ISSN 0327 - 9545 PRINTED IN ARGENTINA



# **ROSARIO BIOLOGY SOCIETY**

# (SOCIEDAD DE BIOLOGÍA DE ROSARIO)

Abstracts from the

# XII CONGRESS – XXX ANNUAL MEETING

December 2-3, 2010

Facultad de Ciencias Médicas Universidad Nacional de Rosario Rosario, Santa Fe, ARGENTINA

Abstracts were revised by a Scientific CommitteeBoard of the Rosario Society Biology 2008

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## EFFECT OF PROANTOCYANIDIN-ENRICHED EX-TRACTS OF *Ligaria cuneifolia (Lc)* ON ERYTHROCYTE SHAPE AND OSMOTIC RESISTANCE IN RATS FED DIETS NORMO AND HYPERCHOLESTEROLEMIC

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The Lc methanolic fraction via ip, increase the osmotic resistance and produced stomatocytosis. Objective: To analyze the effect of proantocyanidine-enriched extracts of Lc on erythrocyte shape and osmotic resistance in rats fed diets normo and hypercholeste rolemic. Methods & Results: Male adult Wistar rats (n=24), 70 days old, were divided into: 1. normocholesterolemic standard diet (SD) 2. Standard diet supplemented with cholesterol (97% purity) 8g/kg diet and corn oil 28% (w/w), hypercholesterolemic (HD). Rats were used as controls (C) (n = 6 SD and 6 HD) injected ip with saline and treated (T) (n = 6 SD and 6 HD) injected ip PLc 3 mg/100g bw every 24 hours for 3 days. On the fourth day the rats were anesthetized, blood obtained by cardiac puncture, determining: Morphological index (MI, microscopy), MI: CSD: -1.53  $\pm$  0.27, TSD: - $2.149 \pm 0.13$  (ns); CHD:  $-2.45 \pm 0.07$  \*\*; THD:  $-2.18 \pm 0.11$  (ns) (\*\*p <0.001 vs. CSD). Osmotic fragility: erythocytes, using the parameters: X50 ([NaCl], which produces 50% hemolysis) and  $\beta$ (homogeneity of the population). X50: CSD:  $0.51 \pm 0.04$ , TSD: 0.55 $\pm$  0.05 (ns), CHD, 0.52  $\pm$  0.04, THD: 0.49  $\pm$  0.02 (ns),  $\beta$ : CSD:  $10.75 \pm 0.84$ , TSD:  $9.50 \pm 0.63$  (ns); CHD:  $9.57 \pm 0.58$ , THD: 9.48 $\pm 0.63$  (ns). Conclusion: The intake of diets rich in cholesterol leads to increased blood lipids that induce a discocyte-to-stomatocyte change. It was possible to obtain a fraction of Lc which produces a decrease of lipid without altering the hemorheological parameters.

#### 2.

# *IN VITRO* EFFECT OF DIFFERENT METHOTREXATE [MTX] CONCENTRATIONS ON ERYTHROCYTE OSMOTIC RESISTANCE

Martino M<sup>1</sup>, Urli L<sup>1</sup>, Svetaz MJ<sup>2</sup>, Luquita A<sup>1</sup>, Rasia M<sup>1</sup>.

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Methotrexate [MTX] is an antimetabolite drug used in rheumatoid arthritis treatment in seric concentrations between 0,1 to 2  $\mu$ M. One of its main adverse effects is anaemia. Previously we have found that MTX produces stomatocytosis and impairs erythrocyte deformability. Objective: demonstrate in vitro effect of MTX on erythrocyte osmotic fragility. Methods: Normal blood samples were fractioned in five aliquots, one being the control (C, n=5) and the other were incubated with growing MTX concentrations (0,5; 0,75; 1,5  $\mu$ M) (n=5) during 120 min at 37°C. In which was determining the osmotic fragility (measured photometrically at 540 nm), obtaining a X<sub>50</sub> value (NaCl mM concentration yielding 50% hemolysis) and a  $\beta$  (degree of response homogeneity). Results (mean ±SD): X50: C:  $0,64 \pm 0,04$ ; Mtx0,5:  $0,66 \pm 0,03$ ; Mtx0,75:  $0,67 \pm 0,02$ ; Mtx1: 0,70  $\pm$  0,03 \*; Mtx5: 0,71  $\pm$  0,03\*\*.  $\beta$  values: C: 4,97  $\pm$ 0,02; Mtx0,5: 4,87  $\pm$  0,02; Mtx0,75: 4,89  $\pm$  0,01; Mtx1: 3,35  $\pm$  $0.01^{**}$ ; Mtx5: 2.88 ± 0.05<sup>\*\*</sup>. MCV values (µm3): C: 90.52 ± 0.5; Mtx0,5:  $89,70 \pm 0,35$ ; Mtx0,75:  $89,68 \pm 0,35$ ; Mtx1:  $89,48 \pm 0,37^*$ ; Mtx5: 88,22 ± 0,93 \*. (\*p<0,05; \*\*p<0,01 respect to C). Conclusion: presence of Mtx in incubation medium increased the osmotic fragility (higher X50) as well as heterogeneity of the population response to hyposmolarity in the 1 and 5 µM doses. Besides, erythrocyte VCM decreased. This effects, added to stomatocytosis and impaired deformability, can be related to the anaemia found in patients treated with this drug.

3.

#### ERYTHROCYTE SHAPE AND OSMOTIC FRAGILITY ASSESSMENT OF RED BLOOD CELLS OF THE IIMb LINE OF RATS OF 300-DAY-OLD

Ruiz MF<sup>2</sup>, Casco C<sup>2</sup>, Visconti M<sup>1</sup>, Gayol M del C<sup>2</sup>, Bollini A<sup>1</sup>, Hernández G<sup>1</sup>, Bazzoni G<sup>1</sup>.

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In 2009, our studies showed that red blood cells (RBC) of 200-dayold IIMb (b) line of rats are significantly more resistant to osmotic changes than those from the beta line. In this study we investigate the osmotic fragility (OF) and the erythrocyte shape (ES) of RBC suspensions of the b and beta line of 300-day- old rats and compare them with the results obtained at the age of 200 day. Males of 300 day from the b and beta lines (n=8) were used. The ES was determined by microscopy and OF by photometry, reporting: X<sub>50</sub> (concentration of NaCl obtaining 50% of hemolysis) and  $\beta$  (cell population response). Statistical studies: U-Mann-Whitney test. The results were expressed as mean $\pm$ SEM. Differences significative if p<0,05. <u>Results</u>: OF: b line: X<sub>50</sub> (mM):74,34±0,96\*\*; β: 0,107±0,009\*\*\* beta line: X<sub>50</sub> (mM):87,04±1,63\*\*; β:0,089±0,005\*\*\*. \*\*p<0,0005 \*\*\*p<0,025. ES: stomatocytes 3 and 4. Conclusion: RBC from the b line are significantly more resistant to osmotic changes (lower  $X_{so}$ ), with a more homogeneous response (higher  $\beta$ ). When comparing it to 200-day-old rats, we observed that the b line does not significantly change its  $X_{50}$  (74,13±1,10) with age but their response is more homogeneous ( $\beta$ : 0,0085±0,002). Regarding the beta line, the OF increase and the homogeneity of its response decrease with age. In both lines, stomatocytes increased with age. The metabolic disorder in beta line influences the osmotic behavior of RBC. The RBC of the b line keeps the same behavior to the osmotic changes.

4.

# STUDY OF ERITROCYTE DEFORMABILITY IN 300 DAYS RATS OF THE 11Mb STRAIN

Visconti M<sup>1</sup>, Ruiz MF<sup>2</sup>, Casco C<sup>2</sup>, Gayol M del C<sup>2</sup>, Bollini A<sup>1</sup>, Bazzoni G<sup>1</sup>, Hernández G<sup>1</sup>.

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After the extinction of the á line, and therefore in 2001, the incorporation of a new eumetabolic line as a witness, the IIMB line (b) developed phenotypic instability evolve the behavior of IIM $\beta$  ( $\beta$ , obese and diabetic). In this paper we will study erythrocyte deformability (ED), hemoglobin concentration (Hb) and hematocrit (Hct) in 300 days animals in b and  $\beta$  line, and analyze the probable breeding behavior exhibited by the  $\beta$  model (n = 7). We determine: a) Hb spectrophotometrically; b) Hct by microcentrifugation, and c) rigidity index: (RI) (high RI low ED) by filtration through pores of 5µm. Also we estimate the middle corpuscular hemoglobin concentration (MCHbC): Hb/Ht. Results: median and range. Statistical analysis: nonparametric Mann-Wihtney. Results: RI (%): β: 12.87 (11.4-15.9) b: 10.4 (6.3-16) ns; Het (%): β: 36 (27-38) b: 42.5 (37.5-43.5) \*; Hb (g / dl): β: 11.04 (9.15-13.25) b: 13.59 (12.5-14.77) \*; CHbCM (g / dl): β: 32.5 (29.66-34.87) b: 33 (29-35) ns. n.s.: not significant, \*: p = 0.000. Our results show no significant differences in the ED and CHbCM, there was significant the increase in Hb and Hct. The latter two variables increased in parallel. We conclude, therefore, that ED did not differ between lines because of the similarity in the CHbCM (a determining factor of the internal viscosity of erythrocytes and thus ED). On the other hand, it is important to note that hemorheological differences were observed in animals of 200 days began to disappear with increasing age of the same. Thus both models, at least in the variables studied in this work, become very similar to the age of 300 days.

# ERYTHROCYTE AGGREGATION STUDY IN RATS OF 300 DAYS OF THE LINE IIMb

Casco C<sup>2</sup>, Visconti M<sup>1</sup>, Ruiz MF<sup>2</sup>, Gayol M del C<sup>2</sup>, Hernández G<sup>1</sup>, Bazzoni G<sup>1</sup>, Bollini A<sup>1</sup>.

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Introduction: In previous work we compared the hemorheological behavior in 200 days animals of line "b" and line IIMFm /  $\beta$  ( $\beta$ ) rat model of Obesity and Diabetes. The values for both variables- ervthrocyte aggregation (EA) and deformability (ED)- in line b were lower than  $\beta$ , had been away from the line alpha eumetabólic. Objetive: compare the behavior of these two hemorheological variables in animals of 300 days. Methodology: We used male rats, line b and  $\beta$  of 300 days (n = 7). The EA was determined by optical method in suspensions of red blood cells (RBs) Dextran 500 2% in saline (Htc: 40%) We determined:  $s_0/n_0$  (estimating the size of the aggregates) and 2k<sub>2</sub>n<sub>0</sub> (estimating the initial rate). Was measured Rigidity index (RI) (RI means more lower ED): RBs suspended in saline-albumin were filtered using filters with pores of 5µm. EA results were analyzed with t Student test, expressed as mean  $\pm$  SD. Results: RI results were analyzed with non parametric Mann-Wihtney and expressed as median and range. Results: EA: (2k2n0)  $\beta$ : 0.45 ± 0.23, b: 0.48 ± 0.19ns; (s0/n0)  $\beta$ : 1.73 ± 0.09, b: 1.72 ± 0.06 ns. RI (%): β: 12.87 (11.4-15.9) b: 10.4 (6.3-16) ns. The results showed no significant differences. We can conclude that the line b developed changes that tend to reproduce the behavior that exhibits the model about  $\beta$ .

#### 6.

# QUERCETIN INHIBITION OF ARSENIC ACTION ON ERYTHROCYTE AGGREGABILITY (EA)

Bollini A, Hernández G, Rasia M, Mengarelli G, Casco C, Ruiz MF, Visconti M, Huarte M<sup>\*</sup>, Phiel L<sup>\*</sup>, Rubin de Celis E<sup>\*</sup>, Bazzoni G Cátedra de Física Biológica. Fac. de Cs. Médicas. UNR; \*Cátedra de Física. Fac. de Bioquímica. UBA.

We have observed in former occasions that arsenic (As<sup>v</sup>) effect on red cell (RC) membrane leads to an increase of the erithrocyte aggregability (EA) probably by lipoperoxidation and cell shape alteration. Recently, it has been stated that quercetin (Qc), protects the cell against oxidative stress. Hypothesis: Qc can prevent As<sup>v</sup>membrane damage, preserving the normal aggregability. Methodology: washed human RC were incubated as follows: I) in PBS, pH: 7.4 10' (control) ; II) in 3µM Qc solution, 10' (Qc); III) in  $3\mu M$  Qc solution, 10' and later in As<sup>v</sup> solution (0,32  $\mu M$ Na<sub>2</sub>HAsO<sub>4</sub>.7H<sub>2</sub>O), 30'(Qc-As<sup>v</sup>); IV) in As<sup>v</sup> solution, 30' (As<sup>v</sup>); determining: (i) cell shape (microscopic) with morphologic index (MI) as result, II) EA (by light transmition) with a T value (aggregate size) and a V value (aggregation rate). Statistic values stipulated in each variable observed; presented as mean  $\pm$  SEM, p< 0.05 was accepted. Results:T values: solution I:1.8913±0.003 (n:89)°; II:1.8677±0.005 (n:31)<sup>a,c</sup>; III:1.875±0.004 (n:40)<sup>a,c</sup>; IV: 1.904±0.003 (n:55)°; V values: solution I: 1.25±0.04 (n:81)<sup>a,c</sup>; II: 1.12±0.09 (n:31)<sup>a,c</sup>; III: 1.32±0.05 (n:43)<sup>a,c</sup>; IV: 1.64±0.06 (n:55)<sup>c</sup>; IM values: solution I: 0.74±0.29 (n:16)<sup>a,c</sup>; II: 1.00±0.32 (n:16)<sup>a,c</sup>; III: 1.33±0.28 (n:16)<sup>a,c</sup>; IV: -1.49±0.11 (n:20)<sup>c</sup> (ANOVA test) a: ns ; b: p<0.005; c: p<0.05. Discussion: The results show that previous Qc treatment inhibits membrane As<sup>v</sup> action on the cell aggregability.

8.

# LIPID PEROXIDATION AND ERYTHROCYTE MEM-BRANE LIPID FLUIDITY IN PATIENS WITH SYSTEMIC LUPUS ERYTHEMATOSUS (SLE)

Zilli ML<sup>1</sup>, Torrente J<sup>1</sup>, Spengler MI<sup>1</sup>, Svetaz MJ<sup>2</sup>, Leroux B<sup>3</sup>, Carrara P<sup>1</sup>, Van Isseldyk F<sup>1</sup>, Petrelli D<sup>1</sup>, Bertoluzzo S<sup>1</sup>

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We studied the lipid peroxidation and the relationship with the erythrocyte membrane lipid fluidity in 39 women with SLE and 50 healthy women. Lipid peroxidation was determined by the concentration of Malonildialdehido (nM of MDA/ml in a globular concentrate) with TBARS, and the lipid fluidity by fluorescence polarization using TMA-DPH (Trimethylammonium-Diphenylhexatriene). The Anisotropy it's inversely related with erythrocyte membrane lipid fluidity and it is calculated: A = (I parallel - I perpendicular) / (I parallel + 2)I perpendicular). I parallel and I perpendicular correspond to the intensity of fluorescence measured by the parallel and perpendicular polarized emission of the excitation wave. The results showed that women with SLE had significally higher values of: MDA (3.64±0.71 vs 2.95±0.61; p<0.005) and of A (0.18±0.02 vs 0.14±0.01; p<0.005). We also found significant correlation between MDA and A (r = 0.45; p<0.05). The oxidative injurie takes place in SLE and probably inducts structural and functional changes on the erythrocyte membrane which determines lower lipid fluidity.

# LIPID PEROXIDATION OF ERYTHROCYTE MEM-BRANE AND ITS INFLUENCE ON ERYTHROCYTE DEFORMABILITY IN PATIENS WITH SYSTEMIC LUPUS ERYTHEMATOSUS (SLE)

Torrente J<sup>1</sup>, Zilli ML<sup>1</sup>, Spengler MI<sup>1</sup>, Svetaz MJ<sup>2</sup>, Leroux B<sup>3</sup>, Van Isseldyk F<sup>1</sup>, Carrara P<sup>1</sup>, Petrelli D<sup>1</sup>, Bosch P<sup>1</sup>.

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We studied lipid peroxidation and its relationship with the erythrocyte membrane deformability in 39 women with Systemic Lupus Erythematosus (SLE) and 50 healthy women of similar age. Lipid peroxidation was determined with the concentrations of Malonildialdehido (nM of MDA/ml of globular concentrate) with TBARS. The erythrocyte deformability was measured by the filtration of erythrocytes with policarbonat membrane with 5µm pores and it was estimated using its inverse: the rigidity index (RI) For the stadistics we used Student's t distribution and the Pearson's correlation. The results showed that women with SLE had significally greater values from healthy women of: MDA (3.64± 0.71 vs 2.95±0.61; p<0,005) and RI (10.03±3.89 vs 7.11±1.26; p<0, 01). We also found significant correlation between MDA and RI (r=0.57; p<0.01). These results suggest that oxidative injury has place in SLE and that it can perturbe the lipid organization of erythrocyte membrane which could modify the deformability of erythrocytes with the consecuente alteration in the microvasculature typical of SLE.

## ANTIPHOSPHOLIPID ANTIBODIES (a-FL) IN SYSTEMIC LUPUS ERYTHEMATOSUS (SLE) PATIENS, AND ITS RELATION WITH BLOOD RHEOLOGY

Spengler MI<sup>1</sup>, Svetaz MJ<sup>2</sup>, Leroux B<sup>3</sup>, Detarsio G<sup>2</sup>, Petrelli D<sup>1</sup>, Carrara P<sup>1</sup>, Van Isseldyk F<sup>1</sup>, Parente F.

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The aim of this study was to determine a possible association between oxidative damage in erythrocyte membrane and the level of a-FL. 39 SLE patients were studied (age 40±5). Serum determinations of anticardiolipin antibodies (ACA) IgG-IgM were done by ELISA method, and in heparinized blood was measured the degree of lipid peroxidation by determining the Malonildialdehido (MDA) concentration (nM de MDA/ml of globular concentrate) by TBARS, besides membrane lipid fluidity of red blood cells by fluorescent polarization using TMA-DPH (Trimethylammonium-Diphenylhexatriene) was informed as anisotropy (r). The Anisotropy it's inversely related with erythrocyte membrane lipid fluidity and it is calculated: r = (I parallel – I perpendicular) / (I parallel + 2 I perpendicular). I parallel and I perpendicular correspond to the intensity of fluorescence measured by the parallel and perpendicular polarized emission of the excitation wave. Two groups were created: A with negative or low ACA IgG-IgM (< 40U) and B with positive levels. The results showed in group B higher values from group A of : MDA  $(4.47 \pm 0.49 \text{ vs } 3.54 \pm 0.64; \text{ p} < 0.01) \text{ y de r} (0.20 \pm 0.03 \text{ vs } 0.17 \pm 0.03)$ 0.02; p<0.05). It seems there is an association between ACA high levels (more than 40 U) and oxidative membrane damaged and lower membrane lipid fluidity of RBC in SLE patients.

#### 10.

# QUERCETINE PROTECTOR EFFECT ON THE ARSENIC (V) – ERYTHROCYTE MEMBRANE INTERACTION: CELL SHAPE AND OSMOTIC FRAGILITY STUDY

Hernández G, Rasia M, Bollini A, Mengarelli G, Casco C, Ruiz MF, Visconti M, Huarte M<sup>\*</sup>, Phiel L<sup>\*</sup>, Rubin de Celis E<sup>\*</sup>, Bazzoni G. Cát. Física Biológica. Fac. Cs. Médicas. UNR. \*Cát. de Física. Fac. de Bioquímica. UBA.

We have observed in former occasions that arsenic oxidative effect on red cell (RC) membrane leads to stomatocytosis and increased cell osmotic fragility. Recently, it has been stated that quercetin (Qc) locates in the membrane, protecting the cell against oxidative stress. Hypothesis: Qc can prevent Asv-membrane damage. Methodology: washed human RC were incubated as follow: I) in PBS, pH: 7.4 10' (control); II) in 3µM Qc solution, 10' (Qc); III) in  $3\mu M$  Qc solution, 10' and later in As<sup>V</sup> solution (0,32  $\mu M$ Na<sub>2</sub>HAsO<sub>4</sub>.7H<sub>2</sub>O), 30'(Qc-As<sup>v</sup>); IV) in As<sup>v</sup> solution, 30' (As<sup>v</sup>); determining: (i) cell shape (microscopic) with morphologic index (MI) as result, (ii) osmotic fragility (photometrically at 540 nm), with a X<sub>50</sub> value (NaCl mM concentration yielding 50% hemolysis). Statistic values stipulated in each variable observed; presented as mean  $\pm$  SEM, difference were considered significant if p< 0,05, <u>Results</u>: MI (ANOVA test):controls: 0.74±0.29 (n:16)<sup>a,c</sup>; Qc: 1.00±0.32 (n:16)<sup>a,c</sup>; Qc-As<sup>v</sup>: 1.33±0.28 (n:16)<sup>a,c</sup>; As<sup>v</sup>: -1.49±0.11 (n:20)<sup>c</sup>. X<sub>50</sub> (mM, U-Mann-Whitney test): controls: 74.96±2.05 (n:8)<sup>a,b</sup>; Qc: 73.51±2.13 (n:8)<sup>a</sup>; Qc-As<sup>v</sup>: 76.01±3.11 (n:8)<sup>a,c</sup>; As<sup>v</sup>: 82.17±1.38 (n:11)<sup>b,c</sup>. (a: ns ; b: p<0.005; c: p<0.05). Discussion: The results show that previous Qc treatment inhibits membrane As<sup>v</sup> action on the studied variables which enables us to claim that Qc in the membrane prevents As<sup>v</sup>-caused monolayer inbalance, preserving cells as well as their life span.

11.

# QUALITY OF LIFE FACTORS IN ADVANCED BREAST CAN-CER PATIENTS (ABCP), TREATED WITH METRONOMIC CHEMOTHERAPY (MCT) WITH CYCLOPHOSPHAMIDE (CY) AND CELECOXIB (CEL). PRELIMINARY RESULTS OF A PHASE I/II TRIAL

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Chemotherapeutic agents (CA) used at maximum tolerated dose (MTD) are limited by toxicity, non-specificity and resistance development, with a detriment of quality of life. MCT consists in the chronic administration, at regular intervals, of low doses of CA, without extended rest periods. Our goal was to analyze the quality of life of the patients enrolled in the trial up to now. ABCP received 50mg Cyclophosphamide+400mg Celecoxib/day, p.o. Clinical and biochemical evaluations were performed every 15-30 days. Quality of life or performance status was determined by ECOG scale. Patients included in the trial remained in treatment 4 to 34 weeks, without showing serious toxicity. A decline in platelet count Grade II in 1/7 patients, an increase in CPK levels in 1/ 7 patients, and the absence of toxicity in 5/7 patients were observed. Quality life evaluation indicated absence of adverse events and the presence of some positive changes, i.e. decrease in the morphine daily dose, improvement in mobilization. A reduction of one point in the ECOG scale in 5/7 patients and no change in the remaining 2 was also observed. We conclude that although in MCT, the cumulative Cy doses per patient in 21 days are similar to MTD schemes: 1) no important toxic effects were detected 2) An improvement in patients quality of life was obtained.

12.

# ERYTHROCYTE AGGREGATION AND PLASMATIC FIBRINOGEN IN PATIENTS WITH DIABETIC COMPLI-CATIONS

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Considering microvascular and/or blood flow alterations in the pathogenesis of type 2 diabetic complications, this report ethically assesses retinal lesions, fibrinogenemia (Fg) and erythrocyte aggregation (EA) in 52 patients (>50 years old) with and without retinopathy (44 RP+, 8 RP -) and in 18 healthy controls (C) for comparing them and for relating them with the simultaneous presence or absence of microangiopathic skin lesions. Fg, through Clauss' method, and EA through ASP (Aggregate Shape Parameter) were analyzed. Higher levels of Fg and EA  $(353 \pm 54,74 \text{ mg}\%)$ and  $0,67 \pm 0,14$  in RP +,  $253 \pm 54,49$  mg% and  $0,59 \pm 0,17$  in RP and  $208 \pm 26,81 \text{ mg\%}$  and  $0,28 \pm 0,13$  in C [p=  $1.10^{-8}$ ; p=  $4.10^{-7}$ ] were respectively registered. Moreover, 25 of 44 RP + patients evidenced microangiopathic skin lesions. In accordance with previous data about the risk of presenting skin lesions with Fg > 295mg% and ASP  $\geq$  0,65, it was observed that 96% and 86% of the patients with Fg > 295 mg% and ASP > 0.65 became RP+. Summing up, Fg and/or EA could have an early participation in the pathogenesis of these lesions due to its augmentation in RP-patients. Finally, exceeding that skin lesions could be of great help for monitoring diabetic patients, ocular compromise seems to appear earlier in this endocrinopathy.

#### MORPHOMETRIC STUDY OF THORACIC VERTEBRAE WITH DIFFERENT PATTERNS OF FRACTURES AND GRADES OF WEDGING DEFORMITY IN WOMEN OVER 50 YEARS OF AGE

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Vertebral fractures (VFx) represent more than 40% of all osteoporotic fractures. Nine thoracic vertebrae (T4-T12) from spinal X-Ray films belonging to 41 women over 50 years of age, were studied. Different fracture patterns and severity were evaluated morphometrically. VFx deformity pattern (Wedging, Biconcavity or Collapse) and fracture severity (Grade 1, 2 or 3) were defined using Genant's Semiguantitative Method. Bone Densitometry was performed using a Lunar DPX equipment. Diagnosis of Osteoporosis was established following WHO criteria. Statistical analysis: Prevalence rates, Student-t test. Sixty of the 369 studied vertebrae, were considered fractured (86.7% wedge, 8.3% biconcavity and 5.0% collapse fractures). Wedge severity proportions observed were: 68% Grade 1; 20% Grade 2; 12% Grade 3. Average age of patients with Wedge, Biconcave and Collapse fractures were  $74.9 \pm 9.0$ ,  $75.8 \pm$ 7.0 and  $83.0 \pm 4.6$  years, respectively. Women having Collapse vertebral fractures were significantly older than the patients who Biconcave (p<0.05) or Wedge (p<0.01) patterns. Osteoporosis was diagnosed in 30% of the patients with Wedge VFx, 40% of patients with Biconcave VFx and in 100% of women who had suffered Collapse VFx. In this sample, Wedge VFx and Grade 1 severity deformities had the highest prevalence rates. Deterioration of bone mass and worse structural alteration of fractured vertebral bodies (Collapse pattern) was observed in older patients. An early diagnosis of these vertebral deformities would be very useful in women over 50 years in our midst.

#### 14.

## DIACEREIN CITOPROTECTION OF GASTRIC MUCOSA (GM) IN RATS. IS NOT ENDOGEN PGs. DEPENDENT

Bedini O, Naves A, San Miguel P, Chaves J, Yulita P, Rosatti A, Serur M, Settecasi J, Sanvido J, Crosio E, Cicero E, Laudanno O, Cesolari J.

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Diacereín (Dia) Wich is an acetylated form of reina, acts by IL I inhibition. Objective: of this study was to evaluate the effect of Dia in GM, and in the GM injury produced by indomethacin (Indo) and if this action is PGs endogen dependent. Method: Group of wistar rats (n=7),  $400 \pm 25$  g 24 hs fasting, water ad libitum., were submitted to the following experiments: 1) Saline, 2 ml OG waited 60 m; 2) Dia 50 mg OG, 2ml, 60 min. 3) Dia 100 mg, OG, 2 ml, 60 min.4) Indo 75 mg /kg, OG, 120 min. 5) Indo 75 mg / kg IM, 120 min. 6) Indo 75 mg / kg, IM, 120 min. luego Dia 100 mg, OG, 60 min; 7) Dia 100 mg, OG 2 ml, 60 min. later, Indo 75 mg/kg IM, 24 hs. 8) PgE, 200µg/kg, OG, 60 min, Dia, 100 mg OG 60 min. The animals were sacrificad by ether overdose, the stomach was removed. The % of the macroscopical lesional gastric area was calculated by planimetry. Histological examination were obtained The stadistic evaluation was obtained de Student t and ANOVA. **Results:** were expressed by  $X \pm DS$  and considered p <0.05 Results: were expressed by  $X \pm DS$  and constanted  $p^{-50,05}$ significativily. Results: % of GM necrotic lesions: 1) 0.0; 2) 0.0 ± 0.1; 3) 0.0 ± 0.1; 4) 22.5 ± 5.5 (p< 0.001); 5) 25.5 ± 5.5 (p< 0.001); 6) 0.50 ± 0.05 (p< 0.01); 7) 2.5 ± 0.5 (p< 0.01); 8) 0.0 ± 0.1 (p< 0.01); 7) 2.5 ± 0.5 (p< 0.01); 8) 0.0 ± 0.1 (p< 0.01); 7) 2.5 ± 0.5 (p< 0.01); 8) 0.0 ± 0.1 (p< 0.01); 7) 2.5 ± 0.5 (p< 0.01); 8) 0.0 ± 0.1 (p< 0.01); 7) 2.5 ± 0.5 (p< 0.01); 8) 0.0 ± 0.1 (p< 0.01); 7) 2.5 ± 0.5 (p< 0.01); 8) 0.0 ± 0.1 (p< 0.01); 7) 2.5 ± 0.5 (p< 0.01); 8) 0.0 ± 0.1 (p< 0.01); 7) 2.5 ± 0.5 (p< 0.01); 8) 0.0 ± 0.1 (p< 0.01); 7) 2.5 ± 0.5 (p< 0.01); 8) 0.0 ± 0.1 (p< 0.01); 7) 2.5 ± 0.5 (p< 0.01); 8) 0.0 ± 0.1 (p< 0.01); 7) 0.5 ± 0.5 (p< 0.01); 8) 0.0 ± 0.1 (p< 0.01); 7) 0.5 ± 0.5 (p< 0.01); 8) 0.0 ± 0.1 (p< 0.01); 7) 0.5 ± 0.5 (p< 0.01); 8) 0.0 ± 0.1 (p< 0.01); 7) 0.5 ± 0.5 (p< 0.01); 8) 0.0 ± 0.1 (p< 0.01); 7) 0.5 ± 0.5 (p< 0.01); 8) 0.0 ± 0.1 (p< 0.01); 7) 0.5 ± 0.5 (p< 0.01); 8) 0.0 ± 0.1 (p< 0.01); 7) 0.5 ± 0.5 (p< 0.01); 8) 0.0 ± 0.1 (p< 0.01); 7) 0.5 ± 0.5 (p< 0.01); 7) 0.5 ± 0.5 (p< 0.01); 7) 0.5 ± 0.5 (p< 0.01); 8) 0.0 ± 0.1 (p< 0.01); 7) 0.5 ± 0.5 (p< 0.01); 8) 0.0 ± 0.1 (p< 0.01); 7) 0.5 ± 0.5 (p< 0.01); 7) 0.5 ± 0.5 (p< 0.01); 8) 0.5 ± 0.5 (p< 0.01); 8) 0.5 ± 0.5 (p< 0.01); 7) 0.5 ± 0.5 (p< 0.01); 8) 0.5 ± 0.5 (p< 0.01); 7) 0.5 ± 0.5 (p< 0.01); 8) 0.5 ± 0.5 (p< 0.01); 7) 0.5 ± 0.5 (p< 0.01); 7) 0.5 ± 0.5 (p< 0.01); 8) 0.5 ± 0.5 (p< 0.01); 7) 0.5 ± 0.5 (p< 0.01); 8) 0.5 ± 0.5 (p 0.001). Histologic examination : 1, 2 y 3) no mucosa lesions; 4) areas of erosion in the surface epithelium and extravasation of red cells; 5) areas of erosion in the surface epithelium and necrosis of the underlying cells; 6) minimal surface erosions; with haemorrhage 7) microerosions in the surface; 8) normal mucosa Conclusion: 1) Dia alone does not produce GM injury 2) Dia gives GM protection over gastric injury produce by Indo. 3) The gastric protection mechanism is not PGs dependent.

15.

#### EFFECT OF CHRONIC ORAL ADMINISTRATION OF SILDENAFIL ON SEVERAL ABDOMINAL AND EXTRA-ABDOMINAL ORGANS IN RAT

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Objective: To evaluate the action and the effect on different abdominal organs and extra abdominal organs of Sildenafil (SIL), administered orally to rats daily, for 35 days. Methods: In a group of Wistar rats N=10, of  $200 \pm 30g$  with feed ad libitum, were performed the following experiments: Group I : physiological saline for 35 days as daily drink. Group II: was administered to each animal 1 tablet of 50 mg of SIL diluted in 60 ml of water daily, placed in a individual drinker. The water was changed daily and a tablet of SIL was added to each drinker. The rats were sacrificed at 35 days by an overdose of ether, median laparotomy was performed and were tested samples of several organs: Stomach, small and large intestine, liver, pancreas, spleen, heart, lung and eyeball. The samples were fixed in formalin 10% and sent for histology Results: Macroscopically the study group showed no alterations in different organs. Microscopically: In the study group was found: vascular congestion of varying degrees in all organs studied, arterial elements have a smaller caliber than the control group. In contrast, we observed the presence of submucosal venous in the colon, with a significant generalized congestion and very strong expansion of its light. At the eyeball was observed foci of subcapsular edema in the lens, without cataracts degeneration of lens fibers. Conclusions: Chronic oral ingestion of 50 mg of SIL administered in 60 ml of water daily to rats for 35 days showed a significant dilatation of submucosal venous in the colon with congestion and very strong expansion of its lights. Was also observed foci of subcapsular edema in the lens.

#### 16.

# GASTRIC CITOPROTECTION INDUCED BY DIACEREIN IN ETHANOL INJURY OF GASTRIC MUCOSA IN RATS

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Diacereín (Dia), is used in the treatment of, osteoarthritis it is and acetylated form of a natural compound, named reina wich is an antraquinone tipe. It inhibit interleukin I (IL 1) production and the production and release of two enzymes collagenase and estromelisina. Objective: was to evaluate the effect produced for Dia in gastric mucosa injury by ethanol absolute (ETOH). Method: Groups of wistar rats (n= 7),  $400 \pm 25$  g fasting water ad lib, were submitted to the following experiments: 1)1 ml saline OG and waited 60 min 2) ethanol 96° (ETOH) 1 ml, OG and waited 30 min 3) Dia 50 mg, OG, 2ml, 60 min 4) Dia 100 mg, OG, 2 ml, 60 min 5) Dia 100 mg, OG 2 ml, 60 min, later, ETOH, 1 ml, OG, 30 min. later The animals were sacrificed by ether overdose, the stomach was removed and apertura of it along the great curvature, was perfomed. The % of the macroscopical lesional gastric area was calculated by planimetry .Samples for histological examination were obtained, PAS, hematoxilin eosin and alcian blue. An stadistic evaluation was done by Student t and ANOVA. The results were expressed by  $X \pm DS$  and p <0.05 was considered like significant. **Results**:% lesional gastric area 1) 0.0; 2)  $35.5 \pm 5.5$  (p< 0.001); 3)  $0.0 \pm 0.1$  (p< 0.001); 4) 0.0 0.1 (p< 0.001); 5)  $0.0 \pm 0.1$  (p< 0.001). Histology: 1) normal gastric mucosa; 2) necrotic area with haemorrhagic in the mucosa; 3, 4 y 5) without histologic lesions. Conclusion: Diacerein, an IL modulator, is a gastric citoprotector against the ethanol injury in rats

# EFFECTS OF ANTAGONIST FAT ACIDS IN OBESE AND DIABETIC ADULT RATS

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It was observed that with chia ( $\Omega$ 3) there was improvement in the glucolipidic profile and obesity decrease. When the lipid consumption increases, it increases the adipose tissue volume and the triglyceridemia. Fat acids  $\Omega 3/\Omega 6$  have antagonist effects that are reflected in pathologies like obesity and diabetes. In this work the supplements were incorporated to the habitual diet of adult male line  $\beta$  rats. It was given: habitual food(C), habitual food+sunflower seeds(G) and habitual food+chia seeds(CH) (n:5/group). Data were analyzed through ANOVA and Bonferroni (media±SEM): daily intake(grs/d), calorie consumption(kcal/d), Gli, and Gli, overcharge, triglyceridemia TG, adipose panicles and encephalon relative weight(g/biomass). C and G daily intake showed differences at 40ds (p<0,0089) being lower in G. At 80ds calorie consumption is higher in G: (C) 83,51±6,70, (G) 102,1±7,08, (CH) 83,26±4,805 (p<0,0053). Adipose panicle relative weight is higher in G: (C) 0,054±0,005, (G) 0,065±0,004, (CH) 0,052±0,007 (p<0,036). Encephalon relative weight is higher in CH: (C) 0,004±0,0003, (G) 0,0036±0,0003, (CH) 0,005±0,0002 (p<0,0153). Glycemia is lower in CH: Gli<sub>a</sub>: (CH) 1,35±0,08; (C) 1,61±0,03; (G) 1,48±0,05 (p<0,0244) and  $Gli_{120}$ : (CH) 1,63±0,04; (C) 2,12±0,05; (G)  $2,91\pm0,09$  (p<0.0001). This shows the effects of an antilipogenic and antidiabetogenic and lipogenic and diabetogenic diet in adults  $\beta$ . It is noticeable the effects on the encephalic mass in only 80ds. When  $\beta$  consumes habitual diet, hipertriglyceridemia, diabetes and obesity, usual in the model animal, appear.

#### 18.

# POLISATURATED FAT ACIDS AND THEIR INFLUENCE IN THE IIMB RAT INSULIN RESISTANCE CELLULAR IMMUNITY AND ENCEPHALON

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The line IIMb male rat habitual diet was supplemented with chia seeds (Ù3) and sunflower seeds (Ù6). Adult animals were fed with: habitual food(C), habitual food+ sunflower seeds(G) and habitual food+chia seeds(CH), n:6/group. At the start, 40 and 80ds of treatment Gli<sub>0<sup>r</sup></sub> and Gli<sub>120</sub> were registered. Macrophages were obtained after peritoneal washing for cellular culture, phagocytosis percentage(%F) and phagocytosis index(IF) were measured throught a leaven revealing system [Candida albicans(CA) and tropicalis(CT)]. This was analyzed with ANOVA-Bonferroni (media±SEM). At 80ds Gli<sub>0</sub>: differs (p<0,0035) and Gli<sub>120</sub>. (p<0.0001), with lower levels for CH and higher for G. In IF: (G)[CA:1,18±0.35; CT:1,24±0.045]; (C) [CA:2,01±0,05; CT:1,80±0,06]; (CH) [CA:1,91±0,12; CT:1,93±0,14] differ (p<0,0056), where G shows the lowest values. CH expressed the highest %F: (CH) [(CA)19,43±2,15; (CT)19,71±2,57]; (C) [(CA)11,50±0,86; (CT)10,00±0,41]; (G) [CA)11,00±0.35; (CT)10,50 ±0,50] (p<0,0448). The encephalon relative weight showed: (CH) 0,0040±0,0001; (G) 0,00374±0,0001; (C) 0,00373±0,0001 (p<0.0125). The supplement with  $\Omega 6$  unchain insulin resistance, elevate glicemia (glucotóxico effect) and lower immunity. The supplement with  $\Omega$ 3 increase insulin sensibility, modulate glicemia and favor the cellular immunity. Besides, this supplement produce an encephalic weight increase.

19.

# INFLUENTIAL FACTORS IN THE DIET OF CHILDREN BETWEEN 6 AND 8 YEARS OLD AND ITS RELATIONSHIP WITH OVERWEIGHT AND CHILD OBESITY

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This paper shows data on regarding the relationship among the factors that may condition the appearing of eating disorders. Objective: find the relationship between data and personal history, eating habits, physical activity and overweight or child obesity. A cross-sectional study is presented. We worked with a population of 238 individuals (124 boys, 114 girls) between 6 and 8 years old from four cities in our region. Data collection was conducted through a survey developed based on variables such as age, weight, height, physical activity, diet, eating habits and previous diseases. The information obtained was used to prepare the Body Mass Index (BMI), and collate this and other data such as weight and height with the Sociedad Argentina de Pediatría (SAP) percentile tables. The following findings of BMI average and  $\pm$  standard deviation (SD) were obtained:  $19.73 \pm 0.64$  in kids,  $19.23 \pm 0.58$  in girls. In all cases, the mean was above the 75th percentile of the SAP tables. In addition, 43% of the population reported physical activity. In the remaining 57% sedentary lifestyle prevailed. It can be concluded that overweight and obesity in children should be studied in greater detail because of its prevalence and its strong association with factors related to lifestyle and eating habits.

#### 20.

# CARIOGENIC FOODS INTAKE AND DENTAL HYGIENE IN YOUNG ADULTS IN ROSARIO, ARGENTINA

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Cariogenic food intake and dental hygiene habits are important risk factors in cavity production and periodontal disease. These pathologies are not uniformly distributed among the population, and it would be important to know the high-risk groups associated with lower income sectors with no access to private dental attention or health insurance. Our objective was to research oral health condition among the young adult men and women who required tooth extractions. Included were 15 to 45 years olds treated in private and public dental services. Analyzed variables: CMO Index (Caries, Missing, Obturated), sweets intake, type and frequency. Average age of the 517 patients was  $26.5 \pm 8.5$ , 34.8%males and 65.2% females. In private patients, concurrence of both sexes was similar, in public attention women were predominant (p=0.001), average age in private was  $30.0 \pm 9.2$  years old and  $25.6 \pm 8.1$  in public (p<0.001). In private, C and M average was lower, (p=0.001) and (p=0.014) respectively, and O average higher (p<0.001). Private had higher intake of sweet potato jam among males (p=0.004), and honey (p=0.001), cakes (p=0.010), chocolate (p=0.01), hard candy (p=0.05) and ice cream (p<0.0001) among females. Comparing intake hours in private patients, women consumed more snacks, while in public services males did. In both groups, patients who brushed sporadically or never were minority (p=0.04), 90% used fluoride toothpaste (p=0.007)and 10% another element. Conclusion: sweets intake was similar in both groups with the only difference in the type of foods. The hours of sweets intakes were not respected, but among the private patients this was the behavior of the minority.

## 21. HISTOLOGICAL COMPARISON: NORMAL RADICULAR CEMENTUM AND PEARL SHAPES HYPERCEMENTOSIS García Montero M<sup>1</sup>, Obelli J<sup>1</sup>, Pezzotto SM<sup>2</sup>, Poletto L<sup>2</sup>, Kohli A<sup>1</sup>. <sup>1</sup>Facultad de Odontologia, <sup>2</sup>Consejo de Investigaciones, UNR.

Secondary cementum is deposited in a root apical third throughout the life span of a tooth to compensate occlusal erosion keeping dental pieces in occlusion contact. Pearls are more difficult to spot, found by X-rays or after extraction. Their ovoid or spherical aspect is well outlined, hard to the touch, and located in different thirds of the root. Objective: histologically comparing some structures of normal roots apical cementum with pearls. We used 20 normal roots and 20 pearl shapes. The teeth were photographed, the roots divided into thirds to classify the location of the pearls. Normal roots were cut transversally through the middle of the apical third, and pearls through the middle of the salient. The cuts were prepared by erosion technique and analyzed histologically by light microscope at 100x and 450x. Variables compared: cementum width, perimeter, Tomes granular layer visibility, number and distribution of gaps. Proportions between histological data of normal pieces and pearls were calculated with Chisquare Test, Fisher's Exact Test, with 5% significance level. Normal pieces cementum width was thin in 63.2%; in pearls, thick 50% and mixed 40% (p=0.002). In 89.5% normal roots cementum perimeter was not preserved, in 85% of pearls, preserved (p<0.001). In 63.2% of normal roots Tomes granular layer was not visible and visible in 83.3% of pearls (p=0.004). In normal roots the gaps was scarce in 95%; in pearls were scarce in 39% and abundant in 50% (p=0.001). In 100% of normal roots gaps were in order and in 89% of pearls in disorder (p<0.001). In pearls cementum width showed variability compared to normal roots, cementum perimeter was better preserved, the granular zone was more visible and gaps and their arrangement were different.

#### 22.

# STUDY OF ASSISTED POPULATION IN F.O.R. WITH THE USE OF ELECTRONIC MEDICAL HISTORY. GEOCODING

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Information is the final result of data processing and helps us to make decisions. Electronic health records, has advantages with respect to attendance data, teaching and research, but also the management, health planning and public health. One of the applications of computers in teaching and research is access to structured databases where electronic health record is an important source of information. The geocoding database links to a map with the help of a software and output is generated with color graphics, or density scale punctate. The aim of this study was to perform epidemiological analysis of data collected in a Model of Computerized Medical Record designed for the care of patients endodontically treated. Data on gender, most frequent pathology and place of residence, according districts division of Rosario's borough, for strategic planning of health programs. We performed the data load of 328 medical records of patients assisted in the Post-Graduate Specialization in Endodontics Career, Faculty of Dentistry, National University of Rosario during the period 2009-2010. Statistical analysis was performed and georeferenced data processing was carried out through the Arc View software. The findings on the gender variable were 37.1% male and 62.9% female. The most frequent pathology corresponded to processes of pulp death by 33.4%. As regards the place of residence. 31.8% live in the southwest of the city including Villa Gobernador Gálvez. Based on these results we conclude that women (62.9%) are those which arise more frequently for care probably a matter of time availability and that the disease most frequently observed was death pulp (334%), perhaps due to delayed dental care, that results on more compromised situations. The geographical distribution (31.8% southwestern and Villa Gobernador Gálvez) coincides with results of previous studies, perhaps coinciding with low-income areas.

# 23.

# EVALUATION OF THE INTERACTION OF SODIUM HYPOCHLORITE AND EDTA IN ITS ANTIMICROBIAL CAPACITY

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Sodium hypochlorite (NaOCl) is widely used as main endodontic irrigation agent. EDTA solutions have been recommended to remove the inorganic component of the smear layer, and creamy (EDTAc) to also lubricate the canal. The aim of this study was to evaluate if the interaction between them affects NaOCl antimicrobial capacity. 60 maxillary central incisors (total length at least 21 mm) were selected. Access cavity was carried out and the length of work determined, teeth were sterilized in an autoclave. An inoculum of Enterococcus faecalis (ATCC 29212) was placed in the pulp cavity. Teeth were incubated 7 days at 37°C, and randomly divided into 6 groups (n = 10) which received different irrigation regimes during biomechanical preparation: A: NaOCl; B: NaOCl + EDTA; C: NaOCl + EDTA final irrigation; D: EDTAc and then NaOCl; E: NaOCl and EDTAc, the F group was not irrigated (control). Canals were dried, and transport medium (Stuart) was injected, collected and bacteriologically processed. On one tooth of the groups B, C and D were recovered E. faecalis (which were measured in colony forming units-cfu-) although in smaller numbers (ufc) than those recovered from the control group (B: 2.2 x10<sup>4</sup>, C: 5.6 x10<sup>3</sup>, D: 2.4 x10<sup>4</sup>; CONTROL: 1x10<sup>5</sup>cfu). No statistically significant differences between experimental groups (p=0.72) was observed. The disinfection achieved is the same whether using only NaOCl or combining this solution with EDTA in any form (liquid or cream).

## 24.

# *Streptococcus mutans* STUDY IN SALIVA FOR THE EVALU-ATION OF A PREVENTIVE COMMUNITY PROGRAM

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S. mutans have been implicated as causative agents of dental caries. Since different substances have been proposed as beneficial for achieving a reduction of S. mutans in the human dentition is necessary to have a technique to assess this potential action. The objectives of this study were to test a technique for collecting and quantifying S. mutans in saliva of children from different school classes, and calibrate the team. 20 children (6-8 years old) attending two different educational establishments were selected. A sample of total stimulated saliva, was taken after chewing a piece of latex tube for 1 minute; it was aspirated with sterile 5 ml pipette, and placed in a sterile test tube with bacteriological lid, kept on ice at 4°C for transport to the laboratory. Bacterial cultures were performed by seeding with calibrated loop with 3 consecutive discharges, identifying S. mutans by biochemical tests and quantifying colony forming units (cfu). With the proposed methodology was possible to count S. mutans in 95% of the sample (n=19), the number of cfu in the individuals tested was between 2,3 x 10<sup>3</sup> and  $9,8 \ge 10^5$ ; no bacterial growth was obtained in one subject whom was, apparently, receiving antibiotic treatment for a systemic disease. Samples from different educational institutions differed in terms of numbers of organisms of different genders who developed. This technique could be used to assess the effect of substances on S. mutans development in children.

# 25. MICROORGANISMS PRESENT IN FRESH COMMON CARP (Cyprinus carpio) AND SALTED CHUNKS

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The aim of this study was evaluating the microorganisms present in fresh common carp (*Cyprinus carpio*) and in salted chunk. Samples were knocked out, gutted and preserved at 2°C until rigor mortis (70 h) ending. After filleting, the chunks were obtained for subsequent dry and wet salting. Salting was done at 17+/-1°C till reach salt equilibrium in muscle. Were determined: Total aerobic viable counts-TAVC-(Plate Count Agar, 35°C,24h), molds and yeasts-M&Y-(YGC agar, 25°C,5d), sulfite reducing Clostridium-SRC-(sulfite iron agar in an anaerobic, 35°C and 45°C,48h); halophilic bacterias (Gibbons, 20% NaCl, 37°C, 14 d) and Pseudomones spp (CDC-Oxid agar, 25°C, 24-48h. Water activity (aw) was determined by gravimetric technique, % NaCl by Mohr method and water content by constant weight at 105+/-1°C. Aw value decreased from 0.94 to 0.80 in dry salted method and to 0.75 in wet salted. Water content decreased in both methods an 18%. The NaCl% increased from 0.03% to 16.98% in dry salting and to 13.28% in wet salting. TAVC was 1.2x103 CFU/g (raw and dry salted) and 2.5x103CFU/g (wet salted). M&Y count in raw material was 9 CFU/g. In salted samples there was not detected: M&Y, halophilic bacteria, total coliforms neither SRC. Pseudomonas spp. count increased from 3CFU/g (raw material) to 64CFU/g (salted chunk). Pseudomonas fluorescens was identified (by biochemical and API 20NE methods) in dry salted chunks. According to other authors, P. fluorescens generates volatile products (methyl mercaptan, dimethyl sulfide) involved in product deterioration.

#### 26.

# MICROBIOLOGICAL STABILITY OF SMOKED COMMON CARP (Cyprinus carpio) FILLETS

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Common carp (Cyprinus carpio) is a freshwater species of greatest biomass in Argentina. The aim of this work was to analyze the microbiological stability of smoked vacuum packed fillets stored at different temperatures. In a previous stage fillets were immersed in a hypertonic solution (salt, sugar and water) for 1h. Muscle values reached were: 81.47gH<sub>2</sub>O/100g, 6.28gNaCl/100g and 0.953 aw. Smoking process at 22°C for 4h, consisted of: air dehydration, smoking and final air dehydration. Final values were: 79.319gH<sub>2</sub>O/ 100g, 7.62gNaCl/100g and 0.919 aw. Total aerobic viable counts-TAVC-(Plate Count Agar, 35±0.5°C,24h) was 5.0x10 CFU/g, there were not detected: molds and yeasts-M&Y-(YGC agar, 25°C,5d), sulfite reducing Clostridium-SRC-(sulfite iron agar in an anaerobic, 35±0.5°C and 45±0.5°C,48h) and Staphylococcus spp (Baird-Parker agar, 35±0.5°C,24h). Samples were stored at 8, 25 and 35°C, collected daily. TAVC obtained at 8th day were: 1.15x106, 5.6x106 and 2.93x107CFU/g respectively and SCR was not detected. M&Y growth was not detected at any of the temperatures till 5<sup>th</sup>d. On 6<sup>th</sup>d at 25 and 35°C was counted: 7.70x10<sup>2</sup> and 3.70x10<sup>3</sup>CFU/g. At 8°C growth was detected at the 8thd. The longest shelf life is obtained at lower temperatures which may exceed 8 days, at 25°C this time is reduced to 5 days and it is considered that 35°C is not an appropriate storage temperature for this product.

27.

# **OSMOTIC DEHYDRATION OF COMMON CARP** (Cyprinus carpio) FILLETS IN BINARY AND TERNARY SOLUTION

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Osmotic dehydration (OD) is used as pre-treatment prior to freezing; freeze drying, air drying or smoking. The aim of this work was to study the influence of solution composition during OD of carp (Cyprinus carpio) fillets in ternary (salt+sucrose+water) and binary (salt+water) solutions. It was determined: water activity (aw), water content and salt concentration in muscle. Binary (0.44gNaCl:0.56gH2O/l) and ternary (0.44 g NaCl, 0.34 g H2O: 0.22 g sugar/l) solutions were prepared with a ratio of solution/ samples in 1/1 at 20°C. Samples were dipped and randomly sampling at: 0, 15, 30, 60, 90, 150, 210, 270, 390 and 480 min. The raw material values of aw, H<sub>2</sub>O and NaCl content were: 0.990, 89.47g/ 100g and 0.22g/100g. Binary solution values at 480 min were: 0.810-0.834 aw, H<sub>2</sub>O content 70.03-73.02g/100g and 14.50-15.57g/ 100g NaCl. Ternary solution presented: 0.845-0.839 for aw, 63.82-65.84g/100g of H<sub>2</sub>O content and 14.68-14.47g/100g NaCl. The NaCl uptake and water removal depend on solutes properties. The solutes combination results in a salt diffusion decrease due to the high molecular mass of sucrose, a layer on surface sample was made. The solutions aw values were not significant different allowing selecting the salting process according to the sensory characteristics desired for the product.

#### 28.

# **BIOCHEMICAL CHARACTERIZATION IN DIFFERENT** TISSUE OF SMALLNOSE FANSKAKE, S.

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The smallnose fanskate, Sympterygia bonapartii, is an endemic skates of south-west Atlantic; and it is one of the major skates species that has been caught along the Buenos Aires coast. The frozen fins are marketed for export mainly to Asian countries; generating large amount of waste (65-70% by weight). The aim of this work was to characterize biochemically the waste generated in the marketing of S. bonapartii to explore the viability to obtain high value added products. The specimens were dissected, gonads and livers were extracted and the fins were tooks off, leaving remains (head, trunk, tail). Moisture, protein and ash were determinate according AOAC (1996); lipids were extracted by Bligh & Dyer and fatty acids were analyzed using gas chromatography. The cartilaginous tissue was quantified after enzymatic hydrolysis using alkaline protease. The tissues analyzed showed a protein content from 14.66 to 22.86%. The lipid percentage in fins and the remains was 1%, in gonads 3.51±1.20% and in liver 32.27 ±5.54%. The liver oil presented a 35-40% of polyunsaturated fatty acids (PUFAs). Among them, those found in the highest proportions were docosahexaenoic acid (DHA n-3, 20-25%) and eicosapentaenoic acid (EPA n-3, 7-10%). The cartilaginous tissue was  $1.66 \pm 0.18\%$  (fins) and  $1.79\pm0.30\%$  (rest). The chemical composition of this material is very interesting nutritional and economically since they contain high percentage of proteins, lipids rich in n-3 PUFAs and cartilage tissue. Have been reported beneficial properties of n-3 PUFAs of marine origin and cartilage glycosaminoglycans in the prophylaxis and treatment of various diseases.

## Halococcus morrhuae PRESENCE IN SALTING FISHERING PRODUCTS OF Engraulis anchoita

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Halococcus morruae belongs to a group of extremely halophilic archaebacteria responsible of the appearence of a pink-coloured slime and off-odours in salted fish products associated with spoilage. The aim was to determine the presence of H. morrhuae in salted-ripened anchovy (Engraulis anchoita) products. 78 samples of anchoita salted-ripened and 22 of fillets in oil were analyzed. 10g of sample was homogenized in 90 ml of salt broth. 0.1 ml was inoculated on Petri dish with Gibbons medium, incubated at 37°C, 12 days. H. morrhuae identification was based on phenotypic characteristics of ATCC 17082. Isolates were tested for: Gram, motility, oxidase, catalase, Indole, Urease, Tween 80 hydrolysis, Nitrate reduction, Sulfhidric production, starch and gelatin hydrolysis, Lysine decarboxylase, Arginine dehydrolase, Proteolytic and lipolytic characteristics, Citrate and acid production from carbohydrates. Phenotypic characteristics were used for grouping the isolates. Hierarchical cluster analysis was performed. The isolates were clustered into four mayor groups at linkage distance 2,1. In The 1st group H. morruae is associated with eight isolates found in salted anchovy samples at a very short linkage distance, indicating that strains are very similar in their characteristics and probably belong to different species of Halococcus. Other group is formed by 24 strains, that belong to the extreme halophilic bacteria. The linkage distance is short enough to be considered as similar genus but not as a species of the same genus. Halococcus was identified, however do not belong to the species H. morrhuae.

#### 30.

# ACUTE TOXICITY OF NITRITE IN THE SHRIMP Palaemonetes argentinus NOBILI, 1901: INFLUENCE OF SALINITY

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Ammonia is the most common toxicant resulting from excretion by cultured animals and mineralization of organic detritus such as unconsumed feed and feces. P. argentinus is an euryhaline species that inhabits at wide ranges of salinity, including brackish water and saline water of 25‰. The main of this work was to evaluate the salinity influence on the acute toxicity of nitrite in the shrimp P. argentinus. Shrimp collected from Los Padres lagoon (37° 57'S; 57°44'O) were acclimated for 48 hours at freshwater and at different salinities, 6‰ and 12‰. Short-term  $LC_{50}$ (median lethal concentration) toxicity tests were carried out. Nitrite test solutions consisted of concentrations of 10; 20; 40; 60; 80; 100; 120 and 140 mg/l in the first experiment; in the second (6‰) and the third (12‰) experiments 140; 180; 220; 260 y 300 mg/l nitrite were used. A group of shrimp was used as control for each salinity test. The  $LC_{50}$  of nitrite at 96hs was significantly different among the different salinities. The LC50 values recorded in freshwater, 6‰ and 12‰ were 62.53mg/l, 422.85 mg/l, and 703.80mg/l, respectively. In conclusion, P. argentinus is more susceptible to nitrite at hypo-osmotic conditions.

# 31.

#### MARGINAL SPHINCTER AS NEUROEFECTOR SYSTEM OF L-GLUTAMATE IN THE SEA ANEMONE *Phymactis* clematis

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The aim of this work is to determine the action of glutamate (Glu) on the contractility of the marginal sphincter. It was placed in a chamber containing aerated sea water at controlled T°, salinity, osmolarity and pH. Three successive assays were made and expressed as doseresponse curves (DRC) 1) at increasing concentrations of KCl (20-200mM); and for each concentration of KCl: 2) with Glu (10-5M) and 3) Glu (10-5M) plus glycine (Gly) (10-4M) as co-agonist. Previously different concentrations of Glu alone (10<sup>-3</sup>; 10<sup>-4</sup> and 10<sup>-5</sup>M) were tested, without response in any of them. ANOVA test were used. P values < 0.05 were considered statistically different. In 1) maximal response was  $1.29g \pm 0.16$  with 200mM KCl (n=24);  $1.59g \pm$ 0.15 in 2) (n=10) and 2.03g  $\pm$  0.25 in 3) (n=8) were obtained with 140mM KCl. In the three assays with 20 and 40mM KCl no statistically differences were found, while in the other concentrations were highly significative. Over 140mM KCl maximal response in 2) and 3) decreased. In the absence of  $Mg^{2+}$  in the medium, Glu increased the contraction (2.95g ± 0.52) (n=11), while in excess of this ion the maximal contraction was  $(1.33g \pm 0.44)$  (n=6). This behaviour resembles the mechanism of ionotropic N-metyl-D-aspartate (NMDA) receptors in mammals, which has many binding sites: a) to Glu, opening the channel when the membrane is depolarized, promoting calcium influx; for this reason there was no contraction with Glu alone; b) to Gly making Glu more effective; c) to  $Mg^{2+}$  that block the pore of NMDA receptor. Our results suggest that this receptor might be used to regulate calcium influx required for the contraction in the marginal sphincter of Phymactis clematis.

#### 32.

# FIRST REPORT OF BARLEY YELLOW STRIATE MOSAIC VIRUS (BYSMV) INFECTING WHEAT IN ARGENTINA

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During the agricultural campaign of 2006/2007, symptoms suggesting virus infection were observed in wheat crops at high incidence in several provinces of Argentina. The symptoms were mainly leaf stripping, dwarfing, rosette forming and yellowing empty ears. The aim of this work was to characterize this new disease by serology, electron microscopy and experimental transmissions. Symptomatic plants were collected from Buenos Aires, Córdoba, Entre Ríos and La Pampa and then were evaluated by serology. The tested virus were: Barley yellow dwarf virus (BYDV-PAV, BYDV-MAV, BYDV-SGV, BYDV-RMV); Cereal yellow dwarf virus-RPV (CYDV-RPV); Wheat streak mosaic virus (WSMV) by DAS-ELISA and, WSMV, Barley stripe mosaic virus (BSMV) and Barley yellow striate mosaic virus (BYSMV) by indirect ELISA. For electron microscope observation, leafdip and ultrathin section were prepared from sympthomatic tissues. Experimental transmission was proved using Delphacodes kuscheli as insect vector. Serology resulted negative for BYDV (BYDV-PAV, BYDV-MAV, BYDV-SGV, BYDV-RMV), CYDV, WSMV and BSMV and positive to BYSMV (in all of the evaluated samples). Bacilliform virus particles (300nm) characteristic of the Rhabdoviridae family, were observed in leaf-dip preparations and in ultrathin sections of symptomatic plants. The virus was transmitted to healthy wheat using D. kuscheli as vector, and the major symptoms were chlorotic streaking and yellowing. Mechanical transmissions were not possible for this virus. This is the first report of BYSMV infecting wheat in Argentina.

# USE OF RAPD (Random Amplification of Polymorphic DNA) FOR IDENTIFICATION OF Mal de Rio Cuarto virus (MRCV) INSECT VECTORS

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"Mal de Río Cuarto" is the most important disease in corn production in Argentina. It is only transmitted by planthoppers (Hemiptera: Delphacidae). We propose the use of RAPD as a complementary tool for identification of delphacid species most frequently found in field: Delphacodes kuscheli, Toya propinqua, Chionomus haywardi and Pyrophagus tigrinus. DNA was extracted individually from insect of laboratory populations. Specimens of D. kuscheli from four localities were included to corroborate the stability of this technique for the studies of populations. Samples were analyzed by RAPD using primers OPA 4, 11 and 18. As result, we obtained diagnostic bands for D. kuscheli (500 and 1500 bp) and P. tigrinus (600 and 2000pb) by OPA11 amplification. Polymorphic bands were obtained for Ch. haywardi and T. propingua with the proposed primers. As relevant data, different populations of D. kuscheli showed reproducible bands, suggesting the potential of this technique to identify insects from different places. To optimize the use of this technique, it remains to prove other primers until finding one that amplify diagnostic bands simultaneously for all four species. We suggest RAPD as a useful tool for identification of planthopper species in the context of molecular studies of field populations.

#### 34.

# OCCUPATIONAL RISK PERCEPTION AND ADOPTION OF SAFETY PRACTICES AMONG RURAL WORKERS

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Risk perception research can clarify the way they are stablished and managed. The objectives of this study were to quantify occupational risk perceptions among rural workers, the uptake of safety practices and its associations. A cross-sectional study was performed in 125 rural workers in Sarmiento, Santa Fe using a structured questionnaire in personal interviews (simple random sampling, 5% error 5%, confidence level 90%). "Risk perception" was defined according to the WHO and quantified with an ordinal scale, as the frequency of adoption of safety practices. Statistical analysis was performed Sperman rank correlation coefficient. Prevalence of "high" risk perception was higher in pesticide and herbicide handling (85%) than working with animals (16%), parturitions (29%), abortions (41%), carcasses (34%), tools (15%), tractors (22%), machinery (18%) or other activities. On in itinere transit prevalence was 92% in major roads and 34% in country roads. Usage of safety practices "always" was higher when operating machinery (detention previous to examination 88%, coverage of mobile parts 64% and manual Redding previous to operation 50%) than when working with animals (gloves on parturitions 41%, carcass 41% and abortion handling: 41%, face masks 0%), chemicals (gloves: 26%, face masks: 2%, specific clothing 2%). Usage of car headlights during daylight hours or safety belts "always" was higher on major roads (88 and 64%) than on country roads (62 and 30%). Risk perceptions were not correlated to adoption of safety practices.

35.

## T REGULATORY CELLS AND DRAINING LYMPH NODE ENLARGEMENT EVALUATION IN MICE BEARING L-DGE LYMPHOMA AND ITS VARIANTS

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L-DGE is an immunoblastic T lymphoma that develops lymph nodes (LN) metastasis in a low frequency. Through directional selection processes two L-DGE variants were obtained: L-DGE/M and L-DGE/ L, with higher and lower growth rate and metastasis incidence than L-DGE, respectively. CD4<sup>+</sup>CD25<sup>+</sup>FoxP3<sup>+</sup> Treg cells are lymphocytes that inhibit T lymphocytes activation. With the aim of studying the enlargement of draining LN and their % of Tregs, Balb/c mice were s.c. challenged with L-DGE, L-DGE/M or L-DGE/L (Day 0). On day 14 mice were sacrificed, LN excised, weighted and a cell suspension prepared for Tregs quantification by flow cytometry. The % of LN Tregs in L-DGE/M bearing mice was lower than in naïve mice (p<0.05), whereas in L-DGE and L-DGE/L bearing mice Tregs levels were intermediate without differing from LDGE/M and naïve mice. The weight of LN in mice bearing L-DGE and its variants was higher than in naïve mice (p<0.01) and in L-DGE/M bearing mice, higher than in L-DGE and L-DGE/L (p<0.05). L-DGE and its variants would not utilize Treg expansion as a mechanism of tumor escape. LN tissue replacement by metastasis in L-DGE/M mice could account for the increased LN weight and decreased Tregs. In L-DGE and L-DGE/L bearing mice LN increase their volume during tumor evolution and the fact that on day 14 these tumors are only at 70 and 50% of the maximum tumor volume, respectively, could explain the absence of increase in Tregs.

#### 36.

# CHARACTERIZATION OF L-DGE LYMPHOMA AND ITS VARIANTS L-DGE/M (HIGH GROWTH RATE) AND L-DGE/ L (LOW GROWTH RATE)

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L-DGE is an immunoblastic T lymphoma that develops lymph nodes metastasis in a low frequency. Through directional selection processes two L-DGE variants were obtained: L-DGE/M and L-DGE/ L, with higher and lower growth rate and metastasis incidence than L-DGE, respectively. Our aim was to characterize variables related to tumor growth as the mitotic index (MI) and the vascular endothelial growth factor serum concentration [VEGF], the main proangiogenic molecule. Balb/c mice (n=5/group) were s.c. challenged with I) L-DGE/M, II) L-DGE and III) L-DGE/L and tumors were measured periodically. When reaching the maximum tumor volume ethically permitted on days 14, 20 and 25 for I, II and III, respectively, blood samples were taken. Mice were euthanized with CO<sub>2</sub>, tumors excised, Bouin fixed, paraffin embedded, cut and Hematoxilin-Eosin dyed. MI (mitosis nY/field) was evaluated in 50 fields. [VEGF] was determined by ELISA. MI (median [range]: I=0,68[0,4-0,75]; II=1,04[0,88-2], III=0,56 [0,28-1,56]) of I and III did not differ between each other but were lower than that of II (p<0.05). [VEGF] (pg/ml) in mice bearing I (median [range]: 48,05[41,93-93,65]) was higher than that of II (26,21 [20,62-32,50]) (p<0.05); III (35,47[7,95-51,02]) did not differ from I and II. MI was not related to growth rate; their peaks may be located at different time points. Additional factors as [VEGF], and others to be determined, would explain the differences in growth rate among the three lymphomas.

EFFECTIVENESS OF A CARE SYSTEM FOR THE HEAR-ING IMPAIRMENT USING MULTIVARIATE ANALYSIS Quaglino M, Vitelleschi MS, Maldonado L.

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The tracking of hearing problems in newborns offers the opportunity to reduce the average age of identification of the hearing loss. The early diagnosis of hearing loss significantly improves the development of language, cognitive, social and emotional abilities of the child. This paper aims at characterizing 3728 children aged 0 to 14 years, assessed from January 1997 to December 2006 by the Comprehensive Hearing Center of the Municipality of Rosario. To this purpose, we analyze together the variables: age at first consultation, diagnosis time, number of risk factors, diagnosis, year of admission and treatment condition. At the time of the study, 63% of the children treated had been diagnosed with normal hearing, 19% had some type of hearing impairment and 18% were in the process of diagnosis. Within the group of hearing-impaired (833 children) the 59% had perceptive hypoacusia, in the 39% it was conductive, and the 2%, mixed. The Multiple Correspondence Analysis identified two principal dimensions. The first dimension allowed us to explain the characteristics that distinguish the children with hearing loss versus the children with normal hearing. The children with normal hearing are associated with younger age at first consultation and the absence of risk factors. The operation years of the Centre are sorted from right to left, crossing the axis from the position of the hearing-impaired those with a normal hearing. This shows a favourable evolution of the Centre. The second dimension shows that the initial years of the Centre functioning are associated with the children of older ages in the first consultation and the latest years of the Centre with younger ages.

#### 38.

## CAMPYLOBACTER JEJUNI: 5 YEARS OF ITS ANTIBI-OTIC SENSITIVITY IN HOSPITAL ESPAÑOL DE ROSARIO

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Camplylobacter jejuni is recognized as causing human enteritis worldwide. Several studies have shown geographical differences regarding antimicrobial resistance, particularly to erythromycin. This is still considered the drug of choice in the outpatient treatment for adults and children. The aim of this study is to compare the sensitivity to antibiotics during the period between 2005 and 2009. We analyzed a total of 71 isolates collected from 976 adults and children with diarrhea. Antibiotic sensitivity test was performed according to CLSI 06 recommendations and cutoff value according to ANLIS C Malbrán against to: erythromycin (ERY), azithromycin (AZI), gentamicin (GEN), ciprofloxacin (CIP) and levofloxacin (LEV). The percentages of resistance to C. jejuni isolated from humans in that period, are shown in the table below. We found high resistance to fluoroquinolones and macrolides, two drugs that have been considered drugs of choice in adults and children respectively for those *Campylobacter* diarrheas that required antibiotic treatment. While the election in outpatients remains erythromycin, azithromycin has been shown to be an effective alternative because of its less resistance, daily dosing and fewer days of treatment. The isolates were mostly sensitive to gentamicin, which is especially suitable for extraintestinal cases.

#### 39.

# CARBON DIOXIDE CHAMBERPROTOTYPE: EUTHANA-SIA SMALL EXPERIMNTATION ANIMALS

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In places where experimentation animals are treated, it is priority to apply the 3 R's: Replace, Reduce and Refine. Refine refers to optimize the existent techniques to reduce or avoid animal pain and disturbance. Among the euthaniac methods used the ethylic ether inhalation was the most used, today disapproved by ethical and security rules. At present it has been proved that the use of carbon dioxide (CO<sub>2</sub>) in small size animals (rats, mice, etc) at an adequate concentration (70%) is more profitable because it is a safe gas and non irritable both for animals according and worker, In this study a chamber for small animals to International Standards has been designed. The material chosen for its building is polypropylene, a thermoplastic of high hardness and stiffness, light, resistant to chemical products, microorganisms, and water at high temperatures an being a recycling material that minimizes environmental impact. The predominant chape is an elongated cylinder, 13 cm diameter, 100cm long. The dimension, specified above, improve the animal position at inhalation time and reduce pieces (25 cm long each) with different shapes ("T" or "Y" among others) In its ends there are internal rubber rings that allow airtight coupling in the closure linds (free ends) there are metallic for hoses coming from the CO<sub>2</sub> cylinder. These movable lids make it possible to adjust the height of gaseous flow entrance (superior, lateral, etc). This is an object open in its final shape for adapting and refining the technique. It es still to be evaluated its output for possible modifications to get a better efficiency.

#### 40.

# **RELATION BETWEEN BODY MASS INDEX, FAT MASS AND LIPID PROFILE IN ADULTS**

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82 voluntary people between 30 and 50 years old of both genders were analyzed. Anthropometric measures: weigh, size, circumference of the wrist, circumference of hip and waist, brachial perimeter, triceps skinfold, biceps skinfold, subscapularis, suprailiac, and abdominal. A body mass index (BMI )was calculated, the risk of cardiovascular disease (RC) according to hips circumference and Fat Mass percentage (%FM). Results: Normal Weight (19%), overweight (%31), I grade obesity (%24), II grade (%11) and III grade (%15) calculated by the BMI. Overweight (%55), obesity I grade (%18), II grade (%8), III grade (%49) corrected by the BMI. As the BMI increases, the percentage of the FM increases as well with a linear correlation of Pearson=0,56 p=0.002 between both and 100% of the people valuated with obesity have increased the percentage of FM. Within the patients with normal BMI, 67% have an increased FM (>30%) with a correlation of Pearson=0.630 p=0.012. As regards the lipid profile, 23% of the total of the people who have been valuated have an elevated level of triglycerides in blood, 38% a level of the total cholesterol above the required value and 57% have a low level of cholesterol. The BMI correlated in a negative way with the Col HDL with a value of correlation of Pearson =-0.316 p =0.008. 63% of the patients with an elevated RC by the waits circumference have low values of ColHDL. From the obtained data we can observe that the increase of the BMI correlates just with the decrease of the ColHDL as regards the lipid parameters.

# KNOWLEDGE OF AGENTS OR INFECTIONS OF SEXUAL TRANSMISSION OF TWO CARRERS IN COLLEGE STUDENTS

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Evaluated the knowledge among students of 1st and 3rd year medical and social sciences on STIs by conducting semistructured interviews between March and May 2010 of a total population of 580 students. We analyzed data in Microsoft Excel. Of the total survey shows that 98.1% are mentioned in 1st place VIH, syphilis in 2nd (75.5%), hepatitis in 3rd (52%), gonorrhea in 4th (49%) and VPH the 5 th (44.6%). There were similarities in the responses cited by 1st year of the chosen careers, being that 3rd year of medical, the spectrum diseases was higher. Thirty-three people of 1st year of Social Sciences cited 2 or less STIs, while 28 people from 3rd year could not complete more than 2 STIs, 50% of the students of 1st year of medical school did not respond more than 2 STI while only 5 of 3rd year, completed 2 or fewer STIs. The number of students who knew only 2 or less decreased in 3rd year social sciences respect 1st year, while in medicine dramatically decreased from 50 to 5% at 1st and 3rd year respectively. The disease agents known to more than 33% of students were VIH, syphilis, gonorrhea and hepatitis. Less than a third of the students knew VPH or herpes, reaching only 1 or 2 students in each group mentioned Chlamydia or Trichomonas, many of them unknown even by sexual transmission, which reveals a serious lack even in the university level. this require planning a strong program of education by the political and university outreach.

#### 42.

# ANTIMICROBIAL RESISTANCE ANALYSIS OF URINARY TRACT INFECTIONS MOST FREQUENT AGENTS IN A HIGH COMPLEXITY HOSPITAL

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Urinary tract infections are among the most common infections worldwide. There have been no studies in our area in the last ten years, so it becomes necessary an epidemiological survey of the etiology and antimicrobial susceptibility (ATM). We studied 9633 patients hospitalized in the period 2004 to 2008, 1595 of them with documented urinary tract infection. Samples were cultured and identified by conventional methods (ASM Handbook). Sensitivity tests were performed according to CLSI recommendations. The most frequently isolated bacteria were E. coli (N:1042), K. pneumoniae (N:152) and Staphylococcus coagulase negative (SCN). In the SCN (N:151) a 10% resistance to oxacillin, a 10% resistance to trimethoprim-sulfamethoxazole, and 1 strain resistant to clindamycin and 3 to gentamicin were found. All isolates were susceptible to nitrofurantoin. There were 4 (11.1%) S. Methicillin resistant Staphylococcus aureus, 8 (25%) Pseudomonas aeruginosa resistant to carbapenems and 12 (37.5%) were resistant to ceftazidime. Three isolates of A. Baumannii (21.4%) were resistant to carbapenems and 12 (85.7%) to aminopenicillin + sulbactam. E. coli showed high resistance to trimethoprimsulfamethoxazole and aminopenicillins, low resistance to gentamicin, nitrofurantoin and fluorquinolones. Cefotaxime resistance was markedly higher in K. Pneumoniae. The SNA and S. Methicillin resistant Staphylococcus aureus were scarce.

#### 43.

# PREVALENCE OF PATHOGENIC BACTERIA ISOLATED IN SAMPLES OF LOWER RESPIRATORY TRACT

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We performed a retrospective study from 2004 to 2009 by analyzing data on microbiological isolation and characterization of the crop obtained from respiratory samples entered in the Service of Bacteriology. We excluded Mycobacterium tuberculosis and atypical mycobacteria. Biological samples were sputum, tracheal aspirate, washing broncheoalveolar, pleural fluid and pleural biopsy, processed according to methodology. Of a total of 420 samples received, 104 were positive, where 103 were Monomicrobial, while a sputum sample revealed the presence of 2. The total of 240 sputum 54 was positive, with the presence of 55 agents, being 27 (49%) non-fermentative bacilli, 14 (26%) Enterobacteriaceae, 9 (16%) S. aureus, 4 (7%) S. pneumoniae, 1 (2%) H. influenzae. The total tracheal aspirates was 51, being 28 positive, 13 (46%) Enterobacteriaceae, 12 (43%) non-fermentative bacilli and 3 (11%) S. aureus. The Bronqueoalveolar washes were 43, with 14 being positive, 7 (50%) non-fermentative, 5 (36%) S. aureus and 2 (14%) Enterobacteriaceae. Of 82 pleural fluid samples were positive only 5, 3 (60%) Enterobacteriaceae; 1 (20%) S. aureus and 1 (20%) S. pyogenes. From 4 lung biopsies, 3 were (100%) positive bacilli non-fermentative. With the clear inequality of the number in the different samples is shown in the sputum, lung biopsy and washing broncheoalveolar predominantly non-fermentative bacilli. In both samples of tracheal aspirate and pleural fluid prevail enterobacteriaceae.

#### 44.

## PRESERVATION OF AVIAN BLOOD FROM SLAUGHTER-HOUSES: EVALUATION OF DIFFERENT ADDITIVES

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The use of avian blood seems to be a useful alternative to the simple discarding of this by-product with high biochemical oxygen demand. Additionaly, blood can be used as raw material in both feed and food industries on the basis of its excellent nutritional value and functional properties. The main problem is the recovery and storage of blood from slaughterhouse until to processing. Different biotechnology strategies were developed with the aim to increase the product shelf-life at room temperature. The objective of this study was to compare the effect of different additives (used as biopreservatives) on plasma proteins. Three samples of avian blood from slaughterhouse with anticoagulant were taken and they were transported to the laboratory. Samples were divided into five equal parts for the different treatments: 1) non-incubated control blood (T1), 2) blood stored at refrigeration (4°C) (T2), 3) blood stored at room temperature (T3), 4) blood + ammonium citrate, beef extract, lactose, Na2HPO3 and NaH2PO3 (T4), and 5) blood + NaCl, beef extract, lactose, Na2HPO3 and NaH2PO3 (T5). T4 and T5 were stored at room temperature for 24 h. Plasma was obtained and the level of proteolysis were analyzed by SDS-PAGE. Differences in the protein degradation were observed according the treatment. T2 showed similar proteolysis than T1. In contrast, T3 and T5 showed significant protein degradation. Proteolysis was minimal in T4. These data suggest that T4 additives could extend the blood shelflife at room temperature for, at least, 24h.

# SELECTION OF HIDROGEN PEROXIDE-PRODUCING LACTIC ACID BACTERIA OF AVIAN BLOOD FROM SLAUGHTERHOUSE

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Blood can be used as raw material in both feed and food industries on the basis of its excellent nutritional value and functional properties. The main problem is the recovery and storage of blood from slaughterhouse until to processing. Therefore, it was necessary to develop different biotechnology strategies for blood conservation. Lactic acid bacteria (LAB) added to the blood can control the growth of several spoilage bacteria by the production of antimicrobial metabolites (lactic and acetic acids, hydrogen peroxide, reuterin and bacteriocins) produced during the fermentation. The objective of this work was to select hydrogen peroxide-producing LAB of avian blood from slaughterhouse which could be used in the future as biopreservative. Two-hundred LAB strains were analyzed to detect their inhibitory action against spoilage bacteria using the agar diffusion method. Thirty-one strains that produced inhibitory substances were detected and an assay to identify the hydrogen peroxide (MRS agar + 0.25 mg/ml tetramethilbenzidin + 0.01 mg/ml horseradish peroxide; incubation at 37°C for 48 h in anaerobic conditions) production was conducted. Ten from thirty-one strains were positive for hydrogen peroxide production, and only three were positive within the first 10 minutes of aerobic exposition. Approximately, 32% of tested LAB strains produced antibiotic effects by hydrogen peroxide production.

#### 46.

# PERCEPTION AND BEHAVIOR IN OBESE UNIVERSITY ADOLESCENTS

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WHO proposes the following BMI cutoffs for Overweight (Ow)  $\geq$ 27 & Obesity (Ob)  $\geq$ 30, that are useful in individual evaluation, but is well known that Non-transmissible Chronic Diseases risks increase from BMI-27. In presence of these facts adolescents implement conducts to cope with either Ow or Ob. Methodology: A cross sectional exploratory study, was carried on, through open & noninvasive anonymous questionnaires administered to 270 adolescents of public and private university institutions. BMI values were stratified in two categories before applying a personal interview. Results: Ow 10% (27) & Ob 4% (11). Adolescents consider Ob as a stigma that is repulsive & unforgivable. The most significant results are, the detection of the most frequent behavior patterns in the Ob group to tackle with this health problem & their perceptions on it. Data collection from a deep observation and analysis of "thorough description", lead us to determine that adolescents consider Ob as "immoral" since they believe that it is a psychological failure, an incapacity to tackle with a charge of offered election possibilities. They feel guilty and blamed, proposing as a solution physical activity and dieting. Only 3 walk and 1 goes to a gym. All the adolescents admitted adherence to diet composed of "healthy food", that enable them to belong to a peer group in which the only acceptable esthetic model "is to be slim". So far, they do not adhere to a healthy food model. Conclusion: We are able to disclose their necessity to cope with a shameful Ob in this age period of life and its "hyper homogenization". Acceptance of diets to lose weight, as part of a life style, shows a clear cut pattern of eating disorders, close related to a slim model perception to avoid discrimination by their peers.

## 47.

# CLASSIFICATION OF THE eSS AND eSMT DIABETIC NEPHROPATHY ACCORDING TO PATHOLOGICAL CRITERIA

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eSS and eSMT diabetic rats develop lesions of nephropathy. In eSS, it has been reported uremia, increased 24 hours proteinuria and decreased creatinine clearance. According to optical microscopy study made on eSS ( $n = 68 \circ$  and  $68 \circ$ ) and on eSMT ( $n = 50 \circ$  and 74 Q) between 3 and 26 months, the most common glomerular lesion was a mild (E+) or a strong (E++) diffuse mesangial expansion. The % of affected glomeruli was positively correlated with age (E+: eSS ñ= 0.873, p= 0.000. eSMT ñ= 0.689, p= 0.000 and E++: eSS  $\tilde{n}$ = 0.832, p= 0.000. eSMT  $\tilde{n}$ = 0.863, p= 0000). Diffuse glomerular sclerosis was observed in elderly individuals of both lines in whom the number of small glomeruli increased with age (eSS  $\tilde{n}$ = 0.774, p= 0.000; eSMT  $\tilde{n}$ = 0.641, p= 0.000). According to a new classification for diabetic nephropathy (C Tervaert et al. J Am Soc Nephrol, 2010), eSMT and eSS rats less than 12 monthold would be in Class I ("not very evident mesangial alterations") and those over one year old, in Class II ("diffuse mesangial glomerulosclerosis"). It is interesting to note that while some rats over 18 months could be classified in Class IV ("advanced glomerulosclerosis"), only one 18 month-old eSMT male showed a lesion compatible with Class III ("at least one node of Kimmelstiel and Wilson" or "nodular sclerosis"). Further studies on these lines could be useful for studying the pathophysiological mechanisms of nodular and diffuse diabetic nephrosclerosis.

#### 48.

# EMPLOYMENT OF INDICATORS TO DETECT OBESITY TRENDS IN MALE AND FEMALE eSS DIABETIC RATS FROM 4 TO 6 MONTHS OF AGE

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The eSS rats spontaneously develop a complex type 2 diabetic syndrome with higher expression in males. In these animals the weight, influenced by the nutritional environment and age, is also a risk factor for the intensity of metabolism. In order to identify possible trends to obesity, a longitudinal study was carried out in 14 males and 16 females at the ages of 4 and 6 months, kept under the usual conditions of breeding. Body weight (BW), length of tail, nasoanal length (NL) and total length were registered and the Lee index (LI: BW/NL) and the BMI (BW/NL<sup>2</sup>) were calculated. Related-sample Student's t-test (between ages) and Student's t-test for independent samples (between sexes) were used. Values of the indicators significantly increased with age in both males and females. At 4 months, males displayed higher values of NL (Q: 18.9±0.6 cm vs. °: 19.2±1.2 cm; p= 0.01), LI (Q: 0.26±0.01 vs. °: 0.28±0.01; p= 0.014) and BMI (q: 0.36±0.02vs or: 0.44±0.04; p= 0.014). At 6 months, males reached higher values of BW (Q: 188.7±9.3 g vs. °: 225.6±23.3 g; p= 0.000), LI (Q: 0.27±0.01 vs. O: 0.29±0.03; p= 0.026) and BMI (Q: 0.41±0.02 vs. O: 0.45±0.04; p=0.001). IL was always below 0.30, considered the cut-off point for obesity. Compared with results in Wistar rats, eSS rats showed similar values of LI (0.26 to 0.30) but lower of BMI (0.45 to 0.60). According with the indicators employed, eSS rats do not develop obesity trends in the considered ages.

# A167

#### 49.

# **DENTAL PULP PROCESSED WITH COLLAGENASE TYPE II IN RATS WITH NORMAL AND CARIOGENIC DIETS** *Kohli A*<sup>1</sup>, *Pezzotto SM*<sup>2</sup>, *Poletto L*<sup>2</sup>, *Dávila H*<sup>2</sup>.

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The dental pulp is a soft conjunctive tissue which constituents are disposed at well defined areas like Weill basal zone, that in normal pulp is poor in cells. Cariogenic diets are important risk factors. Objetive: to compare structural changes in rats dental pulp according to nutrition characteristics. Ten T inbred rats 12 weeks old were divided in two groups: one with a normal diet and the other with a cariogenic diet with a 10% sacarosa solution in water ad livitum. Rats were sacrificed at 7 and 14 days, maxilar and jaw 1°, 2° and 3° molars were lens examined, stained with a Rodamine solution to detect oclusal caries. Maxilars and jaws were surgically separated, fixed in formol at 10%, submitted to demineralization, and treated with type II collagenase. Then, all of the pieces were paraffin-embedded, cut in histologycal sections of 5µ, and stained with hematoxylin-eosin. At 7 and 14 days both rats groups were examined looking for caries and Weill area infiltration. In the treated group a higher caries proportion, a higher vessels size variability and a higher infiltrated Weill area proportion were found with no statistical differences.

# 50. FRACTION OF MESOPARIETAL SHORTENING IN THE CAT

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The systolic function of the left ventricle is traditionally evaluated by endocardic shortening rates as shortening fraction (FA) and ejection fraction (FE), however, these rates can overestimate the systolic function when the ventricular geometry is altered as in the concentric hypertrophy. The aim of this work is to obtain values of this rate in cardiologically healthy animals to be applied on pathologies developing concentric hypertrophy. Twenty cardiologically healthy cats of distinct ages and breeds were studied. A SonoSite Titan ecograph with 5 to 8 multifrequency transductors was used. Imaging was obtained from the right parasternal window. Measurements were made by M Mode on the short axis at level of the tendinous chordae following the specifications of the American Society of Echocardiography. To calculate the mesoparietal shortening fraction (FAm) the following formula was used: FAm = (DDVI + h) - (DSVI + 2 a')/DDVI + h), where DDVI is the left ventricle diastolic diameter; DDVS is the left ventricle systolic diameter; h is the combined diastolic thickness (PPD + SIVD/2), and a' is the mesoparietal point in systole. The data which were obtained showed that the mean represents values ranging from 20% to 22%, with lower values of 17% and higher ones of 25%.

# 51.

# IMMUNOEPIDEMIOLOGY AND PIG ZOONOSES CONTROL

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Zoonoses are a major public health problem and there are many factors involved in its maintenance. An immunoepidemiological study is being undertaken in order to bring knowledge to the prevention of zoonoses in pig farms of the Caseros Department, Santa Fe. The objectives proposed in 2009-2010 were to determine the rate of seropositivity to brucellosis and leptospirosis and characterize the epidemiological risk factors. Serological studies were conducted to determine the rate of seropositivity leptospirosis and brucellosis. An epidemiological survey was applied to producers as well as to employees. By using scalpel puncture 5 ml of blood/animal were obtained. Obteined sera were studied using serological BPA and MAT for the diagnosis of bovine brucellosis and leptospirosis respectively; according to standards established by the OIE. In 8 of the selected farms 1795 samples were obtained, being 12.5% (1/8) positive to brucellosis with a seropositivity rate of 38% and 100% (8/8) to leptospirosis: 2 farms presented seropositivity rate of 20%, 2 of 25% and the other 61%, 35%, 23% and 14% respectively. In 4 farms human brucellosis cases were manifested. Preliminary results show a significant association among leptospirosis seropositivity rate, the number of animals and the quantily of farms employees with a p <0.0001. Data analysis allowed to characterize the epidemiological risk factors of higher signification in zoonoses of studied farms and to further know the importance of complex. interactions among the environmental diverse components in a whole.

## 52.

# pH AND REDOX POTENTIAL IN CORRIEDALE RUMEN: ANIMAL EFFECTS AND SAMPLING TIME

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The use of in vitro systems for the study of the environment and fodder behavior in rumen presupposes the collection of ruminal content under controlled conditions. Knowledge of fluctuation ranges of the physic-chemical and biochemical parameters in in vivo rumen related with the redox intensity and capacity is important as a prior stage in in vitro studies of biochemical processes, such as ruminal methanogenesis, strongly associated to redox state. The aim of this work was to determine the effects of sampling time and animal on variables associated to redox state in *in vivo* rumen of Corriedale sheep. Three female sheep (A,B, and C) were rumen cannulated and fed pure alfalfa hay once daily (8hs). Samples of ruminal fluid (RF) were taken from each animal from 10hs to 16hs corresponding to 2, 4, 6, and 8hs after ingestion. pH and redox potential (ORP, mV) were determined by potenciometry. Simultaneously, RF was collected from a male Hampshire Down ovine (D) fed on the same diet twice daily (8 and 17hs). Results were analyzed by ANDEVA and LSD test, considering animal and sampling time effects. A, B and C animals did not show significant differences in PH value; animal D showed the lowest value though it differed significantly from animal C. No significant differences between animals or the studied times were observed for the redox potential. The work shows that the RF collected offers appropriate conditions and homogeneity for its use in ruminal in vitro studies and poses an interest for carrying out studies to explain individual pH variations.

# ASSESSMENT OF QUALITY OF IMMUNOLABELING OF TWO ANTIBODIES BY MEANS OF DIFFERENT IMMUNO-HISTOCHEMICAL PROTOCOLS

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The aim of this study was to assess the quality of immunolabeling of two antibodies against lymphocytes on mouse's lymph nodes by means of different immunohistochemical protocols. To address this issue, we used samples fixed in 10% formalin, processed by routine histological methods, and embedded in paraffin. Antibodies against T cells (CD3) and B cells (CD79ácy) were used. During the development of the immunohistochemical technique, only the secondary antibody and the antigen recovery buffers were modified, whereas other variables or steps were kept constant. Though the cells immunolabeled as well as its location were the expected, clear differences in the intensity of staining and background were observed, according to the modified variable. Qualitatively, the best IHQ is one that provides intense and clear immunostaining, without background. According to our observations we can conclude that: when antigenic recovery with heat under pressure in Tris-EDTA buffer, pH 9.0 was performed the best results were obtained using EnVision+<sup>™</sup> polymer as secondary antibody. On the contrary, when antigenic recovery in 0.1 M citrate buffer, pH 6.0 was performed, the greatest immunostaining was obtained using ADVANCE<sup>™</sup> HRP to CD3 antibody and, Avidin-Biotin Complex to CD79ácy. For all antibodies the optimal immunolabeling was obtained using a protocol not recommended by the manufacturer. These results strengthen the importance of comparing protocols at the time of standardizing the use of any antibodies in the laboratory.

#### 54.

# PORCINE CIRCOVIRUS-2 ASSOCIATED WITH PORCINE PARVOVIRUS AND INTESTINAL PARASITES

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Porcine Circovirus-2 caused Porcine Multisistemic Wasting Syndrome (PMWS); coinfection with Porcine Parvovirus (PVP) exacerbates the clinic of PMWS. PVP infection is transmitted by feces. Immunosuppressed pigs suffering PMWS are more susceptible to parasites. The aim was to detect associations between PVP, intestinal parasites and PMWS. Pig feces from 44 farms with (25) and without (19) PMWS were investigated; PMWS diagnosis had been based on clinical symptoms and anatomopatology, histopatology and immnunohistochemistry results. Fecal supernadant was obtained from each sample and PVP was investigated using hemaglutination test (HA); inhibition of HA test (IH) was used to cofirm identity of PVP. Fecal samples were processed using sugar floatation and direct microscopy to detect Nemathelmintos, Apicomplexa, Sarcomastigophora and Ciliophora. PVP were detected in 17/25 and in 12/19 samples from farms with and without PMWS respectively. Balantidium coli, Trichuris suis and helminth eggs were detected in 7/25 and in 5/19 feces from farms with and without PMWS respectively. Associations between PMWS-PVP, and PMWS-intestinal parasites (p<0.05) were detected. Association between PVP and PMWS has been detected previously in other countries. HA was efficient to detect PVP in feces. Pigs with PMWS would be affected by intestinal parasites although feed with antiparasitic drugs. The most frequently diagnosed parasite was Balantidium coli.

55.

# BEHAVIOUR OF TOTAL MICROFLORA IN A SYSTEM COMPOSED OF SOIL SUPPLEMENTED WITH ACETATE AND HYDROQUINONE

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Hydroquinone (HQ) is part of the derivatives of agrochemical industries, of oil, textiles, leather, pharmaceuticals, etc. It is considered an environmentally toxic compound. Its degradation produces simple carbonated compounds such as acetate by an oxidative route and hydrogen peroxide and free radicals by a reducing route. It is not well known how it behaves in complex microbial environments such as the Argiudol soil employed in this work, nor is it well known how it behaves at the level of a microbial environment. In this work the effect produced by the supplementation of increasing doses of HQ (100-400 µg.g<sup>-1</sup>) and acetate (Ac, 1 mg.g<sup>-1</sup>) (treatments HQAc), and without acetate (treatments HQ) were studied. Control soils were those with the aggregate of just Ac (Ac) and with soil without aggregates (S). The soil dehydrogenase activity was studied, and it was observed that the number of heterotrophic bacteria followed a decreasing order: HQAc> Ac> HQ>S. The redox intensity of the system by redox potential (Eh) showed that HOAc treatments had a decreasing order in the ranges: 270 and 0 mV, treatments HQ between 330 and 265 mV, Controls Ac:  $310 \pm 2$  mV and S  $324 \pm 4$ mV. Results show that (i) the increase observed in the number of bacteria was associated to the higher availability of carbon in the system; (ii) according to the Eh values and to the dehydrogenase activity it is suggested that the two carbonated sources worked by increasing the reducing capacity of the system.

#### 56.

## EVALUATION OF INFECTION WITH ARGENTINEAN A178 STRAIN OF BOVINE HERPESVIRUS 5 IN EXPERIMEN-TAL RABBIT MODEL

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Bovine Herpesvirus 5 (BoHV-5) induces neurological signs in the experimental rabbit model. Therefore, this experimental animal is successfully used to study biological characteristics of the different strains. The aim of this study was to analyze the behavior of the BoHV-5 A178 Argentinean strain in the experimental rabbit model to determine whether the strain may be useful to use in the proposed model for evaluating the effectivity of antiviral drugs. The A178 strain was propagated and titrated by Reed and Muench method in Madin-Darby Bovine Kidney cells and then stored at -70°C. The 22 old days New Zealand rabbits (n=5), were inoculated intranasally after local anesthesia with lidocaine 10%. The inoculum (500  $\mu$ l of viral suspension of 10<sup>6</sup> CCID <sub>50%</sub> / 50 $\mu$ l) was introduced into each nostril using a disposable pipette. When animals showed clinical signs they were sacrificed in extremis and samples from lung and brain were taken and conveniently processed for histopathological and virological studies. All infected animals showed neurological signs at 8-11 days pos infection and brains showed nonsuppurative encephalitis characteristic of BoHV-5 infections. We conclude that A178 strain was highly neuropathogenic, predictable in its effects on the experimental model proposed and potentially useful in future studies to evaluate the effectivity of antiviral drugs.

#### **METHANE PRODUCTION IN RUMEN BY PASTURES AND ITS RELATIONSHIP WITH CHEMICAL COMPOSITION** *Smacchia A, Ronzano P, Figallo R, Perotti E, Faienza H, Pidello A. Laboratorio de Química Biológica. Facultad de Cs.Veterinarias. CHUNR JUNR O, Lagos y ruta* 33 (2170) *Casilda E mail*

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With the aim of studying CH<sub>4</sub> production in different pastures and its relationship with their chemical composition, pastures from Casilda, Santa Fe were analyzed. Samples of Alfalfa (Medicago sativa) AA; Melilotus (Melilotus alba) MA; White clover (Trifolium repens) WC; Forage Oats (Avena sativa) FO; Cebadilla (Bromus unioloides) CB; were taken. Samples dried at 60°C, grounded and sieved were incubated in vitro in rumen in closed systems 24h at 39°C. Final gas and CO<sub>2</sub> and CH<sub>4</sub> concentration were measured by gas chronomatography. CH<sub>4</sub> produced was from DDM (degraded dry matter) in rumen at 24h incubation in sacco (CH<sub>4</sub>l/kg DMISD<sub>24h</sub>) and in l/kgDM was expressed. Chemical composition was measured by AOAC: Organic Matter: OM, Non-structural Carbohydrates: NSC, Lignine: LIG. Raw Protein: RP; Neutral detergent Fiber NDF and acid detergent fiber ADF. The results were studied by correlation and regression. The following variables were correlated: Gas (ml/g/24h) with NDF (r:-0.906 p $\leq$ 0.033) and with ADF (r:-0.9809 p $\leq$ 0.0032). CH<sub>4</sub> l/kg/ DM with NDF (r:-0926 p $\leq$ 0.025) and with ADF (r:-0.999 p $\leq$ 0.0001); CH<sub>4</sub>l/kg/DMISD<sub>24h</sub> with OM (r:0.9 p $\leq$ 0.03), con NDF (r:-0.84 $\leq$ 0.07) and with ADF (r:-0.94 p $\leq$ 0.017). FO and CB showed higher values of NDF and ADF and produced less CH<sub>4</sub>. MA showed intermediate values in all variables except in OM which was the highest. AA and WC produced more gas and CH<sub>4</sub>, presenting lower NDF and ADF than the others. It is concluded that gas and methane production were inversely correlated with the NDF and ADF concentration, indicating fractions of the chemical components (cellulose, hemicelluloses, lignine, silica) present in the cell wall of vegetables.

#### 58.

# EVALUATION OF THE METABOLIC ENERGY OF WHOLE DRY DOG FOOD FOR ADULT CANINE FEEDING

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The aim of this work was to define the percentage of commercial foods that comply with the metabolic energy (ME) requirements of adult dogs. Twenty-eight whole dry foods commercially available in stores in Casilda and its surroundings were studied. The centesimal composition described by the producer, the ME in the food by ME (Kcal/100g) = (3.5\*proteins) + (8.5\*lipids) +(3.5\*carbohydrates) as determined by the NRC in 1985 was considered. Based on this, the quantity of food necessary to satisfy the ME requirements for the different body weights as stated by the producer was estimated. These values were compared with those given in the pack. Results showed that of all 28 products, only 14% provided adequate EM quantities, while 86% had inconsistencies with the estimated values. Of all the products evaluated, 65% indicate the ME in their pack, and among them only 33% specify that the ME is expressed in dry matter. Thirty-five % of all products show a lower value to 3100 Kcal/Kg.-the lowest value suggested by the AAFCO (America Association of Officers for Feeding Control) for adult dog diets not to be considered a "low calories" food. Both the excess and the deficit of ME may be a predisposing factor for some diseases as well as mislead the veterinarian in seek for a diagnosis based on weight disorders. It is concluded that in most cases it is necessary to regulate the ration suggested by the producer.

# 59.

# RUMINAL GAS PRODUCTION IN CORRIEDALE OVINE: EFFECTS OF ANIMAL AND INCUBATION TIME

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The aim of this work was to study RF behavior in Corriedale sheep and to determine the effects of incubation time and animal on the gas production of RF incubated in vitro. Four sheep were studied: 3 Corriedale (A, B and C) and 1 Hampshire Down (D). All four were rumen cannulated and fed pure alfalfa hay once a day (8h). Ruminal Fluid was collected from each animal 2h after ingestion. Samples were filtered (0.2mm mesh) and incubated in triplicates in closed ruminal systems with 30ml RF at 39°C for 24h. Simultaneously, a system was charged with a mix of RF and alfalfa hay at 1%p/v (ABCD+AH). Gas production was recorded (ml) at 0.5; 1; 2; 4; 6; 12; 20 and 24 h and the pH and redox potential (ORP mV) at the beginning and end of incubation. Results were analyzed with ANDEVA and LSD Test, separating the animal and incubation time factors. Significant differences were observed in gas production except between 0.5 and 1 or 20 and 24h and among animals. RF from animal D produced lower gas quantity followed by A, C (ABCD+AH) and B. RF of animal B produced the highest gas quantity, even surpassing the AH system. The final pH was lower than the initial and showed no significant differences between animals. It is concluded that the RF obtained shows an adequate behavior for its utilization in in vitro systems, requiring the formation of pools to save individual differences. Moreover, further studies tending to account for individual differences in ruminal gas production are suggested.

#### 60.

# BENZNIDAZOL INHIBITS PROLIFERATION OF CELLS LINES DERIVED FROM ACUTE MYELOID LEUKEMIA

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The clinical outcome of acute myeloid leukemia (AML) is extremely variable, surviving only 20 to 30% of patients; these results demand the development of new treatment strategies. We have shown that Benznidazole (BZL), a compound with well documented trypanocidal activity, possesses anti-inflammatory properties and inhibits the nuclear factor  $\kappa B$  (NF- $\kappa B$ ). We have also shown that BZL led to growth arrest in different cell lines, which is mediated as well by NF-kB inhibition. Our aim was to evaluate if BZL is capable of inhibit cell proliferation of THP1 and OCI/ AML3 cell lines derived from AML. Results: BZL induced a significant reduction in DNA synthesis assessed by [3H]-thymidine incorporation 24 h after treatment (p < 0.05), whereas the reduction in metabolic activity (MTT reduction assay) was observed at 48 h (p < 0.05). With regard to cytotoxicity showed no significant differences in LDH activity of supernatants of control cell cultures and treated with the drug, however there was a small increase in apoptosis even though this was not sufficient to explain the detention level observed in cell growth. Conclusion: BZL inhibited cell proliferation without causing a cytotoxic effect on these cells. The growth arrest observed in these cells by BZL could be valuable in the treatment of AML.

# HISTOPATHOLOGIC FINDINGS IN TWO MOUSE LINES OF THE CBI/IGE COLONY DIFFERING IN RESISTANCE TO *Trichinella spiralis* (*Ts*)

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Lines CBi/C (C) and CBi/L (L) show a differential response when infected with Ts. During the enteric phase, the number of adult parasites and the Ts female fecundity decrease in L (P<0.01) but not in C (P>0.05). In the chronic stage, the parasitic load is significantly smaller in L than in C (P<0.01). Our aim was to determine whether this different susceptibility could be explained, in part, by a different host tissue response in the enteric phase of a primary infection with Ts. Adult mice of both sexes, infected with two L1 Ts larvae per g of body weight, were sacrificed on days 6 and 13 post-infection (pi) (n=14 per line and day of sacrifice) and intestine, lung and heart were excised and prepared for histological examination. The results of the histopathologic analysis concur, in general, with each line's resistance to Ts infection. L showed a higher number of mice with intestinal inflammatory signs. The early inflammatory reaction observed in the lungs of L mice, as opposed to C, supports the hypothesis that this organ could be an important site of retention and destruction of newborn Ts larvae (NL), since NL travel through the microvascular lung system in their way to the striated muscle were they finally encyst. The presence, on day 13 pi, of small lymphoid foci in the heart of 30% of C mice and none in L animals (P=0.052, Fisher's exact test) also suggests that C mice are more susceptible to infection with Ts.

#### 62.

## VARIATION IN BLOOD EOSINOPHILS IN TWO MOUSE LINES OF THE CBI/IGE STOCK DIFFERING IN RESIS-TANCE TO CHALLENGE WITH *Trichinella spiralis*

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One of the immune responses characteristic of tissue helminth infections is eosinophilia. The aim of this study was to analize the possible differences in blood eosinophils (BE) in two lines of mice differing in resistance to Trichinella spiralis (Ts), during the evolution of a primary infection. Adult mice of both sexes from lines CBi/L and CBi/C (n=36 per line) of the CBi/IGE colony were used. Mice were infected with two L1 larvae per g of body weight and sacrificed on days 6, 13 and 30 post-infection (pi). BE was measured in May Grunwald-Giemsa stained blood smears before Ts challenge (control) and on days 6, 13 and 30 pi. The parasite load (PL) in the chronic phase (30 days pi) was measured by counting all the larvae encysted in the tongue. No differences were found associated to sex in any of the variables studied. CBi/L mice showed a significant decrease in BE on day 6 pi (P<0.01) followed by an increase on day 13 pi (P<0.05) when compared with the control value. On the contrary, CBi/C mice exhibited a sustained decline in BE as the infection progressed (P<0.01). CBi/L had a significantly lower PL than CBi/C [median (range); CBi/L: 10 (5-40); CBi/C, 43 (7-109); P=0.0024]. Since eosinophils take part in the destruction of Ts newborn larvae (NL) in their passage through the lung, the eosinophilia found on day 13 pi in CBi/L would be in part responsible for the low parasite load characteristic of this genotype. Conversely, the low BE observed in CBi/C mice would allow a greater number of NL to complete their life cycle and encyst.

63.

## MODIFICATIONS IN THE SERUM LEVELS OF CREATINE KINASE AND LACTATE DEHYDROGENASE IN TWO MOUSE LINES OF THE CBi/IGE STOCK DIFFERING IN RESISTANCE TO INFECTION WITH *Trichinella spiralis*

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Trichinella spiralis (Ts) is a nematode parasite with a complex biological cycle that makes use of more than 100 mammals, including man, for its evolution. The larvae deposited by the Ts females on the small intestine mucosa travel through blood and/or lymph to the skeletal musculature where they encyst, leading the parasitosis to chronicity. Encystation produces partial destruction of the muscle cells, thus releasing cytoplasmic enzymes to the blood. Our aim was to determine the variation in serum activity of the muscle enzymes lactic dehydrogenase (LDH) and creatine kinase (CK) during the evolution of a primary infection with *Ts*, in two lines of mice differing in resistance to the parasite. Adult mice of both sexes from lines CBi/L and CBi/C (n=25 per line) of the CBi/IGE colony, were used. Mice were infected with two L1 larvae per g of body weight and sacrificed on days 6, 13 and 30 post-infection (p-i). Serum samples were analyzed for LDH (U/l) and CK (U/l) using a comercial kit (Wiener lab, Rosario, Argentina). Serum levels of LDH (mean  $\pm$  SEM) in the acute stage, (6 and 13 days - L, 2358 $\pm$ 752, 2818 $\pm$ 409; C: 1563 $\pm$ 324, 1717 $\pm$ 214) were significantly higher than in the chronic stage (30days - L: 1516±291; C: 852±84) (P<0.05); contrariwise, CK did not change its mean value as the infection evolved (P>0.05). Though the increase in LDH was observed in both genotypes, the difference between the acute and chronic phases was higher and more significant in C (P=0.001) than L (P=0.028). These results agree with each genotype's resistance to Ts.

## 64.

# EVOLUTION OF TH1/TH2 INTERLEUKINS IN A MURINE MODEL OF SPONTANEOUS CARCINOGENESIS. ANALY-SIS OF THE PRENEOPLASTIC STAGE

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The murine mammary tumor virus (MMTV) is a heritable somatic mutagen that has very few targets; its tumorigenic capacity is generally restricted to the mammary gland whereas it can be found in a wide variety of tissues as an infectious agent. Selection of variants of immune escape as well as inefficient elimination of the virus in susceptible mice may affect immune responsiveness. Since the immune system is also an essential regulator of tumor progression, our aim was to study changes in serum cytokines in the preneoplastic stage, in a susceptible line of the CBi/IGE murine model of spontaneous mammary carcinogenesis. Line CBi+ possesses an exogenous MMTV variant and all the females develop mammary tumors with a median tumor latency of 240 days. The serum levels of IFN-y, IL-2, IL-4 and IL-10 were measured with an ELISA at 160, 200 and 240 days of age (n= 6 per age group). IFN- $\gamma$  and IL-2 had very low or undetectable levels in the period studied. IL-4 had a tendency to increase (0±SEM, pg/ml; 3.8±0.6, 21.9±15.7, 27.8±18.1) while IL-10 peaked on day 200 (22.4±6.5,  $50.8\pm24.8$ ,  $25.2\pm11.0$ ). The magnitude of the response was scarce in the period examined; small changes were observed mostly in IL-4 and IL-10, usually associated with tumor progression. These results were observed during preneoplasia, a finding compatible with the recent hypothesis that the immune system is also capable of inducing tumor promotion in the early stages of carcinogenesis. The low levels of Th1 type interleukins, specially IFN- $\gamma$ , could explain in part CBi+ susceptibility to MMTV infection since resistance to this retrovirus is dependent on IFN-y production.

# **RELATION BETWEEN DEGREE OF HYDROLYSIS AND HYDROLYZED BETA-LACTOGLOBULIN DETERMINED BY ELECTROPHORETIC MEASUREMENTS**

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Enzymatic hydrolysis of bovine whey proteins, whose main component is beta-lactoglobulin ( $\beta$ -LG), is used in order to obtain food additives with increased emulsifying and foaming properties. At pH 8.0,  $\beta$ -LG presents a conformational transition with the subsequent exposition of sites sensitive to protease attacks. A stock 25 mg mL<sup>-1</sup>  $\beta$ -LG solution was prepared in 50 mM phosphate buffer at pH 8.0. This sample was then submitted to trypsin hydrolysis (1/167 enzyme/ substrate ratio) at 37°C. Aliquots were withdrawn at 0, 5, 15, 30, 60, 120, 180 and 240 minutes of hydrolysis time. The reaction was stopped by heating (5 min at 85°C). Electrophoresis in polyacrylamide gels (SDS-PAGE) was carried out using a stacking gel of 10% and a resolution gel of 15%. The digitalized image of the respective stained gels was analyzed using specific software developed by our research group. Remaining  $\beta$ -LG was determined and expressed in percentage related to non-hydrolyzed sample (0 minutes). Hydrolyzed B-LG was obtained by difference. Trimethylbencenosulfonic acid method (TNBS) was applied to determine the degree of hydrolysis (DH) of the different samples. The results obtained showed a linear relation between DH (%) and hydrolyzed  $\beta$ -LG (%). The statistical analysis showed the following parameters: 0.127 (p<0.005) for the slope and -7.0 x 10<sup>-8</sup> (p<0.005) for the intercept, with a determination coefficient, R<sup>2</sup>, equal to 0.9986. It can be concluded, that it is possible to determine DH by electrophoretic measurements.

#### 66.

# IMPACT OF THE CYOSTORAGE TEMPERATURE INCREMENT ON EXTRACELLULAR MATRIX OF HUMAN CARDIAC VALVES

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Cryopreservation of human cardiac valvular allografts (HVAs) could alter the extracellular matrix. So, we studied the effect of a cryopreservation temperature increment (100°C) on HVAs collagen and elastic networks. We compared fresh  $(C_N)$ , cryopreserved  $(C_{Crvo})$ and cryopreserved with temperature increment ( $C_{\Delta T100}$ ) HVAs. Histological studies: HVAs were fixed in 10% PBS-formaldehyde and processed for paraffin embedded. Pieces of 5 µm were coloured with Direct Red/Picric Acid (DR/PA) and DR/Fast Green (DR/FG) to study collagen network. Elastic network was studied with orcein. We have also estimated the total amount of collagen: a- spectrophotometrically, using DR/PA and thawed HVAs. Pieces of valves (10-80 mg) were incubated with DR/PA. The dye was extracted with NaOH-Methanol and the absorbance was measured at 540 nm. Units of Absorbance/valve tissue mass were correlated; b- using image analysis: 16 images were taken of 3 slices from each group (DR/PA). With Adobe® Photoshop 6.0, collagen fibers were selected, and the area occupied by them was estimated with Scion Image. Results were expressed as density of area occupied by collagen. We observed abundant collagen in  $C_N$  with DR/PA, and fewer collagen type I in  $C_{Cryo}$  and its absence in  $C_{\Delta T100}$  with DR/FG. Elastic network was mainly damaged in  $C_{\Delta T100}$  Total collagen amount was significantly higher in  $C_{Cryo}$  than in  $C_{\Delta T100}$ . We concluded that cryostorage temperature increment induces damages on HVAs' elastic contract of the second se tic and collagen networks.

67.

# SERODIAGNOSIS OF TOXOPLASMOSIS AND CHAGAS DISEASE IN DOGS FROM CASILDA AND SANDFORD

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Our country and our province were affected by global climate and socioeconomic changes (migration of people and animals, ie), leading to exposure to comunicables diseases. Determining dog health condition contributes to the epidemiological control. The aim of this study was to determine the presence of Trypanosoma cruzi and Toxoplasma gondii antibodies in sera of dogs from Sanford and Casilda. Students and teachers conducted supportive professional practice involving diagnosis, prevention and zoonoses control in animals. Veterinarians of Cátedra Clínica de Animales de Compañía attended 71 dogs (males and females aged 2 to 5 years old), made epidemiological records and obtained samples. These dogs were clinically healthy. The sera were processed in Parasitology Area, Fac Cs. Bioq. y Farm. ELISA with recombinant antigens of T. cruzi was used. No sera were found positive for this parasite. Toxoplasmosis was diagnosed by indirect hemagglutination, with and without 2 mercaptoethanol (ME). On 71 tested sera, 24 had antibodies to this parasite (33.8%) with a title of 1/64 (19 sera) and a title of 1/256 (5 sera). 2 ME-treated samples showed a lower title than those without ME, indicating IgM. Presence of Toxoplasmosis antibodies in dogs indicated raw meat and vegetables ingestion, contaminated water in the urban areas. The absence of Chagas disease antibodies indicated no infected vectors. Zoonotic diseases reduction in animals, through prevention and controls is one of the pillars of public health.

#### 68.

# BIORHEOLOGIC AND HEMOAGLUTINANT ACTION OF EXTRACTS OF *Phyllanthus sellowianus* BARK

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Phyllanthus sellowianus Müll Arg. (Sarandí blanco) is a bush we can find in humid and warm regions of our country. It grows in damp grounds, by rivers and brooks. It has been used since colonial time in treatment of Diabetes disease. The study of its action mechanism is important to consider it for future therapeutic uses. Vegetal extracts can induce some biologic activities such as agglutination in human and animal erythrocytes and malignant cells. We have studied the biorheologic and hemoagglutinant action in vitro of different extracts of Phyllanthus sellowianus bark on human erythrocytes. It has been prepared three kinds of extracts: Maceration (BM); Controlled Digestion (BD); Decoction (BC). pH of solutions was 7,4 and osmolarity about 300 mOsm. The quantity of proteins was measured. The erythrocytes were incubated in vitro with the BM, BD and BC extracts at 37°C for 60 minutes and washed with PBS solution. The biorheologic parameters (deformability, membrane surface viscosity, elastic modulus and dynamic viscolelasticity) were determined with an Erythrodeformeter. Immunohematological assays show agglutination in human ABO blood group system. The hemorheologic action changed in BM, BD and BC extracts, related with the work conditions employed for the extract preparation. These results are useful to study the action mechanism of extracts from Phyllanthus sellowianus bark to be considered as therapeutic agent in Diabetes, and they test its hemocompatibility with ABO system and its agglutinant power.

#### 69

# EVALUATION OF GENOTOXIC EFFECTS OF TREAT-MENTS IN BREAST CANCER PATIENTS

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This study evaluated the damage in lymphocytes' DNA by the Comet assay (CA) in cells from healthy donors and patients with breast carcinoma, before (PRE) and after (POS) the first cycle of treatment. In addition, a cutoff value for basal DNA damage in PRE was proposed in order to predict the risk of more severe genotoxic damage due to treatments. Samples were obtained from patients with breast carcinoma (PRE = 54; POS = 24) and healthy donors (n = 15). For the CA, lymphocytes were separated by Percoll gradient, included in 1% agarose gels, and subjected to electrophoresis under alkaline conditions. Comets were stained with ethidium bromide and observed in a fluorescence microscope. The CASP program was used to obtain % of DNA remaining at the head of comets (% DNA). For the visual analysis comets were classified into 5 types. Statistical analysis was performed by the student's t test. A value of p < 0.05 was considered significant. The % DNA was significantly higher in PRE than in POS samples (62.0  $\pm$  2.6% vs. 38.9  $\pm$  3.2%, respectively, p < 0.001). The difference between % DNA of samples from healthy donors and from patients PRE was not statistically significant. Based on data from healthy donors and samples PRE, a cutoff value for % DNA of 65% was considered to classify the samples (% DNA < 65% or % DNA  $\ge$  65%). Considering this value, the PRE samples were divided into 2 groups and the averages of % DNA in POS samples from each group were significantly different (49.2  $\pm$ 4.4% vs.  $30.3 \pm 3.1\%$ , respectively, p < 0.01). The results support the usefulness of the CA to monitor the genotoxic damage of the anti-tumor treatment. The CA could contribute to assess the type of treatment for each patient according to the level of initial damage in their DNA, to reduce the excessive genotoxic damage.

Partially financed by a grant from F. Fiorini Fundation.

#### 70.

# TEXTURAL ANALYSIS OF IMAGES OF SODIUM CASE INATE ACID GELS USING CONFOCAL MICROSCOPY

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The sodium caseinate (NaCAS) is an ingredient widely used by the food industry because of its nutritional and functional properties. Acidification performed a gel structure as a result of dissociation and aggregation of casein fractions. In previous studies the results confirm that the degree of compaction and size of the gel pores depend on the gelation rate, which is connected directly with the added amount of gluco-delta-lactone (GDL). The slower the gelation process, there is more possibility of rearrangement of interactions in the mesh of the gel, and therefore, it becomes more compact and smaller pores. In the present study, images of the sodium caseinate acid gels stained with Rhodamine B at different concentrations of GDL were obtained by confocal microscopy. A program specifically developed for us was used to analyze the texture of such images. The images were normalized by transforming it to a grayscale and then were taken to a whole number format of 8 bits. In its numerical representation studied the Shannon entropy, smoothness, variance of gray scale and uniformity parameters were obtained as estimators of the texture of the images. From the results, we conclude that the entropy and uniformity are image parameters that more accurately represent the structural changes that arise in the inner of NaCAS acid gels related to the degree of compaction and pore size.

71.

# SURFACE CHARGE AND CELLULAR AGGREGATION KINETICS BY LASER BACKSCATTERING

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The decrease in anionic charge on the erythrocyte membrane involves a decrease in intercellular electrostatic repulsion, improve the ervthrocyte aggregation. In vascular diseases, the components of the erythrocyte membrane surface, would be altered thereby affecting interactions, with subsequent vascular complications. In order to model in vitro these alterations, we studied the relationship between the decrease in erythrocyte anionic charge (EAC) by the effect of trypsin studied by laser backscattering (Erythroagregameter) in 6 blood samples from donors healthy. EAC was also determined by the method of distribution in aqueous biphasic systems, binding dyes and digital image analysis (ASP) and the distribution of sizes aggregates. The results obtained showed a decrease in red blood cells EAC from treaties related with untreated. There was a significant decrease (p < 0.0001) in the time required to reach 50% of the maximum aggregation with increasing concentration of trypsin, which correlated linearly (R = 0.99026) with the EAC measured, indicating a decrease in electrostatic repulsion. Trypsin treated RBCs showed irregular amorphous aggregates as reflected in the altered levels of ASP, significantly higher (p <0.001) in the treated samples compared to the untreated sample. The methodology demonstrates the loss of EAC by trypsin treatment which modeled the changes in the cell membrane that could be seen in vascular diseases such as hypertension.

# 72.

# THE FAMILY *DIOSCOREACEAE* IN THE PROVINCE OF SANTA FE (ARGENTINA)

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The Family Dioscoreaceae belongs to the Class Liliopsida (= Monocotyledoneae) and is currently included in the Order Dioscoreales, recently segregated from Liliales sensu lato. It comprises about 630 species that inhabit tropical and subtropical regions, whose centers of diversification are Mesoamerica, Indochina, and Madagascar. They are generally climbing geophyte herbs, glabrous or pubescent. Leaves simple, alternate and flowers actinomorphic, unisexual. Several species are cultivated for their great economic importance as food for their tubers and some have important medicinal properties. In Argentina it is represented by a single genus: Dioscorea L. with 31 species. This is an introduction to the knowledge of this family and its geographical distribution in the province of Santa Fe. The methods consist of a bibliographical review, consultation of national herbaria with important collections of the province (SF: Esperanza; SI: San Isidro; UNR: Zavalla), field work experience of the authors and lab work to confirm their identity. The preliminary results show that the genus Dioscorea L. is represented in Santa Fe for only one species: Dioscorea microbotrya Griseb. with only one collected specimen. It is known as "yaco", "sandía del monte" o "alpa sandía". Its tubers are often used as a water reservoir for long journeys. Taxonomic information, distribution map and illustrations are provided.

# LEARNING NUTRITIONAL CONTENTS IN THE COURSE OF BIOCHEMISTRY IN SECOND YEAR STUDENTS OF MEDICAL SCHOOL

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Nutrition is a basic requirement of the individual's health as it has relevance to his life and the environment. The Biochemistry contributes to the acquisition of knowledge, skills and abilities on nutritional aspects, energy expenditure, biological adaptations in the different stages of healthy human being and his environmental influences. The aim was to determine whether students had knowledge of the nutritional contents after an interdisciplinary seminar integrated by health specialists. An anonymous survey was applied to 37 students, before and after a seminar on nutrition in a private university in Rosario. Each student was identified with a single number at both times. The survey developed consisted of 5 questions of yes - no answers under the slogan: You know. The questions were referred to: 1)calculating the daily energy requirements, 2)contribution of calories from the macronutrients; 3) caloric formula and vitaminic value of foods; 4) suitable distribution of foods and 5)average chemical composition of foods. The processing of data was done using a nonparametric test, Mc Nemar's. We used SAS statistical software package. The positive answers before and after the seminar in the first question were 10.8% and 89.2%, 2nd 35.1 and 64.9%, 3rd 2.7% and 97.3%, 4th 45.9% and 54.1% and 5th 24.3% and 75.7% respectively (p<0.001). We concluded that the seminar had a positive impact on students learning nutrition contents, which proves the hierarchy of interdisciplinary activities to strengthen learning.

#### 74.

# VARIATION IN WATER FLUORIDE (F) CONTENT IN SANTA FE PROVINCE THROUGH TIME AND PLACES OF SAMPLING

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F is a common component in tap water. In concentrations lower than 1 mg/l no side effects are present and it is beneficial for dental health. In concentrations higher than 2 mg/l sings of fluorosis appears. Previous studies showed that Santa Fe region have an adequate F content of water. The objective of this work was to evaluate changes in well water F content (WF) in Santa Fe province through time and regions. F was measured with direct potenciometry and results are express in mg/l. The results were compared with a measurement made in 1942. Changes in WF through time were evaluated using a correlation between 1942 and 2010. Variation of WF in the same region was analyzed measuring WF from wells of three regions (Venado Tuerto (VT), Carlos Pellegrini (CP) and Pueblo Ester (PE)). Wells location was found using positioning tools. A significant correlation between 1942 and 2010 was obtained (r=0,52; p<0.05 n=40). WF was significantly higher in 1942 than 2010 (Students't test, n=20, p<0.05): in 1942: 1,27±0,71 and in 2010: 0,69±0,71. WF of the same region differed significantly (ANOVA, Newman Keuls posttest, p<0.05) CP=Well1:0,30±0,03; W2:0,16±0,01; W3:0,11±0,01; PE=W1: 0,14±0,02; W2:0,36±0,03; W3:0,65±0,03; VT=W1:1,02±0,09; W2: 1,34±0,05, W3:1,19±0,08: W4:1,12±0,01; W5:1,12±0,01. Conclusions: no accumulation of WF was observed through 60 years. This has to be proved since 1942 methodology is unknown and WF differs in different wells of the same region.

75.

## TERRITORIAL ANALYSIS OF PALMAR ARTERIAL ARCHESDISTRIBUTION

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There is a deep palmar arch (DPA) and a superficial palmar arch (SPA). The arches are formed by radial and ulnar arteries anastomosis. In this paper we propose the evaluation of the irrigation pattern, through the identification of the palmar arches, number of branches arising from it and determination of the diameter of the vessels found. 36 fetuses hands were used. Results: complete DPA were found in 45.21% of the cases and complete SPA in 94.12% of the sample. Four constant collaterals where found in the DPA and a fifth accessory branch was present in 15.78% of the cases. Four constant collaterals where found in the SPA and a fifth accessory branch was present in 76.47% of the cases. The average diameter and the standar deviation (SD), measured in mm, were: DPA:  $0.68 \pm 0$ , 18; SPA:  $0.96 \pm 0$ , 32. The average branches diameter and SD were: DPA branches:  $1.04 \pm 0, 04$ ; SPA branches:  $0.31. \pm 0.07$ . Anastomoses between both arches were identified at the 4th interosseous space level and between the radiopalmar branch and the 3rd DPA branch. It was concluded that the SPA has bigger diameter than the DPA, and a greater number of collaterals bunches, due to this fact it would had greater responsibility in the irrigation pattern of the palm.

76.

# MORPHOMETRIC STUDY OF THE LYMPHOID COMPARTMENTS FROM THE RABBIT APPENDIX

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Cecal appendix is the major lymphatic organ in the digestive tract of the rabbit, with an immuno-inductive function. In its, lymph follicles with predominant B cells intercalated with T cell areas can be detected, covered by a simple epithelium (FAE) which contains lymphocytes. The aim was to quantify the B and T cell areas and the intraepithelial lymphocytes, when antigen was administered subcutaneously, in a different way from the normal oral route. Twelve New Zealand rabbits were divided into four groups (G). G1: control. G2: adjuvant only. G3: sensitized with ovalbumin (OVA) in incomplete Freund adjuvant (FA). G4: sensitized twice with OVA in incomplete and complete FA. Samples were examined using an image analysis software and results were expressed as mean  $\pm$  standard error. Statistical analysis was performed using the ANOVA test, considering significant p<0,05. T cell areas showed in G1: 133.354,9±9.138,7 µm<sup>2</sup>; 150.055,5±16.844 µm<sup>2</sup> in G2; 196.661,8±22.643 µm<sup>2</sup> in G3 and 285.359,9±24.200 µm<sup>2</sup> in G4. B cell areas were: 473.472,1±26.619 µm<sup>2</sup> in G1; 540.830,9±22.968 µm<sup>2</sup> in G2; 569.935,2±30.456 µm<sup>2</sup> in G3 and 576.759,6±35.528  $\mu$ m<sup>2</sup> in G4, while the FAE lymphocytes: 166,33±12,41 in G1; 168,6±11,38 in G2; 129,5±7,87 in G3 and 123,83±8,70 in G4. We conclude that the subcutaneous immunization may induce both arms of the immune response, predominantly the cellular one. This may be due to the adjuvant used. The systemic route promotes the decrease of the intraepithelial immunocompetent cells indicating a certain compartmentalization in the local immunity.

# MORPHOMETRIC ANALYSIS OF CYSTIC AND COMMON BILE DUCTS AND ITS CLINICAL AGREEMENT

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Intrahepatic bile ducts gather together to conformed the hepatic ducts in the liver hilum, the right and left hepatic ducts, which join and give origin to the main bile duct, which is called the hepatic duct; the later gets the cystic duct that drains the accessory bile ducts and together conform the common bile duct, a terminal segment that run pages in the digestive tract by the second duodenum portion. For this reason, morphometry of bile ducts in our medio will be studied using dissection tecniches. Assessment of the cystic and common bile ducts morphometry is proposed by determining the length, lumen diameter and orifices and termianles. The study was done at the Anatomy Museum. Umlauts and divulsion surgical instruments were required. Samples of 38 adult viscera were used. The ducts to be measure were identified by simple dissection and divulsion, transversals incisions were done at the duct axis to find the lumen. Results: The average measurements and standard deviation (SD) were expressed in mm: cystic duct length  $34.1 \pm$  SD.4.68; common bile duct length  $69 \pm$  DE.11.2; cystic duct lumen 3.4± SD 0.32; common bile duct lumen 5± SD 0.5; cystic duct terminal orifice  $2.8 \pm SD 0$ , 17; common bile duct terminal orifice  $3.3 \pm$  SD.0.25. Conclusions: the lower lumen diameter of all the ones taken in consideration are the cystic and common bile ducts terminal orifices, so it can be infer that they are the places of grater impaction of gallstones.

#### 78.

# FIRST PROGRESSES IN THE ANALYSIS OF PHYSIOLOGI-CAL INTERMALLEOLAR DISTANCE VARIATION OF CHILDHOOD

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The intermalleolar distance (ID) is subjected to normal modifications which are dependent on psychomotion development and walking start.. Evaluation of ID anatomofunctional evolution in kids is proposed, consisting on:to analyze the relationship between intermalleolar distance and walk, to assess the participation of the osteomioarticular apparatus in the physiological variation of the intermalleolar distance and to analyze the possibility of using this parameter as a tool for the early detection pathologies. Children of both sexes between the ages of 12 months and 10 years. The average (A) and standard deviation (SD) of the Internal ID was obtained, with the following results: for children between 1-3 years A 3.83 / SD.1.33, between 3-7 A. 4.21 / SD 1.39, between 7-10 A. 3.49 / SD 2.37. We concluded that the ID constituted a measurement that should be considered in the future as a useful anthropometric measure representing the maturation of the locomotive system. Data obtained shows: normal ID evolution in children population. The normal physiological valgus is more pronounced between 3 and 7 years.

#### 79.

# EXERCISE EFFECT ON RESPONSE TO EXOGENOUS INSULIN IN RATS WITH TYPE I DIABETES MELLITUS (TIDM)

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TIDM is a disease that affects a great amount of people and it is controlled maintaining glucose levels within normality. Exogenous insulin injection is a common treatment and its dosage follows empiric models. Insulin injection combined with exercise is beneficial but it might produce hypoglycemic episodes. Mathematical models for these values are very necessary, there is one that adds exercise, but there are no data to establish when both practices are recommended. The objective of this work was to evaluate the best time separation between exercise and daily insulin dosage in rats. Female IIM/Fm subline "l"rats with TIDM induced by intraperitoneal injection of 20mg aloxane/100g bw were used. After TIDM induction, animals were divided in two groups of 4 animals. Group A received 2 U/day of slow insulin subcutaneously at 12 AM. Group B received the same dose of insulin and run in a treadmill at 4m/min for 30 minutes, at 8 AM. After 4 months glycemia and insulinemia from 8 AM until 2 PM were determined. Results are shown in mean±sd. Group B controlled glycemia better than A, but values were not significantly different. Glycemia (mg/dl): group A: 477±271; B: 447±198. Insulinemia (pmol/L): A: 974±704, B: 947±358. Other treatments were evaluated: 1-insulin injection and then exercise produced hypoglycemia, 2- exercise and then insulin injection decreased glycemia in both groups, but group B controlled it better. Conclusion: controlled exercise 4 hours before injection of insulin improves glycemic control in TIDM rats.

#### 80.

# EVALUATION OF A THERAPY WITH ZOLEDRONATE (Z) AND MONOFLUOROPHOSPHATE (MFP) ON BONE MINERAL DENSITY (BMD) IN OVARIECTOMIZED (OVX) RATS

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Osteoporosis is a disease characterized by low bone mass and increase in fracture risk. Several therapies have been tried combining antiresorptive and anabolic agents. The aim of this study was to evaluate densitometrically the effectiveness of combined use of Z (antirresortive) and MFP (anabolic) to recover bone mass diminished by the effect of OVX. Thirthy-two 70-day-old Sprague Dawley rats were subjected to OVX. After 30 days, the animals were subjected to different treatments 1) Controls (C), 2) MFP: 40 umol/100 g bw/d per os, 3) Z: 1.5 ug/kg/w sc, 4) MFP+Z: 40 umol/ 100 g bw/d per os + 1.5 ug/kg/w sc. Eight rats were sham operated. After one month rats were euthanized, the left tibia was obtained and BMD determined by X-ray absorptiometry. Total, trabecular and cortical BMD was measured and the % of change in controls vs. sham group and controls vs. treated groups were calculated to assess the effect of OVX and treatments respectively.% Atotal BMD: C:- 7.3±1.9<sup>a</sup>, MFP:8.7±3.9, Z:9.5±1.8<sup>b</sup>, MFP+Z: 11.6±2.8<sup>b</sup>, % Δtrabecular BMD: C: -7.9±2.8<sup>a</sup>, MFP: 15.8±4.7<sup>b</sup>, Z: 17.8±5.6<sup>b</sup>, MFP+Z: 20.7±4.6<sup>b</sup>, % Δtrabecular BMD: C: -11.2±3.1<sup>a</sup>, MFP:  $6.1{\pm}4.1,$  Z: 12.9±4.2<sup>b</sup>, MFP+Z: 9.7±5.5, <code>^ap<0.05</code> vs sham, <code>^bp<0.05</code> vs controls, mean±SE, Student's t test for a single sample. These results indicate that the recovery of BMD is predominantly the consequence of an increase in cortical BMD. The effect of the combined treatment with MFP and Z on BMD did not differ from the results found with the drugs administered individually.

# A175

#### 81.

# EFFECT OF DIETARY CALCIUM ON INTESTINAL ALKALINE PHOSPHATASE ACTIVITY IN THE RAT

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Intestinal alkaline phosphatase (IAP) is an enzyme located in the brush border of enterocytes and its physiological role is unknown. IAP binds calcium (Ca) and changes its activity and structure. IAP expression is regulated by vitamin D, suggesting that might be involved in Ca absorption. The aim of this study was to evaluate Ca absorption and IAP activity in rats fed with different Ca content in the diet. Line  $\beta$  rats of 21 days were fed for 30 days with different Ca content: 0.2 g% (n=9), 0.6 g% (n=5) or 0.9 g% (n=9). Ca intake and Ca excretion in feces were measured, and the percentage of Ca absorption was calculated. The rats were sacrificed and the duodenum of each rat were fixed in 10% formalin and histological sections were prepared by conventional histological procedures with the peculiarity that the sections were exposed for 30 minutes to 5-bromo-4-chloro-3-indolyl phosphate to detect IAP. Digital images were obtained and optical density (OD) of the compound formed by the action of IAP were determined with a special software. The results are expressed as median (range) and differences were considered significant when p<0.05 (Kruskal-Wallis; Dunn post-test). The % of Ca absorption was lower in the group receiving more Ca [0.2 g% = 93.7 (70.6-98.0),0.6 g%= 87.3 (86.6-89.8), 0.9 g%= 60.8 (55.8-73.1)\*]. IAP activity was higher in the diet contained more Ca [OD: 0.2 g%= 0.15 (0.14 to 0.21), 0.6 g%= 0.25 (0.15 to 0.29), 0.9 g%= 0.27 (0.18 to 0.36)\*]. These results support the hypothesis that high calcium content increase in IAP activity, and as the consequence of the latter, the fraction of Ca absorption is reduced.

## 82.

# EFFECT OF A THERAPY WITH MONOFLUOROPHOSPHATE (MFP) AND ZOLEDRONATE ON CORTICAL BONE IN RATS Brance ML, Brun LR, Arias L, Rigalli A.

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Zoledronate (Z) decreases bone remodeling and MFP increases osteoblast activity. The treatment of ovariectomized rats (OVX) with MFP, Z, low and high calcium (Ca) diet produced an increase in bone mineral density (BMD) similarly to rats fed with a normal Ca diet. On the contrary, histomorphometric analysis showed an increase in trabecular bone in rats fed with low and high Ca diet group when compared with rats receiving normal Ca diet. This difference could be due to changes in cortical bone. Therefore, the objective of this work was to study the effect of sequential treatment with MFP and Z on the cortical bone. Male Sprague Dawley rats of 7 weeks (n=5/group) were OVX and treated with MFP (400µmol/Kg.day) by orogastric tube from days 30 to 90 and Z subcutaneously (1,5µg/Kg.month) from day 91 to 150 in combination with diet containing different levels of Ca (low Ca diet 0.2% from day 0 to 30 and high Ca diet 2% from day 31 to 150 [T3 group] or normal Ca diet [T4]. There were an untreated group [T2] and a sham group [T1]. Tibias were obtained, a cross section of 2mm at 50% of bone length was made, photographed and analyzed with a software. The results are expressed as mean±SD. Cortical diameter (mm)=  $T1:0.14\pm0.01$ ;  $T2:0.13\pm0.01$ ;  $T3:0.14\pm0.01$ ; T4:0.14±0.01; Cortical area (mm)= T1: 0.9±0.06; T2: 0.87±0.08; T3: 0.91±0.02; T4: 0.86±0.05, p<0.05 ANOVA. It is concluded that the treatment of OVX rats with MFP, Z and different dietary calcium does not change cortical bone. This results could explain the lack of differences in BMD observed in rats treated with Z and MFP with and without changes in Ca content of the diet.

#### 83.

# COMPARISON OF TWO ASSAYS FOR THE MEASURE-MENT OF PLASMA GLUCOSE LEVELS

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Glycemic control in diabetes mellitus (DM) is a common laboratory practice. Blood glucose levels can be determined by spectrophotometry, but sometimes it is impossible to wait the time it takes to know the value and it is necessary to measure it in seconds, for example with the use of test strips. Is common the use of the latter in emergency rooms and experimental works where decisions must be taken in minutes. There is no comparative study of precision and accuracy of both methods. The aim of this study was to evaluate the measurement ranges, accuracy and precision of a spectrophotometric technique based on glucose oxidase (A) and a set of test strips (B). Accuracies were evaluated by 6 measurements of a 100mg/dl glucose standard with Students't test for a sample (mean±SD). A:105±12, B:164±77, values did not differ from the standard value (p>0.05). The precision was evaluated with the coefficient of variation, A:CV=11%, B:CV=47%. Plasma glucose levels were measured in diabetic rats with both methods. When it was necessary, appropriate dilutions were made to measure with A. With B that possibility was impossible and we obtained a measurement range of 35-500 mg/dl. A significant correlation was found between both assays (r=0,82) p<0,01, n=40). We conclude that the spectrophotometric technique has higher accuracy, precision and allows blood glucose measurement at any range of values. In contrast, the technique based on test strips has the advantage of allowing the measurement of glucose in less than 30 seconds, which is a requirement for making a decision in therapies with insulin, hyperinsulinemic euglycemic clamp experiments and closed loop experiments.

#### 84.

# CARDIAC CONDUCTION SYSTEM APPROACH AND BIOMETRIC ANALYSIS OF THE DISTANCES BETWEEN HISS' BUNDLE AND THE VALVE APPARATUS NEXT TO IT Boglioli AR\*, Calgaro GC\*, Cabral ME\*, Francisquelo RD.

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The distance between the Hiss' bundle and the cardiac valves are meaningful to professionals working on these structures, given the closeness between them. Techniques and anatomic features useful to approach the "Conductionis Cordi System", and establishing the distances between the His' bundle and the valvular apparatus are proposed in this paper. 46 pork hearts were used and 37 fresh human hearts. The atrinector segment was dissected, using the irrigating right-ascendant atrial arteries as anatomic features for the approach. We arrived to the atrioventricular portion through the arcuate streak, second-pillar of the right ventricle. Results: The sinus node was identified in 100% of cases, through the dissection of the first ascending atrial artery in 69.23% of hearts and the 2nd ascending atrial artery 30.77%. The atrioventricular segments elements were identified, in 94.52%. The average distances between the His' Bundle (HB) and the aortic valve were determined as 17.81 mm  $\pm$  1.32 SD (standard deviation); 19.2mm  $\pm$  2.35 mm (SD) between HB and the mitral valve; and 10.96 mm  $\pm$  2.76 mm (SD) between HB and tricuspid valve. Only human hearts data was considered. We conclude that the arcuate streak and atrial arteries are valid anatomic features for recognizing the "Conductionis Cordi system". The tricuspid valve is the closest to the HB; the mitral valve is the most distal to the HB.

# CD44 EXPRESIONEN IN EPITHELIAL CELLS OF ORAL LESIONS

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The CD44 protein is involved in normal cell functions. It has been described in some neoplastic tissues is overexpressed and appears with different isoforms, suggesting that is involved in tumor generation. The aim of this study was to investigate by confocal microscopy, the expression of CD44 protein in epithelial cells obtained from saliva samples from patients with oral lesions. We studied 28 patients with various oral lesions (benign, pre-cancerous and cancerous), and a control group (n = 32) who had no alterations. We worked with saliva samples subjected to thermal shock and washed with phosphate buffered saline (PBS). They were concentrated by centrifugation., Then 106 cells were incubated with anti-CD44 antibody suitable dilution for 30 min at room temperature. After washing with PBS, it was incubated with secondary antibody labeled with allophycocyanin (APC). Parallel internal controls were processed for each sample. The different cell suspensions were washed with PBS and observed by confocal microscopy (Nikon C1) using 639 nm red laser. The results obtained showed fluorescence corresponding to the presence of CD44 protein in samples from patients diagnosed with cancer and precancer. A higher intensity was observed in individuals with a pathological diagnosis of squamous cell carcinoma. In contrast, samples from patients with benign lesions showed no fluorescence images as samples of the control group. These findings indicate that overexpression of CD44 molecule analyzed could be considered as a marker of risk in individuals with oral lesions.

#### 86.

## SELF-MEDICATION PREVIOUS MEDICAL CONSULTING IN THE GENERAL GUARD OF THE HOSPITAL PROVIN-CIAL DEL CENTENARIO

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Self-medication (SM) is define by the use of drugs without a previous consult with a health professional and includes not only over-the-counter (OTC) medicine but also under prescription drugs. This abusive use of medicine represents a serious and alarming problem for the society. SM in our country is consider the most frequent way of bad use of drugs and it is probably the most dangerous one. Thousands of people die in Argentina because of the inappropriate use of medicine. Two risk factors have been established: SM and the polypharmacy (simultaneous intake of 6 or more drugs). Many times the shopping of medicine is done in an illegal way. The aim of this study is to determine the prevalence of SM in patients who intake drugs without the doctor's advice before concurring to the Hospital Provincial del Centenario of the city of Rosario, Argentina. A descriptive and transversal research was leaded. 200 patients chosen at random were included in the study. People participated voluntarily in the investigation. SM was detected in 72% of the population studied and no significant differencies were found between men and women. Women were the ones with the major percentage of SM. The most common ages of SM ( 80%) were between 50 and 90 years (p = 0.0001). Among the reasons for consulting the most common complications were: abdominal pain (14%) and multiple trauma (12%). The drugs more often used were: Ibuprofen (38.88%), Paracetamol (13.70%), Diclofenal (6.85%) and Amoxicillin (5.48%). We conclude that the group of drugs used more frequently in SM, previous consultation in the General guard, was that of non-steroidal antiinflamatorioa showing a great difference with other medicines. The fact that these drugs are the most employees can have a direct relation with the type of inquiries served at the General guard. Another finding is the frequent SM associated with amoxicillin and also with Ergotamine, the latter being toxic in high doses.

87.

# THE ROLE OF ALCOHOL CONSUMPTION IN THE PROGRESSION TO FIBROSIS IN CHRONIC HEPATITIS C INFECTION

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Hepatitis C virus (HCV) infects approximately 170 million people worldwide and it is consider as the main cause of chronic hepatitis, cirrhosis and hepatocelular carcinoma. Numerous investigations have been carried out in order to define the principal determining factors of disease evolution. The progression of the histological damage caused by HCV varies between different patients and the factors associated with it are not totally known. The aim of this study was to correlate the severity of histological damage in patients infected with HCV and alcohol consumption. We performed a cohort study from a voluntary testing campaign conducted in the Gastro-enterology Department at the Hospital Provincial del Centenario in the city of Rosario, Argentina. Blood transfused patients before 1993 were called and 1699 of them assisted to the hospital. Among those 1699, 61 (3.6%) were HCV Ac (+) / ARN HCV (+) and 40 (65%) of them underwent a liver biopsy. The population of these research belongs to those patients to whom the METAVIR score. Patients were categorized in two groups: Low Grade (F0-F1) and Intermediate/ High grade (F2-F3-F-4). Alcohol consumption > 20g/dl in womens and > 40g/dl in men was set as a criterion. Our results show that 25 patients (62.5%) had a low grade of fibrosis: F0=8 (20%) and  $F_{1=17}(42.5\%)$ . The 15 remaining showed and intermediate/high grade of fibrosis:  $F_{2=6}(15\%)$  F3=8 (20%) F4=1 (2.5%). In low grades, an 88% (22) of the patients did not drink alcohol and the other 12% (3) did. In (22) of the patients that hot hit and hot hit and bound 12.00 (1) find the patients were drinkers and a 33.3% (5) were not. Those patients whose alcohol consumption was >20 g/dl in women and >40 g/dl in men were included in the intermediate/high grade group. On the contrary, when alcohol consumption was <20 g/dl in women and <40 g/dl in men, low grades of fibrosis were observed. (P < 0.0001). In these research the consumption of alcohol >20 g/dl in women and >40 g/dl in men probed to be a risk factor associated with the progression of liver fibrosis to intermediate/high grades.

#### 88.

## ANALYSIS OF THE EVOLUTION OF THE 6D-SIMPLEX OF PHOTOMETRIC SERIES OF ERYTHROCYTES INCUVATED IN GLUCOSE MEDIUM

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High levels of glycosilation on membrane proteins on diabetic patients induced hemorheologic alterations. The main object of our work was to study the possible relationship between the 6D-simplex corresponding to red blood cell samples and the alterations produced while incubation in vitro with different percentage of glucose. The characterization of the cellular behaviour was made on the basis of fractal approximation for ordinary Brownian motion (oBm) and factionary Brownian motion (fBm.), as the data contain both deterministic and stochastic components. We have the time series but we have not the differential equations for the process so it had to be modelled by a trajectory in phase space. Using delay coordinates we generate two different phase space with the creep and the recovery process to be used in the calculation of the nonlinear parameters. Human blood was obtained from 10 normal controls. Each suspension was divided in 5 aliquots: 1 sample without treatment as control and the other 4, incubated in glucose medium (1%, 2%, 5% and 10%). The results showed significant changes when projecting the 6D-simplex on time. The correlation coefficient was obtained following Pearson and May algorithm: <C (w.t.)  $= 0.0364, <C^{(1\%)} >= 0.1525, <C^{(2\%)} >= 0.3327, <C^{(5\%)} >= 0.7587$ and  $\langle C (surrogates) \rangle = 0,0215$ . The glucose incubation would induce alterations on mechanical erythrocytes properties which are reflected on the nonlinear quantifiers. Finally this technique would be a good tool to analyse the mechanism of non enzymatic glycosilation and to understand the alterations in the microcirculation of diabetic patients.

# ACTIVATION OF *Escherichia coli* SUPEROXIDE RESPONSE REGULON IN ANAEROBIOSIS

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The response to superoxide in *E. coli* partially depends on the sensor SoxR, an iron-sulphur protein that oxidized, enhances transcription of the regulator SoxS, which in turn regulates expression of *soxRS* defense genes. Earlier proposals suggested that the *soxRS* regulon is able to sense the NADPH status, but the nature of the cellular signal sensed by SoxR is still unclear.

The hypothesis that NADPH levels affect deployment of the soxRS response was tested by transforming E. coli cells with genes encoding enzymes and proteins that lead to either build-up or depletion of the cellular NADPH pool. NADPH accumulation by NADP+reducing enzymes, such as malic enzyme or non-phosphorylating glyceraldehyde 3-phosphate dehydrogenase, led to inhibition of the soxRS regulon and enhanced sensitivity to superoxide. Conversely, SoxS expression can be induced by ferredoxin, a redox shuttle that can oxidise NADPH or by MV in anaerobiosis, under conditions in which NADPH is oxidised but no superoxide can be formed, resulting in execution of the soxRS response in the absence of oxidative stress. NADPH decline, favoured an increase in total (NADP+ + NADPH) stocks. The results indicate that NADP(H) pool is the signal for SoxR modulation and could act through the SoxR reductase activities, of which NADPH is electron donor. It is not clear if the anaerobic activation confers any physiological advantage.

#### 90.

# Cr<sup>IV</sup> KINETIC OXIDATION OF D-GALACTURONIC ACID

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Cr<sup>VI</sup>, a dangerous ion because of toxicity and carcinogenicity; is soluble in water and can be transport into cell by ionic transporters. In vitro studies demonstrate that should be a preponderant factor in chromium induced genotoxicity.  $Cr^{\nu}$  and  $C\hat{r^{i\nu}}$  species are propose as oxidation intermediates of several substrates in acidic medium, but the rate process and its dependence on the acidity has been determinate in a few cases. This work try to characterize theses parameters for CrIV oxidation of D-galacturonic acid, using the reaction between both species in specific experimental conditions  $([Cr^{IV}]=5.0x10^{-5}M, \text{ ionic strength}=1.0M, [O_{2}]=1.26x10^{-3}M, T=15^{\circ}C,$ [Substrate]=0.001-0.004M and [H<sup>+</sup>]=0.1-0.3M). Cr<sup>IV</sup> is generate in situ, with Cr<sup>II</sup> and O<sub>2</sub>, according to the fast reaction Cr<sup>II</sup><sub>(ac)</sub>+O<sub>2(g)</sub>  $\leq CrO_2^{2+} + Cr^{IV} + Cr^{III}_{(ac)}$  and oxidize the substrate, reducing it self into Cr<sup>II</sup> by a bielectronic process. Cr<sup>II</sup> is efficiently trapped by O<sub>2</sub> to give superoxochromium(III), CrO<sub>2</sub><sup>2+</sup>, which slowly decompose into Cr<sup>III</sup>. CrO<sub>2</sub><sup>2+</sup> has a characteristic spectra and the intensity change at 290nm can be follow in time. Corresponding graphics of Absorbance vs time were built with these data, observing that kinetic profiles correspond with a simple exponential grow. Experimental velocities constants,  $k_{obs}$ , can be calculated with a non linear fitting of data using the first order kinetic equation: Abs=Abs<sub>inf</sub>+ $(Abs_0-Abs_{inf})e^{(-k4exp t)}$ . Experimental data shows an inverse relation between oxidation rate and [H<sup>+</sup>]; possibly because of the existence of two different species of the substrate, in equilibrium: acid form and anionic form. At high H<sup>+</sup> concentration, predominates the first, less reactive than the other.

#### 91.

# INFLUENCE OF THE LIQUID CRYSTALLINE CHARAC-TERISTICS OF EMULSIONS OF OIL IN WATER ON ITS STABILITY, HLB AND RHEOLOGICAL PROPERTIES

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Objectives: In the medicine and cosmetics industries it continues investigated to increase the stability of the emulsions for obtain stable excipients to assure that the addition of active does not change the viscosity and it will not break the emulsion. In this work we consider as objective to increase the stability of emulsions prepared with stearic acid and triethanolamine, where it modifies only the amounts of stearic acid to achieve a proper HLB and liquid crystalline characteristics. Materials and Methods: We prepared six systems with stearic acid in increasing concentration where the molar ratio of triethanolamine stearate to stearic acid (average molecular mass 270) was between 1:0 and 1:2.5. The systems were prepared by adding the oil phase (stearic acid, mineral oil and propylparaben) to a mixture consisting of 4.14% of triethanolamine and some water by heating and stirring. After mixing, the mixture was diluted with the remaining water and the methylparaben to the total mass corresponding to 100 g. In the proposed systems were analyzed the following parameters: HLB, rheological profile, size and distribution of internal phase droplets, liquid crystals presence by polarizing microscope observation, secondary droplets (droplets aggregates surrounded by a laminar lyotropic structure formed by a surfactant, a longchain alcohol or a fatty acid and water), and stability to centrifugation for 30 minutes at 4000 rpm. Results: Calculated HLB values were between 21 and 8.7. Systems with stearic acid to triethanolamine stearate molar ratio of 1:1 behaved as pseudoplastic with thixotropy . Systems with much stearic acid showed no thixotropic plastic characteristics. Systems No. 3 to No. 6 had liquid crystals and secondary droplets with increasing values and size of internal phase droplets become smaller as they increased stearic acid concentration. The systems 1 and 2 showed phase separation. Systems No. 3, 4, 5 and 6 proved to be stable despite the systems 5 and 6 had an HLB low compared to that required (which is between 10 and 11), but the high viscosity, the presence of liquid crystals and of secondary droplets, contributed to stability. The best systems for their HLB, viscosity, and liquid crystal systems secondary droplets were No. 3 and 4. The presence of secondary droplets increased the stability of systems.

#### 92.

## STUDY OF HAEMOLYTIC AND AGGLUTINATED ACTIV-ITY OF PLANTS LECTINS

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Lectins are proteins of nonimmune origin capable of binding to carbohydrates, which can agglutinate cells or precipitate glycoconjugates. They can also be used as immunologic reagents to identify blood group antigens. The aim of this work was to study the binding and / or hemolytic activity of different seed varieties of legumes. We worked with different types of lectins plant derived from beet, flax, radish, parsley, radishes, carrots, red clover, Amaranthus sp. and Enterolobium contorticilicum. The seeds were pulverized to a fine powder and the obtained extracts were clarified and fractionated. We also controlled the osmolarity and pH of the extract prepared. Fresh human erythrocytes suspensions of blood groups A. B and O were prepared using EDTA as the anticoagulant, washed three times with buffer saline (pH 7.4), and resuspended as a 5% suspension. Hemoagglutinate and haemolytic activity of the crude extracts were determined by conventional immunological techniques in saline and bromelin and papaine medium. The results obtained by titulation of extract of Amaranthus hypochondriacus indicate that it is not specific for glycosylated antigens of the ABO system. The increase in the degree observed after enzyme treatment of erythrocytes is the result of a decrease in electrostatic repulsion favoring agglutination. These findings suggest the need for further studies on the activity of different extracts of lectins for use in the laboratory immunohematological

# DESCRIPTIVE STUDY OF ENZYMES INVOLVED IN OXIDATIVE STRESS IN CHAGASIC PATIENTS

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Few studies reveal the mechanisms that make a chagasic patient develop an irreversible severe disease or remain in the indeterminate phase for the rest of his life. The infected patient can remain asymptomatic until a critical mass of damaged heart tissue begins to show symptoms of irreversible myocardial injury. All living organisms support endogenous and exogenous oxidative stress having many enzymatic and non enzymatic antioxidants defense systems. The aim of this study was to carry out a descriptive study of the activities of enzymes involved in oxidative stress such as superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (KAT) in chagasic patients with heart disease (CconC n:20) and without heart disease (CsinC n:11) compared with healthy controls (CN n:55). The enzymatic activity was determined by spectrophotometric methods. The results were: KAT (K / gHb): CconC  $316 \pm 68$ , CsinC  $332 \pm 41$ , CN  $185 \pm 28$ ; GPx (U / gHb): CconC 98  $\pm$  17, CsinC 102  $\pm$  20, CN 61  $\pm$  11; SOD (USOD / gHb): CconC 3270 ± 333, CsinC 2590 ± 188, CN 895 ± 314. Enzymatic activities showed significant differences (p <0.001) between chagasic patients and normal controls. Trypanosoma cruzi infection could modify oxidative stress contributing with myocardial dysfunction.

#### 94.

# **LEAD REMOVAL FROM WATER BY ADSORPTION ON PRE - TREATED BIOMASS (Saccharomyces cerevisiae)** *Maulión E, Báez G, Ballerini G, Moro A, Delorenzi N, Busti P.*

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Water pollution with heavy metals is an important issue and ordinary methods to remove these contaminants are expensive. This situation has led to the development of new technologies. Bioadsorption is a property of microorganisms to bind metals and it can be improved trough physicochemical treatments. The use of dead Saccharomyces cerevisiae is an attractive alternative because: i) it does not need nutrients, ii) it is not affected by the contaminant toxicity and iii) it is an industrial fermentation waste. In this work,  $Pb^{2+}$  maximum bioadsorption capacity of yeast ( $q_{max}$ ) was optimized. Treated yeast were prepared by suspending cells in H<sub>2</sub>O<sub>2</sub> (pH 4.50) and heating for one hour at 3 atm. The conditions were established through a central composite design by the software Design Expert<sup>™</sup> 6.0. The temperature was ranged from 19.80 to 55.20°C and H<sub>2</sub>O<sub>2</sub> concentration from 1.01 to 199.99 vol. The yeast was washed with distilled water, dried and milled. Batch equilibrium was performed shaking 100 mL of Pb2+ solution with 300 mg of biomass during two hours. Initial lead concentration was ranged between 50 and 400 ppm. Remaining metal concentration was measured by the dithizone method. The parameter  $q_{max}$  was predicted by the Langmuir equation and the value of untreated biomass was 53 mg g<sup>-1</sup>. Surface response method generated a mathematical model that forecasted the  $q_{max}$  of treated biomass. It suggested that suspending yeast in  $H_2O_2$  30 vol at 50 °C would generate an optimal  $q_{max}$ . Batch experiment was assayed with that biomass and  $q_{max}$  value was 72.32 mg g<sup>-1</sup>. Therefore, Pb<sup>2+</sup> maximum bioadsorption capacity was increased 45% compared with untreated biomass.

95.

#### WEAK, PARTIAL AND NULL *RHD* ALLELES. IMPOR-TANCE IN TRANSFUSIONAL MEDICINE

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It is actually considered that patients with reduced expression of the D epitopes develop an immune response after a transfusion with RhD positive units. However, recent studies demonstrated that blood recipients with weak D types 1, 2 and 3 do not produce anti-D. The aim of this work was to characterize the allelic variants of the RHD gene in individuals from Rosario. We studied 18378 blood samples. The Rh phenotype was determined using monoclonal antibodies. Samples with reduced expression of the D antigen underwent molecular characterization. The presence of the RHD gen was investigated using a multiplex PCR strategy. The RHD exons were assessed by SSP-PCR. Weak D type 1, 2, 3 and 4, DVII, RHDø and r's alleles were investigated by PCR. We identified 67 (0.36%) samples with weak D expression and 1315 (7.16%) RhD negative phenotypes. By molecular analysis 16 RHD weak Type 1, 9 Type 2, 7 Type 3, 15 Type 4, 2 Type 59, 1 DVa, 1 DIVa, 16 DVII alleles were determined in the weak D samples. Among RhD negative samples 18 RHD null alleles were detected: 7 RHDø, 6 r's; 4 hybrid alleles RHD-CE(3-9)-RHD; 1 RHD(361del11) and 4 alleles responsible of DEL phenotypes: 3 RHD46(T>C); 1 RHD(M295I). A correlation between the complete Rh phenotype and the different alleles found was observed. The serologic techniques do not precisely determine the different weak variants of the D antigen. The molecular characterization of these alleles would be a useful tool for the selection of Rh compatible units in Blood Banks.

#### 96.

# Ascaris lumbricoides EXTRACTS ( $A_E$ ): SIALIC ACID (SA) CAPTURE (CAP) FROM RED CELLS (RC) AND DESYALATED RED CELLS (RC<sub>2</sub>)

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The RC SA has hemodynamical and hemorheological importance. The aim was to study the effect of A. lumbricoides on RC and RC<sub>E</sub> charge, depending on the treatment (T) time (t) of the globules with the  $A_{F}$ . We worked with 30  $A_{F}$  of adult parasite cuticles and Group O RC (saline medium) and RC<sub>B</sub> (bromelain medium). The T was made by incubating the globule sediment with an equal volume of  $A_E$  for 15', 30', 45', 60', 90' and 120' at 37 °C. There was a Control (C) (globules without any contact with  $A_E$ ) for each Tt. Polybrene Method was simultaneously applied to C, RC and RC<sub>B</sub> for the 6 t. For each obtained aggregation (ag) a Score (S) was assigned. Experimental Coefficient of SA CAP ( $C_{xxp}$ ) was calculated as the quotient between the CS and the Treated S. The experience showed that ag was diminishing for the Tt for RC and  $RC_{B}$ , which indicated that the SA CAP was increasing to a longer t. Though the charge diminished with the t, the RC did not totally lose the ag capacity, not even after 120'; nevertheless, 97% of the same  $RC_B$  were not aggregated after 120', and even 11/30 showed absence of ag after 15'. The statistical analyses demonstrated: 1)Tt had a highly significant effect on  $C_{exp}$  in RC and  $RC_{B}$ . 2) $C_{exp}$  Median was significantly higher for RC with regard to  $RC_{B}$  for all the t. 3)SA CAP from RC did not change between 2 consecutive t, the higher one being observed at 90' and 120', without significant difference, whereas in  $RC_B$ , was significantly lower in the first 3 t with regard to the last 2 t. The results showed that SA CAP increased with the Tt and that the  $A_E$  caused major alteration on  $RC_B$  charge. SA decrease might have relation to thrombosis and anaemia in ascariosis and this effect would have major relevancy in pathologies with erythrocyte SA deficit.

# ANTI-FIBRONECTINE, ANTI-HISTONE ANTIBODIES IN AUTOINMUNE HEPATITIS

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AIH is a chronic inflammatory liver disease of unknown etiology but whose pathogeny is attributed to an immune reaction against hepatocelular auto-antigens. The main characteristics that define AIH are the high titles of auto-antibodies and the polyclonal hypergammaglobulinemia. The aim of this research was to investigate the presence of two auto-antibodies: anti-fibronectina (a-Fn) and anti-Histonas (a-His), in patients diagnosed with AIH and who concurred to the Hospital Provincial del Centenario in Rosario city. 46 samples were analyzed. The study of the autoantibodies was conducted using an indirect ELISA technique. The reading of the Abs was done to a wave length of 450 nm. It were considered positive those samples whose value of Abs was superior to 0.140 for a-Fn and to 0.071 for a-His. Our results showed that 19 (41.3%) patients became positive for anti-Fn, 10 (21.7%) for anti-His and 8 (17.4%) for both auto-antibodies. The statistic analysis was performed using the McNemar test. The results obtained confirmed the presence of a-Fn and a-His in some patients with AIH. Through statistical studies it was concluded that in these patients, the proportion of positive results is not the same for both antibodies (p < 0.05).

#### 98.

## ANALYSIS OF THE EFFECT OF GLYCEMIC CONTROL ON URINARY ALBUMIN EXCRETION IN PATIENTS WITH TYPE 2 DIABETES MELLITUS

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Earliest clinical evidence of diabetic nephropathy (DN) in diabetes mellitus (DM) is microalbuminuria [urinary albumin excretion, (UAE) of 30-300 mg/g creatinine (Cr) in a spot urine collection]. A relationship between microalbuminuria and poor glycemic control has not been clearly shown. The aim of this study was to compare the degree of glycemic control between two groups of patients with type 2 DM over 5 years evolution: normoalbuminuric (NA, UAE <30 mg/g Cr, n= 24) and microalbuminuric (MA, n= 24). UAE was determined by immunoturbidimetric assay. Glycemic control was assessed through glycated hemoglobin levels (% HbA1c), determined by ion exchange chromatography. Cr (mg/dl) was measured by Jaffé method. Results are expressed as mean±SD or median/range. UAE values were higher in the MA group than the NA group:  $77.5\pm58.2$  and  $13.7\pm5.5$  respectively (p < 0.0001). There were no statistically significant differences between both groups in % HbA1c: MA= 9.6/7.2-12.8 and NA= 9.3/6.3-13.2. Prevalence of patients with a poor glycemic control (HbA1c >8%) was similar in MA and NA groups: 91.7% and 79.2% respectively. Serum Cr was higher in MA group than in NA group, but within the reference range: MA= 0.94±0.13 and NA= 0.87±0.1 (p=0.018). MA group had a longer evolution of DM (years) than NA group: 11.7±2.7 and 6.7±3.6 respectively (p<0.0001). In MA group more patients had a DM evolution >10 years than in NA group: 50% and 20.8% respectively (p<0.05). In conclusion microalbuminuria was associated with a longer history of DM but not with glycemic control. Normal levels of serum Cr in MA group indicate that renal function is preserved in incipient DN.

99.

# CHROMIUM (CR) AND VANADIUM(V) BIOSORPTION STUDIES

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Biosortion a process used for heavy metal remediation of water, is passive, because metal accumulation over the biomass surface does not implies metabolic activity. Waste waters from industries, contains Cr and V oxoanions, which implies new remediation technologies must be develop to eliminate them and avoid their environmental incorporation. We realize biosorption studies of Cr and V to obtain an efficient method for their elimination from industrial effluents. Three types of biomass are used: lignocelulosic, pectic and glycoproteic. Biomass interaction, with Cr or V, at a certain acidity value, allows determining which is the most appropriate for its retention and/or reduction. Realized studies: a) biosortion of  $Cr^{\mbox{\tiny III}},$  with different biomass gives an optimum pH value ~ 4.5 and optimum biomass quantity (1.5-5.7 g), b) sorption kinetic studies, allowed us to obtain sorption isotherms for Cr<sup>III</sup> removing, c) determination of optimal conditions to remove Cr and V: spectroscopy techniques of Cr<sup>VI</sup>/biomass, at pH 1.0 indicate at short contact times, presence of Cr<sup>v</sup> and Cr<sup>III</sup> joined to the surface of biomaterials, and at longer times, presence of CrIII. Electronic paramagnetic resonance detection of VIV indicates that biosortion process implies capture and reduction of  $V^{\ensuremath{\nu}}$ and retention of V<sup>IV</sup>, in the same way that occurs with Cr<sup>VI</sup>-Cr<sup>III</sup>.

## 100.

# LACTATE DESHIDROGENASE: ACTIVITY IN HUMAN PLASMA SEMINAL

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The seminal plasma is a complex mixture produced by the testicle, epididymis, the prostate gland and the sperm sac. There is a great variety of present enzymes in the seminal plasma. LDH is in five forms of isoenzyme. Several authors have demonstrated that the activity of LDH in the seminal plasma is higher to the semen. A sixth isoenzyme has been identified, the LDH C4, that is specific of seminal epithelium and is a marking of spermatogenesis, it would be absent in samples of semen without spermatozoa and cells of seminal progeny. Our objective was to compare the total activity of the LDH in seminal plasma in normospermics and azoospermiccs samples. The samples of semen were obtained by masturbation after 3 of 5 days of sexual abstinence, after to realise studies according to norms OMS 2010. We worked with two groups G1: seminal plasma normoespermiccs samples (n:20) and G2: seminal plasma azoospermics samples (n:18). Normospermics samples were considered with more of 20 million spermatozoids by mililiter of sperm, morphology, mobility and vitality within normal parameters. As azoospermics those seminal samples in which spermatozoa in the direct examination were no observed nor after to centrifuge and to dye the extended of sediment with Papanicolaou. The LDH activity was determined by optimized kinetic method UV, diluting the seminal plasma sample to tenth with physiological solution. The following results were obtained G1: 7076+/-4322 and G2:2005+/-1036. We applied the T student test to compare both groups. There are statistically significant differences (P:0,0016). The smaller significants of LDH in seminal plasma of azoospermics samples could be from the absent of fraction LDH C4.

# VALIDATION OF AN "IN HOUSE" ELISA FOR THE QUAN-TIFICATION OF PLASMA LEVELS OF ANTI-ANNEXIN V ANTIBODY

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The aim of this study was to validate an "in house" sandwich ELISA for the quantification of plasma anti-annexin V antibodies (Ab), previously developed by us. The validation parameters studied were: a) exactitude (E), expressed as the percentage deviation between the observed and the true value (acceptance criteria:  $E=100 \pm 20\%$ ); b) precision (P), expressed as either the intra- or the inter-assay coefficient of variation (CV; acceptance criteria: < 20%); c) limit of detection (LD), calculated by adding three times the SD to the mean value of the reagents blank; d) robustness (R), obtained by deliberately introducing slight variations in different parameters. The E was assessed by measuring in the same plate a sample of known Ab titer (n=7); the P was estimated by titrating three plasma samples containing different amounts of Ab (high, medium and low; n=4); the LD was determined by measuring the reagents blank (n=4). To study the R, variations in BSA concentration in the blocking solution (1, 2 or 3%), blocking time periods (60 or 90 min), temperature of the blocking process (room or 37°C) and time periods for the measuring of the final color optical density (5, 15, 30 or 45 min) were introduced. The E was within the  $100 \pm 20\%$  range; both CV were below 20%; the LD was below 1 U/ ml and the slight variations introduced in the technique did not produce any significant variations in the assessment of Ab titers (p>0.05). The method developed by us satisfactorily accomplishes the parameters of validation for an immunoassay.

#### 102.

# STUDY OF THE INTERACTIONS OF VANADIUM WITH POLYHYDROXY COMPOUNDS

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The study of the interactions of metal ions with organic compounds has been of great interest in recent years, because of the potential use of these ligands as pharmacological agents. Vanadium is an essential element in some organisms. In humans is not proven its essentiality, although there compounds that mimic and enhance the insulin activity. The study of the interactions of vanadium with polyhydroxy compounds and the subsequent stabilization of VO species are of great interest in relation to the metabolism of vanadium. To enrich the knowledge of the bioinorganic chemistry of this element, we studied the kinetic of the reduction of V(V) with D-fructose, D-glucose, oxalic, salicylic and citric acids, and the characterization of complexes of V(IV) obtained. This investigation includes kinetic measurements, intermediates detection, identification and characterization of the reaction products in solid state and in solution. The reactions were performed under conditions of isolation, monitoring the formation of V(IV) at 765 nm, with [V(V)]=15mM and [S]:[V(V)]≥10, [HClO<sub>4</sub>]=0.01-0.10M, at constant ionic strength (I=1.5M) and temperature (37°C). We calculated the rate constants of pseudo-first order from the linear portion of graphics lnAbs vs. time. Free radicals was evidenced by the polymerisation of acrylamide. The solid state complexes were synthesized from the reaction between NH<sub>4</sub>VO<sub>2</sub> (5 mmol) and substrate (15 mmol), at pH~2, stirring at 60°C, to verify the total consumption of V(V). Complexes were precipitated with ethanol, washed and recrystallized, yielding bluish powders (yields: 10-50%, depending on the substrate).

103.

# FIBROBLAST MITOCHONDRIAL ACTIVITY OF HUMAN CARDIAC VALVES THAT HAVE SUFFERED AN INCRE-MENT OF THE CRYOSTORAGE TEMPERATURE

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Cryopreservation of human cardiac valves could alter cellular viability, and this may be aggravated by sudden changes of the cryostorage temperature. For that reason, we studied the effects that a cryostorage temperature increment (100°C) could exert on the mitochondrial activity of human cardiac valve fibroblasts. Two groups were evaluated: conventionally cryopreserved valves (C<sub>cryo</sub>) and those that have suffered the cryostorage temperature elevation ( $C_{AT100}$ ) (n = 5), using: a- MTT reduction technique to determine the amount of formazan synthesized per mg of valvular tissue. Valves pieces (20-80 mg) were incubated with Krebs-Henseleit solution and MTT (pH=7.40, 1 mg/mL) at 37°C, 60', under carbogen atmosphere. Then, they were treated with a solubilizing agent, sonicated and incubated 30' to extract formazan. Absorbance was measured at  $\lambda$ =570 nm. b-Respiratory activity evaluation to assess basal oxygen consumption rate, and after succinate and CCCP addition. A thermostatized camera with an O<sub>2</sub> voltamperometric sensor and an analogical register was used. The measured oxygen consumption rates did not showed significant differences between groups, but VO<sub>2SUCC</sub>/VO<sub>2BASAL</sub> was significantly higher for C<sub>AT100</sub> (p=0.0000), while VO<sub>2CCCP</sub>/VO<sub>2SUCC</sub> was significantly lower (p=0.0240). Besides, less amount of formazan was extracted for  $C_{\Delta T100}$  (p=0.0000). These results let us conclude that mitochondrial activity is altered for  $C_{2T100}$ , and that its inner membrane is damaged.

#### 104.

# MUTAGENICITY AND FITOTOXICITY STUDIES OF DERIVATIVES OF SULFA DRUGS BY MEANS OF THE AMES AND ALLIUM TEST

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DNA can be damaged by N-nitroso compounds, which are formed by reaction of amides and amines with nitrites in the stomach. Sulfonamides have such functions in their molecules. The aim of this work was to determine the mutagenicity and fitotoxicity of mixtures formed by nitrite and two derivatives of sulfa drugs (glibenclamide and the complex sulfathiazole-Co(III), Co(III)-ST), and the action of L-ascorbic acid (asc.) as antimutagen (AM). Mutagenicity/ antimutagenicity were evaluated by means of Ames test, using the Salmonella typhimurium TA98 and TA100 strains. In the Ames test a compound would be a mutagen if the reversion coefficient, R.C. (R.C.: revertant number per tested plate/revertant number per control plate) is  $\geq 2$ . The % of inhibition was calculated with the following equation: % inh. =  $[(RC_{without AM} - RC_{with AM})/(RC_{without AM} - 1)]$ 100. Fitotoxicity was evaluated with the Allium test, exposing bulbs of onion to increasing doses of the systems in study. Length and morphology of the roots were evaluated as macroscopic parameters and mitotic index (MI) as microscopic one. The observed mutagenicity of the ST-Co(III)-nitrite mixture was inhibit by asc. The glibenclamide-nitrite mixture didn't show direct mutagenicity in the Ames test. With the Allium test, the behavior of glibenclamidenitrite was similar to glibenclamide (range: 0-20 mg/L), with MI like to the negative control (4-5%).

# EXPRESSION CLONING OF A PROTEIN FROM HUMAN OVIDUCTAL SECRETION WHICH INTERACTS WITH SPERMATOZOA

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We have recently identified a protein from conditioned medium (CM) of human oviductal tissue culture with the ability to interact with spermatozoa (SP) as calgranulin B. In this study, human calgranulin B was cloned and obtained as recombinant protein. In addition, the protein was immmunolocalized in oviductal tissue and on human SP. Human calgranulin B was amplified by PCR using a commercial plasmid which contains the protein cDNA as a template DNA. The amplified sequence was inserted (next to a sequence of glutathione S-transferase) into the plasmid pGEX-2T. The plasmids were cloned by transformation on E. coli. Bacteria containing the plasmid were induced to express the fusion protein, which was purified using the batch method with glutathione Sepharosa 4B. The protein was analyzed by SDS-PAGE and Western Blot. Human oviductal tissue was obtained from pre-menopausal women with no clinical history of infection or cancer disease, scheduled for routine hysterectomies. Human SP were obtained from normozoospermic samples of healthy donors after 3 days of sexual abstinence. Motile SP were recovered by swim-up and were incubated under capacitating conditions in the presence of proteins from CM, and then, calgranulin B was immunolocalized using specific antibodies and a second antibody conjugated with Cy3. The protein was detected mainly in the head and the mid piece of SP. Calgranulin B was detected, by indirect immunofluorescence with specific antibodies in sections of fixed oviductal tissue, in the epithelial cells. In conclusion, recombinant human calgranulin B was obtained and the protein was immunolocalized in oviductal epithelial cells, and the head and mid piece of human SP, confirming binding to these gametes. Funded by grants from FONCyT and SecyT UNR.

106.

# IgG IN ERYTHROCYTES SUBPOPULATIONS OF DIFERENT AGE

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After a life span of 120 days, senescent red blood cells (SeRBC) expose removal markers that account for their selective recognition by macrophages and clearance from circulation. There is evidence that RBC ageing leads to the binding of autologous immunoglobulin G (IgG) followed by recognition and phagocytosis. The purpose of this study was to investigate the RBC-IgG bound to membrane in SeRBC and Young (Y) RBC suspensions obtained by 2 different techniques. ACD anticoagulated blood samples were obtained from normal volunteer donors (n=7). Cell subpopulations were used (YRBC and SeRBC): I) separated by Percoll gradient centrifugation and II) evaluated by flow cytometry using light scatter parameters. All suspensions were incubated with Alexa 488-antihuman IgG. Flow cytometry analysis of the labeled samples was performed on a FACSAria II (Becton Dickinson). RBCs were selected using FSC and SSC gates and read on a dot plot (FL1 vs. FSC). The percentage of IgG positive cells were YRBC: 0.04 and SeRBC 2.60 in red cell populations separated according to I and YRBC: 0.16% and SeRBC: 1.5% evaluated according to II. These findings demonstrate a significant increase in membrane-bound IgG in populations of SeRBC. The erythrocyte populations of different ages could be studied without making a physical separation of cells. Direct assessment of the presence of IgG by flow cytometry allows the use of small amounts of samples and avoids the manipulation of separation techniques.

# 107.

# CHROMAGAR *MALASSEZIA* MEDIUM USED TO TEST ANTIFUNGAL SUSCEPTIBILITY

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At present there are no standardized in vitro tests to determine the susceptibility of Malassezia yeasts to antifungals. The aim of our study was to evaluate the utility of chromogenic, CHROMagar Malassezia (CHR-MM) medium to testing susceptibilities of Malassezia species using a disk diffusion method. Four reference strains: M.sympodialis (CBS 7222), M.slooffiae (CBS 7956), M.furfur (CBS 7019) and M.pachydermatis (CBS 1337), 15 strains isolated from Pityriasis Versicolor patient, and quality control (QC) strains Candida parapsilosis (ATCC 22019) and C.krusei (ATCC 6258) were tested. Following the guidelines M44-A document issued by CLSI, inocula were prepared and inoculated in CHR-MM. The QC strains were also inoculated in Mueller-Hinton agar with methylene blue and 2% glucose (MH). Tablets Neo-Sensitabs TM Rosco of antifungals voriconazole (VZ), fluconazole (FZ), ketoconazole (KZ), itraconazole (IZ) and terbinafine (TF) were evaluated. The studies were performed in duplicate; sterility and growth controls were performed. Plates were incubated at 32°C (Malassezia spp) and 28°C (QC) for 48h. Inhibition zone diameter to reference strains, QC and clinical isolates, showed on average the following diameters (in mm): VZ ( $43 \pm 2$ ); FZ ( $35 \pm 5$ ); KZ ( $25 \pm 2$ ); IZ  $(28 \pm 3)$  and TF  $(25 \pm 2)$ . The tablet zone diameters for QC strains in CHR-MM were comparable with those obtained in MH and accepted by the CLSI for these OC strains. The medium CHR-MM was suitable for the homogeneous development of yeast and it was not antagonist to any tested antifungals. Our results show that the tablets sensibility tests are feasible with the CHR-MM medium used. Further studies of correlation with quantitative tests should be carried out to classify the susceptible or resistant strains.

#### 108.

#### MOLECULAR IDENTIFICATION OF *Cryptosporidium* parvum FROM PATIENTS OF MIRA Y LOPEZ HOSPITAL, SANTA FE

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The genus Cryptosporidium consists of Apicomplexan parasite species that infect a variety of vertebrate hosts including humans, dogs, cats and other animals. Infected individuals show a spectrum of clinical presentations but the pathogenicity varies among parasite species and the type, age, host immune status. Patients from Hospital Mira y López (Santa Fe) belong to low socioeconomic status, mostly lacking water and sewage network. The aim of this study was to characterize Cryptosporidium spp. genotype isolated from stools of patients from Mira y Lopez Hospital, Santa Fe, using PCR-RFLP. During the period of 01/01/2008 to 31/12/2008 faeces of 4663 patients were analyzed with a prevalence of 40.36% for at least one parasite. Stools were processed with direct microscopic examination, concentration techniques: flotation (Zinc Sulfate 33%) and sedimentation (formalin-ether), acid alcohol resistant staining. Of the analysed samples, 32 were positive for Cryptosporidium spp. and were confirmed by IFA using monoclonal antibodies. The molecular identification of 32 positive samples was carried out in Parasitology Area, (UNR) using nested PCR (Xiao ., 2000) with modifications. A fragment of 1325 bp were amplificated, followed by treatment with restriction enzymes, SspI, and DdeI VspI. Bands of 635 bp were found, corresponding to C. parvum. The results indicate that these cryptosporidiosis cases may be frequently associated with zoonotic transmission and are pioneers in the city of Santa Fe regarding Cryptosporidium spp.

#### 109

## BODY MASS INDEX AS A POSSIBLE RISK FACTOR ASSOCIATED WITH THE PROGRESSION TO FIBROSIS IN CHRONIC HEPATITIS C INFECTION

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Hepatitis C virus (HCV) infects approximately 170 million people worldwide and it is consider as the main cause of chronic hepatitis, cirrhosis and hepatocelular carcinoma. The study of the natural history of the infection caused by this virus has been very challenging due to the fact that in most cases it is impossible to identify the identify the onset of the disease since it is asymptomatic. Among the different associated risk factors, the BMI =25 kg/m<sup>2</sup> has been described as a frecuent finding in patients with chronic hepatitis. However, it's role has not been vet established. It has been suggested that it could be related with the pathogenesis of steatosis in chronic hepatitis c infection and the possible contribution of the latter in the progression of fibrosis. The aim of this study was to assess whether there is correlation between BMI and the various stages of fibrosis. We performed a cohort study from a voluntary testing campaign conducted in the Gastroenterology Department of the Hospital Provincial del Centenario in the city of Rosario, Argentina. Blood transfused patients before 1993 were called and 1699 of them assisted to the hospital. Among those 1699, 61 (3.6%) were HCV Ac (+) / ARN HCV (+). In 40 (65%) infected patients a liver biopsy was performed. The population of these research belongs to those patients with liver biopsy. The degree of fibrosis was assessed using the METAVIR score. Patients were categorized in two groups: Low Grade (F0-F1) and Intermediate/ High grade (F2-F3-F-4). BMI were obtained and a BMI =  $25 \text{ kg/m}^2$  was fixed as a criteria. Our results show that 25 patients (62.5%) had a low grade of fibrosis: FO=8 (20%) and F1=17 (42.5%). The 15 remaining showed and intermediate/high grade of fibrosis: F2=6 (15%) F3=8 (20%) F4=1 (2.5%). Regarding grades of fibrosis and the association with BMI, it was found that in low grades a 68% (17) of the patients had a BMI =25 kg/m<sup>2</sup> and the other 32% remaining presented a BMI = 25 kg/m<sup>2</sup>. Concerning intermediate/high grades, a 40% (6) showed a BMI =25 kg/m<sup>2</sup> and the other 60% (9) had a BMI = 25 kg/m<sup>2</sup>. (P=0.0001). In conclusion, the BMI did not play a role as a risk factor in the progression of liver fibrosis towards intermediate/high grades.

#### 110.

# ANALYSIS OF CATALASE ISOFORMS ACTIVITY AND EXPRESSION IN TOMATO PLANTS

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Plants are sessile organisms that have acquired a series of mechanisms to adapt to the changing environment in which they live. One of these mechanisms is provided by catalase that catalyzes the decomposition of hydrogen peroxide produced under physiological and stress conditions. Up today, in Solanum lycopersicum it has been described three genes that encode catalases: cat1, cat2 and er60. In this research, we analyze the activity and expression profile of catalase in different organs of tomato plants (cv Micro-Tom). It was observed that the catalase activity depended on the organ studied being the greater value in leaf. A gene sequence that encodes a putative catalase was identified by in silico analysis of available databases. This sequence was named cat3. The transcript level of each component of the family was analyzed by Real Time PCR. cat1 was the most abundant transcript in all tissues analyzed except leaf blade where the most abundant was cat2. er60 was expressed at low levels in all cases and the higher expression of cat3 was observed in flower. In addition, it was noted that catalase activity decreased during fruit ripening while the transcript level of the four CAT genes increased. These results suggest a postranscriptionally regulated process. Our results suggest that expression of the four CAT genes is tissue and developmental regulated. This information may be valuable for further analysis of catalase profile and function diversity in tomato plants.

#### 111.

#### EFFECT OF PHAGE BIOCONTROL ON STRAINS OF SHIGA TOXIN-PRODUCING *Escherichia coli* IN MEAT PRODUCTS

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It is especially important in our region to control the spread of pathogenic Enterobacteriaceae producing food-borne disease, such as Shiga toxin-producing Escherichia coli strains (STEC). STEC are emerging human pathogens that can cause diarrhea, as well as severe clinical manifestations including hemolytic uremic syndrome. In this paper, we propose bacteriophages as a tool for the biocontrol of these pathogenic bacteria present in meat. Two phages (DT1 and DT6) were selected and it was determined their lithic effect using meat as a matrix contaminated with previously characterized strains that have virulence factors. We evaluated the reduction of viable cells (VC) of DH5 $\alpha$  on meat after the exposure to DT1 at 5, 24 and 37°C. It was also tested the effect of both phages against STEC and enteropathogenic strains (EPEC). Based on experiments in meat with DH5 $\alpha$ , we obtained a significant difference in the percentage of VC with the three temperatures tested, observing the further reduction within the first hours of the assay. This reduction was also influenced by the concentration of phage used, being the biggest used more effective. When STEC and EPEC were tested, a significant reduction of bacterial contamination was observed up to 3 hours of incubation and in some cases up to 6 hours of incubation. In all cases, when we worked with EPEC, the VC reduction was significant at 5 and 24°C and not significant at 37°C. These results indicate that tested phages may be useful in the biocontrol of STEC and EPEC in meat products at refrigeration and ambient temperatures.

#### 112.

#### INVESTIGATE THE KNOWLEDGE OF EMERGING DISEASE DENGUE TRANSMITTED BY *Aedes aegiypti* IN STUDENTS OF THE AREA OF HEALTH

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Due to the impact of Dengue infectious disease transmitted by Aedes *aegivpti* in our community in 2009, a diagnostic work was carried out in health area university students since these students are not only members of the population but also potential agents of Community Health. The aim was to investigate the knowledge of these students about this disease so as to carry out activities of promotion and prevention. We administered an anonymous survey to 232 students of a private university in the city of Rosario. The survey consisted of seven questions, some of multiple choice and others of dichotomous answers about knowledge of the disease, ways in which information was reached, ways of transmission, causative organism, clinical manifestations, notion of prevention and treatment. The results were expressed in percentages. 97% of the students proved to have knowledge of the disease, 80% knew it through mass media, 88% knew about transmission, 63% knew the causative agent, 1% knew which were the clinical manifestations, 81% had knowledge about prevention and 29% knew the right treatment. The majority of those who were polled knew of the disease by the mass media and it was important the percentage who knew the causative agent, transmission and prevention of the disease. However they were not aware of the clinical manifestations and knew very little about treatment. We concluded that it was necessary to create an academic space of discussion at the Institution by specialists from different disciplines in an integrated way and systematic in time, to guarantee appropriate promotion and prevention of an emerging diseases such as Dengue.

## GENOTYPIFICATION OF *Giardia lamblia* CYSTS OF DOGS FROM THE INFLUENCE AREA, VETERINARY SCIENCES FACULTY, UNR

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Giardia lamblia is a flagellate protozoan, whose cysts and trophozoites were recovered from mammals feces, including humans and domestic animals. Contaminated water and fomites are the most effective vehicles for transmitting the infection. The amplification of the triosephosphate isomerase has confirmed the human strains division in two genotypes: genotype A (gA) and genotype B (gB), more pathogenic and also found in dogs. The aim of this study was to investigate the genotypes of Giardia spp. isolated from dogs faeces, in the influence area of Fac. Cs. Veterinarias (UNR), using PCR-RFLP. Feces of 34 adult dogs were processed in Parasitology Area, Fac. de Cs. Bioq. y Farm. (UNR), by direct microscopic examination, permanent stainings and macroscopic examination. Cysts of G. lamblia were found in six samples, purified by sucrose gradient and were subjected with 6 cycles of freezing/thawing and incubation with proteinase K and lysis buffer (NaCl, EDTA, SDS, pH 7.8) at 37°C for 24 hours. DNA extraction was performed with phenol/chloroform. PCR1 amplified a fragment of 576 bp (gA) and 208 bp (gB), in PCR2 a 476 bp (gA) followed by RsaI and 140 bp (gB). PCR2 products were observed by gel electrophoresis. Genotype B was found in all samples. This would explain the transmission of G. lamblia cysts through a common source of infection, for example, water, between patients and dogs living in the same area.

#### 114.

# EFFECT OF DIFFERENT DOSES OF NITROGEN AND SULFUR ON THE YIELD AND QUALITY OF TWO CORN GENOTYPES (Zea mays L.)

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The objective of this experiment was to analyze the effect of different doses of nitrogen and sulfur on the yield and quality of two corn genotypes. The trial was conducted in Zavalla (60° 53' W, 33° 01'S.), Argentina. The hybrids sown were: ACA 2000, which has a low yield potential and vitreous texture, and ACA 417 RR2, with a high yield potential and soft texture. The treatments consisted in three rates of nitrogen (N<sub>0</sub>: control; N<sub>75</sub>:75 kg ha<sup>-1</sup> N, and N<sub>150</sub>: 150 kg. ha<sup>-1</sup>N), and two rates of sulfur (S<sub>0</sub>: 0 kg ha<sup>-1</sup> S y S<sub>40</sub>: 40 kg. ha<sup>-1</sup> S). The grain yield at 14% humidity (YLD), the grain weight by hectoliters (WH) and the weight of 1000 grain were evaluated. Data were subjected to ANOVA and the means were compared through Duncan's test. During the critical period (CP), mean temperatures were 21.4°C and rainfall was approximately 200 mm. As regards YLD, both hybrids differed (P=0.01) from the control with the addition of 150 kg of N (with and without S); while the ACA 2000 hybrid had a proportionally higher increase in YLD than the ACA 417 hybrid (272% and 101%, respectively). As to the weight of 1000 kernels, both hybrids differed (P=0.01) with doses of 150 kg of N (with and without S) from the treatment without N and with S. thus the ACA 2000 increased an 18% and the ACA 417 a 20%. As a consequence of nitrogen being tied up by the stubble, the response to N fertilization on yield was given with the highest doses and varied according to the grain texture.

115.

#### FIRST REPORT ON PHYSIOLOGICAL RESPONSES INDUCED BY FROG EYE LEAF SPOT (*Cercospora sojina*) IN SOYBEAN

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The objective of this work was to detect changes in the content of Total Protein (TP) and Peroxidase Activity (PA) induced by the natural infection of *C. sojina* on soybean leaves. Thirty asymptomatic (Asy) and symptomatic (Sy) leaves per plot, of a cultivar sown in random blocks with 3 replications were evaluated in the South of Santa Fe, Arg. Leaves were cut in 2 sections: apical (Ap) and basal (B). The treatments were: (Ap-Asy; Ap-Sy; B-Asy; B-Sy). Protein crude extracts (Pce) were obtained from tissue sections (2,5 g) of each treatment and plot. The TP were analyzed by Biuret method and expressed as mgTP / mlPce. PA was calculated meassuring the absorbancy of the tetraguayacol (460 nm: 26.6 mM-1cm-1) produced by the oxidation of guayacol with the O2 releadsed by the peroxidase action on the hydrogen peroxide (H2O2) (3 min. reaction). PA was expressed as specific enzimatic activity: µmol of product / min.mg of enzime. Treatments were duplicated and analyzed with a factorial ANOVA: 3 plots x 2 reactions (Asy e Sy) x 2 foliar sections (Ap y B) x 3 observations. According to previous results, the TP was significantly higher in leaves Sy (40,1 mg.TP / ml.Ec) than in leaves Asy (31.4 mg.TP / ml.Ec). Also, TP was significantly major en sections Ap (38,07 mg.PT / ml.Ec) than in sections B (33,44 mg.PT/ml.Ec). Whilst PA was major in leaves Asy (15,3 µmol / min.mg.enz) than in leaves Sy (11,7 µmol / min.mg.enz.), its could point out that the peroxidase activity induced by C. sojina avoided the infection or the symptom expression in the leaves of this soybean cultivar. New Proteinograms will be done to identify other active Proteins related with the defense reactions against Frog Eye Leaf Spot.

#### 116.

# INTRODUCTION TO THE STUDY OF THE FAMILY PONTEDERIACEAE IN THE PROVINCE OF SANTA FE

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The objective of this work is to begin the taxonomic study and the geographical and ecological distribution of the Pontederiaceae Family for the province of Santa Fe. The Pontederiaceae Family is classified inside the order Bromeliales (according to the system of Melchior) or of Commelinales (APG III System) in the class Liliopsida (=Monocotyledoneae). This family is constituted by 4 genera distributed all over the world in tropical and subtropical regions, with few temperate species. In Argentina it consists of 3 genera with a total of 10 aquatic herbaceous species. They present vegetative shoots of undetermined growth, submerged or emergent, with terminal inflorescence, generally protected by a spathe. Leaves of two types: the sessile submerged and arranged in a basal rosette, filiform or linear; and the petiolate, emergent or floating cordiform or reniform, with articulate petioles or not, thick or thin and septate. Flowers perfect, sessile, hypogynous or periginous, with petaloid perigone, in panicles or spikes, rarely solitary. Stamens 3-6; gynoecium 3 carpelled, uniloculate, with one to numerous seminal rudiments of parietal or basal placentation. Fruit: capsule with persistent perianth or utricle with crests. We worked with specimens preserved in the herbaria UNR (Rosario), SF (Esperanza) and SI (Darwinion, San Isidro); together with a bibliographical revision and the field experience of the authors. The preliminary results show that in Santa Fe the family is represented by 3 genera, 7 species and a variety: Eichhornia azurea (Sw.) Kunth; E. crassipes (Mart.) Solms; Heteranthera limosa (Sw.) Willd.; H. multiflora (Griseb.) Horn; Pontederia cordata L. var. cordata; P. rotundifolia L.f. and P. subovata (Seub.) Lowden. Illustrations and keys of the genera and species are presented based on morphological character of diagnostic value; as well as distribution maps of the species.

# PLANT STAND REDUCTION OF ARTICHOKE (Cynara cardunculus L. var. scolymus L) IN THE ROSARIO AREA

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In recent years the crops of Rosario artichoke area was reduced. Among the major problems were the diseases. The objective of this work was to determine the annual reduction of the plants stand and the pathogen presents. The evaluation of a 1 year crop of cultivar Concerto® (Nunhems Co.). On 2010 was measured the incidence of dead plants. Material was collected to identify the pathogens. The symptomatic plant tissue sections were sown in Petri dishes in 2% APG. Prior to the desinfection of plant material in a 2% NaClO<sub>4</sub> then was rinsed in sterile distilled water. Petri plates were incubated at 20-22°C for 7 days with light / dark for 12 hours. The identification of pathogens was done under binocular stereoscopic microscope and microscope. To determine the presence of Cucumber Mosaic Virus (CMV) and Tomato Spotted Wilt Virus (TSWV) the DAS-ELISA method with alkaline phosphatase was used. The incidence of death plants was 30%. The pathogens identified were: Fusarium spp., F. equiseti, F. verticilloides, and Verticillium spp. The ELISA tests were negative fom CMV and TSWV. All the fungic pathogens isolated death the plants by basal rot. We will continue the studies on twice times on the year and to by continue on the next year with the objective to propouse different alternativs of management of this problem.

# 118.

# RADIOGRAPHIC PATTERNS OF 10 SHRUBS AND TREE NATIVE AND CULTIVATED SEEDS AND FRUITS SPECIES

Alzugaray  $C^1$ , Carnevale  $N^1$ , Craviotto  $R^2$ , Gallo  $C^2$ , Arango  $M^2$ , Seguetti  $J^1$ , Salinas  $A^{1*}$ .

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The X-Ray technique is greatly known and used in medicine, biology and industries and it is very useful in studies of seeds and fruits quality by its anatomy and morphology. The objective of this work was to create radiographic patterns that allowed to characterize woody native and cultivated seed and fruit species by its morphological and anatomical characteristics, physical damages and abnormalities in the maturation process. Radiographic plates of fruits and seeds were obtained with the X Ray Equipment RUTAX and traditional revealed of the plates were made. There were taken 100 radiographic plates of the fruits of each one of the shrubs and forest species studied. This radiographic images were compared with their respective digital photographic images. The patterns were made with radiographic images and its corresponding digital photographic images considering: whole seeds and seeds completely developed; seeds not completely developed; empty seeds; seeds damaged by insects and broken seeds. Forest species generally have seeds with hard teguments and a great quantity of empty seeds. The X-Ray Rutax equipment allowed to make an efficient and quickly characterization of seeds and fruits of forest species

119.

# THE FAMILY *EPHEDRACEAE* IN THE PROVINCE OF SANTA FE (ARGENTINA)

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The objective of this work is to begin the taxonomic study and the geographical and ecological distribution of the Ephedraceae Family for the province of Santa Fe. This family, belonging into the Order Efedrales (=Gnetales), presents only one genus: Ephedra L. with some 40 species distributed in both hemispheres, 10 of which grow in Argentina. They are generally dioecious plants, shrubs or subshrubs, erect, climbing or creeping with equisetoid branches. Reduced scale leaves in whorls of 3-4, or decussate, connate at the base in a small sheath. Male structures gathered in sessile or pedunculate strobilus, single whorls formed with 3-4 bracts or compound ones formed by 1-16 whorls with decussate bracts. Female strobilus generally single, sessile or pedunculate, arranged in 3-10 whorls of decussate bracts, or whorls with 3-4 connate bracts, only the upper ones are fertile with 2-4 ovules. Seeds with two filiform cotiledons. We worked with specimens preserved in the herbaria UNR (Rosario), SF (Esperanza) and SI (Darwinion, San Isidro); together with bibliographical revision. Our preliminary results show that the family is represented in Santa Fe by only one taxon: Ephedra triandra Tul. emend. J. H. Hunz. Taxonomic information, illustrations and a distribution map are provided.

# 120.

# VASCULAR FLORA OF THE SANTA FE PROVINCE: *MELIACEAE*

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The Family Meliaceae (Magnoliopsida, Order Sapindales), comprises 51 genera and some 550 species of pantropical distribution. In Argentina it is represented by 4 genera, 11 native species, and one exotic species widely cultivated. It comprises trees, rarely shrubs, with bitter and astringent bark. Alternate leaves, pinnate to bipinnate, occasionally trifoliolate or unifoliolate, usually with entire leaflets. Flowers bisexual, if unisexual often with rudiments of the opposite sex, hypogynous and actinomorphic, in axillary inflorescences. Calyx and corolla 4-5-merous, petals free or basally connate. Androecium with 4-10 stamens, usually atop a tube with or without appendixes. Fruit a capsule, berry or drupe, seeds winged, with or without aril. The present contribution provides keys for the identification of taxa based on morphological characters and a map of geographical distribution. The methods consisted in bibliographical review, analysis of important plant collections of the province (Herbaria SF, SI and UNR), field observations and lab work to confirm the specimens' identity. In the Santa Fe province the family is represented by two genera: Guarea Allam. ex L. [G. guidonia (L.) Sleumer y G. macrophylla Vahl subsp. spicaeflora (A. Juss.) T. D. Penn.] and Melia L. The later is represented in many departments by the naturalized tree M. azedarach L.

## INCIDENCE OF Xanthomonas arboricola pv. juglandis and Fusarium spp. IN BUDS AND SHOOTS IN DIFFERENT VARIETIES OF Juglans regia IN SOUTH OF SANTA FE PROVINCE. ARGENTINA

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Brown Apical Necrosis (BAN) is caused by Xanthomonas arboicola pv. juglandis, Fusarium spp. and other pathogens. X. arboricola produce shoots necrosis, fruit drop and looses in comercial quality. The purpose of this work was to evaluate presence of X. arboricola pv. juglandis and Fusarium spp. in buds and the incidence and severity of necrosis in terminal shoots on different varieties of Juglans regia. The evaluation was made on experimental plant field in Zavalla. Three replications of 20 buds of Franquette, Chandler, Tulare and Davis varieties were sown in Petri dishes. The selective medium for X. arborícola pv. juglandis Brilliant cresyl blue starch (BS) was used. The dishes were incubated at 28°C during 72 hs. Incidence of shoot terminal necrosis in plants (infected plants/total evaluated plants x 100) and severity of shoots terminal necrosis (infected shoots/total evaluated shoots x 100) were visually evaluated over the same plants. Results were statistically analysed by ANOVA and Duncan 0.05%. In Petri dishes buds with opalescent colonies that hidrolizyng starch (X. arboricola py. juglandis) were counted. Five days after on the same medium the presence of Fusarium spp. was determinated. Results showed bud infection of Xanthomonas between 94 and 84% without significant differences between varieties. No significant differences for Fusarium (values between 42 and 28%) appeared. The incidence on plants with terminal shoots necrosis were 100%. Significant differences were observed on varieties with shoots terminal necrosis; Franquette (25%), Chandler (31%), Tulare (15%) and Davis (35%) were observed.

#### 122.

# GROWTH OF M-406 MAMMARY ADENOCARCINOMA IN THE F2 GENERATION OF A CROSS BETWEENN MICE SUSCEPTIBLE AND RESISTANT TO TUMOR CHALLENGE

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M-406 tumor spontaneously appeared in CBi a line used as control of an experiment of artificial selection for body conformation from which CBi- was derived. CBi- mice challenged with M-406 show 100% of takes and 100% of regressions (resistance) while in CBi mice, 100% of tumors grow exponentially (susceptibility). F1 reciprocal hybrids challenged with M-406 showed dominance of susceptibility. With the aim of studying the behavior of mice from the F2 generation, 24 mice/sex of the four subpopulations obtained by crossing the reciprocal F1 hybrids [(CBi-xCBi)X(CBixCBi-)] were challenged with M-406 The tumor grew exponentially in 145 mice. From the exponential growth model Vt=Vi.ek.t derives Tumor volume Doubling Time (TvDT=0,69/k) which is used as a growth parameter. TvDT differences were only observed between males (M) and females (F) in F2A [(CBi-xCBi)x(CBi-xCbi), F:5,34±0,44; M:4,27±0,26; p=0,038] and F2D [(CBixCBi-)x(CBixCBi-), F:4,04±0,14; M:4,85±0,38; p=0,041] populations. In F the tumor grows faster in F2D than in F2A (p=0,0097), a difference not seen in M (p=0,102). These results suggest a relationship with the maternal genotype, as F2A and F2D have double CBi- and CBi genetic load, respectively which is also associated to sex.

123.

## GROWTH OF M-406 MAMMARY ADENOCARCINOMA IN DIFFERENT F2 SUBPOPULATIONS DERIVER FROM CROSSING MICE SUSCEPTIBLE AND RESISTANT TO TUMOR CHALLENGE

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CBi is a mouse line used as control in an artificial body conformational selection experiment from which CBi- was derived. M-406 spontaneously arose in a CBi female When CBi is challenged with M-406 the tumor grows exponentially in both sexes showing 100% of progression (susceptibility). In CBi- mice the tumor grows in 100% of the animals and then returns (resistance). The tumor grows exponentially in all F1 animals derived from reciprocally crossing both lines. The aim of this work was to analyze the tumoral behavior in the filial two (F2). 24 individuals of each sex, from each of the four reciprocal combinations among F1 hybrids were inoculated with M-406, and the progression/ return ratio was analyzed as well as the survival time of those animals who did not return. The tumor grows in 100% of the F1 animals. Non significant differences was evident neither between sexes in each of the four combination, nor among combinations within sex (P>0.05) in the percentage of animals in which the tumor returns or grows until the ethical limit. Moreover the animals show equal survival time with non significant differences, no maters sex or combination considered. It can be concluded that the four combinations of F2 animals are equivalent and can be considered as a unique population. These results confirm previous evidence from the F1 related with the autosomical localization of the resistance-susceptible genetic determinants and the absence of maternal effects.

# ERRATAS

# **ROSARIO BIOLOGY SOCIETY**

(Sociedad de Biología de Rosario)

Abstracts from the

# XI CONGRESS – XXIX ANNUAL MEETING

December, 3-4, 2009

Sede de Gobierno de la Universidad Nacional de Rosario

Rosario, ARGENTINA

E2.

# E1. DESCRIPTIVE STUDY OF DENTAL ROOTS WITH NON-APICAL CEMENT FORMATIONS

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Secondary cement is deposited in radicle apex during the entire life of tooth. Its function is to compensate the oclussal wear in order to keep them in the oclussal plane. In some dental roots nonapical cement deposits can be found after an extraction. The aim was to analyze the anatomical characteristics of these formations. Were included twenty teeth with roots presenting cement deposits with non-apical localization. Were analyzed: age (29-79), sex, previous general and periodontal pathology, reason for extraction and anatomical characteristics of root and particularly of lesion, in which the following were considered: consistency, volume of saliency, quantity, shape, limits, size, color, appearance and texture. The average age was  $61\pm16$ , women 75%, prevalent general pathology was diabetes 50%, which was also associated with Paget's disease in 20%. Cavities were the reason for extraction in 55%, and 40% they were associated with dental mobility due to inflammation of the periodontal ligament. Root color was light 85%, with a pearly appearance 70% and smooth texture 80%. Non-apical cement formations showed a hard consistency 100%. The volume of saliency was: -slight 45%, -moderate 15%, and -prominent 40%. Lesions were observed -one 55%, -two 30%, -three 5%, and -more than three 10%. The shape was -round 70%, -oval 20%, and irregular 10% remaining. They were -well defined 90%, -large 70%, -white 70%, and the same color as the root 30%; the -appearance was pearly 75%, and the -texture smooth 90%. Patients with chronic cavities and dental mobility as a consequence of periodontal ligament inflammation, these non-apical cement deposits are a biological response aiming to stabilize the dental piece given that the characteristics of appearance are the same along the entire radicular surface, including the lesion.

# DISINFECTION OF GUTTA-PERCHA CONES: ITS EFFECT ON THE APICAL ADJUSTMENT

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Endodontic filling with gutta-percha cones is based on the conformation of an apical matrix in which the main cone must fit to diminish the film of the sealer and the risk of leakage. We have found that, when coming from tubes in use, cones are usually contaminated (prevalent microorganism Staphylococcus). It is suggested to disinfect the cones with sodium hypoclorite (SH), a rinsing with ethylic alcohol 96° (EA) would eliminate hypoclorite crystals. The aim of this study was to evaluate if the disinfection with SH of gutta-percha cones affects its apical adjustment, and to determine if a rinsing with ethylic alcohol modifies the results. 30 extracted upper incisors were selected, access cavity was performed and they were instrumented (sequential technique, master apical file # 50). Teeth were divided into 3 groups (n=10) A: cones were disinfected with 5.25% SH - 1 minute; B: after disinfection cones were rinsed with EA; C: cones did not receive any treatment (control). The adjustment of the cones was evaluated in three ways: visual, tactile, and x-ray. Gutta-percha cones of the three groups fit satisfactorily before their disinfection. After the treatment on group A two of them did not pass the tactile test (20%). In group B six (60%) did not pass the tactile test, three (30%) the visual, and two (20%) the x-ray one. Cones of the control group were pull-out of the teeth and returned to place, passing all the tests. Proposed methodology did not demonstrate to affect, significantly, cones apical adjustment; nevertheless it was modified when a rinsing with EA was used. The time of disinfection with SH should not surpass the minute and soon they would have to be dried in sterile gauze.

#### E3.

## MICROSTRUCTURE AND MICROHARDNESS OF HUMAN MOLAR TOOTH ENAMEL

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In every type of tooth enamel, the structure of prisms and crystals makes it possible to meet the biomechanical demand. The radial enamel type, with parallel prisms, presents higher abrasion or wear resistance. The enamel type with Hunter Schreger bands and the irregular enamel type, with prism crossings, avoid fracture propagation. Enamel hardness is important since it has a direct relationship with abrasion resistance. The purpose of this work was to identify the enamel types and to establish their relationship with human tooth microhardness. Twelve inferior molar crowns were longitudinally cut, embedded in epoxy resin, grinded, etched with acid and observed under a Scanning Electron Microscope in free faces and cusps in the outer third (A) and in the inner third (B), where microhardness was also measured with a Vickers test. Radial enamel type was identified in the outer zone of the medial third of the free faces and enamel type with bands was identified in the inner zone. On both free faces, the bands occupied the thickest portion of the enamel, while the radial enamel occupied the thinnest portion. The radial enamel type was the only one present in the cervical third. In the lingual cusp the inner enamel was of the irregular type with marked prism intercrossing and it was completed with radial enamel as far as the outer surface. In the vestibular cusp the inner enamel showed bands and the outer enamel was of the radial type. Microhardness value was  $Hv_{100=}$  312,0Vk in (A), and  $Hv_{100=}$  284,33Vk in (B). The outer and harder radial enamel type, combined with the inner enamel type, either irregular or with bands, constitute a biomechanical adaptation of the functional areas.

## E4.

# SOCIAL SAMPLING OF BUCCAL HEALTH CARE PRACTICES AND KNOWLEDGE

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Education for Health (EFH) as a social practice addressed to the population is a great tool to promote health practices in the community. Establishing a social representation implies determining what people know, what they believe, how they interpret what they believe, what people do and how they behave. Our objective was to describe parents' buccal health care practices and knowledge. These parents, whose children attend a public school in the central area in Rosario, agreed to participate in a buccal EFH program. An analytical observational epidemiological study was conducted with open surveys. The data was processed with SAS System for Windows 6.12. The responses about care, practices and knowledge about buccal health were related to gender and schooling level (SL). Written consents to participate in the program were distributed together with 150 surveys. 94 surveys were completed by parents of an average age of 38,01±6.95, out of which 74 were mothers and 20 fathers. Regarding buccal health care, the most appreciated and performed practice by parents and their family group were self-care preventive practices (63,83%). Said responses showed significant differences regarding genderand SL (p=0,001). 34,04% considered the dentist as the main communicator while 30,85 % failed to respond. These responses showed a significant difference by gender and SL (p=0,001). 38,30% of the respondents said they have no difficulty in keeping their buccal health, while 20,21% failed to respond. There were differences by gender or SL (p=0,001). Bacterial illnesses were considered as the best-known illnesses (58,51%). However, there was no difference by gender (p=0,505) or SL (p=0,729). The etiology of illnesses was associated with lack of individual care in 54,25%. A significant difference was found by SL (p=0,001) but not by gender (p=0,277). It can be concluded that both gender and SL determined most of the responses. The group has self-care habits and is aware of the importance of the dentist as a communicator of knowledge, as someone who communicates the appearance of prevailing illnesses and their basic forms of prevention.

#### E.5

## **TOOTH BRUSHING HABITS IN DIFFERENTS SUB-POPULATIONS OF ROSARIO CITY** *Kohli A<sup>1</sup>, Pezzotto SM<sup>2</sup>, Poletto L<sup>2</sup>.*

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Caries and periodontal disease are causes of dental loss. Contributing the bacterial plaque that is not regularly removed from the dental surface and food remains in oral cavity after a meal. The aim was to determine the application of dietary norms and customs in three subpopulations of Rosario. Were analyzed: age (15-45), after-dinner brushing habits, meals following after-dinner brushing, and information about oral health received by patients attending private and two public services. Variance analysis, chi-square test, and correlation coefficients were applied. In all brushing frequencies 71% of total (517) brush their teeth after dinner and do not consume cariogenic food or drinks afterwards (p<0.0001). Who eat and drink before bed and do not brush are majority in publics, but have the habit 31% in the private (p=0.01). After-dinner meals not followed by dental hygiene was private 50% and public 64% (p=0.013). Received oral health information 88% private and 72% public services (p=0.004). In following order -school: private 50%, municipal 43%, provincial 59%; -dentist's: private 48%, municipal 33%, provincial 23%; -home: private 29%, municipal 16%, provincial 18%, -television: private 33%, municipal 22%, provincial 13%. Unfavorable results were attributed -erroneous concepts: private 15%. municipal 20%, provincial 15%; -dentist's responsibility: private 36%, municipal 37%, provincial 24%; -economic reasons: private 31%, municipal 24%, provincial 40%, -personal negligence: private 29%, municipal 49%, provincial 43%. After-dinner meals not followed by brushing, along with personal negligence, are risk factors that contribute to dental loss in individuals 15 to 45 years old. In public odontological services, providing information on how detrimental these behaviors are to oral health would be a useful tool for reducing dental loss.

E6.

# STUDY OF THE ERYTHROCYTE AGGREGATION IN RATS OF THE LINE B

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In previous works we used IIMFm/ $\beta$  line ( $\beta$ ), rat model of obesity and diabetes, which showed hyperaggregation to eumetabolic  $\alpha$ line, extinct today. The line IIMb (b), incorporated as a new eumetabolic line, developmed glucose intolerance and obesity tendency. Objetive: study erythrocyte aggregation (EA), and eryhrocyte deformability (ED), in animals of the b line and analyze the development of modifications that spread to the reproduction  $\beta$ model's behavior. We used b line males rats of 200 days (n=4). AE, was determined by optic method in suspensions of red blood cells (RBC) in plasma and in Dextrán 500 2% in saline (Htc: 40%). We determined:  $s_0/n_0$  (estimates size of aggregates) and  $2k_2n_0$  (estimates initial rate) Rigidity index (RI) was measured (highest RI smaller ED): RBCs in saline-albumin was filtered through 5µm pores. EA results were analyzed with t Student test and expressed as medial±SD. RI results were analyzed with Mann-Wihtney test and expressed as medium and range. Results:EA Plasma (2k,n<sub>0</sub>):β:  $0.69\pm0.35$ ; b:  $0.36\pm0.21$  \*\*\*;  $(s_0/n_0)$ :  $\beta$ :  $1.77\pm0.11$ ; b:  $1.26\pm0.31$ \*\*\*; EA Dextran  $(2k,n_0)\beta$ : 0.62±0.28; b: 0.48±0.09\*\*; (S0/N0)  $\beta$ : 1.77±0.11; b: 1.68±0.11\*\*\*. IR(%):β: 9.15 (6.13-12.92); b: 12.96  $(9.42-17.84)^{**}$ . Both parameters are smaller in b line to  $\beta$  -if well they are bigger than that of  $\alpha$  line (published data)-; as much in plasma as in Dextrán. This would indicate that observed differences are mainly due to factors related with mechanical properties of cellular membrane. The smaller ED in line b it would be conferring sustenance to this hypothesis.

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