhoa, 1988			
		1979						
	a	b	с	a	b	c	lag	
CS	17.96	64.25	0.0165	25.02	57.18	0.0165	7.04	
WS	3.81	83.52	0.0112	18.04	69.3	0.0112	16.6	

In both models CS showed a soluble fraction more important regarding WS. The b fraction evidenced inverse comportment in two models, lower in CS regarding WS. Degradation rates were 1.65 and 1.12 %/h, and potential degradability were 82.2 and 87.3 % for CS and WS, respectively. R² were 0.98 and 0.96 for CS and WS. The best fit determined by f test were Orskov and McDonald (1979) model. The studied samples presented low soluble, high slowly degradable fractions and slow degradation rates. Although the Orskov and Mc Donald (1979) model was recommended, the Danhoa (1988) model determine the latent phase (lag), an interesting parameter for these samples.

### 104.

### CESTODES IN THE RURAL ZONE CANINES

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Cestodes are not characterized, trough to cause clinical syntomatological in hosts, dogs can support important loads but not show a cestodosis. The main disadvantage that presents is that they can be transmitted to the human being and cause a zoonotic disease. Visits to farming establishments of the south of the province of Santa Fe, Argentina (period 2001–2002) to establish cestodes that prevail in canine were made. Test arecoline diagnostic was used, according to guidelines of Schantz, carmine coloration with acetic, and compression of proglotids. The test independence of attributes was used based on the ditribution  $\chi^2$  to establish the relation between cestodosis and age and cestodosis and sex. The visited establishments were 12, being cestodes in 9 (75%). The drug to 45 was administer and responded same to the 41, of that total 19 (46,34%) eliminated one or cestodes such were identified like Echinococcus granulosus 10.52%, Dipylidium caninum 73,68%, and Taenia hydatígena 68,42%. Was not significant association between cestodosis and age (p<0.05) and cestodosis and sex (p<0.05). Although the work has not concluded, in the establishments where were etiologic agents of zoonosis (E. granulosus and D. caninum) administrered the specific drug to the dogs and corresponding prophylactic measures were advised.

### A DEVICE FOR CONTINUOUS MEASUREMENT OF BIOLOGICAL FLUIDS REDOX POTENTIAL

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Redox potential is considered an important parameter to characterise a biological system. In spite of the great amount of information available, the measurement of this parameter in biological systems still presents some problems. Among these, we can mention the problems related to spatial heterogeneity of a biological natural system, where a number of factors reduce the reliability of such measurement (releases of gases, air contamination, liquid junction effects, the presence of sub-systems out of equilibrium). This study was conducted to develop a method for multiple continuous and stabilised redox potential measurements using Pt electrodes in chemically heterogeneous solutions or suspension. Eh evolution was assessed using a device made up of 4 acrylic microcosms (50 mm diameter, 20 mm hight) equipped with 3 10 mm Pt disks. The sample was connected to a reference electrode through salt bridges inserted into the cells. Each Pt disc and the reference electrode were connected to a data adquisition system with twelve analog channels. This system allows to register Eh values at chosen set times through a sequential measurement software. The use of developed microcosm allowed to obtain Eh values with low standard errors compared to the values obtained with conventional Ptelectrodes. There are two reasons to explain this fact: (i)the cell surface/Pt surface ratio is low; (ii) the solution-atmosphere system can stabilise rather quickly. Under this conditions, measured Eh values can clearly show electrochemical changes in complex biological systems as ruminal fluid, spermatic suspension and soil or sediment suspension.

### 107.

## HISTOLOGICAL AND HISTOCHEMICAL STUDY OF INTESTINE IN JUVENILE OF Odontesthes bonariensis

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In this study the tissue arrangement and the different cells that make up the intestine of *Odontesthes bonariensis* are determined. Samples from fifty specimens were taken, fixed in 10% formaldehyde and embedded in paraffin. Sections of 2 µm thickness were stained with histological and histochemical techniques. Results showed that the digestive tract had three tunicae: mucosa, muscular and serosa. The muscular lamina of mucosa was not observed. Histologically, intestinal mucosa showed large primary villi from which secondary ones branched off. Microscopically, these villi were made up of a pseudostratified epithelium with mucous cells and a central axis of lax connective tissue that derived from the lamina propria. In the centre of this structure a blood vessel was observed. The lamina propria did not show glands. The muscular layer had smooth muscular fibres in two strata: circular internal and longitudinal external. The serosa was typical. Based on the mucosa structure an anterior, transitional and posterior region were observed. In the former one primary and secondary villi were high and numerous occupying the lumen almost completely. The epithelial cells found on the lumen were column-like, had a brushy edge and were P.A.S. positive. Mucous cells showed a heterochromatin basal nucleus with large vacuole positive to Alcian blue in their apical portion. Rodlet cells with cytoplasmatic acidophilic granules, were found intercalated between epithelial cells. In the transitional region, the mucosa presented thinner primary villi and shorter secondary villi. In the posterior region no secondary villi were seen. The number of calceiform cells decreased considerably in this region.

#### 106.

### HISTOLOGICAL AND HISTOCHEMICAL STUDY OF APPENDED GLANDS OF THE DIGESTIVE TRACT IN JUVENILE PEJERREY (Odontesthes bonariensis)

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In this study, the histological structure of appended glands of the digestive tract in juvenile of *Odontesthes bonariensis* is determined. Samples from fifty specimens were taken, fixed in 10% formaldehyde and embedded in paraffin. Sections of 2 µm thickness were stained with HE, P.A.S., Orcein Acid-Giemsa, Prussian Blue Stain and Gallego Trichrome. Results showed that the liver presented a thin capsule of connective tissue covered with serosa. Hepatocytes appeared separated by capillaries and radially distributed from central veins. Liver cells had a polyhedral shape, a large nucleus centrally located, lax chromatin and an evident nucleolus. Its cytoplasm showed a perinuclear zone intensely basophilic, P.A.S. positive and a marginal region with lipid vacuoles. Among hepatocytes small ducts formed by a simple cubic epithelium were observed. Prussian Blue reaction did not show a high activity. The pancreas possessed two portions, an intrahepatic one and extrahepatic one. Both portions were represented by pancreatic acini. The intrahepatic portion was formed by acinic nodules. These acinic groups were distributed around a central blood vessel. The extrahepatic portion had a lobular distribution. Acinar cells showed a pyramidal shape. The nucleus, basally located, had lax chromatin and a prominent nucleolus. A large quantity of acidophilic and apically located granules of secretion were seen. The structures of appended glands of the digestive tract of Odontesthes bonariensis here described are in agreement with those described for other teleosts.

### **08.**

## NUMBER OF CORPORA LUTEA IN FEMALE MICE SELECTED FOR BODY WEIGHT

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From a *t* population of CF1 strain mice without selection, two pairs of divergent selection lines were founded for weight at 49 days of age (W), s and h: negatives; s' and h': positives. This directional selection modified the fertility coefficient (FC= 7x LS/(D+21), where LS is litter size and D the number of days from breeding to parturition), age at vaginal opening and number and size of ovarian follicles. With the aim of further studying the differences between lines, the averages of the number of corpora lutea (CL) produced per female were compared. The largest number of corpora lutea was found in line s', which was also the most heavy line. Line t had intermediate values between selected lines showing no significant differences with them. The associations between CL and the other variables were:  $r_{CL-TC}$ = 0.19 n.s.,  $r_{CL-CF}$ = 0.75 n.s. and  $r_{CL-W}$ =0.95 p<0.05. Weight selection seems to have differentiated positive lines from negative ones for the CL, LS and FC variables. Moreover, the average number of CL as well as the other fertility parameters compared vary significantly between positive selection lines, not being important the difference found between negative selection lines. Therefore, it was confirmed that directional weight selection has also modified the number of corpora lutea.

## CHARACTERIZATION OF CANINE VAGINAL CELLS AND DETERMINATION OF OESTRAL CYCLE PHASES WITH HEMATOXYLIN-EOSIN

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The exfoliative vaginal cytological technique is a simple and economic technique that allows observation of the oestrus cycle phases. Several staining techniques are mentioned in literature but no haematoxylin-eosin (H-E) reference can be found. In this study cells found in oestrus cycle phases were characterized using H-E staining. Swabs from the mid part of the vagina were sampled, dried in the open air and stained. A morphologic description was done of each cell and a mean and standard deviant of the largest and smallest diameters (LD and SD respectively) of the cytoplasm as well as the nuclear diameter (ND) were estimated. Phases were determined considering cells and the aggregate of neutrophils, erythrocytes, detritus and mucus. The following cells were seen:

a) Parabasal: small, round or oval, intensely basophilic and high cytoplasm-nucleus relation; b) Intermediate: of varying sizes, basophilic and acidophilic, of irregular margins and lower cytoplasm-nucleus relation; c) Superficial with nucleus: larger in size and acidophilic, abundant cytoplasm, folded margins. Small and pycnotic nucleus; d) Scales: superficial, anucleate and with maximum keratinisation; e) Metaoestral: intermediate cells with one or more neutrophils in their basophilic cytoplasm. H-E staining allowed a clear characterization of cell types and cycle phases. This, together with its simple protocol and low cost, is considered to make it a good complementary diagnostic method for veterinary clinic.

## 111. PRELIMINAR EVALUATION OF STRAIN RB51 BRUCELLA ABORTUS VACCINE IN PORCINE

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Porcine brucellosis causes abortions and infertility in porcine, along with Public Health alterations. Our objective was to evaluate the efficacy of RB51 vaccine in a naturally infected herd from a farm with 85% (102/120 females) of brucellosis prevalence (B. suis biovar I) and reproductive failures. The animals received 2ml of RB51 vaccine (Schering-Plough). Blood samples were obtained at 0, 6, 12, 24 and 36 months after vaccination and standard serologic test for brucellosis were developed. Also, the reproductive parameters were obtained. At 6, 12 and 24 months 100% of the animals, including those seropositive at day 0, were seronegative. At 36 months, on a total number of 80 pigs, only 5 (6,25%) were seropositive, corresponding to non-vaccinated gilts, daughters of vaccinated mothers. During the whole period, the number of piglets weaned per sow/delivery, increased from 5 to 8, and abortions decreased from 80% (96/120) to 5% (4/80) in the last year. Abortions occurred to 4 of the 5 seropositive non-vaccinated piglets, and B. suis biovar I was isolated of one of them. The infection source could be the boars which were not vaccinated, or the infected females which could have maintained a latent infection, a possibility that deserves further study. This results show that RB51 vaccine could be a good immunogen for utilization in infected herds.

#### 110.

### ORIGIN OF COMMON CAROTID AND RIGHT SUBCLA-VIAN ARTERIES IN THE CAT (Felis Domesticus)

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To describe the origin of these arteries, thirteen adult, male and female cats were used, they were from private clinic and their deaths were due to different causes which neither comprised thoracic organs nor cervical region. Firstly, common carotid artery was found in its cervical situation, then it was saline flushed and latex injected. The sample was refrigerated during 72 hours at 2 C. After that, the thoracic cavity was opened and arteries were dissected and photographed "in situ". Length, depth and distance between origins were measured. Schematic drawing were made and variations were analyzed. From these observations it was determined that left subclavian artery and brachiocephalic trunk arise separately from the aortic arch and that right and left common carotids and right subclavian arteries arise from the brachiocephalic trunk. It was found that (1) the brachiocephalic trunk gives three branches: right common carotid artery, left common carotid artery and right subclavian artery, (2) the brachiocephalic trunk divides into right subclavian artery and a short bicarotidean trunk which divides into the two common carotids and (3) the brachiocephalic trunk divides into left common carotid artery and a common trunk which after a short way divides into right subclavian artery and right common carotid artery. From 13 cases, 5 (38,46%) showed Model 1; 3 (23,08%) showed Model 2 and 5 (38,46%) showed Model 3. It is concluded that in cats the origin of these vessels may present three different behaviors that must be remembered.

### 112.

### STRUCTURAL AND HISTOCHEMICAL STUDY IN GILLS OF JUVENILE SILVERSIDE (Odontesthes bonariensis)

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The aim of the present study was to characterize the gills of juvenile of Odontesthes bonariensis structurally and histochemically. The gills of 50 fishes were dissected out, fixed in 10% formaldehyde solution and embedded in paraffin wax. Sections of 2 µm were stained with haematoxylin-eosin, P.A.S., alcian blue (pH 2,5), acid orcein-Giemsa and Jones methenamine. Branchial filament stroma was composed of hyaline cartilage surrounded by a dense connective tissue with prevalence of collagen and reticular fibres. Over this a plane stratified epithelium resting on a thick basal membrane rich in reticular fibres was observed. Chloride cells (CC), rodlet cells (RC) and mucous cells were observed in this epithelium. The latter cells were located in the most superficial strata, presenting a basal euchromatin nucleus and apical vesicles containing acid mucins. CC were located in the interlamellar space in 2-3 cell clusters. They showed a polygonal shape, presenting a round euchromatin nucleus and abundant weakly basophile cytoplasm. RC revealed an outlying nucleus and abundant P.A.S. positive cytoplasmic granules. The lamellae stroma was poorly developed, represented by scarce collagens and elastic fibres surrounding capillaries distributed parallel to the lamellae base. Those vascular spaces were defined by lateral pillar cell projections. These cells showed a small heterochromatin nucleus that occupied most of the basophile cytoplasm. Both sides of the capillaries were covered by a simple flat epithelium that would improve the efficiency of gas diffusion through the water-blood barrier. The biochemical composition and general structure of gill tissues in this species are similar to those described for other teleosts and mainly for fresh water fishes.

### ACTION OF AN ETHYLENE INHIBITOR ON SUNFLOWER IN VITRO ORGANOGENESIS

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Hyperhydricity is a physiological malformation that affects the quality of micropropagated plants. This phenomenon is associated with a high production of ethylene. The aim of this work was to analyse the effect of silver nitrate, an inhibitor of ethylene's action, on sunflower in vitro organogenesis. Cotyledonary explants from the inbred lines S74B and LxN621B were evaluated in vitro. Two culture media were formulated on MS saline base: M1=2 mg/l KIN + 1mg/l IAA; M2=M1 + 5,0  $\mu$ M Ag NO<sub>3</sub>. Cultures (N=130) were grown at 25  $\pm$  2 °C with an 12-hr photoperiod for 35 days. The traits analysed were regeneration percentage and percentage of hyperhydrated shoots. Data were analysed by the non-parametric Chi-square test and by the Sokal's G test. Both genotypes developed shoots and primordia showing that they have regeneration potential. Significant genotypic differences were observed on M1. Between media, significant differences were observed only for S74B inbred line. Non-significant 'genotype x media' interaction was detected by the Sokal's G test. The percentage of hyperhydrated shoots was lower (p < 0.01) on M2 for both genotypes. Even though this ethylene's inhibitor reduced the regeneration capacity of S74B inbred line, it was useful for improving the quality of the regenerated organs. As a conclusion, silver nitrate is efficient was controlling hyperhydricity on shoots and primordia regenerated from sunflower cotyledonary explants.

### 115.

## THE EFFECT OF CLUSTER LOCATION WITHIN PLANT AND FRUIT POSITION WITHIN CLUSTER ON TOMATO FRUIT QUALITY TRAITS

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Weight and shelf life are traits that define the commercial quality of tomato fruits (Lycopersicon esculentum Mill.) and they are particularly dependant on the genotype. The crop canopy determines different environmental conditions (as light incidence) in the different clusters of plants and in the fruits of these clusters and could influence these quality traits. The aim of this work was to evaluate the effect of these locations for weight and the shelf life in five genotypes. The genotypes were lines Caimanta (C) and Nor (N) of L. esculentum var. esculentum, accession LA 1385 of L. esculentum var. cerasiforme (Ce) and the hybrids between C and N (F<sub>1</sub> CxN), and N and Ce (F<sub>1</sub> NxCe). Weight (W, in g) and shelf-life (SI, in days) were evaluated at either the first, the second and the third clusters of each plant, discriminating according to the fruit position on each cluster (basal, middle and apical). The experimental design was a nested ANOVA with three source of variation, considering fixed effects (genotype, cluster within genotype and fruit within cluster). Highly significant differences (p <0,01) were found among the genotypes for W (F = 334,2) and SI (F = 18,3). No significant differences were found for W (F = 1,3) while significant differences were found for SI (F = 2,1; p < 0,05) in respect to the position of cluster within in plant. The effect of fruit position within cluster was not significant for SI (F = 1,1), but it was highly significant for W (F = 3.1; p < 0.01). These results suggest that it is possible to obtain a uniform fruit ripening from the same cluster in spite of the different fruit weights.

#### 114.

### CHARACTERIZATION OF THE IN VITRO RESPONSE IN SUNFLOWER BY PROTEIN PATTERN

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The successive steps in the regeneration process are coincident with events on the molecular and biochemical levels. The aim of this work was to analyse changes in the protein profiles of sunflower explants cultured on two culture media that induced the formation of either shoots or callus (media M1 and MA respectively). Cotyledons from the Ñ-834 inbred line were used as explants. Three regions were identificated from the axis embryo area to the distal one: proximal (p), central (c) and distal (d). The M1 consisted in Murashige and Skoog, 1962 (MS) salts, 1 mgl<sup>-1</sup> IAA, 2 mgl<sup>-1</sup> KIN while MA consisted in MS + 1 mg/l 2,4-D. Cultures (N=252) were grown at  $25 \pm 2^{\circ}$ C with an 12-hr photoperiod for 28 days. Proteins were extracted from cotyledon samples cultivated for 0, 2, 4, 7, 14, 21 and 28 days and separated by SDS-PAGE. All explants cultivated on MA developed friable callus (%C=100%). Regeneration was observed in cotyledons on M1, however its amount varied according to the region. A significant decreasing morphogenic gradient was observed from proximal to distal cotyledonal regions (94, 77 and 10% for P, C and D respectively). It was found that a 28-kDa protein is accumulated on cotyledons cultivated on M1 after 7 days of incubation. This protein was not observed on MA culture condition. This results suggest that this 28-kDa protein could be used as potential marker for shoot organogenesis in this species.

### 116.

## FIRST REPORT OF TWO SPECIES OF Colletotrichum ASSOCIATED TO Commelina erecta L.

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The productive model based on no tillage, transgenic soybean growing, and the intensive glyfosate employment caused the increase of *Commelina erecta* population. During 2000 in Marcos Juárez (Córdoba Province), the incidence of plants showing necrotic foliar blotches with chlorotic margin was 70%; in Zavalla (Santa Fe Province) the incidence in leaves was  $63 \pm 4\%$ . The objects of study were: i) to identify causal agent of foliar blotches observed, ii) to determine the weed-microorganism interaction, and iii) to infer the epidemiological role of *C. erecta* in the cycle of soybean pathogens. Fungi were isolated on PDA 2% from the infected tissue previously disinfected with sodium hypoclorite 1%. Two species were identified based on the morphology of colonies, reproductive structures, and the size of 40 conidia each one: a) Colletotrichum truncatum with unicellular curved conidia of  $27 \pm 1.9 \,\mu m$  of length and  $4 \pm 0.5 \,\mu m$  of width, b) C. gloeosporioides with unicellular straight conidia of  $16.5 \pm 1.2 \mu m$  of length and  $4.8 \pm 0.6 \mu m$  of width. Both species were separately inoculated in the foliar sheaths and under of stem nodes of Commelina plants. Control plants were similarly processed but without mycelium. Both Colletotrichum species caused disease on *C. erecta*, and were re-isolated from the infected plants showing blight stems and leaves but not the typical blotches. C. truncatum and C. gloeosporioides are also pathogens associated to the end of soybean growing cycle, therefore it is necessary to determine if Commelina erecta would act as alternative host of these pathogens.

### PATHOGENICITY EVALUATION OF Fusarium graminearum ON A SOYBEAN COMMERCIAL VARIETY

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Fusarium graminearum is an important pathogen of wheat and corn. In soybean, the fungus was mentioned as a saprophytic microorganism colonizing fruits and seeds. Previous reports of artificial inoculations showed negative pathogenicity. The object of study was to evaluate if two F. graminearum isolates are capable of establish a nutritional association and develop symptoms on soybean The isolates were obtained from symptomatic soybean plants of A-4910 variety and inbred line seeds of U.S.A. Seedlings of the A-4910 variety were inoculated separately with both isolates, at V1 to V2 stages. Inoculations were done by including a mycelium piece into a small wound caused in hypocotyl with a scalpel. The experiment was a Completely Randomized Design with not balanced three treatments (neck mycelium, seeds mycelium and control without mycelium). A seven levels scale (0=without symptoms and 7=plant death) was used to evaluate the symptoms, and the number of plants/category was registered at four observation times. Re-isolation of the fungal mycelium was done on PDA 2% from the infected tissues but not from the control plants. Results were analyzed with the Kruskal-Wallis non parametric analysis. This study, conducted as described, showed that F. graminearum acted as a pathogenic organism in the evaluated genotype by reproducing the disease. Significant differences were detected between isolates and observation times. In this host-pathogen interaction, biochemical defensive mechanisms were not effective to stop the fungal development. The effectiveness of cultural control practices based on Maize, Soybean and Wheat-Soybean crop rotation should be re-evaluated.

## MICROBIAL POPULATIONS IN Bt AND NON Bt CORN RHIZOSPHERE

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The present work was make in order to study the effect of the endotoxin of transgenic corn on microbial populations. The rizospheric effect was estimated in two cultivars of Bt corn (Chalten TD event 176), Dekalb 696 MG (event MON810) and its not transgenics (Ch. and Dk. 696). Seeds were sown in pots with soil of a corn field. Samples were taken of the rizospheric soil to 7, 14 and 21 days after the sowing (plants with 2, 3, and 4 leaves). On each sample aerobic heterotrophic bacteria, pseudomonads and soil fungi were quantificated using solid media. The culture media employed were Plate Count Agar, King B Medium and Potato Dextrose Agar for each group of microorganisms. The results were expressed as CFU. g-1 dry soil. The rhizosphere effect was positive for heterotrophic bacteria and pseudomonads in all cases, and for fungi in 14 and 21 days soil samples. Results indicated that, when comparing heterotrophic bacteria and pseudomonads, differences between Chalten and Chalten TD were significative in 14 and 21 days samples, but Chalten showed greater values. Also differences between Dekalb 696 and Dekalb 696MG were significative, but, in this case, greater values were shown by Dekalb 696MG. When considering fungi, Chalten showed greater numbers of CFU xg-1 soil than Chalten TD, but for Dekalb hybrids, the transgenic one showed greater values The results obtained let us conclude that rhizospheric soil estimulates the microbial populations considered, indicating that they would not be affected by the exudates of transgenic plants roots, but the performance of the two transgenic hybrids is different probably owed to the different structure of the introduced gene.

#### 118.

### EVALUATION OF CHEMICAL TREATMENTS TOWARDS WALNUT BLIGHT

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Walnut Blight, a common disease all over the world is caused by Xanthomonas campestris pv. Juglandis. Growing and herbaceous organs are attacked. Rain, wind and cultural practices help in the bacterial dispersion. The easier alternatives for the control of this disease are varietal resistance and the use of chemical products. The purpose of the present work was to evaluate different varieties and different preventive chemical treatments to control Walnut Blight. The assay took place in Zavalla, Santa Fe, during 2001. The used varieties were Chandler, Davis, Tulare and Franquette, grafted on Juglans nigra L, as rootstock. A completely aleatory with factorial arrange experimental design was used. Dates were analyzed by Proc. GLM SAS 6.12. The disease severity was evaluated by affected foliar area/ total foliar area x 100. Three treatments were evaluated: 1-T1 Pentahidrated cupper sulfate (Phyton) in doses of 100 cm<sup>3</sup> of p.c. to each 100 l. of water. 2-T2 Kasugamicina (Kasumin) in doses of 250 cm<sup>3</sup> of p.c. to each 100 l. of water 3-T3 a blank. The products were applied in the phenological stages of: 1-resting buds, 2-blowing buds, 3-flowering, 4-filled up fruits. The statistical analysis showed high significant differences (p <0.01) between varieties, being Davis, Tulare and Franquette the best ones, with severity of 8%, while Chandler showed severity of 26%. Duncan test showed Phyton and Kasumin with severity of 12%, in comparison to the blank, with 35% of severity. As conclusion, the use of good performance varieties and preventive chemical treatments is the best alternative to control this disease.

### 120.

## EFFECTS OF SALINITY ON GERMINATION IN SCHINOPSIS BALANSAE (ENGL)

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"Quebracho Colorado chaqueño" is an heliofil specie, and its seeds start to mature on the third week of February. Germination tests submitting seeds to different CINa concentrations, where made to explain which are the causes that affect germination and the establishment of seedling of this specie. The place where recollection took place was in Las Gamas, 15 km from the city of Vera (Santa Fe, Argentina). Four treatments with different salinity concentration where made: 0.2, 0.3, 0.4 and 0.6M, and bidistillated water was used for the tester. Then they were putted in incubation chambers at 25°C with a photoperiod of 12 hours. The germination speed index (GSI) was determined and the average germination time (AGT). The abnormal number of plants was determined, considering the roots and the cotyledons. The results show significant differences in germination, IVG and TMG between the treatments. In the germination the percent varied from 45% in the tester to 0% in the 0.4 and 0.6 M concentrations, the IVG and TMG diminished considerably when the concentrations increased. On major CINa concentrations the seedling showed thinner roots, opaque and dark, with less o even no radical capilars. A 2,22% of abnormal seedling was registered in the tester, 4% in the  $0.2\mbox{M}$ treatment and 22,2% in the 0.3M. This results could show that the salinity not only limits the germination in S. balansae, but also affects the seedlings.

## DIFFERENCES BETWEEN MAGRARIO AND HAMPSHIRE DOWN LAMBS FOR PUBERTY WEIGHT AND LONGISSIMUS DORSI FAT DEPOTS

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Ovine meat production of good quality could be an interesting alternative for the small farmers. *Magrario* is a new ovine genotype that was obtained through several backcrosses from the Ideal Breed to Texel Breed and their lambs have lean carcass even in feed lot conditions. After weaning (3 months old) Magrario lambs (M) were compared with Hampshire Down lambs (HD), the most frequent meat breed in the area. These lambs were reared during two months in two different environments: Feed Lot Conditions (FLC) and Range Conditions (RC). After this period, the final weight (FW) was registered and four ultrasonic measurements (cm) were obtained from the Longissimus dorsi: SF (Subcutaneous Fat), FP (Perimuscular Fat), DLD (Deep Longissimus dimension) and WLD (Width Longissimus Dimension). No significant differences were found between genotypes in any environmental conditions for FW, but M and HD lambs had significant differences for SF (M=0.47  $\pm$  0.02 and HD  $=0.83 \pm 0.10$ ; p<0.05) and FP (M=0.23  $\pm 0.02$  and HD=0.33  $\pm 0.01$ ; p<0.01). The broad sense Heritability (H) was also estimated in FLC and RC. A significant H was only found in the FLC for SF and FP (H=0.70; p<0.05 and H=0.50; p<0.01 respectively). Apparently the M and HD carcass differences can only be detected in good environmental conditions. Phenotypic correlation's (r) between FW and SF was also evaluated in each genotype through the two environmental conditions. In HD, r was 0.88 (R<sup>2</sup>=0.77; p<0.01) and in M was 0.28 (p>0.05). These results suggest that the increase of puberty weight in the HD is depending of an increase in the fat level.

### 123.

## PRELIMINARY STUDY OF THE *URTICACEAE* IN THE SANTA FE PROVINCE (ARGENTINA)

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The family *Urticaceae* L. is represented in Santa Fe by three tribes: *Urereae, Parietarieae* y *Boehmereae*, all comprising annual or biannual herbs. The aim of this contribution is to communicate the taxonomic study and the geographic and ecological distribution of this family in our province.

Most species were observed and collected in their natural environment. A deep bibliographic revision and visits to Rosario, Esperanza and Darwinion Herbaria were carried out. Keys and distribution map are provided.

This family is represented by four genera and seven species. In the genus *Urtica* they are: *U. spatulata* Sm., known as "ortiga brava", "ortiga crespa"; *U. urens* L. "ortiga", both widely distributed, and the more restricted *U. circularis* (Hicken) Sorarú. In the genus *Urera* there appears only one species: *U. aurantiaca* Wedd., occurring in riparian forests in the river Paraná valley. Both the previous genera present stingy trichomes. In the genus *Parietaria* the species are *P. officinalis* L. and *P. debilis* G. Forst. The genus *Boehmeria* shows only *B. cylindrica* (L.) Sw. Both these two last genera lack stingy trichomes.

#### 122.

### POLLEN MORPHOLOGY IN THE GENUS JUGLANS (JUGLANDACEAE) IN ZAVALLA, SANTA FE PROVINCE

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In our country the genus *Juglans* is represented by *J. australis* Gris., autochthonous in the Tucumano-Oranense Forest. In the Villarino Experimental Field, Rosario University, other species of the same genus are cultivated. The purpose of this work is to study the pollen morphology of three species of the genus *Juglans* occurring in this field in Zavalla: *Juglans nigra*, *Juglans regia* and an undetermined *Juglans* sp. Fresh male inflorescences were collected, and their pollen was subjected to acetolysis with conventional techniques, and later studied with LM and SEM. The *Juglans* pollen presents characteristics with taxonomical value both at the genus and species level.

Observed with LM, the pollen of the genus *Juglans* presents the following characteristics: surfaces psilate-scabrate, tectate-perforate or microechinate without any verrucae. Each porus with a differentiated area around it. This area may have either sexine or nexine thicker or thinner than the rest of the mesoporium. Pori usually circular with edges well-defined, perhaps with a ring or band. Exine tectate-perforate, with or without microechinae. Pori never surrounded by well defined, irregular intectae-granulate zones. Margins of pori slightly protruding in optical section of the grain, tectum with regularly spaced microechinae or scabrae. Nexine fragmented or dissolved in a broad zone around each porus. Columellae distinct, uniform and fine. Grain with 6-20 pori, sometimes aggregated towards one part of the grain.

With SEM differences in pori and pollen grain sizes appear. Pollen sizes: *J. regia*: 325  $\mu$ , *J. nigra* 350  $\mu$ , *J. sp*, 375  $\mu$ .

### 124.

## FLORA OF THE SANTA FE PROVINCE (ARGENTINA): PRELIMINARY REPORT OF THE GERANIACEAE

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The *Geraniaceae* family comprises five genera and 700 species throughout the world, though scarce in the tropics. The object of the present contribution is to study this family for the Santa Fe province of Argentina.

Both in this country and in the province the family is represented by only two genera: *Erodium* L'Herit. and *Geranium* L.. They consist of annual or perennial pubescent herbs, with peciolate alternate leaves subtended by stipules. Flowers perfect, persistent calyx with five sepals; corolla pentamerous; stamens 10 in two cycles; superior gynoecium, pentalocular, with five concrescent styles. Fruit schizocarp with a conspicuous rostrum, later dividing into five uniseminate mericarps.

Most species were observed in their natural environment and collected. A deep bibliographic revision and visits to Rosario, Esperanza and Darwinion Herbaria were carried out. Keys to the genera and species are presented.

Six species occur in the Santa Fe province: *Erodium cicutarium* (L.) L'Herit.; *E. malacoides* (L.) L'Herit.; *Geranium albicans* St. Hil.; *G. carolinianum* L.; *G. dissectum* L. and *G. molle* L. Occasionally *Pelargonium* X *hortorum* Bailey has been observed as escaped from cultivation in certain urban areas.

### 125. PRELIMINARY STUDY OF THE ACANTHACEAE FAMILY

## IN SANTA FE PROVINCE (ARGENTINA) McCargo JC, Oakley LJ, García RCA, and Prado DE.

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The *Acanthaceae* in Argentina are represented by 17 genera and 73 species, 13 of which are endemic. So far no study of the family for the Santa Fe province has been performed. Some of the species have been observed in their habitat, but the majority were studied as conserved specimens in the herbaria UNR (Rosario), SF (Esperanza), SI (Darwinion, San Isidro), together with a thorough bibliographic revision. The *Acanthaceae* present simple opposite leaves, without stipules, solitary flowers or in racemes, spikes or cyme of racemes, with large floral bracts. Calyx pentalobate, large corolla usually brightly colored, gamopetalous, pentalobate, frequently bilabiate.

Seven genera of Acanthaceae occur in Santa Fe: Carlowrightia A Gray, Dicliptera Juss., Dyschoriste Nees, Hygrophila R. Br., Justicia L., Ruellia L., Stenandrium Nees. These comprise 18 species: Carlowrightia sulcata (Nees) C. Ezcurra, Dicliptera tweediana Nees, D. niederleiniana Lindau, Dyschoriste humilis Lindau, H. guianensis Nees, Justicia axillaris (Nees) Lindau, J. comata (L.) Lam., J. goudotii V.A.W. Graham, J. laevilinguis (Nees) Lindau, J. tweediana (Nees) Griseb., Ruellia brevifolia (Pohl) C. Ezcurra, R. coerulea Morong, R. erythropus (Nees) Lindau, R. geminiflora Kunth, R. hygrophila Mart., R. sanguinea Griseb., Stenandrium diphyllum Nees, S. dulce (Cav.) Nees. They are distributed in nine departments of the province, particularly to the center and north: Gral Obligado, Iriondo, La Capital, Nueve de Julio, Rosario, San Cristóbal, San Javier, San Jerónimo, y Vera.

### 127. SOYBEAN: OPPORTUNITY OF GROWING MATURITY GROUP IV CULTIVARS IN LATE PLANTED DATES

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To respond to local demands, a field trial was carried out to evaluate the opportunity of growing maturity group (MG) IV cultivars (cv) in a double cropping, no tillage, system after wheat on a Vertic Argiudoll soil. Seeds of A4902RG (MG IV), A5634RG (.MG V) and A6445RG (MG VI) were sown on 2 December 2001, at 0.38 m row width in Zavalla (33° 01' S, 60° 53' W). Final density was 32 plants.m<sup>-2</sup>. Phenological stages were recorded. Three samples of 1 m2 per plot were harvested and dry matter (DM), harvest index (HI), grain yield (Yld) and its components: seed number.m<sup>-2</sup> (SN) and seed weight (SW), were determined at maturity. The experimental design was a randomized complete block with three replications. Data were subjected to analysis of variance and means were compared by Duncan's Test (P≤0.05). A4902RG and A6445RG had the highest Yld (2400 kg.ha-1, both cv). A4902RG had shorter vegetative and reproductive periods and lower SN than A6445RG; but SN was compensated by greater SW. A5634RG had a similar length of phenological stages than A6445RG. Spatial plant arrangement allowed 95% light interception at flowering in all cv. Canopy growth was adequate for A4902RG but it was excessive in A5634RG and A6445RG and lodging was produced. Because of this stress, A5634RG had the lowest SN and SW. There were no differences in DM and HI. Our data indicate that MG IV cv, with an adequate plant arrangement, are an interesting alternative to optimize grain yield in time-constrained systems to avoid early fall frost and high humidity damage at harvest.

#### 126.

## CONTAMINATION OF THE WORKER APPLYING PHYTOSANITARY PRODUCTS TO HORTICULTURAL CROPS

Panelo MS, Bulacio LG (ex-aequo), Giuliani SL, Lenardón AM, Giolito I. Cátedras Sistemas de Producción Hortícola y Terapéutica Vegetal. Facultad de Ciencias Agrarias, Consejo de Investigaciones UNR.

The objective of the work was to determine the areas of the worker's body that receive product when performing leaf treatments with hand operated equipment on horticultural crops of different heights. The work was done on crops of Swiss chard (plantation frame 0,70 x 0,30 m, double row/ridge; average crop height 0,60 m) and French beans (plantation frame 1,40 x 0,70 m, paired lines, average crop height 2,10 m). White cotton cloth patches (ten by ten centimeters each) were located over the body of the worker. A manual sprayer with a single 8002 flat fan nozzle was used, replacing the phytosanitary products for a phenolphthalein solution (0,5 g/l). Four applications were performed on each crop, along 100 m between the rows of plants. Later at the laboratory 10 cm<sup>2</sup> pieces of cloth were cut from each patch, the colouring stuck was recovered by means of a solution of (NAOH) 0,1N and was read in a spectrophotometer at 540 nm. Results were analysed on a completely randomized design, with four repetitions, and they showed that the whole body was exposed at the time of the applications. The treatment on Swiss chard left 1057,90 µg/cm<sup>2</sup> of colouring over the patches on the worker's body: 2% of the residue on the head, 10,58% on trunk and abdomen, 16,35% and 71,07% on upper and lower limbs, respectively. The treatment on French beans left 370,80 µg/cm<sup>2</sup> of phenolphthalein: 20,24% on the head, 16,30% on trunk and abdomen, 37,30% on upper limbs and 26,16% on lower limbs.

### 128.

### QUALITY AND DRY MATTER YIELD OF TWO ALFALFA CULTIVARS WITH DIFFERENT WINTER REST

Verdura L, Cechetti S, Acebal MA, Calvo FP, Spiller L<sup>1</sup>, Wirsch G and Figallo RM<sup>2</sup>.

Nutrición Animal, <sup>1</sup>Forajes, Cs.Agrarias y <sup>2</sup>Química Biológica, Cs. Veterinarias. U.N.Rosario.

Alfalfa (*Medicago sativa* L.) is an essential resource for animal production systems. This study was conducted to evaluate dry matter (DM) yield and morphologic and nutritional characteristics of two alfalfa cultivars (cv) with different degree of winter rest (latency degree: LD). On samples of cv P.5683 (LD 6) and Siriver (LD 8) in vegetative stage were measured: plants/m2 (PLT), crop hight (H, cm) and leave/stem relation (L/S as DM, in a subsample of 30 stems); and percent of DM, crude protein (CP%) and neutral detergent fiber (NDF%) were determined. DM yield (kg DM /ha) was calculated. Data were subjeted to Analysis of Variance.

Var	LD 6 $x \pm SE$	LD 8 $x \pm SE$	p
PLT /m2	$30.00 \pm 4.08$	57.00 ± 5.00	0.005
H cm	24.61 ± 0.68	$34.20 \pm 0.65$	0.000
L/S Rel	$1.69 \pm 0.16$	$1.17 \pm 0.07$	0.024
DM %	19.40 ± 1.71	$20.10 \pm 0.72$	0.718
CP %	15.95 ± 1.30	$13.79 \pm 0.26$	0.150
NDF %	$28.51 \pm 0.86$	$30.64 \pm 0.64$	0.094
DM /ha	1401.2 ± 285.7	1711.0 ± 112.9	0.350

Although differences were not significant, in LD 8 the CP% was minor and NDF% greater possibly dued to the smaller relation L/S in cv with short latency. Same reason would explain the similar dry matter yield by hectare although in cv with intermediate latency (LD 6) the stand of plants and the crop hight were minor. The different degree of winter rest did not affect the nutritional quality nor the production of the cv studied, although it modified some important morphologic characteristics for animal production.

## 129. EFFECT OF SALINITY ON GERMINATION IN *Prosopis nigra* (GRISEB.)

López D and Carnevale N. Cátedra de Ecología. Fac. de Cs. Agrarias. U.N.R.-CIUNR.

Prosopis nigra (algarrobo) is one of the 29 species on this gene amply spread in the center or the country, and occupies the lower spaces with high flood probably in the woods. Some germination studies were made on the gene Prosopis, but not on this specie. To obtain more information about the conditions that let it's installation in the quebrachal, the percentage of germination of this specie under different CINa concentrations was considered. The fruits were harvested on their maturity before their falling from the trees on May 2002. The harvest took place in Las Gamas, 15 Km from Vera City (Santa Fe, Argentina). The seeds were separated from the legume and four treatments with different saline concentrations: 0.2, 0.3, 0.4 and 0.6 M, and the tester. Four repetitions of 25 seeds each one were made on each treatment. They were putted on plastic trays of 10cm deep using sand as substrate with each solution; and for the tester bidestillate water was used. They were placed in incubator of 25° and 12 hours photoperiod. The percentage of germinated seeds, the germination speed rate (GSI) and the average germination time (AGT) were determined. The abnormal seedlings percentage produced from their emergence over the total germinated seeds was determined. The results on germination and AGT showed significant differences between all treatments (p<0.05), but not on the GSI values. The major germination percentage was on tester and there was no germination in concentrations 0.4 and 0.6 M. The abnormal seedlings considered so because of the absence or roots or with anomaly  $\bar{\text{in}}$  cotyledones, represented a 3.9% . In the 0.3 M treatment seedlings with enlarged hypocotyl and thinner were detected, an in tester the seedlings were normal. Although there are other Prosopis species with tolerance to salinity (P. chilensis and P. flexuosa) this isn't confirmed for P. nigra. Although the soils where this specie grows are characterized for the presence of salt the germination of P. nigra shows limits with the major concentrations of CINa used here.

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