BIOCELL
2004, 28(2): 201-233

ISSN 0327 - 9545
PRINTED IN ARGENTINA

ROSARIO BIOLOGY SOCIETY

(Sociedad de Biología de Rosario)

Abstracts from the

XXIII ANNUAL MEETING

December 4-5, 2003

Sede de Gobierno de la Universidad Nacional de Rosario
Rosario, ARGENTINA

1.

ANDROGENS AND ACUTE HEPATIC FAILURE PREVENTION INDUCED BY PARACETAMOL IN RATS

Laudanno O, Páez S, Cesolari JA.
Gastroenterología Exp., Fac. Cs. Médicas. UNR, Rosario, Argentina.

Liver constitutive receptors (CAR) are agonists against paracetamol (Pa) liver toxicity. In case of androgens blocking comatose Pa doses. Randomized female Wister rats groups (n=10), 200-400 g weight, 24 hs fasting, water d-libitum were submited to the following experiments: 1 Vehicle: 1 ml 1% Carboxy-methyl cellulose (IP). 2 1.8 g/kg Pa (IP) (comatose doses). 3 250 mg/kg (sc) testosterone (T) (4 esters): a) 60 min pre-Pa; b) 60 min post-Pa and c) 5 hr post-Pa. 4 25 mg/kg (sc) Nandrolone: a) 60 min pre-Pa; b) 60 min post-Pa and c) 5 hr post-Pa. After 24 hs in coma control rats were sacrificed with ether overdose. Laparatomy and thoracotomy were performed and blood withdrawn by cardiac puncture to assess transaminase. The liver was removed and samples were obtainde (HE). Hepatic necrosis scores (HNS) were tabulated. Rat survival (S) was determined. Student's t test and ANOVA were performed. 1 Vehicle: TGO 11±3, TGP 7±4, HNS 0% and S 100%. 2 Pa TGO 1845±185, TGP 710±3, HNS 3.2 and S 20%. 3 T: a) pre-Pa TGO 135±19, TGP 41±8 (<0.001), HNS 1.2 (<0.001) and S 90% (<0.001); b) 60 min post-Pa TGO 215±21, TGP 86±7 (<0.001), HNS 1.3 and S 90% (<0.001); c) 5 hr post-Pa TGO 260±21, TGP 74±11, HNS 1.5 and S 80% (<0.001); 4 Nandrolone: a) TGO 124±12, TGP 39±7, HNS 1.1 and S 90% (<0.001); b) TGO 190±18, TGP 60±14, HNS 1.5 and S 80% (<0.001) and c) TGO 215±21, TGP 86±28, HNS 1.6 and S 80% (<0.001). Conclution: androgens blocking CARs prevent acute hepatic failure, therefore could be useful in human hepatic coma treatment due to paracetamol.

2.

***IN VITRO* EFFECT OF HIALURONIC ACID (HA) ON ERYTHROCYTE MEMBRANE FLUIDITY**

Luquita A[1], Gennaro AM[2], Urli L[1], Svetaz MJ[3], Fernández MC[4], Caferra D[4], Rasia M[1].
[1]Cát. Biofísica, Fac. Cs. Médicas, UNR, CIURN; [2]Fac. Bioq. y Cs. Biológicas UNL,Conicet; [3]Fac. Cs. Bioq. y Farm.; [4]Cát. Bioq., Fac. Cs. Médicas, UNR.

Previous results lead us to the hypothesis that serum HA decreases erythrocyte membrane fluidity and deformability. In order to demonstrate this, HA (SIGMA Chem. Co.) was purify by applying cold chromatography and its concentration in the elution was determined by colorimetry. Washed erythrocytes have been employed, Hemoglobin free erythrocyte membranes were obtained from a fraction of them. These ghosts (n=5) were divided in two samples: the first one (HA group) was treated with purified HA in a similar concentration to patient serum, while the other (control group) was treated with the same volume of elution solution. After 30 minutes at 37°C, changes in conformational membrane protein (W/S) were estimated by electronic paramagnetic resonance. In the remaining fraction of whole erythrocyte, the degree of ordering (S) of the acyl chains were measured at different depths. The statistical analysis of the results was performed by Wilcoxon test for paired data. Results are expressed as median (M) and confidence interval (IC 95%). Group HA: [HA]s = 103 (100-105) µg/ml, W/S = 3,20 (3,10- 3,30), Control group (CG): W/S = 2,65 (2,60-2,70), $p<0.05$. Group HA: S_5 = 0,690^{ns} (0,677-0,707), S_{12} =0,521^{ns} (0,520- 0,524), S_{16}=0,229^{ns} (0,225-0,233), CG. S_5=0,693 (0,685-0,703) S_{12}=0,525(0,524-0,527), S_{16}=0,230(0,228-0,230). In HA group W/S was significantly decreased but S_5, S_{12} and S_{16} were no modified. These results point out that AH did not alter the degree of ordering of lipid chains, but it did interact with cytoskeletal proteins.

3.

ERYRHROCYTE AGGREGABILITY IN β RAT STRAIN. ***IN VITRO* EFFECT OF INSULIN**

Cinara L[1], Bollini A[1], Gayol M del C[2], Hernández G.[1]
[1]Cátedra de Biofísica, [2]Cátedra de Biología, Facultad de Ciencias Médicas, U.N.R.

β rat strain, a hypertriglyceridemic obesity and diabetes model, has shown hemorheological alterations associated with triglyceridemia and glycemia levels, in a similar way as obese diabetic patients do. In the present work it has been studied the *in vitro* effect of two physiological insulin concentrations (120 and 480 pmol/l) upon erythrocyte aggregability (EA), in the β strain and in rats of b strain (eumetabolic control) aged 200 days (n=9). Blood was obtained by cardiac puncture. Red blood cells (RBCs) were separated by centrifugation, washed in saline, divided in three aliquots, and incubated during 15 minutes: 1)PBS containing 100 mg/dl glucose (PBSg) without insulin; 2) PBSg plus 120 pmol/l insulin; 3) PBSg plus 480 pmol/l insulin. The supernatant was removed and RBCs were suspended in Dextran500 for AE estimation. The statistical analysis was performed through Student Student's *t* test for unpaired data. It was observed a significant increase in the AE in β strain respect to eumetabolic b strain in insulin absence and in both lines by the *in vitro* effect of insulin. These results suggest that insulin modifies the erythrocyte membrane, influencing the EA positively. These changes might be mediated by erythrocyte deformability. As observed in previous studies, cell deformability is increased by the *in vitro* effect of insulin.

4.

ERYTHROCYTE AGGREGATION MODIFICATIONS IN EQUINES BEARING SPONTANEOUS LAMINITIS

Catalani G, Dottavio ME, Rasia M.
Cát. Física Biol. Fac. Cs. Méd. UNR. Cat. Fisiología. Fac. Cs. Veterinarias. UNR.

Laminitis is an acute/subacute disease affecting the hoof capsule and soft tissue between hoof and 3rd. phalanx, producing sometimes its rotation. It is a secondary response to alterations in other parts of the organism. Terminal vascular ischemia in limbs due to microthrombosis has been observed. There are modifications in plasma proteins, mainly fibrinogen, which is important in aggregation. High ceruloplasmin, could explain the increased aggregability in comparison with human beings and other mammals. Objetive: to study aggregability in RBCs and its relationship with [fibrinogen] and [ceruloplasmin] in anticoagulated (EDTA) blood samples in 6 racing horses with spontaneous laminitis. Samples obtained at 2, 3 and 10 days disease-onset. Assays: fibrinogen (F, mg/dl, gravimetry); ceruloplasmine (Cp, mg/dl, turbidimetry). RBC-aggregation, registering optic density changes in whole blood samples automatically shaken each 2 min. Curve fitting yields two parameters: 1) estimation of average aggregate size (S); 2) aggregate rate (R). RBCs in autologous plasma (40% hematocrit). Statistic analysis: Wilcoxon's and Spearman Tests. Results (median, range): R no change ($p>0.05$) between day 2 [1.95 (2.2-1.6)] and day 3 [1.89 (1.8-1.9)]. There are significant differences ($p<0.05$) at day 10 [1.17 (0.9-1.3)]. No S modifications were observed during time evolution ($p>0.05$), neither in [F] nor in [Cp] at days 2 and 3 respectively. At day 10 decrease of both proteins ($p<0.05$). Significant correlations for R, F and Cp ($p<0.05$) during follow-up: [$r_{V,F}$=0.16; $r_{V,F}$=0.34; $r_{V,F}$=0.46; $r_{V,Cp}$= 0.16; $r_{V,Cp}$=0.27; $r_{V,Cp}$=0.16; $r_{V,Cp}$=0.17]. The laminitis show [F] and [Cp] during the first days onset, thus leading to higher aggregation, not observed at day 10.

5.

OSMOTIC FRAGILITY AND RED CELLS SHAPE IN ALUMINIUM (AL)-INTOXICATED RATS

Chiarotto M, Contini M del C, Graffione S, Bazzoni G, Rasia M*
*Cát. de Biofísica. Fac. de Cs. Médicas. UNR. *Cát. de Fisiología. Fac. de Bioq. y Cs. Biol. Santa Fe.UNL.*

The object was to study the *in vivo* effect of aluminium administration upon the osmotic response and shape of red cells, taking into account its possible relation with the anemia observed in Al intoxicated patients. Wistar male adult rats were used: one half were controls (n=4) and the other half were injected with $Al(OH)_3$ 80mg/ml PC i.p. 3 times a week for 3 months (n=4). Red blood cell resistance to osmotic haemolysis was determined photometrically and two parameters were obtained (x_{50}: NaCl concentration for 50% haemolysis; β: sample homogeneity). The shape of red cells was quantified through a morphological index (MI) (according to Bessis). Statistical analysis: Mann-Whitney U test for unpaired groups, data are presented as median and range. Osmotic fragility: x_{50} (mM): control:81,07 (80,80-82,80); intoxicate rats: 68,25* (67,70-69,40); β: control: 0,107 (0,075-0,119); intoxicated rats: 0,065* (0,052-0,072). MI: control: -0.9 (-1,1/ -0,7); intoxicated rats: -2,60* (-2,90/ -2,40). *p<0,001. Stomatocytosis in intoxicated rats suggest that Al enters the lipid bilayer, modifying cell shape. Furthermore, Al insertion in the membrane allows its expansion, stabilizing the cell against hypotonic lysis and could explain the diminution in deformability measured, whereas the lesser deformability is congruent with the anaemia observed in intoxicated patients.

6.

EFFECT OF THE ALUMINUM ON THE OXIDATIVE STRESS ON REGENERATED LIVERS

González MA[1], Mahieu S[1], Bernal C[2], Contini M del C[1], Catalín A[1], Millen N[1], Carrillo MC[3].
[1]Fisiol Humana., [2]Brom. y Nutrición. Fac. Bioq. y Cs. Biológicas. UNL. [3]Instit. Fisiología Exper. UNR.

The aim of this work was to study the effect of the chronic exposition of Aluminum (Al) on parameters related to oxidative stress in regenerated livers after 48 hs of hepatectomy (HP). Wistar male rats were utilized, the average weight being 300 g divided into 4 experimental groups (n=5 each group) A: controls; B: treated with Al(OH)3; with a dose of Al elementary 27 mg/Kg PC administered i.p. 3 times per week for 3 months; C: 48 hs HP; D: Al + HP 48 hs. The enzymatic activity of catalase (CAT); Glutathione Peroxidase (GSH-Px) and the content of Glutathione (GSH).were analyzed. The lipid peroxidation level (LPO) was determined through the determination of the substances reactive to tiobarbituric acid. Results were expressed as mean ± SEM; *significant difference p<0.05: LPO (nmolmda/g tej. Húmedo) A:275±38.05; B:573±75*; C:842±61* D:1455±155.* GSH (μmol/g tejido) A:3.90±0.14; B:2.52±0.12*; C:4.22±0.21; D:3.17±0.003*. GSH-Px (nmol NADPH/ min.mg prot). A:82.84±7.05; B:45.19±3.31*; C:61.36±4.5*; D:57.08±6.6*. CAT (U/mg prot): A:0.49±0.031; B:0.08±0.003*; C:0.21±0.02*; D:0.072±0.01*. In animals with partial hepatectomy, it was observed, as it was expected according to studies previously done, an increase in the lipid peroxidation index and a decrease in the activity of the antioxidant enzymes, what would reflect an increase in the free radicals production , without being modified the levels of GSH. The Al per se causes similar changes, with very significant decreases in catalase and in the levels of cellular GSH. In hepatectomized animals a summation in the effects is observed, being detected a marked LPO. This can be directly related to the decrease of GSH. Besides, the Al would cause an important inhibition in the catalasa activity that would contribute to explain the pronounsed increase in LPO.

7.

EFFECTS OF THE ALUMINUM (AL) ON THE ANTIOXIDANT STATE AND THE LIPID PEROXIDATIÓN IN RENAL TISSUE IN RATS WITH PARTIAL HEPATECTOMY

Mahieu S[1], Millen N[1], González MA[1], Burns P[1], Contini M del C[1], Elías MM[2]
[1]Fisiología Humana. Fac. Bioq. y Cs. Biológicas. UNL. [2]Farmac. Fac. Cs. Bioq. y Farm. UNR. E-mail: smahieu@fbcb.unl.edu.ar

In previous works we reported abnormalities in rat renal function during the process of liver regeneration after a partial hepatectomy (HP), which are accentuated in animals treated chronically with Al. This study was done to explain 96 hs after a HP (65%): a) if the oxidative changes observed at the level of the hepatocites during the cell regeneration process can induce similar changes in kidney, b) if the presence of Al that affects the liver and renal function would condition such changes. Four groups of animals were studied: Control C (n=8); Al (n=5), treated with aluminum lactate during 3 months (6,2 mg/100g weight, i.p. 3 times per week); HP (n=8); HP+Al (n=5). Glutathione (GSH) and lipid peroxidation (LPO) levels, the activity of the antioxidant enzimas Glutathione peroxidase (GSH-Px) and catalasa (CAT) were assayed in renal tissue homogenates. Serum lactate dehydrogenase (LDH) and alanine amine transferase (ALAT) were used to evaluate the liver injury and the glomerular filtration rate (GFR) was measured to evaluate the renal function. Results given as mean ± SEM (*significant difference p<0.05). LPO nmol MDA/g tej.hum.: C:233.1±9.6; HP: 465.9±39.6*; Al: 420.9±5.2 *; Al+HP: 450.1± 21.5*. GSH μmol/g tej.hum.: C: 2.37±0.1; HP: 1.89±0.1*; Al:1.78±0.1*; Al+HP: 1.39±0.2*. GSH-Px nmol NADPH/min.mg prot:C:51.6±3.4; HP:25.0±0.1*; Al: 22.5± 0.9*; Al+HP:13.9±1.7*. CAT U/mg prot. C:0.47±0.03; HP: 0.32± 0.005; Al: 0.10 ± 0.01*; Al+HP: 0.12± 0.03*. The Al did not modify, either the GFR, or the activity of the liver enzimes. A reduction in GFR can be observed in HP, which was greater in HP+Al. In both groups LDH and ALT increased. Results indicated that the HP induce lipid peroxidation in renal tissue, which can be linked to the decrease of the GSH levels and of the GSH-Px activity. The Al conditions these effects to reduce, besides GSH and the activity of GSH-Px, that of CAT.

8.

EFFECT OF ORGANICS COMPOUNDS ON RODAMINE B (RB) EFFLUX IN ISOLATED HEPATOCYTES

Sayago G, Agüero R, Figueroa N, Rodríguez Garay E.
Cátedra de Fisiología, Fac. Cs. Médicas, UNR. Inst. Fisiol. Exp. (IFISE) CONICET-UNR. E-mail: raguero@citynet.net.ar

RB is an organic cation which is transported by the liver probably employing transport mediated mechanism. In influx and efflux experiments, nearly saturation behavior and temperature dependence at low compound concentration (< 1 μM) was observed. Present work bring near evidence on transport mechanism involved. Male Wistar rat (n:4) hepatocytes isolated by collagenase-perfusion technique (> 80% viability, assessed by Tripan blue exclusion) was loaded with RB (15 min. incubation; 37ºC). Cellular pellet was washed several times to removed extracellular RB. Initial RB load was: 85 ± 5 pmoles x 10^6 cel. Efflux (between 6 s to 30 min; 37 ºC) were evaluated resuspending hepatocytes ($5x10^6$ cells/ml) in free RB medium alone (control) or containing another organic compounds (*trans* position) which possessed identified hepatic mediated transporter us Quinine (**Q**); basolaterally located transporter OCTP1/SLC22A1 and apical located transporter MDR1/mdr1 (ABCB1/abcb1), Ouabain (**Ou**) basolaterally located transporter Oatp 2/Slc21a5 and Cyclosporine A (**CPA**) apically located transporter MDR1/mdr1 (ABCB1/abcb1). At each time efflux was stopped by silicone oil centrifugation technique. RB remaining in the cells was extracted and fluorimetrically evaluated. RB efflux was *trans* stimulated in presence of **Q** and **Ou** while was not affected or *trans* inhibited by **CPA**. *Trans*- stimulation phenomenon (increases compound efflux in the presence of analogous compound in opposed side of membrane), suggest the participation of membrane transporters in the RB movement. **Q** (cation) and **OU** (neutral) share the same basolaterally located transporter. Difference in magnitude of effects could be attributed to different molecular charge or affinity of these compounds for the transporter. **CPA** is a highly lipophilic cation which could interferred inespecifically with RB efflux or perhaps Rb has not affinity by the apically located transporter.

RB load released at 8 min
(% with respect to control value (mediana; n:4)

Compound (*100μM*)	*Trans* *i*nhibition	*Trans* stimulation
free	99,38 %	99,38 %
Quinine	-	226,78 %
Ouabain	-	184,26 %
Cyclosporin A	96,83 %	

9.

A COMPLEMENTARY MORPHO-INFORMATIC AID FOR DIAGNOSING ARTERIAL STENOSES

Idelsohn S[1], Miguel JC[1], Mattara M[2], Ponso RR[2], D'Ottavio AE[1,3]
[1]Facultades de Ciencias Médicas y de [2]Ingeniería, Agrimensura y Ciencias Exactas. [3]Consejo de Investigaciones, Universidad Nacional de Rosario.

This communication presents a complementary morpho-informatic aid for diminishing a reported 15% of re-obstructions after human femoral artery revascularizations. It is based on a simulator computational program where data obtained from portions of femoral arteries with different grades of stenoses are incorporated. In this case, data were derived from 6 necropsies and 8 amputations performed in adult men (55 ± 5 years old). Specimens were fixed in formalin, glycerin and alcohol and then sagittally divided into two hemi-arteries where the vessel and the luminal diameters were measured 3 mm each through a caliper under a Zeiss binocular microscope. Likewise, the program processed a lot of simulated data. Results, expressed as maximal curves, allow interpreting most of the possible regimens and are visualized as graphics whose abscises, measured in mm, belong to the post stenotic lenght the turbulences reach and whose ordinates register the relationship: stenosis diameter / vessel diameter, calculated half millimeter each. These distinct graphics were summarized in an only one. Thus, the professional by obtaining the aforesaid relationship through arteriography or eco-doppler may establish the proper location for sectioning the artery. These results, corroborated anatopathologically, may lead to a more complete diagnosis together the other available methods.

10.

PLASMATIC VISCOSITY AND BLOOD FIBRINOGEN IN DIABETIC PATIENTS

Carrera LI[1], Foresto P[2], Etchepare R[4], D'Arrigo M[2], D'Ottavio AE[1,3], Valverde J[2], Rasia R[2].
[1]Cátedra de Histología y Embriología, Facultad de Ciencias Médicas, UNR. [2]Laboratorio de Inmunohematología y Hemorreología, Facultad de Ciencias Bioquímicas y Farmacéuticas, UNR. [3]Consejo de Investigaciones, Universidad Nacional de Rosario. [4]Hospital Provincial Rosario.

As occurs in nerves, kidneys and eyes, diabetes impacts the skin, from a microcirculatory standpoint. Thus, parietal and hemorheological disturbances may be suspected. Since no noteworthy microscopic lesions appeared, hemorheological disorders became relevant. Consequently, we analyzed plasmatic viscosity and blood fibrinogen in 60 diabetic (19 with skin lesions and 41 without it) and 20 healthy patients. Plasmatic viscosity was determined with a cone-plate viscometer at a 2.30 seg.$^{-1}$ shear rate whilst blood fibrinogen was determined following Clauss method. Kriskal-Wallis test or ANOVA were statistically employed as needed. Subsequently, Pearson and Spearman correlation coefficient were used for comparing both variables. Significant differences were found (1) *in plasmatic viscosity* (p=0.008) (controls: 4.23 ± 1.53, diabetics with no skin lesions: 13.14 ± 12.30 and diabetics with skin lesions: 8.12 ± 5.47) and (2) *in blood fibrinogen* (p=0.0003) (controls: 209 ± 26 mg %, diabetics with no skin lesions: 318 ± 68 mg % and diabetics with skin lesions: 353 ± 79 mg %). The referred correlation did not show significant differences. These results could demonstrate that these alterations may proceed to skin lesions and that both parameters could contribute to its development. However, in relation to plasmatic viscosity, other factors distinct to fibrinogen could be involved.

11.

THE HUMAN INTESTINE DURING ITS EARLY FETAL DEVELOPMENT

Tellez TE[1], Carrera LI[1], D'Ottavio AE[1,2].
[1]Cátedra de Histología y Embriología, Fac. de Medicina, UNR. [2]CIUNR.

Taking into account the relevance of digestive system development, this paper deals with the differentiation of the intestine in 9th and 10th week-old six human fetuses obtained from spontaneous abortions in public hospitals. Serial 7 µm thick sections from the abdominal-pelvic zone were sequentially exposed to varied histological and histochemical procedures. Afterwards, they were planimetrically processed and superposed for spatial reconstruction. At these ages, the intestinal tube was primordially located in the caudal portion of the abdominal-pelvic cavity being the cephalic one occupied by the liver. The tube presented a proximal zone much more flexuous than its distal one. A significant part of the flexuous zone was situated into the umbilical cord. From a histological standpoint, of all the components usually described in the intestinal wall it was only observed the inner circular layer of smooth muscle of the muscularis externa, the myenteric plexus and a dense condensation next to the mesothelium. The longer proximal zone revealed maximal tubular and luminal diameters. Besides, this zone showed numerous villi covered by columnar epithelium lying on a highly vascularized lamina propria, very rich in reticular fibers. Conversely, the shorter distal zone evidenced the same structure but in a lesser extent. These results lead to suppose that the morphological pattern of the proximal zone may be functionally linked to the luminal content (absorption of the amniotic fluid?).

12.

RENAL LECTIN HISTOCHEMICAL STUDY IN 1 YEAR-OLD DIABETIC RATS

Frontini AV[1], Hisano N[1], D´Ottavio AE[1,2].
[1]Cátedra de Histología y Embriología. Facultad de Ciencias Médicas. [2]Consejo de Investigaciones. Universidad Nacional de Rosario (UNR).

This communication completes previous reports performed in renal cortex and medulla of diabetic rats intending to register data compatible with its demonstrated nephropaty in evolution. 15 male rats (5 eSS, 5 eSMT and 5 Wistar –controls-) were weighed and sacrificed at 12 months of age. Kidneys were weighed, fixed in Bouin's fluid and subsequently in 10% neutral formalin, embedded in paraffin and cut. Sections were exposed to several lectins [Arachis hypogaea (PNA), Canavalis ensiformis (Con-A), Dolichos biflorus (DBA), Glycine max (SBA), Ricinus communis (RCA), Triticum vulgaris (WGA), Urex europeus (UEA-I)], afterwards to the Complex Avidin-Biotin and to Diaminobencidin and finally visualized in a Zeiss microscope. Diabetic rats were less reactive than controls in superficial and juxtamedullary glomeruli as well as in superficial and juxtamedullary basal membrane of convoluted proximal and distal tubules with WGA and in superficial and juxtamedullary Bowman's capsules with RCA and UEA-1. Conversely, diabetic animals were more reactive in the apical border of the straight distal tubules with WGA and RCA and in the collector tubules with UEA-1. Obtained data appear consistent with the nephropathy in evolution revealed in former communications through other techniques.

13.

INFLUENCE OF A SOY FLOUR DIET ON THE CECUM OF PREPUBERAL DISMETABOLIC RATS

Rodríguez G[1], De Ascencao GR[1], D'Ottavio AE[1,2].
[1]Cátedra de Histología y Embriología. Facultad de Ciencias Médicas. [2]Consejo de Investigaciones. Universidad Nacional de Rosario.

The present report focuses in the cecum morphology and completes previous communications related with the influence of a soy flour diet on other digestive organs of prepuberal ß rats (genetically obese and developing late type 2 diabetes). 10 male ß rats were fed *ad libitum* from 22 to 55 days of age with 2 diets differing only in its protein source (soy flour with inactivated antinutritional factors in the experimental group and casein in controls). Ceca were dissected, measured and weighed, fixed in 10% neutral formalin, embedded in paraffin, cut at 6 µm and stained with con Periodic Acid Schiff and Hematoxylin- Eosin. Specimens were visualized in a Zeiss microscope with a linear scale in the eyepiece. Significant higher values were detected in the soy flour group in ceca lengths (4.40 ± 0.63 cm vs.3.55 ± 0.10 cm, $p<0.05$) and in its absolute (916.50 ± 103.35 mg vs. 530.25 ± 85.92 mg, $p<0.05$) and relative weights (511.03 ± 55.07 mg/g body weight vs. 311.47 ± 71.85 mg/g body weight, $p<0.05$). Taking into account that soy beans contain proteins acting as antigens and the cecum, particularly in determined animals, is related with immunological processes the obtained results may be compatible with this link.

14.

TESTICULAR AND EPIDIDYMAL MORPHOLOGY IN ONE YEAR-OLD DIABETIC RATS

Feresin N[1], D'Ottavio AE[1,2], Hisano N[1].
[1]Cátedra de Histología y Embriología, Facultad de Ciencias Médicas. [2]Consejo de Investigaciones, Universidad Nacional de Rosario.

Completing a previous report based on the modifications produced by nutritional and metabolic factors in male reproductive system, testicles and epididymal tails in 1 year-old eSMT diabetic rats were morphologically analyzed. At 12 months of age, 5 eSMT and 5 Wistar rats (controls), receiving a commercial diet *ad libitum*t were sacrificed with ether overdose. Testicles and epididymes were dissected and weighed. Epididymides were fixed in 10% neutral formalin and testicles in Bouin's fluid and subsequently in 10% neutral formalin. Specimens underwent a histological routine procedure being sections stained with Giemsa, Hematoxylin-Eosin, Masson's Trichrome and Periodic Acid Schiff. A not significant descendent tendency was registered in eSMT in body weight and in absolute and relative testicular and epididymal weights. Both groups revealed a well conserved interstitial testicular structure. However, atrophic tubules appeared more severe in eSMT rats. Proximal epididymal tubules showed a regular shape with spermatozoa content in every animal whilst distal ones were irregular in eSMT rats and showed persistence of spermatozoa. Likewise, these animals evidenced tubules with necrotic epithelium and larger interstitial spaces. The aforesaid descendent tendency in eSMT and its congruence with histological structures compatible with ageing match with the already reported higher severity and duration of the diabetic syndrome in these rats.

15.

N-ACETYL-CISTEINE AND ACUTE HEPATIC FAILURE DUE TO PARACETAMOL IN RATS

Paez S, Laudanno O, Cesolari J, Paez M, Aramberry L, Sambrano JS, Basa E, Savio L, Calvi B, Catalano J.
Gastroenter. Exp.- Cát. Histol. y Embriol, Fac. Cs. Méd. UNR.

Paracetamol overdose (PARA) produces liver central lobular necrosis due to the saturated glucuronization, thus deviating the PARA metabolism to chromosome P450, which generates a reactive metabolite (N-acetyl-para-benzoquinone-amide, (NADPQ)). The N-acetylcysteine (NAC) is the antidote, increasing the hepatic glutathione and neutralizing the NADPQ. Objective: to study NAC at 5 and 24hr after comatose doses in an experimental rat model with high mortality rate. Randomized female Wistar rats (n=10 each group), 200-230g wgt, 24hr fasting, water ad lib. were submitted to the following experiments: I- Vehicle: intraperitoneal 1ml 1% Carboxymethylcellulose. II- 1.8 g/kg IP PARA, 48hr (dependent comatose dose: 1.1; 1.2; 1.4; 1.6; 2 g/kg). The rats were sacrificed during hepatic coma at 24 and 48hr. III- PARA and 250 mg/kg NAC after 5hr by oral gavage. IV- PARA and NAC 24hr later. The rats were sacrificed by ether overdose to performed laparotomy and thoracotomy, withdrawing blood by cardiac puncture to assess transaminases. Right and left lobe liver samples were obtained for histological examination (HE). Liver necrosis score: 0-4. Rats survival estimated at 48hr. Student's t test and ANOVA was calculated. Results: 24 hr, I- TGO 11±3, TGP 7±2. II- TGO 1810±130, TGP 723±16 (<0.001). III- TGO 312±16, TGP 121±15. IV- TGO 725±21, TGP 214±16 (<0.001). Liver hepatic necrosis score at 24hr: I-0. II-3.2. III-1.7. IV-2.1. Survival at 48hr: I-100%, II-20%, III-90%. IV-50%. Conclusion: N-acethylcysteine 5hr post-PARA showed 90% survival, whereas 24hr later yielded 50% survival.

16.

BIOMASS IN EARLY AGES AND GLUCIDIC INTOLERANCE EXPRESSION: FETAL HEFTY PHENOTYPE IN THE DIABETIC LINES OF RATS eSS AND eSMT?

Romeo A, Romera L, Azpeitia N, Martínez SM, Montenegro S, Tarrés MC.
Cát. de Biología. Fac. de Ciencias Médicas. CIUNR. PIAD. UNR.

Evidences have been reported establishing a relation between biomass at birth and subsequent development of diabetes, thus formulating two hypotheses: thrifty and fetal hefty phenotype respectively. In two murine models of spontaneous diabetes, eSS and eSMT, we studied the relation between biomass in early ages and the intensity of hyperglycemia in adulthood, registering in eSS and eSMT males the litter size (LS), weight at birth (WB), weight at weaning (WW) and adult weight (AW) and the values of glucose tolerance curve at 6 months of age (G0, G30, G60 and G120). A factorial analysis of main components was carried out, considering as active the 8 mentioned variables and as illustrative the line (eSS and eSMT). Classification techniques on the calculated factorial axis were applied, grouping the animals in 3 clusters, being line the main differentiation variable (Class 1: formed by eSMT rats, with WW, AW, G0, G30, G60 and G120 above the general average; Class 2: eSS rats with WB higher than the general mean and WW higher than Class 1; Class 3: rats with bigger LS).
These results might contribute with some elements in favour of the fetal hefty phenotype hypothesis indicating that, in these murine models of diabetes, an augmented weight in early ages might increase the defects of glucidic homeostasis that could be maintained until adulthood.

17.

STABILITY IN GLUCOSE INTOLERANCE AND ADULT WEIGHT IN GENERATIONS OF DIABETIC eSMT RATS

Demartis P, Abellán von Farkas D, Biga L, Giancola E, Ferraro JI, Martínez SM, Tarrés MC, Montenegro S.
Cátedra de Biología. Facultad de Ciencias Médicas. CIUNR. PIAD. UNR.

We have generated eSMT, a recombinating line of rats, diabetic and hefty. Since type 2 diabetic syndromes are influenced by biomass variations, we found it interesting to carry out the analysis of growth in the eSMT rat, seeking possible associations with the expression of diabetes in adult age, as well as to evaluate if such characteristics were maintained with increase of homocygosis due to consanguinity. In two distant generations we registered, in animals of both sexes, biomass and tail length - as an indicator of skeletal length - weekly from 21 to 70 days of age. At 7 months we measured biomass, basal glycemia and glucose oral overload test. Growth curves were adjusted according to von Bertalanffy model. Significant differences were verified between sexes, generations, males and females and, in adult weight and glycemias after glucose overload, stability between generations and sexual dimorphism. Since accelerated growth is a risk factor in susceptive genotypes, basal normoglycemia in males of the control generation could be related to their lower growth speed; higher glycemias of females from advanced generations might be related with their faster growth that, together with their smaller length, would produce more compact animals.

18.

GLOMERULAR LESIONS IN KIDNEYS OF MALES IN THE SPONTANEOUSLY DIABETIC LINES OF RATS, eSS AND eSMT

Fixman M, Felizia S, Furlano M, Ramonda M, Frenquelli F, Dalmau A, Montenegro S, Tarrés MC, Martínez SM, Picena JC.
Cátedra de Biología. Facultad de Ciencias Médicas. CIUNR. UNR.

In animal models of diabetes, the study of glomerular alterations is interesting because the vital prognosis depends largely on them. The evolution of glomerulopathy was studied in two lines of rats that spontaneously develop type 2 diabetes: eSS and eSMT, the latter with an earlier and more severe expression. 68 eSS and 50 eSMT males, from 3 to 24 months of age, were weighed and sacrificed with ether overdose. Both kidneys were removed and weighed, underwent fixation and embedding, and were stained with H-E and H-PAS for optic microscopy.
The following characteristics were registered: small glomeruli less than 80 microns in diameter (SG); thickening of the glomerular basement membrane (TGBM); mesangial expansion, slight (ME+) or severe (ME++) and partial and total fibrohyalinosis (FH). Significant correlations were verified in eSS and eSMT between age and: biomass, SG, TGBM, ME+, ME++ and FH. Kidney weight, SG and ME+ were higher in eSMT. Only the amount of SG was higher in eSS.
These results allow to suggest that in eSMT rats the more intense metabolic disturbances went along with an increase of SG and ME+ and that, as in many other murine models, glomerular anomalies worsen as age advances.

19.

STRESS PERCEPTION IN THE ONSET OF RHEUMATIC DISEASES. FINAL RESULTS

Estrella V[1], Barraza SC[1], Leroux MB[1], Paris L[2], Leiva M[3], Bottai H[3]
[1]Cát. Dermatología, Fac. Cs. Médicas. [2]Cát. Met. Inv. Psicológica II. Fac. Psicología. [3]Área Estadística. Fac. Cs. Bioq.

Stress is based on the perception of individuals regarding the interactive balance between demands and their ability to cope whit them. The relationship between the onset of autoinmune diseases and stress has already been studied. The present work takes as objectives 1) the perception studies of the effect of stressful events in the appearance of the first symptoms of the collagen diseases. 2) To analyse which are the most frequent stress indicators 3) To analyse the relation between the demographic variables sex, age and marital status of the patients and the perception of the unchaining events. There are studied 189 adult patients who consulting the service of dermatology (Hospital Provincial del Centenario) with diagnosis of Scleroderma, 95, (SLE), 60, Arthritis Rheumatoid (RA), 17 and others answering a questionnaire elaborated for this purpose. Of the whole of patients: 64 do not recount unchaining (30 with ESC.:22 with SLE; 4 with RA and 8 with other diceases). Of the 125 that they recognize some psychosocial stressor influencing the appearance of the symptoms prevails the death of some nearby relative, 45 patients. The economic problems, labour or familiar type are observed secondly. The time between the unchaining circumstance and the appearance of the first symptoms is in the main one year. They do not exist significant association between the sex and the perception of unchaining event. Clear indications of relation exist between the studied pathology and the precedent of stress. The patients recount in a high percentage the existence of circumstances of emotional intense impact as unchaining of rheumatic diseases.

20.

NEW MECHANISMS IN GASTRIC PROTECTION DUE TO HEAT SHOCK PROTEINS

Basa E, Cesolari JA, Laudanno OM, Aramberry LJM, Sambrano JS, Savio L.
Gastroenterol. Exp., Catedra de Histologia y Embriologia. Fac.Cs Medicas. UNR.

Heat shock proteins (HSP) induced by hyperthermia protect the gastric mucosa against ethanol. Objective: to verify if gluthation depletion and blocking of the following receptors (Rc) :minergic HT1; 5-HT2 and 5-HT3 serotoninergic μ, δ and κ opioids and axon-Na-channels Rcs affect gastric protection mediated by HSP. Wistar rats, (n=7 each group), 200-230g, 24hr fasting, water ad lib, avoiding coprophagy were submitted to the following experiment: Group1) 1ml saline (S), by orogastric gavage in bolus, waiting 60 min. 2) 1 ml 96% Ethanol (ETOH) OG, waiting 20 min. 3) Hyperthermia (HT): rats were vertically immersed, in 42°-43°C water during 20 min. 4) HT 1 hr; then 1ml ETOH, OG, 20 min. Pre-treatment: 5) 1m 1%, Lidocaine OG, 30 min. 6) Hg dichloride (HgCl2) -gluthation depletor- 1ml 1‰, SC, 30 min. 7) Cinitapride 1mg/Kg OG 30 min selective 5-HT2 inhibitor. 8) Ondansentron, 4mg/kg IP 30 min 5-HT3 selective inhibitor. 9) Naloxone, 0.2mg/kg, SC, 30 min. 10) Desloratadine 2.5mg/Kg, OG, 30 min HT1 selective inhibitor. HT, 1hr, then ETOH 20 min. Rats were sacrificed by ether overdose. Laparatomy, gastrectomy opening the stomach along the great curvature to tabulate lesion area % by computerized planimetry. Histological exam (H.E.).Macroscopy (%lesional area):1) 1.0±0.1, 2) 35.5±5, 3), 1.0±0.1, 4) 5.1±0.7 ($p<0.001$), 5) 2.1±0.6, 6) 30.1±4.6 ($p<0.001$), 7) 32.3±4.5 ($p<0.001$), 8) 1.2±0.3, 9) 10.1±1.5 ($p< 0.03$), 10) 1.2±0.3. Gastric necrosis, except 1) and 3). Gastric protection induced by HT, gluthation and opioid and 5-HT2 serotoninergic Rcs might take part in HSP inducing mechanisms.

21.
PREVALENCE CHANGE IN ARTERIAL BLOOD PRESSURE ACCORDING TO VII-JNC-2003 CUT-OFFS

Raynaudo P, Martos I, Tunkiewiez I, D´Andrea J, Sosa L, Bravo Luna M.
Cat. Bioestadistica. FCE, Cat. Biofis. F.Cs. M. UNR, CIUNR.

The new VII-JNC-2003, suggests changes in arterial blood pressure (ABP) classification and cut-offs. Normal high BP (NHBP) is replaced by pre-hypertension (Pre). We hereby present a sample comporting 148 healthy volunteers both sexes classified according to the VII-JNC-2003 standards in comparison with the V-JNC-1993 ones. Basal BP measurements according to the V-JNC recommendations. Cut-offs: 1) NHBP, according to V-JNC: sBP=135-139mmhg; DBP=85-89 mmhg, 2) Pre, according to VII-JNC: SBP=120-139mmhg; ABP=80-89 mmhg. Stratification by sex and age (yr): <=34; 35-55; >=56. Prevalence estimation. Association: Cochran-Mantel-Haenszel's test, ORs calculated stratifying by sex and age, Breslow-Day's test (homogeneity ratios). There was an increased Prevalence (HNBP vs Pre) according to the new standards, mainly in males. Association between BP and sex, taking into account sex, support this observation, e.g.: sex, <=34 and 35-55yr, df=1, value=9.11, p=0.0025. ORs ratio value=9.0455, 95% CL=1.83-44.59. SBP: the chance of higher Prevalence in men for HNBP-Pre is rather similar according to V-JNC vs VII-JNC standards (9 to 10 times higher for males than females respectively). DBP: according to V-JNC, males HNBP chance is 14-fold higher than in females, whereas according to VII-JNC for Pre the chance is only 8-fold higher, suggesting a relative increase in HNBP-Pre in females regarding males, according to the new standards. We consider that the exclusion of HNBP from the classification in the VII-JNC, that leads to label as Pre-hypertensive, individuals with BP values that according to the V-JNC were normal, is not a suitable Prevention policy since might increase the stress factor in people with these values.

22.
GESTATIONAL WEIGHT AND THAT OF THE BREEDING AT THE BIRTH TIME IN TWO LINES OF INBRED RATS

Gioiella L, Labourdette VB, Gayol M del C, Posadas MD.
Cátedra de Biología. Facultad de Ciencias Médicas U.N.R.

In human beings and in some murine models, the pathologies as well as the morfometric parental variables have been related to the foetal growth. Inbred rats β are obese and diabetic, so it was of interest to make a comparative follow-up between line β and control b of pregestional weight and the mother weight winning during pregnancy, evaluating the relationship with the foetal growth estimated for the weight at time of birth.
The study was done with primiparous females of 150 days of age from line b (n=13) and line β (n=10). The information obtained was analysed with Student test. The results were the following (mean ±SEM): Pregestational weight (g) : β : 264.3 ± 10.30 vs b: 255.7 ± 4.64 ($p>0.05$). Mother weight at pregnancy ending (g) : β : 367.0 ± 12.38 vs b: 338.3 ± 7.13 ($p<0.05$). Mother weight winning during pregnancy (g) : β : 102.3 ± 5.23 vs b: 82.15 ± 7.41 ($p<0.05$). Breeding average weight at birth time (g) : β : 6.99 ± 0.80 vs b: 6.71 ± 0.39 ($p>0.05$). Average of born breeding: β : 9.00 ± 1.09 vs b: 5.92 ± 0.84 ($p<0.05$). In β the number of born breeding is not related to the mother gestational weight winning ($r= 0.119$; $p>0.05$), in b that relationship was significant ($r=0.584$; $p<0.05$). There was no connection between the breeding average weight at birth time and mother gestational growth winning in neither line (β: $r=0.238$; $p>0.05$; b: $r=0.026$ $p>0.05$).
In obese and diabetic line β the mother morphometric variables (pregentational weight, final pregnancy weight and mother weight winning during pregnancy) would affect neither the breeding average weight at birth time nor the born breeding average. Perhaps, β insulin resistance, increased during gestation, would produce a weight winning, due to a greater deposit of corporal fat without affecting the breeding growth.

23.
PUBERTAL DEVELOPMENT OF REPRODUCTIVE FUNCTION IN TWO INBRED LINES OF RATS

Blanco ML, Labourdette VB, Gayol M del C, Posadas MD.
Cátedra de Biología. Facultad de Ciencias Médicas U.N.R.

Obesity is related to a high number of organic alterations including reproductive features, specially those concerning pubertal development.
Our intention is to compare reproductive features between females from an obese (β) and control (b) line.
Daily after weaning 8 β females and 10 b females were assessed for vaginal opening (VO). From VO on, vaginal samples were taken and were examined through a microscope in order to get first estrus (FE). These animals were weighed at both VO and FE ages (VOW and FEW).
Results are shown as media ± SEM:
VO (days): β: 28.13 ± 0.90 vs b: 32.18 ± 1.29 ($p<0.05$)
FE (days): β: 30.88 ± 0.58 vs b: 36.36 ± 0.91 ($p<0.001$)
VOW (g): β: 70.85 ± 7.09 vs b: 73.63 ± 7.24 ($p>0.05$)
FEW (g): β: 87.58 ± 1.75 vs b: 94.75 ± 4.26 ($p>0.05$)
Results reflect the hypothesis by which a critical weight needs to be achieved in order to reach pubertal development.
β rats achieve this critical weight faster than b ones, which might then explain its anticipation in reaching puberty, measured in this case by vaginal opening and first estrus ages.
Leptin, produced by adipose tissue, is probably an important factor in timing puberty due to a control mechanism over gondotrophins.

24.
EFFECTS OF A PHYSICAL TRAINING PLAN AND FOOD RESTRICTION IN GENETICALLY OBESE RATS

Ronco J, Posadas M, Labourdette VB, Gayol M del C.
Cátedra de Biología. Facultad de Ciencias Médicas. UNR.

In previous researches we found that in pubescent rats a moderate physical training plan modificated neither body weight nor plasmatics parameters. The aim of the present work was the evaluate, in β rats, the effects of the combinations of both caloric restriction and physical training on body weight and plasmatics parameters. We worked with 17 β male rats (60 to 120 day old). They were at random separated in three groups: ER food restriction and training group; C control group and R food restriction group. The training was done three times a week, 15 minutes a day employing an eco 3/6 rodent threading machine at 35 m/min. The food restriction was made by feeding them in alternate days. The data were analyzed by ANOVA test and the results were expressed with mean ± SEM:
Body weight (g): **ER**:311.9 ± 9.3 vs **R**:303.5 ± 9.5 ($p>0.05$); **ER**:311.9 ± 9.3 vs **C**: 416.2 ± 7.2 ($p<0.001$); **R**:303.5 ± 9.5 vs.**C**: 416.2 ± 7.2 ($p<0.001$). Fatty panicles weight (g/100 of body weigth): **ER**:4.34 ± 0.35 vs **R**:4.61± 0.18 ($p>0.05$); **ER**:4.34 ± 0.35 vs **C**:6.40 ± 0.24 ($p<0.01$); **R**:4.61±0.18 vs **C**:6.40±0.24 ($p<0.001$). Trygliceridemia g/l): **ER**: 1.43 ± 0.09 vs **R**:1.02 ± 0.11 ($p>0.05$); **ER**:1.43 ± 0.09 vs **C**: 2.46 ± 0.16 ($p<0.001$); **R**:1.02 ± 0.11 vs **C**:2.46 ± 0.165 ($p<0.001$). Glycemia after 120 min. of an oral glucose overload (g/l): **ER**:1.10± 0.07 vs **R**:1.27± 0.07 ($p>0.05$); **ER**:1.10 ± 0.07 vs **C**:2.00± .30 ($p<0.01$); **R**:1.27± 0.07 vs **C**:2.00 ± 0.30 ($p<0.05$). Fasting glycemia and Cholesterolemia: There was no significant difference.
The differences found in body weight and tryigliceridemia level were attributed to food restriction. This plan of physical training did not aded any improvement to the effects of the food restriction.

25.

ONSET OF SIGNIFICANT SEXUAL DIVERGENCE OF BODY WEIGHT AND TAIL LENGTH ON RATS FROM LINES b AND β

Alet N, Labourdette VB, Posadas M, Gayol M del C.
Cátedra de Biología, Facultad de Ciencias Médicas, UNR.

The differences among secondary sexual characters include body weight and tail length. Our aim is to include rats of both sexes indistinctly in future experimental designs. To attain that we decided to precise the age when sexual differences become significant on rats from the inbred lines b and β.
The values of body weight and tail length were measured every other day on 11 rats b (6 male, 5 female) and 22 rats β (11 male, 11 female) from 22 to 60 days of age. All along this period they were located in individual cages. Ambiental temperature and humidity, light dark period and food and water access were controled as usual. We compared the mean values between sexes for each variable in each day of measurement within lines (mean ± SEM).
We found a significant difference ($p<0.05$) on body weight between male (M) and female (F) older than 32 days for line b (M: 94.9 ± 2.2 vs F: 80.1 ± 3.4) and older than 38 days for line β. Tail length was significantly divergent upon 60 days old on line b (M: 14.1 ± 0.3 vs F: 12.9 ± 0.4) and 54 days old on line β (M: 15.5 ± 0.4 vs F: 14.2±0.3).
The variable that showed earlier difference between sexes has been body weight for both lines. Therefore we decided to include both sexes not further than 30 days of age for line b and 36 days of age for line β. Using rats under these limits will assure absence of significant differences between sexes for the variables studied.

26.

GLUCOLIPIDIC PARAMETRES AND BODY WEIGHT EVALUATION IN IIMb INBREED LINE

Garnedi LB, Gayol M del C, Posadas MD, Labourdette VB.
Cátedra de Biología. Facultad de Ciencias Médicas. UNR.

Due to the extinction of the IIMα line which was used as control, we had the necessity of being provided with a new eumetabolic model, the IIMb line from Rosario Medical Investigation Institute. We know that highly inbred lines are sensible to environmental changes, so we studied their adaptability to the new environment. To do that we measured body weight, triglyceridemia, blood cholesterol, fasting glycemia and glycemia after 120 minutes of an oral glucose overload at the age of 50,100,200 and 300 days; on 10 male rats IIMb and 10 male rats IIMß. The IIMb line glycemia (g/l) was lower than IIMß, they had significant differences at the age of 200 and 300 days b:0.93±0.01 vs ß:1.55±0.07 ($p<0.001$); b:0.84±0.04 vs ß:1.18±0.06 ($p<0.001$). The glycemia after 120 minutes of an oral overload (g/l) also showed significant differences between lines at the same age b:0.86±0.08 vs ß:2.23±0.17 ($p<0.001$); b:1.08±0.07 vs ß:2.28±0.14 ($p<0.001$). The triglyceridemia (g/l) has significant differences at the age of 50 days: b:0.96±0.07 vs ß:1.51±0.17 ($p<0.01$); 100 days: b:0.77±0.04 vs ß:2.07±0.23 ($p<0.001$); 200 days: b:1.15±0.07 vs ß:2.33±0.38 ($p<0.05$) and 300 days: b:1.05±0.13 vs ß:3.40±0.47 ($p<0.001$). We found no statistical difference in blood cholesterol. In relation to body weight (g), both lines had similar results at weaning and had significant differences at 200 and 300 days of age b:348,36±7.61 vs ß:382.6±8.48 ($p<0.01$) and b:374±12.58 vs ß:436.30±8.72 ($p<0.001$). These results belong to inbreed animals from the first generation in the new environment. We found they have eumetabolic values for all those variables so they can be used as control to compare them with ß.

27.

EFFECT OF TIME AND CONCENTRATION OF ENZYME ON THE ACTIVITY OF RAT INTESTINAL ALKALINE PHOSPHATASE (PA), IN PRESENCE OF CALCIUM (Ca^{++})

Brun LRM, Brance ML, Rigalli A, Puche RC.
Lab. Bioogía Ósea. Facultad Medicina. UNR.

Calcium interacts with PA and modifies the activity of the later. Concentrations of Ca^{++} below 10 mM activate PA. However, concentrations higher than 10 mM inhibit the enzyme *in vitro*. Polyacrylamide gel electrophoresis (PAGE) of PA treated with $^{45}Ca^{++}$ showed that PA bound to Ca^{++} has lower electrophoretic motility. This result suggests an increase in molecular weight. In order to get evidence whether the process induced by Ca^{++} involves aggregation, the effect of Ca^{++} was investigated at different times and PA concentrations. PA activities were measured *in vitro*, with p-nitrophenylphosphate (pNFF) as substrate, at the beginning of incubation with Ca^{++} 50 mM, and at 30, 60, 90 y 180 seconds. The experiment was repeated with four purified PA concentrations. PA activity was expressed in pmoles of $pNFF \cdot seg^{-1} \cdot L^{-1}$. Enzymatic activity decreased as a linear function of time at any PA concentration. The slope of enzymatic activity as a function of time (SEA, pmoles $pNFF \cdot seg^{-2} \cdot L^{-1}$) measures the inactivation rate. The effect was more evident at high concentrations of PA.

PA: $pmol.L^{-1}$	SEA	r	n
2.20	0.129±0.044	-0.82	10
1.10	0.102±0.024	-0.81	10
0.55	0.067±0.020	-0.77	10
0.28	0.024±0.010	-0.71	10

Conclusions:1) Effect of Ca^{++} depend of time. 2) Inactivation rate is dependent of the PA concentrations. Both observations indicates that inactivation would involve diffusion and aggregation.

28.

EFFECT OF CALCIUM (Ca^{++}) ON CITOPLASMIC AND BRUSH BORDER ACTIVITY OF ENTEROCYTE ALKALINE PHOSPHATASE (PA), IN THE RAT

Brance ML, Brun LRM, Rigalli A, Puche RC.
Lab. Biología Ósea. Facultad de Medicina. UNR.

PA binds Ca^{++} and modifies its activity depending on Ca^{++} levels. This effect was shown *in vitro* and in the intestinal lumen of rats *in vivo*. The aim of this work was to investigate the effect of Ca^{++} on PA in brush border and cytoplasm of enterocyte of rats *in vivo*.Three centimetres of the duodenum of adults rats were isolated *in situ* through two ligatures. Filling solutions were flown through a catheter placed at one end. Four rats (Controls) were given 2 ml of NaCl 9 g/L and 4 rats (Treated) received 2 ml of $CaCl_2$ 50 mM. After 20 minutes, duodenums were treated with conventional histologyc techniques. Cellular activity of PA was measured with 5-bromo-4-clorine-3-indolyl phosphate as substrate. Absorbance was measured on digital images with a specific software. In a separate experiment, histologycal specimenes of Controls were treated with Ca^{++} and PA activity was measured as stated above. Without Ca^{++}, PA activity in brush border (0.10±0.04) was not different from that of the citoplasm (0.11±0.04). With Ca^{++}, PA activity in brush border increased (0.20±0.15) and was higher than in citoplasm of the same cell (0.04±0.02,$p<0,001$) and brush border of controls ($p<0,01$). In contrast, PA activity in cytoplasm with Ca^{++} decreased in comparison with controls, $p<0,0001$. Controls in presence of Ca^{++} did not show changes in citoplasmatic PA activity (0.11±0.08, $p>0,05$) whereas the activity increased in brush border (0.20±0.12, $p<0,01$), and it was similar to Treated. Conclusions: 1) High lumenal levels of Ca^{++} increased brush border activity and decresed citoplasmic activitity of PA. B) the effect on brush border activity seems to be a direct effect of Ca^{++}.

29.

RISK FACTOR (RF) STUDY FOR OSTEOPOROTIC HIP FRACTURES (HF) IN INPATIENTS OLDER THAN 50

Tomat MF, Masoni AM, Morosano ME.
Cátedra de Química Biológica, Facultad de Cs. Médicas, Universidad Nacional de Rosario, Argentina.

HFs are a serious problem for Public Health because of the strong socio-economical impact as regards surgery, material and human resources for later recovery, and patient impairment. The aim of our work was to study the different RFs related to HF in 172 inpatients older than 50, from the PAMI II, Rosario. The case-control study involved HF patients (**cases**, n=86; 65 women, 21 men), and other patients (**controls**, n=86, keeping the 3:1 ratio women over men). Data were collected twice a week by a member of the research group. The outcomes were analyzed through average consequences, standard deviations, Chi square and Student´s t test. The HFs are more frequent in women (W/M 3:1). Average age of the sample under study: 80.2±0.76; n: 172. Among the most frequent pathologic previous histories there are: Type II Diabetes, COPD and Feeding Behavior Disorders. There was no significant difference in women from both groups in: Age of Menarchia (12.3±0.12); Age of Menopause (50.1±0.25), and Nulliparity. There was no significant difference in Immobility, Tobacco and Alcohol Consumption, Solar Exposure, Physical Activity and BMI in both groups. Frequency of Previous Falls significantly greater in **cases** (x^2=16.02p<0.0001, n:172). Difficulty in sitting up (x^2=3.92p<0.05); difficulty in getting up and down vehicles (x^2=8.4p<0.001); Visual disorders (x^2=5.50p<0.01). Cognitive Distortion (x^2=3.85p<0.05; n=172). Significantly lower consumption of dairy products in HF patients.

30.

HISTOPATHOLOGICAL CHANGES IN CECAL AND PEYER'S PATCHES FROM OVALBUMIN SENSITIZED AND CHALLENGED RABBITS

Roma S, Campi P, Pérez F, Dlugovitzky D.
Cát. Histología y Embriología y Sección Inmunología. Cát. de Microbiología. Fac Ciencias. Médicas. UNR.

In rabbits, Peyer's and cecal patches are immuno-inductive sites from gut, where luminal antigens are transported in a controlled manner and presented to lymphatic cells in order to induce the secretory immune response. The aim of the present work was to describe the histopathological changes in ileal and cecal patches from ovalbumin (OVA) sensitized and orally challenged rabbits. Twenty New Zealand rabbits were divided into four groups (G). G1: normal control. G2: OVA sensitized. G3: not sensitized and orally challenged and G4: OVA sensitized and orally challenged. Sensitization was performed subcutaneously with OVA. Rabbits were OVA orally challenged by an intragastric canula. Specific anti-OVA IgE levels were evaluated by positive passive cutaneous anaphylaxis test (PCA). Samples from both patches were fixed in formaldehyde, paraffin embedded and H&E stained. Animals from G2 showed edema, lymphangiectasis and eosinophils leucocytes in villus. Lymphatic follicles presented prominent germinal centers. In G4 the eosinophilic infiltration was intense with germinal centers with tangible macrophages. No histopathological changes were found in G1 and G3. In sensitized animals the soluble mediators determines the vasoactive phenomena and the prominent germinal centers indicate a immune stimulation. In animals from G4 the macrophagic activity found in follicles and the lesser edema may downgregulate the immune response.

31.

LECTINS REACTIVITY IN IMMUNO-INDUCTIVE SITES FROM THE RABBIT GUT. QUALITATIVE STUDY

Pérez F˙, Roma S˙, Barbeito C˝, Gimeno E˝, Dlugovitzky D≈.
˙Cát. Histol y Embriol y ≈Cát. Microbiol. Fac Ciencias Médicas. UNR. ˝Cát Patología General. Fac. Ciencias Veterinarias. UNLP.

The rabbit appendix, cecal patches, Peyer's patches and sacculus rotundum constitute immuno-inductive sites from gut that deliver antigens in a controlled manner. To transport luminal matter, the initial step is antigen attachement to glycoconjugates on surfaces of cells. Glycoconjugates can be detected with certain lectins. The aim of the present work was to determine the glycosilation pattern in gut from normal, ovalbumin (OVA) subcutaneously sensitized and OVA orally challenged rabbits. Twenty New Zealand rabbits were divided into four groups (G). G1: normal control. G2: OVA sensitized. G3: non sensitized and OVA challenged. G4: OVA sensitized and challenged. In all groups, samples from the four immuno-inductive sites were stained with Ulex Europaeus I (UEA-I) that evidences L-fucose and Wheat Germ Agglutinin (WGA) for N-acetil-glucosamine. In appendix and sacculus rotundum, UEA-I stained membranous cells from the epithelium associated to follicles (FAE). In cecal and Peyer's patches the same lectin stained all the FAE, the dome lymphatic cells and follicles. WGA showed no specific results. With both lectins, no differences were detected between normal and experimental groups. Fucose seems to be implicated in macromolecules transport. The differential glycosilation pattern probably represents a special adaptation of cells for sampling antigens. In Peyer's and cecal patches cells are in close contact to luminal antigens while in appendix and sacculus rotumdum there are the villus tissues interposed.

32.

MAFOSFAMIDE (MF) INHIBITS PROLIFERATION AND MODULATES THE EXPRESSION OF IL-10 AND IL-10 RECEPTOR (IL-10R) OF METASTATIC CELLS OF A RAT B CELL LYMPHOMA (L-TACB)

Rico MJ[1], Matar P[1], Scharovsky OG**
Inst. Genética Experimental, Fac. Ciencias. Médicas.
**Consejo de Investigaciones, U.N.R. Rosario.*
[1]Contributed equally to this work.

We have previously demonstrated that L-TACB metastatic cells (Mc) produce significantly more IL-10 than primary tumor cells (Tc) and that IL-10 increases *in vitro* Mc proliferation rate. Also, we have shown that a single-low dose Cyclophosphamide (Cy) has an antimetastatic effect on lymphoma (L-TACB) bearing rats. Our aim was to investigate the effect of MF (a compound giving the same active metabolites as Cy) on cell proliferation, IL-10R expression and IL-10 secretion. Cell suspensions from L-TACB Tc and Mc were prepared. Cells were incubated 96h with 0, 5 and 10 μM MF in RPMI + 10% FCS and cells and conditioned media (CM) were obtained. Mc proliferation was inhibited by both doses of MF, while Tc were not affected by them. [IL-10], measured by ELISA, showed non-detectable levels in Tc CM, while for Mc it was higher (p<0.001) in control cells (mean ± SE: 493±7,6 pg/ml) than in 5μM (127±1,5 pg/ml) and 10μM MF (26±3 pg/ml) treated cells. [IL-10R], determined by CELISA, was for Mc control cells (O. D.:1488±11,8) higher (p<0.001) than for cells incubated with MF (236±13,9 and 76,5±3,6, respectively). No variations were detected for Tc. We conclude that MF down-regulates the expression of IL-10R and IL-10 secretion of L-TACB Mc, decreasing an important growth factor for these cells and, hence, inhibiting their proliferation. MF and, by extension, Cy, would inhibit the autocrine loop that stimulates metastatic cells growth.

33.

WEIGHT VARIATIONS OF NATIVE KIDNEY IN "L" LINE OF RATS, UNDERGOING UNILATERAL NEPHRECTOMY

Palmisano EM, Vogliotti L, Castellana N, Talarn AA, Tamae Kakasu M, Tejedo DA, Gorosito MD, Pérez B, Picena JC, Diez J de la C.
Instituto de Cirugía Experimental "Prof. Dr. J. J. Boretti", HEEP, Facultad de Ciencias Médicas, Universidad Nacional de Rosario.

Unilateral nephrectomy modifies the native kidney, probably due to a compensatory response of hypertrophy and hyperplasia. One of the most sensitive index to estimate hypertrophy is kidney weight. We studied kidney weight variations depending on age and sex in "L" line of rats undergoing unilateral nephrectomy. We used 64 "L" rats, classified according to: 1) Sex: 1a- ♀ (n=32) and 1b- ♂ (n=32), 2) Age: 2a- Young (Y) 50 days (n=32) and 2b- Adults (A) 85 days (n=32) and 3) Treatment: 3a- Nephrectomized (N) and 3b- Control (C). The animals of group (N) underwent a left nephrectomy. 45 days later the animals were euthanasied, and the right remaining kidney was removed. The animals of group (C) were treated under the same food and care conditions of group (N). 45 days later the animals were euthanasied, and the right remaining kidney was removed. To study a possible treatment effect on the rats' kidney, as weel as sex and age we used ANOVA at 3 factors of 2 levels each. Since the results of the triple interaction *sex(s)*treatment(t)*age(a) were not significant (F=0,077; p=0,783) we made ANOVA at 2 factors (s)*(t), (a)*(t) and (s)*(a). We concluded that the kidney's average weight is significantly heavier for (N) rats and males too. There were no significant differences in weight average between females (Y) and (A) rats (F=0,18; p=0,67), but there were differences in males (F=16,3; p=0,0003).

34.

COLCHICINE IN THE PREVENTION OF GASTRO INTESTINAL INJURIES INDUCED BY ANTI-INFLAMMATORY NON STEROIDS IN RATS

Catalano JBA, Laudanno OM, Cesolari JAM, Aramberry L, Basa E, Calvi BJ, Savio L, Paez S, Sambrano JS, Godoy A.
Gastroenterología Experimental. Cát. Histología y Embriología Fac. Ciencias Médicas. Universidad Nacional de Rosario.

The Colchicine (Co) inhibits the migration of granulocytes toward the inflamed area and it diminishes their metabolic and phagocytic activity. The objective of this work was to verify if the Co, at leucopénic dose, protects the gastrointestinal mucous membrane in the presence of anti-inflammatory non steroids (AINES) not selective inhibitors of ciclooxigenasas 1 and 2 (COX-1 and COX-2). The following experiments were carried out with random groups of rats Wistar (n=7 per group), 200-230g, fasting 24hs, except for water ad líbitum and avoiding the coprofagia,: Group 1 - Normal saline, 1ml for orogástric catheter (OG) and then wait for 24hs.. 2 - Co 2 intraperitoneal mg/kg (IP), 24hs. 3 -Ketorolac (Keto), 30mg/kg, OG and then wait for 24 hs. 4-Co, 2hs, then Keto, 24hs. The rats were sacrificed by eter overdose. Laparotomy, gastrectomy, enterectomy, were carried out tabulating the percentage (%) of the gastric macroscopic and intestinal erosive area (mm2), and cuts were obtained for histological studies (H-E) and biopsy for mieloperoxidasa (MPO). The results were evaluated by" t" of Student and ANOVA. The% of the gastric necrotic area:1 and 2-1.0±0.1 the same as the intestinal erosive area. 3-7.5± 1.3 and 18±15 ($P< 0,001$) and the MPO in the intestinal area 450 ± 40 U/100mg/protein ($P < 0,001$). 4 - 0.7 ± 0.2 (n.s.), in intestine 1.0±0.1(n.s.) and the MPO of 35 ± 4 U/100mg/protein ($p<0.001$). Colchicine, when blocking the leukocityc margination prevented the gastrointestinal injuries induced by Ketorolac.

35.

PHYSICAL ACTIVITY AS PROTECTING FACTOR IN CARDIOVASCULAR DISEASES IN THE SCHOOL OF MEDICINE

Palmisano EM, Macagno MA, Acosta AM, Barchetta YA, Cena PS, Vargas DA, López ML, Ybáñez MA, Pérez B, Cucurullo MH.
Docentes del Lab. de Habilidades y Destrezas. FCM. UNR.

Daily aerobic physical activity (PA) for 30 min, four times / wk at least decreases the risk of occurrence of cardiovascular disease (CVD). Objective: to assess the practice of physical activity in adolescents a transversal descriptive study in students of the second year of the School of Medicine (UNR), from August to September 2003, was carried on. An anonymous and voluntary questionnaire in order to evaluate the variables related to PA was applied. Variables considered: 1) PA practice; 2) type of PA; 3) duration, in minutes per day; 4) time per week. Results expressed as percentage. Total population: n=2072, questionnaires 418 (20.17%).
♂ 22.97% (96); a) PA: 42.70% (41), a1) Aerobic PA: 85.37% (35), a2) Anaerobic PA: 14.63% (6); a3) over 30 min/day: 100% (41), a4) less than 30 min: 0% (0); a5) more than 4 times/week 26.83% (11), a6) less than 4 times /wk: 73.17% (30), and b) non-PA 57.30% (55). ♀ 77.03% (322); a) 42.24% (136), a1) 94.85% (129), a2) 5.15% (7); a3) 95.59% (130), a4) 4.41% (6); a5) 45.59% (62), a6) 54.41% (74) and b) 57.76% (186).
It can be concluded that: 1) there is no physical activity in a high percentage of students, both sexes. 2) there is a higher prevalence in aerobic PA over 30 min daily and less than 4 times/wk. It is clear the importance of implementing such a healthy practice, that adequately performed might provide protection against the development of cardiovascular disease.

36.

CHARACTERIZATION OF A MURINE MODEL OF SPONTANEOUS BREAST CARCINOGENESIS

Suárez C, Varoli F, Hinrichsen L.*
*Inst. Genética Experimental, Fac. Cs. Médicas, U.N.R. - *Consejo de Investigaiones, U.N.R.*

Susceptibility to hereditary breast cancer is a complex phenomenon, in which multiple genes might be involved. It is currently accepted that there is a direct correlation between plasma estrogen levels at one point in time and breast cancer risk, but little is known about a continued exposure to high estrogen levels. Since high estrogens levels and high peak bone mass are associated, the susceptibility to development of spontaneous breast tumors was studied in female mice of two lines (CBi+ and CBi/C) characterized by high peak bone mass. Females, either virgin or after a complete reproductive cycle (n=40, per group), were studied. Virgin mice from the reciprocal crosses (F_1, n=25 per cross) were also analyzed to estimate the pattern of inheritance of susceptibility and the presence of maternal effects. Length of life free of tumor was calculated with the Kaplan-Meier method and comparisons between groups were done with the log-rank test. Significance of the differences en the percentage of mice that develop a tumor was estimated with the χ^2 test. The genotypes differed in the age at which tumor was detected (median, age in days; CBi+=243, CBi/C=504; $p<0.001$) and the percentage of mice with tumors (CBi+=100%, CBi/C=24%; $p<0.001$); a complete reproductive cycle did not affect these relationships (CBi+=220, 100%; CBi/C=484, 17%; $p<0.001$). F_1 had different medians ((+ x C)=230; (C x +)=401; $p<0.001$) but did not differ in the percentage of animals with tumor ((+ x C)=68%; (C x +)=77%; $p>0.05$). These results support the hypothesis that susceptibility is genetically determined and that the expression of this trait might also be influenced by hormonal and/or viral factors.

37.

THE CLINICAL DIAGNOSIS OF BACTERIAL VAGINOSIS IS CORRELATED WITH MICROBIOLÓGICAL DIAGNOSIS

Ruiz Abad I[2], Belmonte A[1], Nogueras M[1], Ombrella A[1], Dlugovitzky D[1].
[1]Cát. de Microbiología, Parasitología y Virología. [2]Cát. de Ginecología. Fac. de Cs. Médicas. UNR.

The bacterial vaginosis (BV) is characterized for a great production of a white-gray, homogeneous and adherent vaginal flow, with a pH>4,5 and amines smell. On the Gram -Nicolle (GN) stained smears it could be observed displacement of the Lactobacilus flora by GN negative cocobacili and vibrios. Sensitivity and specificity of Amsel *et al* criteria was evaluated (pH>4.5, positive amine test (AT), clue cells presence (CC) and vaginal flow macroscopic characteristics for the diagnosis of BV, related to its characteristic bacteria isolation, *Gardnerella vaginalis* (GV). 100 women, sexually active, in reproductive age who attend to Hospital Provincial del Centenario during 2002, was studied. Samples of cervix and endocervical exudate, were taken for the microbiological diagnosis of genital pathogens and the application of Amsel criteria. For G.V. isolation agar CNA was used. The obtained data show that pH discard assessment has a screening value, since a pH of 4.5 or less discards VB diagnosis certainly (VP 98%). pH detection and amines smell combination, added to microscopic observation are easily evaluated characteristics at the gynecological consult, specific sensible and cheap.

38.

EVALUATION OF THE INTEGRIN CD11b FROM PMN AND MN CELLS STIMULATED WITH INACTIVATED *M. tuberculosis* IN TUBERCULOSIS PATIENTS TREATED WITH *M.vaccae*

Dlugovitzky D, Fiorenza G, Farroni MA, Aita J, Stanford J.
Sec. Inmunol. Cát. de Microbiología. Hosp. Carrasco Fac. Cs. Médicas. UNR. Dto de Microbiología. Esc. de Medicina Univ. de Londres.

In previous studies *M. vaccae* immunostimulatory activity was demonstrated. The effect of triple dose of *M. vaccae* on the integrin, CD11b of CMN and PMN from TB was evaluated, in order to get a shorter treatment. The cells were stimulated or not with heat inactivated *M. tuberculosis* (H37 Rv). 21 patients TB (HIV-) of both sexes and variable age were studied. On day 0 a sample of blood was extracted, via i.v (M0), for the immunological studies, and the patients received the 1st dose of *M. vaccae*. In the two subsequent months they received the same *M. vaccae* dose. During the three following months, several exams were done and M1, M2, M3 were extracted for immunological studies. CMN and PMN were separated with Ficoll- Hypaque and cultured in RPMI with and without H37 Rv stimuli. Cd11b data were obtained by Flow Citometry. *M. vaccae* therapy increased basal and stimulated CD11b expression, on CMN and PMN, in M1, M2 and M3.This data suggest an increasing immunostimulatory effect when three doses of *M. vaccae* are inoculated.

39.

ASSESSMENT OF RESPIRATORY BURST FROM PMN AND MN CELLS STIMULATED WITH INACTIVATED *M. tuberculosis* IN TUBERCULOSIS PATIENTS (TB) TREATED WITH *M.vaccae*

Dlugovitzky D, Fiorenza G, Farroni MA, Aita J, Stanford J.
Sec. Inmunol. Cat. Microbiología. Hosp. Carrasco Fac. Cs. Médicas. UNR. Dpto. de Microbiología, Escuela de Medicina, Univ. de Londres.

M. vaccae immunemodulatory effect has been demonstrated in previous trials. In this work the effect of triple dose of *M. vaccae* on Respiratory Burst (R.B) of stimulated or not CMN and PMN from TB patients was evaluated. The stimuli was a heat inactivated *M. tuberculosis* (H37Rv). 21 patients TB (HIV-) of both sexes and variable age were studied. On day 0 a sample of blood was extracted, i.v (M0), for the immunological studies and patients received the 1st dose of *M. vaccae*. In the two subsequent months they received the same *M. vaccae* dose. During the three following months, several controls were done and M1, M2, M3 were extracted for immunological studies. CMN and PMN and cultured in RPMI with and without H37 Rv stimuli. R.B data were obtained by Flow Citometry, and expressed as index R *M. vaccae* therapy increased basal and stimulated RB on CMN and PMN in M1, M2, and M3. R of CMN and PMN was increased by *M. vaccae* triple dose treatment.

40.

MONOCLONAL ANTIBODIES CHARACTERIZATION BY PHOTONIC TECHNIQUES. APPLICATION TO RED CELL GLYCOPHORINS

de Isla N[1], Valverde J[2], Stoltz JF[1], Rasia R[2], Riquelme B[2]
[1]Méc. et Ing. Cell. et Tiss - Fac de Méd. U.H.P. Nancy I, Francia.
[2]Areas Inmunohematología y Física., Fac. Cs. Bioq. y Farm., UNR, e Inst. de Física Rosario (CONICET-UNR), Argentina.

A frequent problem in clinical immunohematology is to define the antibody quality, which is related to its affinity. The possibility of determining the monoclonal antibody quality opens significant perspectives in biophysics. In this work, different bio-photonic technologies have been applied to the characterization of monoclonal antibodies against epitopes on human red blood cells glycophorins (anti-GPA, anti-GPB, anti-GPC, anti-Wrb, anti-EnaTS, anti-EnaFS). The distribution of the antibodies on the erythrocyte membrane was analyzed by 3D fluorescence microscopy. The quantification of the antibodies bound to the membrane and their affinity constant were studied by flow cytometry. The effect of the antibodies on the rigidity of the erythrocyte membrane was analyzed by light transmission and by laser diffraction. Antibodies analyzed were characterized by means of the antigenic site number recognized (mr), the affinity constant (Ka) and their effect on the membrane rigidity.

Antibody	Specificity	mr ($x10^5$)	Ka ($x10^7 M^{-1}$)
NaM167-8A2	Anti-GPA/GPB	0.65 ± 0.13	8.07 ± 4.89
NaM228-2E2	Anti-En^{a}TS	2.90 ± 1.10	4.6 ± 3.4
CBC-124	Anti-En^{a}FR	0.96 ± 0.24	5.14 ± 2.9
64-4G11	Anti-GPC	1.57 ± 0.71	13.46 ± 5.31

Studies were realized on 40 normal samples (intra-test error <15%). Given the sensibility and precision obtained these techniques can be used as complement of the classic techniques current used for the quality control of monoclonal antibodies.

41.

IDENTIFICATION OF CANDIDA-SPECIES BY PCR FINGERPRINTING

Giro LG, Ramadán SS, Bulacio LC, Ramos LL, López CE.
CEREMIC (Centro de Referencia de Micología). Facultad de Ciencias Bioquímicas y Farmacéuticas. Universidad Nacional de Rosario.

Systemic infection by fungi is an increasing problem, especially in immunocompromised and leukemic patients.
Our purpose was to study the applicability of the PCR fingerprinting as an easy method in order to unequivocal and fast discrimination of the major human pathogenic yeasts (*Candida albicans, C. krusei. C. parapsilopsis, C. tropicalis, C.glabrata, C. guillermondii, C. lusitaniae, C. utilis y C. dubliniensis*).
Sensitivity to antifungal drugs varies among species and strains. Correct identification of clinical yeast isolates has become essential for optimal clinical management, detailed epidemiological studies, and prevention and containment of outbreaks.
The reference species were subject to phenotypic methods of identifications: morphological and biochemical analysis and also commercially available identification systems: API-20C and CHROMagar-Candida and one genotypic method: PCR fingerprinting, based on the detection of DNA polymorphisms between minisatellite specific sequences with the primer M13.
Sufficient polymorphisms among the total set of banding patterns was observed, with adequate similarity in the set of mayor patterns obtained from a given species, to allow each isolates to be assigned unambiguously to a particular species. This was found to be rapid, reproducible and was well suited for the identification of *Candida* species.

43.

INCREASE OF NITRIC OXIDE IN HEPATECTOMISED RATS: EFFECT ON BAX, BCL-X_L AND P53

Ronco MT, Alvarez ML, Monti J, Carrillo MC,Carnovale CE.
Inst. Fisiología Experimental Fac. Cs. Bioq. y Farm. UNR.

In a previous work we found that an increase of nitric oxide (NO) 5 hours post hepatatectomy modulate liver regeneration. In order to investigate the precise role of NO in liver regeneration, we study the effect of NO increase on the expression of pro-apoptotic proteins (Bax, p53) and anti-apoptotic protein (Bcl-x_L). Two-thirds hepatectomy male Wistar rats were randomized in three experimental groups (n=5): Control (PH-C), treated with iNOS inductor, LPS (2 mg/kg body weight, i.p.) (PH-LPS) and treated with a NO donor, sodiumnitropruside (2,5mg/kg body weight, i.v. at a rate of 1ml/hour) (PH-SNP). Animals were killed at 5 hours after surgery. Significance in differences was tested by one-way ANOVA, followed by Tuckey´s or Dunnet´s test. Hepatic cytosolic iNOS showed an increase of 24% in PH-LPS animals respect to PH-C analysed by immunobloting. Cytosolic nitrate (expressed as nitrates nmol / 100 mg protein) were higher in treated groups respect to PH-C (PH-C: 8.9±1.2, PH-LPS: 16.1±1.9*, PH-SNP:20.5±2.6*). Lipoperoxidation (LPO) levels was measured as nmoles of MDA / 100m g of microsomal protein (PH-C: 112±7; PH-LPS: 144±6*; PH-SNP: 170±2*) (*$p<0.05$). The expression of liver mitochondrial Bax protein, in PH-LPS and in PH-SNP, showed an increase of 56% and 45% respect to PH-C, respectively. Levels of anti-apoptotic protein Bcl-x_L, not shown difference between the groups studied. In liver total lysates, the expression of p53 protein (check point of cellular cycle) showed an increase of 40% at 5 hours post-PH in treated animals respect to PH-C. The increase of NO by both, LPS and SNP post PH increase LPO levels and produce increase of the pro-apoptotic protein Bax and p53.

42.

STUDY OF α AND β THALASSEMIC ERYTHROCYTES: SENSITIVITY TO INITIAL CONDITION

Korol A[1], Perez S[2], Rasia R[3].
[1]Depto. de Matemática, [2]Lab. de Hematología, [3]Depto. de Física, Facultad de Cs. Bioquímicas. UNR. E-mail: akorol@ifir.ifir.edu.ar

The α and β thalassemia are inherited disorders, which are characterized by a decrease of a fixed chain of α and β globins respectively. A numerical method formulated on the basis of fractal approximation for ordinary (OBM) and fractionary Brownian motion (FBM), is proposed to evaluate sensitive dependence on initial conditions. This approach is based on the assumption that diffractometric data involves both deterministic and stochastic components, so it could be modelled as a system of bounded correlated random walk. A laser beam perpendicularly traversing the layer of shear deformed erythrocytes diffracts producing a Franhoufer pattern and diffracted intensity that falls onto a photomultiplier tube, constitutes the time series. Here we report studies on 30 donors: 11 α thalassemic, 9 β thalassemic and 10 healthy donors non smokers and non alcoholic individuals.
The results suggests that the time series of β thalassemic are chaotic while the photometrically recorded series which belongs to healthy donors and also to α thalassemic patients are white noise. This information, which can not be found by a linear approach, could be used not only in analysing these nonlinear parameters, but also in evaluating clinical aspects of erythrocyte rheological properties. It should be noted, that we have observed different results for patients who have been treated for short or interrupted times. Further studies with larger and well defined patient populations are in process.

44.

EFFECT OF OXIDATIVE STRESS ON THE SEMINAL QUALITY IN INFERTILE MEN

Bouvet BR, Paparella CV, Brufman AS, Farias M, Feldman R, Gatti VN, Solis EA.
Departamento de Bioquímica Clínica. Fac. Cs Bioq. y Farm., Servicio de Reproducción Fa. Cs. Médicas. U. N. Rosario.

The disbalance between the pros and antioxidant is called oxidative stress (OS). Our objetive was to study in infertile men the relationship the OS, with seminal cytomorphology and sperm functional test. Sperm samples from 40 men that presented no sperm agglutination or hyperviscosity were chosen. To evaluated OS, the modified sperm stress (MOST) was applied. The value 0.40 (final motility over initial motility) was used to separate the samples in two groups, G1 (n=14) MOST>0.40 (normal) and G2 (n=26) MOST<0.40 (abnormal).The seminal cytomorphology and sperm functional test, were evaluated according to WHO criteria 1999). The statistical analysis were determined by using *Student t* test, obtainig the following results:

Variable	p	statistical significant
Sperm morphology	0.8500	no significant
Germinal cells	0.0082	significant
Nuclear DNA state	0.0090	significant
Conc. Macrophages	0.0031	significant
Function sperm memb.	0.0093	significant

With exception of the morphology, significant statistically difference excists between both groups. The presence of macrophages, as spermatogenic arrest (germinall cells), induce OS that alter the functionality of sperm membrane and nuclear DNA, essential structures for the fertilization process.

45.

EFFECT OF QUERCETIN EXTRACTED OF *LIGARIA cuneifolia* (MUÉRDAGO CRIOLLO, *L. cuneifolia*) ON ERYTHROCYTE SHAPE IN RATS TREATED BY VIA INTRAPERITONEAL

Crosetti D[1], Ferrero M[1], Dominighini A[1], Alvarez L[3], Ronco MT[3], Wagner M[2], Gurni A[2], Carnovale C[3], Luquita A[1].
[1]Cátedra de Biofísica, Facultad de Ciencias Médicas, UNR. [2]Cátedra de Farmacobotánica, Facultad de Farmacia y Bioquímica, UBA. [3]Cátedra de Fisiología, Facultad de Ciencias Bioquímicas y Farmacéuticas, UNR-CONICET, Suipacha 570. Rosario. Argentina.

In a previous work, we found that *L cuneifolia*-treatment by i.p. via, produces an increase of rigidity index (RI) and a diminution of erythrocyte morphologic index (MI). In this work we analysed the effect of quercetin (one of the compounds extracted of *L cuneifolia*) on both rat erythrocyte shape and deformability. The *L cuneifolia* was collected in Córdoba and processed to obtain quercetin. Male Wistar rats were injected by i.p. via once a day during 3 days with: saline solution [Control (C) (n:6)] or quercetin [230 µg/100g body weight; Treated (T_Q); n:5]. Significance in differences was tested by one-way ANOVA, followed by Dunnet´s test. Blood was obtained by cardiac punction. The RI (measured by filtration through nucleopore membrane) obtained were: C: 6,13± 0,60; T_Q: 7,88± 2,40. The MI (estimated by the Bessis classification) were: C: -0,80± 0,05; T_Q: -1,96± 0,20*, (*p<0,05 vs C). No changes were observed in Mean Corpuscular Hemoglobin (C: 32,48± 0,15) nor in Mean Corpuscular Volumen (C: 58,29± 1,44). These data suggest a direct effect of quercetin on the erythrocyte membrane that produces a change of cellular morphology without alteration the erythrocyte deformability (RI).

46.

TUMORAL PLASMA CELLS AS SOURCE OF FERRITIN IN MULTIPLE MYELOMA

Solís EA[1], Theiller ER[2], Minella KT[2], Cuestas V[2], Fuentes M[2], Costamagna A[2], Gómez Ayet M[2], Giugni MC[2].
[1]Cátedra de Bioquímica Clínica, Área Inmunología. Facultad de Ciencias Bioquímicas y Farmacéuticas. UNR. Rosario.
[2]Departamento de Bioquímica Clínica y Cuantitativa, Áreas Inmunología y Hematología y Cátedra de Morfología Normal. Facultad de Bioquímica y Ciencias Biológicas. UNL. Santa Fe.

High serum ferritin levels, which did not correlate with iron marrow stores, were found by the authors in a group of Multiple Myeloma patients. The aim of this study was to determine the origin of the increased serum protein concentration investigating ferritin expression in marrow tumoral plasma cells.
In 48 patients, enrolled in the study at different states of the disease, the authors measured serum ferritin by IRMA- mat Ferritin Byk-Sangtec Diagnostica, plasma cell ferritin by immunohistochemistry (DAKO LSAB2 system, HRP-DAB and rabbit anti-human Ferr antibody-DAKO) and iron marrow stores by Perls's reaction. The mean serum ferritin concentration was significantly higher (p<0,0001) in this group ($\bar{x}$ = 504.6 ng/ml) compared with the contrl group ($\bar{x}$ = 86,7 ng/ml). As statistics method it was used Student's test for mean difference and assuming unequal variances. Evaluation of iron marrow store (hemosiderin) showed absence of it (grade 0) in 90% of the cases. On the other hand, tumoral bone marrow plasma cells showed ferritin expression in 98% of the samples.
The results suggest that high serum ferritin concentration is related to increased synthesis by tumor cells in response to others agents or circunstances, besides iron, involved in the carcinogenesis process and that should be investigated.

47.

FERRITIN REGULATION BY INTERLEUKIN-6 IN MULTIPLE MYELOMA

Solís EA[1], Theiller ER[2], Minella KT[2], Ghío HL[2], Garnero N[2], De la Vega CD[2], Denner S[2]
[1]Departamento de Bioquímica Clínica, Facultad de Ciencias Bioquímicas y Farmacéuticas. UNR. Rosario. [2]Departamento de Bioquímica Clínica y Cuantitativa, Facultad de Bioquímica y Ciencias Biológicas. UNL. Santa Fe.

In addition to iron, ferritin (Ferr) expression has been reported to be altered by a number of other agents and circumstances, such as cytokines, oncogenes, transcriptional regulation and a pathway involving cAMP. In previous studies, the authors found a significantly increased of serum Ferr concentration in Multiple Myeloma (MM) patients with low or absent iron stores. Interleukin-6 (IL-6) is the major tumor growth factor for myeloma plasma cells proliferation. This fact suggests a probable Ferr transcriptional regulation by IL-6 in MM. The aim of this study was to investigate Ferr/IL-6 correlation in MM. In 24 MM patients with significantly high serum Ferr levels [$\bar{x}$ patients (ng/ml) = 504,6 ± 386,2 and $\bar{x}$ controls (ng/ml) = 86,7 ± 30], low or absent iron marrow stores and without known indicators of acute inflammation, it was determined serum IL-6 concentration, which was also found in a control group. Serum IL-6 was measured by assay IRMA Bio Source International Inc. The mean serum IL-6 concentration was statistically different between *patients* and *control* groups ($\bar{x}$ patients = 30,6 pg/ml and $\bar{x}$ controls = 7,8 pg/ml), using Student's test for mean difference and assuming unequal variances.
The results suggest a probable positive regulation of Ferr expression by IL-6 in MM and show Ferr as a tumor protein with clinical importance of this disease.

48.

FERRITIN AS INDICATOR OF CELLULAR PROLIFERATION IN MALIGNANT COLONIC TISSUE WITH HIGH CARCINOEMBRYONIC ANTIGEN EXPRESSION

Solís EA[1], Theiller ER[2], Minella KT[2], Giugni MC[2], Denner S[2].
[1]Fac. Cs. Bioq. y Farm. UNR. [2]Fac. Bioq. y Cs. Biol. UNL. Santa Fe.

As tumors growth faster, they need more iron than normal cells. An excess of intracellular iron was shown to be beneficial for the survival and growth of tumor cells; tumors are often associated with the sites of deposition of the metal, which is principally stored in ferritin (Ferr). To examine the biological significance of Ferr expression in colorectal cancer a retrospective immunohistochemical study was performed in colorectal carcinomas poorly or moderately differentiated (n=31) with high cellular carcinoembryonic antigen (CEA) expression and in normal colonic mucosae (n=31). It was used an immunohistochemical staining procedure with DAKO LSAB2 system- HRP and rabbit anti-human Ferr antibody. Weak cytoplasmic Ferr expression was observed in specific areas of non-neoplastic colorectal epithelium. Carcinoma cells had a significantly higher proportion of Ferr expression than those in normal controls (p<0,001); they showed strong and diffuse cytoplasmic Ferr immunoreactivity in 78% of the cancer samples and a positive correlation with the histologic grade of the tumor and with cellular CEA expression, which has been useful as a prognostic marker closely correlated with advanced disease in colorectal cancer patients. The results suggest that cytoplasmic Ferr expression is associated with malignant neoplastic transformation and cellular proliferation, and show that Ferr, like CEA, may be a useful tumor marker for disease progression in colorectal cancer patients.

49.

COMPARATIVE STUDY OF DNA EXTRACTION METHODS FOR MICROSATELLITE AMPLIFICATION FROM BUCCAL CELLS

Landi C, Fornes C, Callero M, Chialina S, Solis E.
Histocompatibility and Mol Biol Lab. Italian Garibaldi Hospital. Rosario.

STR (Short Tandem repeats) loci consist of short, repetitive sequence elements of 3 to 7 base pairs in length**.** These abundant repeats are well distributed throughout the human genome and are a rich source of highly polymorphic markers, which often may be detected using PCR. The aim of this work was to compare three DNA extraction methods from buccal cells (saliva and mouthwash) for STR amplification. The quantity and quality of 15 DNA samples were evaluated by extraction with: 1) organic solvents (Phenol / Chloroform), 2) Chelex-100 (Biorad) and 3) Chelex-100 after enzymatic digestion. (Modified Chelex).
The amount of DNA was estimated by electrophoresis in agarose gels. The R (Abs260/280) and the amplification of STR system-D5S346 were used to confirm the purity and quality of the DNA. The percentage of DNA samples with concentrations more than 4ng/ul was: 1) Phenol / Chloroform 86.7% in saliva and 60% mouthwash. 2) Chelex -100, 66.7% in saliva and 20% mouthwash. 3) Modified Chelex 86.7% in saliva and 40% in mouthwash. Cochran and Mc Nemar statistical tests were used.
The results suggest that greater amount of DNA, with less protein contamination (R >1.60) was obtained by the Phenol/Chloroform method. Nevertheless, the modified Chelex method using saliva specimens was equally efficient for STR amplification by PCR.

50.

D ANTIGEN EXPRESSION IN SENESCENT RED BLOOD CELLS

Biondi C, Ensinck A, Cotorruelo C, Marini A, García Borrás S, Racca L[1], Racca A[2].
Área Inmunología. [1]Area Estadística. Facultad de Ciencias Bioquímicas y Farmacéuticas. [2]C.I.U.N.R. U.N.R.

Human erythrocytes have a well-defined lifespan of 120 days. Certain blood group antigens may play a role in the removal of senescent red blood cells (SeRBC) from the circulation. Recentely we observed a decreased ABO and MN antigenic expression in the population of SeRBC. The aim of this work is to investigate the effect of red cell aging on the D antigen. Blood samples were drawn by venipuncture from 12 hematogically normal volunteer donors. Density separation was effected by short-duration, high speed centrifugation of concentrated (80% hematocrit) RBC at 10.000 g for 15 minutes. Following centrifugation the top and bottom 10% fractions were removed and designated as the young RBC (YRBC) and SeRBC. Standard serial dilutions of an IgG anti-D serum were prepared and the titre and score with SeRBC, YRBC and unseparated RBC (URBC) were obtained by hemagglutination. Friedman's test and multiple comparisons were used to analyse the results. Titres obtained were: SeRBC: 853.3 ± 252.1; YRBC: 245.3 ± 101.5 and URBC: 448.0 ± 115.8. Observed scores were similar among the three groups. Titres obtained with URBC were significatively higher than those found with YRBC, but lower than SeRBC ($p<0.05$) indicating that the D antigen reactivity is increased with cell aging. This result may be related to modifications observed in membrane proteins that might lead to the selective removal of aged red cells from circulation.

51.

PLASMATIC CHOLESTEROL, ERYTROCYTE SHAPE AND DEFORMABITILY IN ALUMINIUM (Al) TREATED AND HEPATECTOMISED RATS

Contini M del C[1], Bazzoni G[2], Carnovale CE[3]
[1]Cát. Fisiol, Fac. Bioq. Cs Biol., UNL. Santa Fe. [2]Cát. Biofísica, Fac. Cs. Médicas. [3]Cát. Fisiol., Fac. Cs. Bioqca y Farm., UNR-CONICET. Rosario.

Other authors have demonstrated that Al-treatment produce a diminution in erythrocyte deformability and changes in red blood cell (RBC) morphology. Partial hepatectomy in rats produce a reduction of plasmatic cholesterol (Co).The aim of the present study was to evaluate both erytrocyte shape and deformability in Al-treated and hepatectomised rats as well as theirs relationships with the level of Co. Adult male Wistar rats were divided into four groups (n=4 each one): Sham (SH) simulated surgery, hepatectomised (HP) remotion of 65% of the liver; SH and HP with 8 mg $Al(OH)_3$/kg b.w, i.p, 3 times a week during 3 months. 48 hs post-surgery anticoagulated blood was extracted. Cholesterol concentration was determined by enzimatic method, index of rigidity (IR) by filtration and morphologic index (IM) was calculated (IM= Σ [shape index multiplied by cell number/ whole number of cells]). Results (mean ± SEM): Co (g/l) SH:1.36 ± 0.06; HP: 0.77 ± 0.02* ; SH -Al: 0.72 ± 0.01* ; HP-Al: 0.85 ± 0.03 *. (IR %):SH: 9.23 ± 0.40; HP: 17.2 ± 0,44 *; SH -Al: 29.59 **+; HP–Al: 35.53 **+. IM: SH: -0.90 ± 0.07; HP: -2.13 ± 0.29* ; SH-Al: -2.64 ± 0.06** ; HP-Al: -2.70 ± 0.05**. (*p<0.05; **p<0.0001 vs SH; + p<0.05 vs HP). In Al-treated and hepatectomised rats an increase of erytrocyte rigidity (increase of IR= decrease of deformability) associated with the diminution of the plasmatic cholesterol (r:-0.6475 p<0.05; n:19) was observed. Co would modify the membrane cholestrol content decreasing erytrocyte deformability. The increase of stomatocytes (IM more negative) in HP animals agreed with the increased IR. On the other hand, the Al-treated rats (SH and HP) showed a greater change in IR and IM, probably due to two effects on the properties of the RBC membrane: 1. the diminution of Co; 2. the metal- interaction in a direct form with erythrocyte membrane components.

52.

LIPID PROFILE VALUES IN CHILDREN AND ADOLESCENT

Corbera MB, Lioi SA, Pituelli NE, Caferra D, Turco M, Cid H, Rosillo I.
Cátedra Química Analítica Clínica, Fac. Cs. Bioquímicas y Farmacéuticas UNR.

Lipids are currently considered as a main cardiovascular risk factor in young people. Objective: We attempted to determine the lipid profile in a children and adolescent population through a new non-HDL-Cholesterol (non-HDL-Co) parameter, analyzing lipid parameters and their global behaviour according to age and sex. 1) Total Co (Tco), 2) triglycerids (TG), 3) HDL-Co, 4) LDL-Co, were assessed in a scholar population composed of 728 children: 476 females (F), 252 males (M), aged 5-17 yr. Enzyme methods for 1 and 2; lipoproteins selective precipitation for HDL-Co; and calculation by Friedwald equation for LDL-Co; non-HDL-Co was calculates as: TCo – HDL-Co. Means, SD and 25, 75 and 95 percentiles of the parameters under study were obtained separately on M and F for 5-11 and 12-17yr intervals. International cut-off values: TCo >176 mg/dl, TG >=91 mg/dl, LDL-Co >112 mg/dl and HDL-Co <=50mg/dl. Risk percentage in the population; 5-11yr Fs: TCo 32%, TG 20%, HDL-Co 15%, LDL-Co 22%, non-HDLCo 26%; 12-77yr: TCo 26%, TG 17%, HDL-Co 26%, LDL-Co 19%, non-HDL-Co 22%; 5-11yr Ms: TCo 21%, TG 12%, HDLCo 18%, LDLCo 14%, non-HDL-Co 14%: 12-17yr interval: TCo 17%, TG 12%, HDL-Co 19%, LDL-Co 9%, non-HDL-Co 11%. Prevalence of risk factors in our population is higher in females than in males. With the new parameter non-HDL-Co a higher percentage is detected in comparison with LDL-Co and TG. Our results emphasize the importance of detecting and identifying earlier risk factors in young populations in order to implement prevention measures.

53.

ROLE OF *hrp* GENES OF *Xanthomonas axonopodis* pv. *citri* IN THE PLANT-PATHOGEN INTERACTION

Dunger RG, Orellano EG, Ottado J.
IBR-CONICET, Area Biología Molecular, Fac. Cs Bioq. y Farm. UNR.

Citrus canker is caused by the phytopathogen *Xanthomonas axonopodis* pv. *citri* (*Xac*) and affects different regions in our country where citrus are produced. *Xac* is a Gram-negative bacterium that depends on the secretion system type III (SSTIII) for the translocation of pathogenicity factors. This system is encoded by the *hrp* cluster (HR (hypersensitive response) and pathogenicity). The HR is a plant response characterized by rapid cellular death around and in the initial site of the infection. In this work we had studied the *hrp* cluster, specifically the *hrpB5* and *hrcN* genes and components of the SSTIII of *Xac*, and their role in plant-pathogen interaction, either compatible or incompatible. *hrcN* codifies for a putative ATPase whereas the functions of HrpB5 is still unidentified. A mutant in the *hrpB5* and *hrcN* genes was constructed using a suicidal mobilizable vector, and by conjugation a mutant bacteria in SSTIII was obtained. Infections of orange (*Citrus sinensis)* leaves were carried out with *Xac* wild type and mutant strains and was determined that the mutant do not trigger disease on the plant. It were performed growth curves of mutant and wild type *Xac* on host plants and it was observed that mutant strain could not grow on this leaves. Infections of non host cotton plants (*Gossypium hirsutum*) with the mutant strain of *Xac* did not trigger HR on the plant. Biochemical studies using trypan blue stain and diaminobenzidine demonstrated that there is neither bacterial colonization nor cellular death of plant tissue due to HR produced by plant-pathogen interaction. The results showed indicate that HrcN and HrpB5 proteins would be indispensable for bacterial colonization on host plants and therefore the disease establishment as well as the development of HR in non host plants.

55.

DERMATOMYCOSES IN ROSARIO: PERIODS 1959-1988 AND 1991-2000

Sortino M[1], Amigot S[1], Bulacio L[1], López C[1], Luque A[1], Ramadán S[1], Ramos L[1], Tosello ME[1], Leiva M[2], Biasoli M[1].
[1]CEREMIC. [2]Cát. Estadística, Fac. Cs. Bioq. y Farm., UNR.

From 1991 to 2000, 6117 specimens from patients with clinically suspected dermatomycoses were studied. The results were compared with those belonging to the period 1959-1988 Direct examinations and cultures were carried out to each sample. Fungal identification was based on the macro, micromorphology and biochemical characteristics. The rate of positive samples between 1991 and 2000 was 42.42%, whereas between 1959 and 1988 it was only 37.16%. In the most recent period 62,84% of patients were female, and 37.16% were male. Positive results for men were significant higher ($p<0,05$). The positive results showed the following distribution: Dermatophytosis 53.96%; Candidiasis 23.66%, and Pitiriasis 16,99%. Pitiriasis were localized mainly on glabrous skin (91.7%); its diagnosis was made merely by direct observation in the 93.34% of the cases. 70.36% of superficial candidiasis were localized in nails and *Candida albicans* was isolated only in 23.36% of the cases. The dermatophytosis localization were: *Tinea pedis* (25.52%), *T. corporis* (23.88%), *T. unguium* (22.14%) and *T. capitis* (20.02%). This distribution differs from that of the 1959-1988 period, when the most frequent syndromes were *T. capitis* and *T. corporis*. Over this period *Micorsporum canis* and *Trichophyton rubrum* were the most frecuently isolated dermatophytes (53.16% and 28.65% respectively). During the 1991-2000 period a significant increase of *T.rubrum* and a decrease of *M.canis* were observed ($p<0,001$). In Candidiasis the recover from cultures was significantly higher than in Dermatophytosis ($p<0,001$). The increase of *T.rubrum* as ethiological agent of tinea may be related with changes in people's way of life.

54.

CHEMOMETRIC METHODS FOR THE SIMULTANEOUS DETERMINATION OF CHLORTHALIDONE AND ATENOLOL IN COMMERCIAL TABLETS

Castellano PM[1], Ferraro MCF[2], Kaufman TS[1,2].
[1]Área Análisis de Medicamentos; [2]Instituto de Química Orgánica de Síntesis (CONICET-UNR). Facultad de Ciencias Bioquímicas y Farmacéuticas. Universidad Nacional de Rosario.

Modern Pharmaceutical Analysis requires fast, simple and economic analytical methods for bulk drug and drug product quality control. Chemometrics-based data processing is a valid tool to achieve this goal. Because of its wide use, we decided to study the resolution of the diuretic-antihypertensive mixture of Atenolol and Chlorthalidone, commercially available as tablets having a mass relationship (Atenolol/Chlorthalidone) of 4.

We developed and evaluated different spectroscopic (UV) analytical procedures, which included zero crossing second derivative spectroscopy and chemometric data analysis (normal and first derivative spectra) employing partial least squares, classical least squares and principal component regression.

Calibration models were built employing a factorial design, with spectra recorded in the 250-350 nm region. Derivation was carried out with the Savitzky-Golay algorithm and data processing was done with a series of appropriate routines written for Matlab. Internal validation was carried out by the "leave one out" method and analytical validation was carried out for accuracy and precision with three sets of independent synthetic samples. The appropriate validated methods were applied to the simultaneous determination of the analytes in a commercial formulation, providing recovery data which indicated full compliance with the corresponding amounts of analytes declared in the label.

56.

FUNGICIDAL NON-CHITINOLYTIC ACTIVITY OF PROTEIC COMPOUNDS BY *Streptomyces* C/33-6

Acosta MG[1,2], Amigot SL[1], Magni C[2], Fulgueira CL.[1,3]
[1]CEREMIC, [2]IBR, [3]CIUNR. Fac. Cs. Bioq. y Farm. UNR. Suipacha 531. 2000-Rosario. Argentina. E-mail: cfulgueira@yahoo.com.ar

Antifungal proteins are of great biotechnological interest because of their potential use in medicine, as food and seed preservative agents, and for engineering plants for resistance to phytopathogenic fungi. After extensive screening experiments to search for biocontrol agents against toxigenic fungi, we found a strain, Streptomyces C/33-6, that secretes a novel antifungal proteinaceous compound in the culture supernatants.

Streptomyces C/33-6 was grown in Waksman broth at 30ºC for 4 days. Culture supernatants were dialysed and concentrated 10-fold through an YM10 cellulose membrane. Proteins precipitated with cold acetone were suspended in NaAc buffer. This concentrate (100-fold) inhibited the conidial germination of toxigenic Aspergillus parasiticus, Fusarium graminearum, F. sporotrichioides and F. proliferatum. We used a fluorochromatic method with fluorescein diacetate-ethidium bromide, usual colony count method and, a germination inhibition assay to characterize the antifungal activity of the Streptomyces concentrate against F.graminearum conidia. The number of viable propagules decreased from 2.5 x 105 CFU/ml (in the control) to less than 10 CFU/ml with supernatant addition. Conidia showed no lysis by they didn't germinate in spite of successive washes. Streptomyces C/33-6 didn't degrade colloidal chitin after incubation for up to 20 days. Then, we conclude that the antagonistic effect was due to compounds with fungicidal and not chitinolytic activity. The use of two contrasting fluorescent reagents provides clear results not obtained in other viability assays.

57.

FIRST IDENTIFICATION STUDY OF *Candida dubliniensis* AMONG HIV POSITIVE PATIENTS IN ARGENTINA AND ITS DIFFERENTIATION FROM *Candida albicans*

Binolfi A[1], Biasoli MS[1], Luque AG[1], Tosello ME[1], Racca LL[2], Magaró HM[1].
[1]CEREMIC, [2]Area Estadística. Fac. Cs. Bioq. y Farm. UNR, Suipacha 531, (2000) Rosario, Argentina.

Candida dubliniensis is a novel yeast specie that is phenotypicaly related with *Candida albicans*. It has been associated with oropharyngeal infections in HIV(+) patients and fluconazole resistant strains have been recovered.
The objective of this study is to detect *C. dubliniensis* in the oral mucose of HIV(+) patients and to evaluate the usefulness of several methods for the differentiation of both species.
A total of 131 strains were isolated from the oral cavities of HIV(+) patients. These strains grew as green colonies in CHROMagar Candida media and produced chlamydospores in Corn meal agar with Tween 80. Then, the following differentiation test were carried out: differential growth at 45ºC in Potato Dextrose Agar; assimilation of D-xylose; chlamydospore production in Sunflower Seeds Agar (SSA); carbohydrate assimilation profiles using the API 20 C Aux commercial kit and PCR using the *C. dubliniensis* specific primers DUBF and DUBR and the universal fungal primers ITS_1 and ITS_4 as internal control.
From the 131 strains recovered, 21 (16,03%) were identified as *C. dubliniensis* and 110 (83,97%) as *C. albicans*. The PCR, the API 20 C Aux kit and the morphology in SSA are the most efficient methods to differentiate this yeasts, being the latter the most simple and inexpensive one.It is important to correctly identify this new specie in order to understand its epidemiological and clinical role in human infections.

58.

ECOLOGY OF THE GASTROINTESTINAL TRACT IN IMMUNOCOMPROMISED PATIENT

Tosello M[2], Echenique C[1], Luque A[2], Bogino B[1], Indelman P[1], Biasoli M[2], [3]Servicio de Microbiología and Magaró H[1,2].
[1]Area de Parasitología. [2]CEREMIC. Fac. de Cs. Bioq. y Farm. UNR.
[3]CEMAR. Municipalidad de Rosario.
E-mail: me_toselloar@yahoo.com.ar

The objective of this work was to analyze parasites, bacterias, yeasts and physiochemical characteristics in faeces samples of immunocompromised patient with gastrointestinal transtorns. Parasitological, mycological and bacteriological analysis were carried out on 26 diarreics faeces samples. Four samples were negative and 22 were positive to parasites, yeasts and/or bacterias. Yeasts were isolated from 19 samples (84,62%), of which 12 had more than 5.000 UFC/g and only 7 had less than 5.000 UFC/g. The species were: *Candida albicans* (48%), *C. parapsilosis* (21%), *C. tropicalis* (16%) and other (15%). In 8 samples (36,4%) were detected parasites: *Strongyloides stercoralis* larvae (12,5%), *Giardia lamblia* cysts (25%), *Entamoeba coli* cysts (25%), *Chilomastix mesnilii* cysts (12,5%), *Blastocystis hominis* (37,5%) and *Cryptosporidium parvum* oocysts (12,5%). A patient presented enteropatogen *Escherichia coli* associated to *C. parapsilosis* with 9.000 UFC/g. Most of the faeces samples were yellowish clear brown color, with fatty acids, soaps, starch, dextrins and fibers, and 3 samples presented sacharolitic flora. The pH varied between 4,5 and 7,5. *C. dubliniensis* was isolated of a boy with AIDS. In 3 cases there was association of parasites with more than 10.000 UFC/g of *C. albicans*. A high parasitic load of *S. stercoralis* corresponded an undernourished patient with eosinophilia. *Cryptosporidium parvum* was marker of AIDS in another of the studied patients. The association of *Blastocystis hominis* (10 / 400x) and *Enterococcus faecalis* was observed in a neutropenic patient with acute myelogenous leukemia.

59.

PCR IDENTIFICATION OF STRAINS BELONG TO *Trichophyton* GENUS

Bonino GS, Tartabini ML, Luque AG.
CEREMIC Fac. Cs. Bioq. y Farm. UNR, Rosario, Argentina.

The aim of this study was to confirm the phenotypic identification of species of *Trichophyton* by PCR fingerprinting. Fifty-six strains isolated from clinical samples at the CEREMIC and reference strains of *Microsporum canis*, *Epidermophyton floccosum*, *Trichophyton rubrum*, *Trichophyton mentagrophytes* var. *erinacei*, *Trichophyton mentagrophytes* var. *interdigitale*, *Trichophyton mentagrophytes* var. *nodulare* and *Arthroderma benhamia* were studied. The morphological characteristics were examined, and the following physiological tests were performed: urease activity, hair perforation *in vitro* and alkalinization of bromocresol-purple casein glucose agar. A minipreparation for DNA extraction was used. The PCR fingerprinting was carrier out using the simple repeated sequence $(GACA)_4$ as a single primer. Amplification reactions were performed in volumes of 50 µl containing 3 µl of template DNA, reaction buffer (10 mM Tris-HCl PH 8.3, 50 mM KCl), 2,5 mM $MgCl_2$, 200 µM of each dNTP, 160 ng of primer and 2,5 U of Taq ADN polymerase. PCR was performed in a DNA Thermal Cycler with an initial denaturation step of 5 min at 94°C followed by 39 cycles of 1 min at 94°C, 1 min at 50°C and 1 min at 72°C and then a final extension of 7 min at 72°C. Species-specific profiles were observed for *M. canis, E. floccosum, T. rubrum* (39 strains) and two profiles for *T. mentagrophytes* and *A. benhamiae*, profile I (13 strains) and profile II (4 strains). Phenotypic identification was confirmed and defined by PCR fingerprinting. It is a simple and rapid technique and can be applied in cases where it is necessary to identify strains that don't present typical morphological features. The only disadvantage is its high cost.

60.

STUDY ON RAYNAUD PATIENTS. INTERRELATION AMONG CAPILLAROSCOPY , HAEMORRHEOLOGICAL VARIABLES AND CONCENTRATION OF SOME PLASMATIC PROTEINS

Spengler MI[1], Svetaz MJ[2], Leroux B[3], Leiva M[4], Bottai H[4].
[1]Cát. de Fís. Biol, [3]Cát. Dermatología. Fac. Cs. Médicas, [2]Sec. I. Celular, Dpto. Bioq. Clínica, [4]Cát. Estadística. Fac. Cs. Bioq. y Farm., UNR.

Blood rheological properties, fibrinogen (fp) and immunoglobulin plasmatic concentrations (Ig) as well as nailfold capillaroscopy were studied and the results correlated, based on a possible participation of rheological disturbances in the Raynaud physiopathological mechanism. The explicative variables were blood and plasmatic viscosity (which were measured at a share rate of 230 s^{-1}, using a cone – plate viscosimeter); the erythrocytic aggregation rate (V) by a method which measured transmitted light through a blood sample; fp was measured using a gravimetric method and the Ig (IgG and IgM) by radial immunodifussion. The response variable was the nailfold capillaroscopy pattern (determined with a magnifying glass at magnifications of 40x), categorized as normal pattern (NP) or pathological pattern (PP). The fp; V and IgM variables were considerably greater ($p< 0,05$ for all of them) in PP patiens compared to NP patiens. No significant difference in blood and plasma viscosity was found in both groups. Data statistical analysis (dichotomous logistic regression model) allowed us to model the chance of PP presence in terms of fp. The odds ratio was 2.18, which shows that the chance of showing PP increases 2.18 times for each 100 mg/dl fp increase. We conclude that the fp and IgM increase in these patients results in a V increase, which determines a slowdown in the flow and the presence of stasis and acidosis localized areas.

61.

Cr^{3+}, Hg^{+}, Fe^{3+} BINDING TO BOVINE SERUM ALBUMIN

Bertoluzzo SM[1,2], Bertoluzzo MG[1], Rigatuso R[1], Quattrin F[1], Godoy NC[2], Corchs J[3].
[1]Taller de Física, Area Física, Dpto. Química Física, Fac. Cs. Bioq. y Farm., UNR. [2]Cátedra de Biofisica, Fac. de Cs. Médicas, UNR. [3]Cátedra de Fisiología, Fac. de Cs. Médicas, UNR.

The chromium, arsenic and mercury contamination is a very worrying subject because of their adverse effects on health and their carcinogenic properties. This might be a consequence of the binding of these ions to biological macromolecules. In the present work Cr^{3+}, Hg^{+}, Fe^{3+} affinity to the serum albumin is studied. Bovine serum albumin (BSA) and anilino-8-naphtalene sulfonate (ANS) were used. The BSA concentration was 20 μM in phosphate buffer, pH= 7.4. The ions were obtained from $CrK(SO_4)2.\ 12H_2O$) 5 mM, $Fe(SO_4)_3$ 5 mM, $HgCl_2$ 5 mM. The ANS binding to the BSA was followed by fluorescence emission when the ANS is bound to the protein in presence and in absence of the ions. From the isotherms, the affinity constant and binding sites were calculated and analyzed with Scatchard graphics. From the results obtained it could be concluded that there is a competition between ions and the ANS for the binding sites to the albumin. There is one site of high affinity and another one of low affinity for the three ions. The high affinity sites are: (1000 ± 400) M^{-1}, (4500±800) M^{-1} and (1500±500) M^{-1} and the low affinity sites are (140 ± 10) M^{-1}, (1000±300) M^{-1} and (170±30) M^{-1}, for Cr^{3+}, Hg^{+}, Fe^{3+}, respectively. In order to find out if there is cooperativity the Hill graphics were drawn. As these graphics resulted to be linear with a slop about 1, we can conclude that the sites are independent from each other. Then we deduce that Hg has more affinity to the BSA than Fe and Cr. However, the BSA can carry some of these ions.

62.

EFFECT OF THE NIFEDIPIN ON RED BLOOD CELL MEMBRANE IN PATIENTS WITH SYSTEMIC SCLEROSIS. PRELIMINARY STUDY

Contesti JF[1], Bertoluzzo SM[2], Leroux B[3], Svetaz MJ[4], Spengler MI[1]
[1]Cát. Física Biológica; [3]Cát. de Dermatología, Fac. Ciencias Médicas; [2]Cát. de Física; [4]Sección Inmunidad Celular, Depto Bioq. Clín., Fac. Cs Bioquímicas y Farmacéuticas, UNR.

The objective of the present work was studying the effect of treatments with nifedipina on the red blood cell membrane fluidity in patients with systemic sclerosis. The measures were done on 13 patients with systemic sclerosis in treatment with nifedipina and on a group of 15 sane controls, by the spectroscopic method with fluoresce polarization. Red blood cells were isolated by centrifugation and suspended in PBS. Then TM-DPH (Trimethylammonium-Diphenylhexatriene), a fluorescent component was added. This fluorophore ables to measure the membrane freedom grade between the phospholipidic molecules and infers the membrane fluidity. The measures permits calculate the anisotropy coefficient (r), which relates inversely with the membrane fluidity. The mean value of r obtained for patients in treatment with nifedipina was 0.159±0.020, meanwhile the one obtained for normal patients was 0.212±0.015. From the results we can conclude that nifedipina increases the erythrocyte membrane fluidity. We propose that this drogue blocks the Ca^{++} channels diminishing its intracell concentrations and the interaction between this and the membrane components. That is why these patients would have double benefit: the vasodilator effect and the increase in the deformation erythrocyte capacity improving in this way the tissue perfusion and diminishing the illness symptom.

63.

A TLC BIOAUTOGRAPHIC METHOD FOR THE DETECTION OF XANTHINE OXIDASE INHIBITORS

Ramallo IA, Zacchino SA, Furlán RLE.
Cát. Farmacognosia. Fac.Cs Bioquímicas y Farmacéuticas. UNR.

Natural products are an important source of new drugs (D.G. Newman *J. Nat. Prod.* **2003**, *66*, 1022-1037). To screen plant extracts avoiding the time consuming isolation of known substances, assays able to detect active compounds present in complex plant matrices are needed (*J. Chromatogr. A* **2001**, *915*, 217). We describe here a bioauthographic assay suitable to localize Xanthine-Oxidase (XO) inhibitors absorbed on chromatography plates (TLC). This enzyme catalyzes the oxidation of hypoxanthine to xanthine and, in the presence of molecular oxygen, to uric acid and superoxide anions. Therefore, XO inhibition is a therapeutic approach for treating gout, kidney stones, and myocardial ischemia (H. Li *J. Nat. Prod.* **1999**, 1053).
For the development of the assay, enzyme and substrate were spread on the plates under different conditions, and several revealing agents for enzyme activity were tested. The best results were obtained when TLC plates were layered with agar solution containing XO and nitroblue tetrazolium chloride (NBT). After solidification the system was immersed in xanthine solution at 35°C for 20 minutes. Enzyme oxidation of xanthine produces superoxide which reduces soluble NBT to a purple precipitate that results in gel staining. Inhibitors of the enzyme are detected as white spots in a purple background. The detection limit for the commercial inhibitor allopurinol was 0.01 μg. The bioassay is rapid and allows the screening of several samples at the same time. It is particularly suited for dereplication of plant extracts.
Acknowledgement. Fundación Antorchas, TWAS, FONCYT, UNR y CONICET.

64.

ELIMINATION OF P-AMINOHIPPURATE (PAH) AND SULFOBROMOPHTHALEIN (BSP) IN RATS WITH EXTRAHEPATIC CHOLESTASIS (EHC) OF 21 HOURS. A COMPARATIVE STUDY

Brandoni A, Villar SR, Quaglia NB, Torres AM.
Area Farmacología. Fac. Cs. Bioq. y Farm. U.N.R. CONICET.

We have described alterations in the pharmacokinetic of organic anions in rats with EHC. The aim of this study was to compare the systemic and renal handling of two organic anions, PAH and BSP in rats with EHC. EHC was produced by bile duct ligation during 21 h (L) in adult male Wistar rats. A group of Sham rats was also processed (S). One group of S (n=6) and L (n=4) received a single dose of PAH (30 mg/kg b.w.). Other group of S (n=7) and L (n=8) received a single dose of BSP (10 mg/kg b.w.). From these studies, systemic clearance of PAH and BSP (ClsPAH; ClsBSP, mL/min/100g) were obtained. The quantity of each drug excreted in urine was also determined (PAHo, mg; BSPo, μg). Basolateral membranes were isolated from renal cortex in order to investigate the abundances of the Organic Anion Transporter 1 (OAT1, %) and BSP/Bilirubin Binding Protein (BBBP, %). In other group of rats (S, n=4; L, n=4), renal plasma flow (RPF, mL/min/100g) was measured by fluorescent microspheres. ClsPAH: S=2.82±0.22, L= 3.55±0.06*; ClsBSP: S=0.81±0.08, L=0.19±0.03*; PAH_o: S=2.92 ±0.11, L=3.67±0.31*; BSP_o: S=1.92±0.34, L=32.40±11.93*; OAT1: S=100±7, L=139±11*; BBBP: S=100±10, L=173±3*; RPF: S=3.81±0.32, L=2.02±0.30* (*$p<0.05$). An increase in ClsPAH, anion excreted mainly by kidneys and a decrease in ClsBSP, anion excreted preferentially by liver were observed in rats with EHC. An increment in the quantity of PAH and BSP in urine was found although the lower RPF. This could be explained, at least in part, by the higher abundances of the renal carriers studied in the group of rats with EHC.

65.

RENAL HEMODYNAMICS PARAMETERS IN RATS TREATED WITH VITAMINA D3

Quaglia NB, Brandoni A, Nowicki S, Torres AM.*
Area Farmacología. Fac. Cs Bioq. y Farm. UNR. CONICET.
**Centro Inv. Endocrinol. (CEDIE). CONICET.*

We have observed modifications in the renal clearance of drugs in rats treated with vitamin D3. The aim of this study was to evaluate some renal hemodynamic parameters. Adult male Wistar rats treated with an overdose of vitamin D3 (300000 UI/Kg. b.w., i.m., 10 days before) (T) and control rats (C) were used. Glomerular filtration rate (GFR) was evaluated by conventional clearance techniques, renal blood flow (RBF) was determined using fluorescence microspheres then, filtration fraction (FF) was calculated. Renal arterial calcium (RAC) was evaluated by atomic absorption spectrophotometry and epinephrine (E), norepinefrine (NE) and dopamine (D) concentrations were assayed in renal cortex using HPLC. Results (*p<0.05):

	Control	Tratado
GFR (ml/min/100 g.b.w.)	0.782 ± 0.08	0.471 ± 0.039*
RBF (ml/min/100 g.b.w.)	5.32 ± 0.75	1.58 ± 0.39*
FF	0.245 ± 0.022	0.490 ± 0.012*
RAC (umol/g dry weight)	17 ± 1	22 ± 1*
E (%)	100 ± 10	73 ± 5*
NE (%)	100 ± 5	92 ± 5
D (%)	100 ± 9	98 ± 9

The diminution of RBF justifies the lower GFR in T rats. The increased calcium in renal arteries could explain RBF modifications. It could exist a preferential constriction in efferent arterioles as a result of the higher FF observed in T rats.

66.

RENAL EXCRETION OF ORGANIC ANIONS IN RATS WITH CHRONIC RENAL FAILURE (CRF)

Torres AM, Brandoni A, Villar SR, Ottaviano G, Muller A*, Picena JC[#], Martinez AJ[&], Mac Laughlin M**
*Area Farmacología. [&]Area Morfología. Facultad Ciencias Bioquímicas y Farmacéuticas. UNR. CONICET. [#]Cátedra de Anatomía y Fisiología Patológicas. Fac. Ciencias Médicas. UNR. *ININCA, Fac. de Medicina. UBA. CONICET.*

We evaluated some variables of PAH excretion in CRF. We induced CRF in (n=6) male Wistar rats, by removing 5/6 of their renal mass. A control (C) group (n=4) was also processed. We measured, systolic blood pressure (SBP, mmHg) in awake rats, plasma creatinine (Cr, mg%) and excreted, secreted and filtered PAH loads (EL,SL,FL, µg/min/100g b.w.). The remnant kidney masses were processed for histological studies, and to evaluate the amount of the organic anion transport protein (OAT1) in the basolateral membrane of proximal tubular cells (S2 segment) by immunohistochemistry. Statistycs was done by the un-paired t-test (*p<0.05). We obtained: SBP: C=126±2, CRF=150±4*; Cr: C=0.94±0.04, CRF: 2.10±0.02*; EL: C=103±20; CRF: 46±12*; FL: C=27±5, CRF=10±2*; SL: C+75±15, CRF=34±10*. The CRF group had lymphocitic infiltrate in the interstitium, a moderate dilation of proximal tubules, decreased cell height and tubular tiroidization, and a reduced amount of OAT1. Thus, in CRF, there is a decrease in the renal excretion of PAH load which could be explained by either decreased PAH filtration load, or by a reduction in PAH secreted load, which, in turn, could be attributed, at least in part, by an altered expression of the OAT1 protein.

67.

RENAL ELIMINATION OF ORGANIC ANIONS IN RATS AFTER 48 H RELEASE OF BILATERAL URETERAL OBSTRUCTION

Villar SR, Brandoni A, Torres AM.
Farmacología. Fac. Cs. Bioq. y Farm. UNR. CONICET.

The obstructive uropathy is a disease produced by obstruction of the urinary tract. One of the proteins that allows renal detoxification of organic anions from the body is the Organic Anion Transporter 1 (OAT1).
The aim of this study was to evaluate the renal capability to excrete organic anions in rats with bilateral ureteral obstruction (BUO).Adult male Wistar rats were employed. Both ureters were ligated during 24 h, then they were released and the studies were done after 48 h (BUO, n=6). A parallel group of sham rats (Sham, n=5) was employed. Plasma urea leves were evaluated by spectrofhotometric technique (Up). P-aminohippurate (PAH) renal clearance (Cl_{PAH}), excreted (EL_{PAH}), filtrated (FL_{PAH}) and secreted (SL_{PAH}) loads were determined employing conventional clearance techniques. The abundance of the renal OAT1 was determined by Electrophoresis and Western Blot. (* p<0.05). Results:

	Sham	OUB
Up (g/L)	0.62 ± 0.07	1.78 ± 0.25*
Cl_{PAH} (mL/min/100g b.w.)	4.12 ± 0.09	0.83 ± 0.15*
EL_{PAH} (µg/min/100g b.w.)	165.3 ± 6.3	89.7 ± 12.4*
FL_{PAH} (µg/min/100g b.w.)	22.9 ± 1.1	20.3 ± 3.6
SL_{PAH} (µg/min/100g b.w.)	134.2 ± 8.8	69.3 ± 10.2*
OAT1 (%)	100 ± 6	44 ± 4*

In this experimental model of BUO a diminution in the Cl_{PAH} was observed after 48 h release of the obstruction. The lower EL_{PAH} was determined by the lower SL_{PAH}. This could be explained, at least to some extent, for the diminution in the abundance of the OAT1.

68.

PARACETAMOL (APAP) CHANGES THE TRITON X-100 SOLUBILITY OF Na^+, K^+ ATPASA (NKA) FROM RAT RENAL CORTEX

Wayllace N, Molinas S, Monasterolo LA, Elías M, Trumper L.
Farmacología Facultad de Ciencias Bioquímicas y Farmacéuticas. CONICET-CIUNR.

NKA is an integral membrane protein composed by two subunits: α and β. The α_1 subunit interacts with membrane associated cytoskeletal proteins leading to the formation of a detergent-insoluble complex. The basolateral localization of NKA is essential for the efficient Na^+ reabsorption. In previous work, we described the development of acute renal failure after the administration of a toxic, non-lethal dose of APAP to male Wistar rats. In this study we analyzed the possibility that APAP could weaken the membrane anchorage of NKA. The following experimental groups used were: i) kidneys obtained from control rats (c), ii) kidneys obtained after one hour of APAP administration, 1000 mg/kg b.w., i.p. (APAP 1h), iii) idem ii) but after 16 hs of APAP administration (APAP16h). The cortex was isolated and homogenized in a buffer with 0.1% Triton X-100. After centrifugation (35000 xg, 14 min, 4°) supernatants representing the Triton-soluble (TS) were separated from pellets representing the Triton-insoluble (TI) fractions. α_1 and β_1 subunits were detected by Western blot in both fractions. The signal obtained for α_1 subunit was referred to the sum of TS+TI. α 1% in TS was: C= 48.0 ± 0.15, APAP1h= 65.8 ± 2.7**, APAP 16h= 81.4 ± 8.1 **, ** p< 0.01. A trend to decrease β_1 was observed in the TI fractions in APAP16h. These results could suggest that APAP promote the weakening of NKA anchorage to the basolateral membrane, the first step for the milocalization of the enzyme.

69.

NA^+, K^+ ATPase (NKA) INTRACELLULAR DISTRIBUTION AFTER ISCHEMIC-REPERFUSION INJURY IN RENAL CORTEX

Molinas SM, Trumper L, Pelourson L, Elías MM.
Area Farmacología. Facultad de Cs. Bioquímicas y Farmacéuticas. Universidad Nacional de Rosario. CIUNR. CONICET.

The purpose of the present study was to analyze renal function and distribution of NKA in plasma membranes (PM) and microsomes (Mi) after an ischemic-reperfusion injury in renal cortex from postischemic (IR) and contralateral (CL) kidneys. Adult male Wistar rats were submitted to 40 minutes of unilateral renal ischemia followed by 24 hours of reperfusion. At the end of this period, we studied renal function in IR and CL kidney separately by clearance techniques. Renal cortex from both kidneys was separated. PM and Mi were prepared by differential centrifugation. NKA activity was measured and α and β subunits abundance was evaluated by western blot. IR presented an altered renal function, while CL was not different from controls. In IR increased abundance of α subunit in PM and Mi was found, while β subunit abundance was normal. This could indicate an increased synthesis or a decreased degradation of α subunit, its accumulation in vesicles and an increased membrane insertion. NKA activity was decreased in PM and Mi, both in IR and CL kidneys. In CL the decreased NKA activity would be enough to maintain a normal Na^+ excretion. In IR tubular epithelia damage, evidenced by decreased alkaline phosphatase in PM, could be in part responsible of the elevated Na^+ excretion found in those kidneys. A systemic signal should be responsible for the changes found in CL.

70.

AGGLUTINABILITY AND ELECTROPHORETIC MOBILITY OF RED BLOOD CELLS' TREATED WITH PROTEOLITIC ENZYMES

Foresto P[1], Grandfilts C[2], Sondag D[3], Rasia R[1], Valverde J[1].
[1]Laboratorio de Inmunohematología y Hemorreología. Dpto. Bioq. Clínica. Fac. Cs. Bioq. y Farmac. UNR. [2]Centro Interfacultades de Biomateriales, Facultad de Química, Universidad de Lieja. [3]Centre Hospitaller Universitaire de Liége (Bélgica).

In this study we have used different enzymatic treatment to modify the erythrocyte membrane structure in order to investigate the surface charge modifications by electrophoretic mobility measurement. We work with human red blood cells (type O R1r) which were treated with neuraminidase and three proteolytic enzymes for use in blood group serology (bromelin, papain and trypsin). The electrophoretic mobility of RBC was measured using Coulter Delsa (*Doppler Electrophoretic Light Scattering Analysis*) 440SX. The graphic of electroforetic mobility, which is plotted as a function of protease concentration, shows a steep decrease around the critical protease concentration at which the cells become agglutinable and the mobility shows a drastic decrese. Our results showed that the decrease in electrophoretic mobility was inversely proportional to the increase in cell agglutinability by anti-D antibodies. The use of one or more units of the enzyme activity resulted in a low-level plateau of mobility. From these relationship between electrophoretic mobility and agglutinability there is an important factor which is common to both properties which is the cell surface charge. It is important to emphasize that the rheological properties of RBC change after enzimatic treatment which depend of the stability and deformability of their membrane.

71.

VANADIUM MEDIATED FREE RADICALS PRODUCTION IN CENTRAL NERVOUS SYSTEM OF ADULT RATS

Quiroga A, Biancardi ME, Alvarez S, Martinez A, Garcia G.
Area Morfología. Facultad de Cs. Bioq. y Farm. UNR.

Altered levels of noradrenaline and other biogenic amines in experimental animals exposed to vanadium and higher blood levels of this element in the manic-depressive syndrome in humans, suggest the neurotoxic effect of vanadium. Since free radicals would be involved in several nervous system disfunctions and pathologies, this work studied the oxidative effect of vanadium (+5) on adult rats´ central nervous system (CNS) using biochemical and histologic methods. Thirty-four male adult rats were divided into 2 groups: Treated Group: i.p. injected with 3 mg/kg/day of sodium metavanadate during 5 days and Control Group: injected with saline solution during the same period. At the end of treatment, the following studies were performed: lipoperoxidation in prefrontal cortex, hippocampus, striatum, midbrain, hypothalamus and cerebellum by the thiobarbituric acid (TBA) reaction; enzymehistochemistry for NADPH diaphorase (NADPHd) and immunohistochemistry with anti heat shock protein 70 (HSP-70) and anti gliofibrillar acid protein (GFAP). An increase of 50% in the final concentration of TBA-reactive material was observed in cerebellum and hippocampus homogenates in the treated group. Cerebellum, striatum, cortex and hipothalamus (supraoptic nuclei) neurons´ NADPHd staining was higher in the treated group. GFAP immunohistochemistry in cerebellum and hippocampus showed a reactive gliosis in the exposed animals. HSP-70 immunostaingning showed the presence of reactive neurons in cerebellum, hipothalamus (supraoptic nuclei) and cortex of treated animals. Data obtained suggest an oxidative effect of vanadium (+5) on adult rats´ CNS.

72.

BEHAVIORAL AND CENTRAL NERVOUS SYSTEM MYELIN ALTERATIONS IN VANADIUM EXPOSED ADULT RATS

Biancardi ME, Quiroga A, Soazo M, Martinez A, Garcia G.
Area Morfología. Facultad de Cs. Bioquímicas y Farmacéuticas. Universidad Nacional de Rosario. Argentina.

Alterations by heavy metals and environmental pollutants on the CNS myelin membrane structure in animals and humans have been observed. High lipoperoxidation levels together with phospholipids, cerebrosides, cholesterol and protein levels diminution in different brain areas of vanadium exposed animals were reported. The aim of this work was to study the effect of vanadium on myelin from central and peripheral nervous system of adult rats, using behavioral and histologic methods. 17 male adult rats (Wistar) were treated with 3 mg/kg/day (i.p.) of sodium metavanadate during 5 days. Equal number of animals (control group) was injected with saline solution. At 6th day, general motor activity in the open field and equilibrium with the rotarod test, were measured. Then, animals were perfusion-fixed through the abdominal aorta, brains and sciatic and optic nerves were removed and processed according to the histologic studies to be performed: myelin histochemistry by Schmued´s gold chloride (AuCl) and Klüver-Barrera´s Luxol Fast Blue methods and immunohistochemistry using an antibody anti-myelin basic protein (a-MBP) by Sternberger´s indirect PAP method. Behavioral alterations in the open field, with a significative decrease in the number of crosses, rearings and grooms, were observed in the treated group. In the rotarod test treated and control animals showed similar performances. A decrease in CNS myelin AuCl staining was observed in the treated group. Results suggest that vanadium as metavanadate would alter CNS myelin structure likely acting on myelin lipid components.

73.

EFFECT OF REGULATORY ANIONS ON THE THERMAL STABILITY OF THE SOLUBLE MITOCHONDRIAL ATPase (F_1)

Gastaldi LM, Lodeyro AF, Roveri OA.
Area Biofísica, Depto de Química Biológica. Facultad de Ciencias Bioquímicas y Farmacéuticas. Universidad Nacional de Rosario.

Mitochondrial F_1F_oATPase catalyzes the synthesis of ATP driven by an electrochemical proton gradient generated through the inner mitochondrial membrane by the respiratory chain. When F_1 is isolated from the membrane, it only can hydrolyze ATP, it is stable at 30ºC and it becomes cold labile. Its ATPase activity is modulated by regulatory anions. Kinetic studies performed in our laboratory have shown that bicarbonate and sulfate bind at different sites on F_1. Accordingly, it has been postulated that sulfate binds to a catalytic site and bicarbonate to a non-catalytic one.
The kinetic of inactivation of F_1 incubated at temperatures ranging from 0 to 50ºC was determined in the absence and in the presence of sulfate and bicarbonate. A single exponential decay to a residual activity significantly different than zero was observed, either in the absence or in the presence of sulfate or bicarbonate. However, sulfate increased the residual activity (half-maximal effect was obtained with 8 and 13 mM sulfate at 20 and 40ºC, respectively). Instead, bicarbonate decreased almost completely the residual activities (50% of the effect was produced by 14 and 5 mM bicarbonate at 20ºC and 40ºC). These results show that sulfate increases whereas bicarbonate decreases the thermal stability of the enzyme and provide another experimental evidence that sulfate and bicarbonate bind to different sites on F_1.

74.

INTERACTION OF SULFATHIAZOLE-COBALT COMPLEXES WITH BOVINE SERUM ALBUMIN

*Molina G[1], Bellú S[1], Hure E[1], Trapé M[1], Trossero C[1], Drogo C[1], Nerli B[2], Picó G[2], Campagnoli D[3], Rizzotto M[1]**
[1]Áreas Inorgánica y [2]Fisicoquímica, Fac. Bioq. y Farm., UNR, Rosario; [3]Fac. Ing. Qca, UNL, Santa Fe.
**E-mail: mrizzott@fbioyf.unr.edu.ar*

The binding of drugs to plasmic proteins, principally albumin and α-glicoproteins, is one of the factors that affects the availability of drugs in the human body. In the present work we analyzed the interaction of the complexes: sulfathiazole-Co(II), ST-Co(II); sulfathiazole-Co(III), ST-Co(III) (which were obtained and analyzed previously by us), and sulfathiazole (sodium salt), NaST, with bovine serum albumin (BSA), by mean of fluorescence spectroscopy. It was observed that both complexes quenched partially the native fluorescence of BSA at 340 nm (λ excitation: 300 nm), suggesting a specific interaction with the protein. The mathematical procedure (Scatchard equation corresponding to two equivalent and independent sites) suggests an interaction with BSA of similar affinity for NaST and for ST-Co(II), while the affinity could be little high for the interaction with ST-Co(III). The values of the affinity constants (k) and n, mean number of ligand molecules binding by mol of protein, are in the following table:

Ligand	NaST	ST-Co(II)	ST-Co(III)
k x 10^{-5}	1.40 ± 0.12	1.21 ± 0.08	2.60 ± 0.28
n	2.9 ± 0.1	2.30 ± 0.09	2.4 ± 0.2

The native fluorescence of BSA is not zero for high concentrations of the ligands, so, it is possible to conclude that the ligands accede to only one of the two triptofans of the BSA.

75.

INTERACTION SULFATHIAZOLE-COBALT: POTENTIOMETRIC DETERMINATION OF SPECIES

*Bellú S, Rizzotto M**
*Área Inorgánica, Fac. de Ciencias Bioquímicas y Farmacéuticas, UNR, Rosario; *E-mail: mrizzott@fbioyf.unr.edu.ar*

Actually, there are many areas of interest for sulfanilamide metal complexes. "Sulfa" drugs bind in different grades to plasmic proteins. The grade of this binding is related to the pKa of each sulfa, so, it is very interesting to study this kind of systems. The biological importance of cobalt is due to its participation in the family of vitamin B12. Besides, cobalt is important in industrial catalytic processes too. In the present work we determined the species present in solution and its respectively stability constants (β) in the system sulfathiazole-cobalt(II), in molar ratio ligand/metal = 2, by potentiometric titration. It was titrated sulfathiazole in presence and in absence of Co(II), with ionic strength, I = 0.1 M, (KCl), T = 25ºC, with carbon dioxide-free NaOH 0.1 M, under a flowing of nitrogen free of CO_2. All the titrations were made by duplicate. The data were processing employing the programs BEST (A.E. Martell and R.J. Motekaitis, Determination and Use of Stability Constants, VCH, New York, 2nd ed., 1992) and ERRBEST (A. Olivieri, G. Escandar, Anal. Lett. 1997, vol 30, pp.1967). It was observed the formation of one specie sulfathiazole-cobalt (LM) and another hidroxo: LM(OH). The following table shows the best values of log β obtained by fitting the experimental data.

Specie	β expression	log β	σ fit
LM	[ML]/[L][M]	15.1076	0.014
LM(OH)	[LM(OH)]/[L][M][OH]	7.6688	0.014

76.

RHD ZYGOSITY STUDY BY MOLECULAR METHODS

Cotorruelo C, Biondi C, García Borrás S, Racca A.*
*Área Inmunología. Dpto. de Bioquímica Clínica. Fac. Cs. Bioquímicas y Farmacéuticas. *CIUNR. UNR.*

The *RHD* gene is flanked by the 5' and 3' *Rhesus boxes* which contain a completely identical sequence termed the "identity region". The deletion of the *RHD* gene, found in most RhD negative Caucasians, is thought to be due to an unequal crossing-over of the 5' and 3' *Rhesus boxes* resulting in the formation of a hybrid *Rhesus box*. The aim of this work was to evaluate a PCR-based method for the determination of *RHD* zygosity by amplification of the hybrid *Rhesus box*. We studied blood samples of 82 trios (father, mother and child). The Rh phenotype was performed by hemagglutination. The *RHD* deleted allele was determined by PCR using a forward primer complementary to the 5´ end of the identity region of the upstream and hybrid *Rhesus boxes* and a reverse primer complementary to the 3´end of identity region of the downstream and hybrid *Rhesus boxes*. These primers amplify a 1981 bp segment of the hybrid *Rhesus box* in RhD negative and RhD positive heterozygous samples. RhD negative individuals (n=44) and all RhD positive members whose heterozygous status was confirmed by family studies of the RhD phenotype (n=35) were PCR positive for the deleted allele. Among the rest of the RhD positive samples (n=167) the zygosity assigned by PCR was concordance with the most probable genotype except for 8 cases. One sample was homozygous and seven were heterozygous according to serology but they typed PCR positive and PCR negative respectively. The discrepant results might be attributed to low frequency haplotypes that confound the zygosity determined serologicaly. This assay is a reliable and simple method for *RHD* zygosity analysis and could allow a better management of sensitized pregnant women.

77.

DAILY VARIATIONS OF GLUCOSE AND INSULIN PLASMATICS IN *Holando argentino* BULL CALVES

Moreyra E, Romano G, Sgüerzo W, Kiener M, Viano D.
Cátedra de Fisiología- Facultad de Ciencias Veterinarias.
Financiado por: CAI+D 2000 Nº 14/1/183 - UNL.

The variations of glucose and insulina plasmatics concentrations was studed throughout the day and its relationship with the food intake in order to make a profile of these variables in bull calves. A jugular blood sample, was obtained every three hours time, during 24 hours, to 7 *Holando argentino* calves, 3 to 7 days old, with ingestion of matinal and vespertine milk substitute. Glucose and insulin were determined from blood plasme. According the variance analysis the animal group was homogenous. No significant difference were found between posprandial sample of glucose. Comparing the interprandiales periods, the nocturnal one registered a low level of glucemia and both showed lower values than the posprandiales periods. This variable displayed a pattern of two tips, with significant difference (P<0,05) between the greater and smaller average. About insulin concentration the morning posprandial period registered higher values (P<0,05) than the afternoon one. The insulin level in the daylight interprandial period were higher than the one at nigth (P<0,05). The morning ingestion induces higher and more lasting concentrations of insulin. Both variables presented the smaller value at 02:00AM, right in the valley of the nocturnal posprandial period and varied significantly throughout the day. The tight relationship between the pancreatic secretion and the levels of glucose in calves of 3 to 7 days old it will be interesting in relation to the physiological changes that take place during the development of these animals and the appearance of a new temporality of these rates.

78.

PROTECTION GIVEN BY STRAIN RB51 *Brucella abortus* VACCINE IN PORCINE FROM A FARM NATURALLY INFECTED WITH *Brucella suis*

Arestegui MB[1], Gualtieri CAS[1], Delgado GJ[2], Peralta L[1], Scharovsky OG[3].
[1]Cátedra de Sueros y Vacunas; [2]Cátedra de Obstetricia, Fac. Cs. Veterinarias, Casilda; [3]Inst. Genética Experimental, Fac. Cs. Médicas, Consejo de Investigaciones, U.N.R., Rosario, Argentina.

Porcine brucellosis causes reproductive alterations like abortions and infertility. We have shown that RB51 vaccine significantly diminishes the prevalence of infection in a farm naturally infected with *B. Suis*, after 36 month post-vaccination (PO-v) and improves productive variables like the increase in the number of piglets weaned per pig/delivery and the diminution of abortions. Aiming to continue the evaluation of the efficacy of RB51 vaccine, 23 reposition piglets from the infected farm, daughters of vaccinated mothers, were vaccinated and blood samples for serologic diagnostic (BPA, Wright and 2-Mercapto Ethanol) were taken every three months, including the animals vaccinated 3 years before. The prevalence of infection pre-vaccination 20.8% (11/53) did not differ from the values obtained at 9 months PO-v: 16.9% (10/53). Also, the serologic titles of seropositive animals did not change during that period of time. It is concluded that even when the vaccination of adult animals diminished the prevalence of infection and improved productive indicators up to 36 months after it, infection remained latent and was transmitted to the non-vaccinated pigs. The response to the vaccination of the young animals, 9 months PO-v, was different to that obtained in adults in a longer time. The follow-up of this herd during a larger period of time will enable us to evaluate any change of the observed behavior.

79.

OBTENTION OF SPERMADHESINS TYPE HBP BY SUBCELULAR PARTING OF BOAR'S SPERMATOZOA

Dapino D[1], Marini P[2], Cabada M[2].
[1]Cát. Fisiología. F.C.V.; [2]División Biología del Desarrollo (IBR) y Área Biología. F.C.B. y F. U.N.R.

The spermadhesins are glycoproteins synthesized by the seminal vesicles, that are bound to plasma membrane of spermatozoa at ejaculation and interact with substances in oviduct. Among these substances is the heparin, a glycosaminoglycan that bounds to spermadhesins called HBP, inducing, in some species, capacitation and acrosome reaction. In boar, this proteins have been studied only in seminal plasma. Objective: develop an efficient method for HBP extraction. Assays: a) Incubation in hipotonic media containing fructose/sodium citrate (FC), b) Incubation with calcium ionophore A 23187 (I) (5, 10 and 20 µM) in TALP, in both, 0, 30 and 60 minutes of incubation. The acrosome was stained with Wells & Awa. Statistics: ANOVA. There were no significant differences in acrosome lost (P> 0.01), incubation time showed significant differences (P<0.01). The best conditions were 60 minutes of incubation in (I) 10 µM and (FC), so FC was selected. The product was centrifugated to separate a supernatant (S2) and membrane´s fractions. This, was treated with salt and then with Tritón X-100, obtaining by ultracentrifugation, periferic (S3) and integral proteins (S4) respectively, and a final pellet (P4). (S2), (S3), (S4), (P4) and BSA as negative control, were applied in nitrocelulose sheet, incubated in biotynilated heparin, streptavidin/peroxidase and later developed by a colorimetric reaction. There is HBP in S2, S3 and S4, proving its presence in acrosomal content as well as in periferic and integral proteins. The efficience of the extraction method was shown by the absence of HBP in P4.

80.

KINETICS OF DRY MATTER RUMINAL DEGRADATION OF RESIDUAL CROPS

Colabianchi BA, Smacchia AM, Faienza HL, Figallo RM, Pidello A.
Lab. de Química Biológica. Fac. Cs.Veterinarias. CIUNR. UNR.

The aim of this work was to study the kinetics of dry matter ruminal degradation of residual crops in the rumen of sheep fed alfalfa hay. Wheat (*ws*), corn (*cs*), sorghum (*ss*) and soybean (*ys*) straw samples were dried at 60°C, ground and sifted with a 2 mm mesh. Samples of three g dry matter (DM) in nylon bags (17mgDM/cm^2) were incubated within the rumen of two sheep during 0, 2, 4, 8, 12, 18, 24, 48, 72 and 96h, in three different periods. The observed results in DM ruminal degradation (DMRD) were fitted at (1) Orskov & McDonald (1979): $DMRD = a + b\,(1 - e^{-ct})$ and (2) Danhoa (1988) models: $DMRD = a + b\,(1 - e^{-c(t-lag)})$; where **a** is the soluble fraction; **b,** the slowly degradable fraction; **c,** the degradation rate of b; and **a + b,** the potential degradability and **lag** latent phase. The best fit was determined with f test (P>0.05).

	Model 1			Model 2			
	a	b	c	a	b	c	Lag
cs	3.6a	93.1b	0.010a	17.6b	79.1b	0.010a	15.7b
ws	17.8c	68.3ab	0.016a	18.93b	67.2ab	0.016a	1.04ab
ss	14.8b	58.4ab	0.017ab	13.24ab	59.9ab	0.017ab	-1.54a
ys	16.9bc	29.9a	0.031b	2.97a	43.8a	0.031b	-12.23a

Note: a, b and c, in the same column indicate significative differences ($p \leq 0.05$).

The potential degradability was 96.7, 86.1, 73.2 and 46.8 %, and the R^2 was 0.96, 0.97, 0.99 and 0.95 for *ws*, *cs*, *ss* and *ys,* respectively. The best fit determined by f test was for model 1. The studied samples presented low soluble and high slowly degradable fractions, and slow degradation rates. Although model 1 was recommended, model 2 determined the latent phase (lag), an interesting parameter for samples such as wheat straw.

81.

KINETICS OF DRY MATTER RUMINAL DEGRADATION OF PASTURE HAYS

Figallo RM, Smacchia AM, Faienza HL, Pidello A.
Laboratorio de Química Biológica. Facultad de Cs.Veterinarias. CIUNR. UNR.

The aim of this work was to study the kinetics of dry matter ruminal degradation of pasture hays in the rumen of sheep fed alfalfa hay. Samples of festuca (*Festuca arundinacea*), setaria (*Setaria italica*) and alfalfa (*Medicago sativa*) hays were dried at 60°C, milled and sieved through a 2 mm mesh. Three g dry matter (DM) of sample in nylon bags (17mgDM/cm^2) were incubated within the rumen of two sheep during 0, 2, 4, 8, 12, 18, 24 and 48h, in three different periods. The observed results in dry matter ruminal degradation (DMRD) were fitted at Orskov & McDonald (1979): DMRD = a + b (1 - e^{-ct}); where: **a** soluble fraction, **b** slowly degradable fraction, **c** degradation rate of b, and **a + b** potential degradability.

Pasture Hays	*Orskov & McDonald. 1979*				R^2
	a	b	c	a + b	
Setaria	11.9a	67.5d	0.048a	79.4d	0.91
Festuca	14.8a	51.8c	0.048a	66.5bc	0.97
Alfalfa 1	16.8b	39.9a	0.096ab	56.8ab	0.95
Alfalfa 2	25.9d	49.2abc	0.128b	75.2cd	0.92
Alfalfa 3	14.0a	40.6ab	0.067ab	54.5a	0.96
Alfalfa 5	22.8bc	46.5bc	0.115b	69.2c	0.99
Alfalfa 6	21.6c	45.9abc	0.128b	67.5c	0.99
Alfalfa 7	23.8c	43.2abc	0.096b	67.0c	0.99

Note: a, b, c and d, in the same column indicate differences ($p \leq 0.05$).

Setaria and festuca hays showed lower soluble fractions, higher slowly degradable fractions, and slower degradation rates than alfalfa hays.

82.

EPIDEMIOLOGIC CHARACTERISTICS OF BOVINE MASTITIS CAUSED BY *Staphylococcus aureus*

François S[1], Limansky A[2], Sutich E[2], Ebner G[2], Comba E[1], Meregalli S[1], Pidone C[1], Pereyra N[1], Anthony L[1], Viale A[2].
[1]Cát. de Microbiología, Fac. de Cs. Veterinarias, Ruta 33 y Bv. Spangenberg, Casilda. [2]Dpto. de Microbiología, Fac. de Cs. Bioq. y Farm. UNR. Suipacha 531, Rosario, Sta. Fe.

Staphylococcus aureus (*S. aureus*) is the most frequent bovine mastitis associated species. The aims of the present work were: i) to analyze the dissemination of *S. aureus* in a herd, and ii) to establish the genomic diversity of the strains that cause clinical (CM) and sub-clinical mastitis (SC). Two hundred milk samples from cows with CM and 85 from cows with SM from herds of Santa Fe were analyzed. To perform the *S. aureus* phenotypic characterization, conventional biochemical tests and a biotypification pattern which characterises specific ecovars and non-specific biotypes of host (NHS) were employed. To perform clonal characterization, PCR with degenerated oligonucleotides was used. Eight *S. aureus* from CM and 50 from SM were recovered. Biotypification characterized 6 of the CM isolates in bovine ecovar and the rest in NHS. SM isolates corresponded mostly to bovine ecovar (40) while the remaining to avian, ovine, NHS1, NHS2, NHS3 and NHS4. This phenotypic methodology showed a greater ecovar variety within the SM isolates as a predominance of bovine ecovar. PCR detected 1 clone (A) and 6 clones (A, E, F, G, J, H) among CM and SM isolates respectly. Both methodologies showed that clone A corresponded mostly to bovine ecovar while clones E, F, G, J and H to avian, NHS1, and NHS2 biotypes. Clone A was highly disseminated in the herds and recovered at different times of the year. The results suggests the high virulence and contagious capacity of these *S. aureus* strains.

83.

HISTOLOGICAL AND HISTOCHEMICAL STUDY OF THE STRUCTURE OF HAEMOLYMPHATIC NODES IN SWINE

Milone D, Salvatore S, Tobin M, Cerutti P, Ugalde JA.
Histología II y Embriología Especial. Fac. de Cs. Vet. U.N.R.

In reference to the structure and ultra structure of the haemal and haemolymphatic nodes, a typical hemolymphnode was found in pigs. The observations demonstrate that this organ has a capsule of dense connective tissue, with a lot of isolated smooth muscular fibers. The hilium was observed to receive blood and lymphatic vessels which then branch off into smaller vessels through the trabeculae and into the parenchyma. Below the capsule, the subcapsular sinus where blood circulates was seen. This sinus is made up of a fibrillar structure and reticular cells with a cytoplasmatic prolongation that appear from a central body where the nucleus is. The distribution of these elements determines small intercommunicated cameras ending close to the lymphatic tissue. The parenchyma, with a cortex and a medulla, consists of diffuse, nodular lymphatic tissue. The nodules of the cortical region were seen to be well delimitated and separated from the surrounding tissue as well as from the blood flow of the subcapsular sinus by one or two layers of long, fusiform cells with acidophilic cytoplasm, a large central nucleus, slack chromatin and evident nucleoli that could be reticular cells. They were immersed in an intercellular and amorphous substance. In the medullar region, lymphatic nodules like those observed in the cortical region and in the diffuse and reticular cells were seen. Lymphatic sinuses with undefined limits were detected. No blood sinuses were observed. In conclusion, the structure of these organs is believed to allow for the coexistence of the two circulations: the blood one which is in the cortex and the lymphatic one in the medulla without there being any communication between them.

84.

MINERALIZATION OF ORGANIC MATTER IN PRESENCE OF DIFFERENT ELECTRON FINAL ACCEPTORS IN AN ZERO TILLED SOIL

Perotti EBR, Menéndez LT, Gaia OE, Pidello A.
Cátedra de Química Biológica, FCV-CIUNR, O. Lagos y Ruta 33 (2170) Casilda, Pcia. Santa Fe. E-mail: eperotti@fveter.unr.edu.ar

The CO_2 is the final product of the soil organic matter (SOM) mineralization. The aim of this work was to study the SOM mineralization in presence of the different electron final acceptors (e$^-$FA). The soil was an Argiudol (Zavalla, Pcia Santa Fe, Argentine) sampled at 0-5 cm, 5-15 cm and 15-25 cm, during four seasons in a year. The CO_2 production was studied in aerobic (A) and anaerobic conditions, with (B) and without (C) KNO_3 (1 mg.g^{-1} soil). The SOM was evaluated through their major components: the oxidizable carbon (OC) and the total nitrogen (tN). The results indicated that SOM at the uppest stratum was the highest, in coincidence with the number of total microorganisms. The MOS quality was evaluated through the quotien OC:tN, there were no differences between strata. The CO_2 production of the uppest stratum was the highest too, but it was not affected by the nature of e$^-$FA. While the CO_2 production at lower strata had the order: C >B >A. Because the SOM was different only in quantity, not in quality (the quotien OC:tN was not different), the different mineralization capacity observed suggests that: (i) there is a microbial population at strata with different functional capabilities, and (ii) its different behaviour is associated with different e$^-$FA presence. The results show the need of studying the impact upon the structure of the functional microbial population when the type and the quantity of e$^-$FA were changed. This will allow to determine the level of the functional stability presented by the microbial structure of each stratum.

85.

OXIDIZABLE CARBON IN FORAGES AND RELATION WITH THE KINETICS OFRUMINAL DEGRADATION

Figallo RM, Smacchia AM, Faienza HL, Pidello A.
Laboratorio de Quim. Biol. Fac. Cs.Veterinarias. CIUNR. UNR.

The main of this work was to determinate if the carbon concnetration in forages fed to ruminants can be considered an indicator of ruminal degradation (DMRD). A large range of dietary substrates was studied –**Presserved forages**: alfalfa, setaria and fstuca hays, and corn silage; pastures: oat, bromus, alfalfa, trifolium repens and mellilotus pastures; **Straws**: wheat, corm, sorghum and soybean straws and **Cereal grains**: sorghum and barley (n=19). Oxidizable carbon (Ox-C%) was determined in duplicate and Walkey & Black (**WB**) abd Harris (**HA**) methods were employed differing in the concentration of oxidizing ($K_2Cr_2O_2$) and valuation solution (SO_4Fe). DMRD was determined by *in sacco* method; ressults were fitted to Orskov & Mc Donald model; and the fast (a), slowly (b) and potentialy (a+b) degradable ruminal fractions and degradation rate (c) were obtained.The WB presented lower mean values ((34.15±0.55) than HA (36.88±1.51) and lower variation coefficients (7.11 vs 17.90) respectively.

Substrates	Ox-C%		DMRD			
	WB	HA	a	b	c	a+b
Pastures	31.19a	33.13a	34.18c	47.15	0.102b	81.89
Forages	34.02ab	35.78a	19.21ab	50.20	0.083ab	69.42
Straws	36.23bc	37.37a	13.30a	62.40	0.018a	75.71
Cereals	37.26c	52.35b	28.58bc	68.67	0.170b	81.34

Note: a, b and c in the same columm indicate differences ($p \leq 0.05$).

Ox-C% of forages studied by both methods was correlated ($0.631 p \leq 0.004$). WB was related with the fast ($-0.633\ p \leq 0.036$) and slowly fraction ($0.563\ p \leq 0.0012$), while HA was only rrelated with the slowly degradable fraction ($0.58\ p \leq 0.06$). Results show that WB was found best indicator to determine the carbon fraction in ofrages. It was found to be inversely related with the fast and directly related with the slowly ruminal degradable fractions. WB can be effective in ruminal methane prediction.

87.

REPRODUCTION OF CHINCHILLAS AND ITS RELATION WITH PHOTOPERIOD

Nistal AJ[1], Catalani G[1], Wojdyla D[2].
[1]Cátedra de Fisiología. Facultad de Ciencias Veterinarias. [2]Cátedra de Bioestadística. Escuela de Estadística. Universidad Nacional de Rosario. Rosario, Argentina.

Chinchilla (Chinchilla lanigera) is a rodent bred as a fur animal. Interest on chinchilla began owing to its valuable fur, to be an endangered species and as a possible experimental model for laboratory studies Its reproductive cycles are continous along the year, with an oestral cycle of 30 a 50 days. Gestation is of 111 days and thay can produce 1- 4 offsprings. In comercial farms, reproductive cycles shows a non homogenous distribution along the year. The relevance of the photoperiod as trigger of the oestral cycle is well documented for other species. The objective was to characterise the presentation of deliveries, matings and number of offsprings during each station of the year and the possible influence of the photoperiod in different departments (Rosario, Constitución, San Lorenzo, Caseros, Gral. López) of the south of the province Santa Fe. Information from 1671 females, 1626 deliveries and 2811 offsprings was gathered. Statistical analysis was performed using nonparametric tests. Results show that mating during an increasing photoperiod resulted in more deliveries (61.8±10.6 *vs* 28.5±10.2, $P<0.0001$) However, proliphicity (expressed as offsprings per delivery) was influenced by the duration of the day. Higher proliphicity was observed in days with less than 12 h of light (1.78±0.2) compared with more than 12 h of light (1.57±0.2)($P<0.01$). Although further studies are required, considering other variables, the present work shows that the photoperiod, increasing or decreasing, is an important factor that determines reproductive cycles and fertility.

86.

ULTRASTRUCTURAL STUDY OF RODLET CELLS DEVELOPMENT IN THE GUT OF TURBOT

Vigliano FA[1], Quintáns ML[2], Quiroga MI[2], Nieto JM[2].
[1]Histology and Embryology, Sch. Vet. Med., U.N.R., Argentina.
[2]Vet. Clin. Sci. Department, Sch. Vet. Med., U.S.C., Spain.

The aim of this work was to characterize the developmental stages of rodlet cells found in gut epithelia of turbot (*Scophthalmus maximus*) in order to clarify their origin and function. Small pieces from several portions of gut were dissected out, fixed in 2.5% glutaraldehyde, and processed by routine techniques for transmission electron microscopy.

Three cellular stages: immature, intermediate and mature rodlet cells were observed. Immature cells were located near the basement membrane and had a poor organelle development. Beneath their plasma membrane a granular region were seen. In the intermediate stage, this region was organized in a fibrous coat with microfilaments. The rough endoplasmic reticulum (RER) cisternae were enlarged by electrolucent material. Mature rodlet cells were seen in the most superficial strata and showed desmosome junctions with adjacent enterocytes. In their thick fibrous coat, peripheral dense plaques, similar to those described for smooth muscle fibers, were identified. RER and Golgi apparatus remained at the basal cytoplasm while mitochondria and their characteristic electrodense granules occupied an apical position. The morphology of these granules suggests that they were synthesized in the RER cisternae. Rodlet cells were frequently observed to be contacting the lumen organ through an apical plasma membrane projection.

The presence of a capsule with a putative contractile property, as well as the cellular polarity, indicated that rodlet cells might have a secretory function.

88.

GONADOTROPHINE-INDUCED OVULATION IN WEIGHT-SELECTED MICE

Bernardi S[1], Brogliatti G[2], Oyarzabal MI[3].
[1]Cátedra de Histología y Embriología, Fac. Cs. Veterinarias. UNR.
[2]Actividad Privada. [3]Cátedra de Producción Animal II, Fac. Cs. Veterinarias y Consejo de Investigaciones. UNR.

A two way selection of body weight at 49 days of age was carried out in two pairs of lines (***s*** and ***h***: negative lines, ***s'*** and ***h'***: positive lines). They were founded from a control population (***t***) of unselected CF1 mice with Ne˜40. Response to selection was followed by modifications in other unselected characteristics related to fertility: age at vaginal opening and at first estrus, estral cycle duration, number and size of ovarian follicles and number of corpora lutea. Significant differences were detected between the two positive and negative lines. The response to stimulation of ovarian function in each line was compared through corpora lutea (CL) count with the aim of confirming if ovulation induction partly inhibits these differences. The results were then associated to the selected characteristic (P). Overrovulation was achieved with the administration of 5UI of Gce and 5UI of Gch. The positive lines had a higher number of CL than the negative ones in both the group under hormonal treatment (WT) and the group without treatment (NT) ($p<0,001$). The same relation was observed in the NT group when CL was related with P ($p<0,01$). Induction invalidated the differences between positive and negative lines for CL_{WT}/P due to a higher relative increase: $RI=\{|(CL_{WT}-CL_{NT})/CL_{NT}| \times 100\}/P$ in the CL number of the negative lines.

89.

DETERMINATION OF GLYCOPROTEINS IN GERMINAL EPITHELIUM OF *Genypterus blacodes*

Díaz AO[1], Freijo RO[1], Garcia AM[1], Macchi GJ[2].
[1]Depto. de Biología. Fac. Cs. Ex. Nat. UNMDP. Funes 3250 3° piso. (7600) Mar del Plata. [2]INIDEP. E-mail: adiaz@mdp.edu.ar

The ovaries of *Genypterus blacodes* (pink cuskeel) correspond to the cistovarian type, hollow organs into which numerous lamellae project to the central lumen, supporting follicles in maturation. Lamellae are covered by a germinal epithelium (GE), which shows secretory activity of glycoproteins (GPs) during spawning.
The purpose of this study was to examine the histochemical properties of the GPs secreted by the GE cells in order to know aspects of the reproductive biology of *G. blacodes.* Following techniques for the visualization and identification of GPs were used: 1) PAS (GPs with oxidizable vicinal diols); 2) Selective periodate oxidation method (PA*S) (sialic acid without substituents or substituted in C7 or C9); 3) K(OH)/PA*S: (total sialic acids); 4) K(OH)/PA*/Bh/PA (neutral sugars); 5) PA/Bh/K(OH)/PAS (sialic acid substituted in C7 and/or C8 and O-acil sugars); 6) Alcian Blue (AB) pH 2.5 (GPs with carboxyl groups and/or with sulphated esters); 7) AB pH 1.0 (GPs with O-sulphated esters); 8) AB pH 0.5 (very sulphated GPs); 9) Toluidine Blue pH 4.2 (acid sulphated GPs) and 10) Toluidine Blue pH 5.6 (acid sulphated and carboxilated GPs) GE cells showed the same morphological characters of the cells with secretory activity and contain a mix of neutral and acidic GPs. The GPs play an important role in mucous secretions and are the main components of the ovarian fluid. Also, this secretions could form gelatinous masses of eggs attached to substrate during spawning. Such reproductive strategy is a differential characteristic from that of others teleost of Southwest Atlantic.

90.

STRUCTURE OF THE GILLS OF THE "CATFISH" *Rhamdia quelen* (OSTEICHTYES, PIMELODIDAE)

Petcoff GM, Díaz AO, Escalante AH, García AM, Goldemberg AL. Dep. Biología. Fac. Cs. Exactas y Naturales. UNMdP. Mar del Plata. E-mail: gpetcoff@mdp.edu.ar

Gills are multifunctional organs and the knowledge of their normal features is necessary for the understanding of pathological alterations. The aim of the present work is to study the histological structures of the gills of the "catfish" *Rhamdia quelen* (Pimelodidae). The specimens were collected from Los Padres Lake, a very attractive place for tourism and fishing, placed in Buenos Aires province. The gills were fixed with 10% formalin, and the histological techniques carried out were: H/E, Mallory Tricromic, PAS and Alcian Blue (pH 2.8). The gill arches possess stratified epithelium with numerous mucous PAS and Alcian Blue positive cells and taste buds. The underlying submucosa contains blood vessels, striated muscular fibres and hialine cartilage. The gill filaments consist of cartilage rays supporting the filament, blood vessels and a stratified epithelium with numerous mucous and chloride cells. The chloride cells are limited to the epithelium of afferent, efferent and interlamellar surfaces of filaments, as well as near the basal region of secondary lamellae. These cells are columnar with a round nuclei and eosinophilic cytoplasm. The mucous cells are found dispersed among epithelial cells and contain neutral and acid mucosubstances. Rodlet cells are characterized by the presence of many acidophilic granules. The lamellar epithelium consists of two epithelial cell layers. The outermost layer is structurally adapted to gas exchange. The lamellar epithelium surrounds the vascular space formed by the pillar cell flanges. The mucous cells are similar to those found in the filament epithelium. No chloride cells were found in the secondary lamellae. The results obtained allow to conclude that the general structure of the gills of *R. quelen* is similar to that found in other freshwater teleosts.

91.

EFFECT OF DOPAMINE ON GLUCOSE LEVELS IN HEMOLYMPH OF *Cyrtograpsus angulatus*

López Mañanes AA.
Dpto. Biología, FCEyN, UNMDP, Funes 3250 (B7602AYJ), Mar del Plata. CONICET. E-mail: mananes@mdp.edu.ar

In decapod crustaceans, biogenic amines (i.e dopamine) appeared to be involved in the regulation of several physiological processes (i.e osmoionoregulation and carbohydrates metabolism). Our previous work has shown a differential effect of dopamine (Dopa) on Na^+,K^+-ATPase and alkaline phosphatase activities in muscle of *C. angulatus* suggesting a role for Dopa in the regulation of mechanisms of adjustments upon hypo-osmotic stress. The aim of this work was to study the effect of Dopa on both glucose levels in hemolymph and the release of glucose from hepatopancreas of *C. angulatus* upon hypo-osmotic stress. Adult male crabs were acclimated for at least for 30 days at 10 ‰ salinity. "*In vivo*" experiments: Crabs were injected with saline (control) or saline + 10^{-4} M Dopa. "*In vitro*" experiments: Sections of hepatopancreas (100 mg) were incubated with 10^{-4} M Dopa (control: saline solution). Glucose release was determined at different times (t min).Three independent experiments were carried out. T-test and ANOVA RM were used as statistical analysis ($p<0.05$). 10^{-4} M Dopa increased "*in vivo*" glucose level in hemolymph at 30 min (mg/100 ml hemolymph) (control:5.5±0.78; Dopa:15.7±1.9) ($p<0.01$) and induced at 60 min the release of glucose from epatopancreas (mg/gtissue) (Control: to=0.86±0.17;t15=1.3±0.34; t30=1.69±0.21; t60=1.73±0.27; Dopa;t15=1.67±0.13; t30=1.41±14; t60=5.5±0.68). The results show that dopamine exhibits an hyperglicemic effect in *C. angulatus* upon hypo-osmotic stress and that part of this effect would involve its direct action on hepatopancreas, suggesting the role of dopamine in glucose homeostasis in hyperegulating conditions of this crab.

92.

ALKALINE PHOSPHATASE ACTIVITIES WITH DIFFERENTIAL RESPONSE TO ENVIRONMENTAL SALINITY IN MUSCLE OF *Chasmagnathus granulata* AND *Cyrtograpsus angulatus* FROM MAR CHIQUITA LAGOON

Pinoni SA[1], López Mañanes AA[1,2].
[1]Dpto. Biología, FCEyN, UNMDP, Funes 3250 (B7602AYJ) Mar del Plata; [2]CONICET. E-mail: mananes@mdp.edu.ar

Alkaline phosphatase (AP) has been little studied in decapod crustaceans. The aims of this work were: a) to characterizate AP activity in muscle of *Chasmagnuthus granulata,* b) to study the long-term response to environmental salinity (S) of AP activity in muscle of *C. granulata* and *Cyrtograpsus angulatus*. Male adults crabs were acclimated for at least 18 days to 35 or 10‰S. AP activity was determined in chela muscle homogenates (0.25M Sucrose/ 0.5mM EGTA-Tris pH 7.4) by measuring pNPP hydrolisis (9.5mM) in the presence of 100mM Tris (pHs 8.0 and 9.0) with or without $MgSO_4$. Three to 6 independent experiments were carried out. ANOVA was used as statistical analysis ($p<0.05$). In *C. granulata*, AP activity (nmoles pNPxmin^{-1}xmg prot^{-1}) was detected at both salinities -Mg^{++} at pH8.0 (35‰S: 203±49, 10‰S: 371± 36) and 9,0 (35‰S: 314±13, 10‰S: 289±26). Activity +Mg^{++} at pH8.0 was higher (35‰S: 723±50 y 10‰S: 765±83) but not at pH 9.0. Acclimation to 10‰S only affected on AP at pH8.0 –Mg^{++}. In *C. angulatus*, AP at pH8,0 + Mg^{++} (35‰S: 625±103, 10‰S: 572±49) and -Mg^{++} (35‰S: 185±58, 10‰S: 83±48) was not affected by acclimation to 10‰S. The results show the existence of AP activities in muscle of chela of both crabs with differential response to Mg^{++} and reduced salinity, suggesting its role in muscle mechanisms secondaries to osmoionoregulation.

93.

Na^+,K^+-ATPase AND IONOREGULATION IN *Cyrtograpsus angulatus* FROM MAR CHIQUITA COASTAL LAGOON

Elhalem LM[1], López Mañanes AA[1,2].
[1]Dpto. Biología, FCEyN, UNMDP, Funes 3250 (B7602AYJ), Mar del Plata; [2]CONICET. E-mail: mananes@mdp.edu.ar

We have previously shown the differential response of Na^+,K^+-ATPase activity in individual gills (g) of *C. angulatus* upon acclimation and after a short-term change from 35 a 6‰ salinity (S). In this work we studied the effect of a short and long-term change from 35 to 10‰S on Na^+,K^+-ATPase activity in g4 (anterior) and 6 (posterior) and on hemolymph ions concentration. Male crabs acclimated for 10 days (T_0) at 35‰S were transferred to 10‰. One (T_1) and 8 (T_2) days after change branchial Na^+,K^+-ATPase and hemolymph ions were determined. 10000 xg supernatants were used as enzyme extract (homogenization medium: 0.25 M sucrose/0.5 mM EGTA-Tris pH 7.4). Activity was assayed by measuring ATP hydrolysis (5 mM) in (mM): 20 imidazol (pH 7.4)/100 NaCl/30 KCl/0.5 EGTA; control: without KCl, with 1 mM ouabain). Na^+ and K^+ were determined by flame photometry and Cl^- by colorimetric method. ANOVA was used as statistical analysis ($p<0.05$). At T_1 activity (nmoles Pi x min^{-1} x mg $prot^{-1}$) increased (100%) in g4 (T_0=99±16; T_1=195±20; T_2=180±32). In g6 a no significant increase occurred (T_0=312±17; T_1=351±49; T_2=442±52). Na^+ and Cl^- (mEq/l) decreased at T_1 being essentially constant at T_2 (T_0=376±7; T_1=332±11; T_2=299±11) (T_0=469±8; T_1=389±22; T_2=319±12). K^+ did not change (T_0=9±0.3; T_1=8±1; T_2=8±0.4). Ions concentration were higher than those in medium (10‰S: Na^+=179±3; Cl^-=152±3; K^+=4±0.3). The results show a differential short and long-term response of Na^+,K^+-ATPase in g4 and 6 to salinity and strong hyperregulatory ability of *C. angulatus* suggesting a distinct role of individual gills in short and long-term ionoregulatory proccess.

94.

CONTRACTILE FORCE IN SEA ANEMONES (ACTINIARIA) FROM SUBTIDAL OF MAR DEL PLATA

Patronelli DL[1], González Olivera E[1], Zamponi MO[2].
[1]Lab. Fisiología Animal. Dpto. Biología; [2]Lab. Biología Cnidarios, Dpto. Cs. Marinas. FCEyN. UNMdP. E-mail: dlpatron@mdp.edu.ar

It was compare the muscular activity of marginal sphincter of two species of sea anemones from subtidal with the sames species from intertidal* *Aulactinia marplatensis* and *Aulactinia reynaudi* were collected from the port of Mar del Plata, Argentina (38º 08´ 17´´ S and 57º 31´ 18´´ W), between 3-5 metres depth. The sphincters were placed in a chamber containing sea-water, connected to a poligraph and stimulated with KCl solution. Dose-response curves expressed in tension in grams vs. concentration were performed. Student´s t-test were used. P values < 0.05 were considered statistically different. The maximal developed force obtained of anemones from intertidal were: *A. marplatensis* 1.33 g ± 0.13 and *A. reynaudi* 1.29 g ± 0.10 whereas the specimens from subtidal developed maximal contractile forces significant lower ($p < 0.05$): *A. marplatensis* 0.73 g ± 0.02 and *A. reynaudi* 0.71 g ± 0.03, without significant difference between themselves ($p > 0.05$). In contrast with intertidal, subtidal environment is characterized for greater stability, because the pressure of column of water, originated from waves, doesn´t impact directly on their lower levels. The strong tidal currents is on the upper level of intertidal. These qualities could justify the differences on contraction force of sea anemones from subtidal compared with the same from intertidal. This comparative information, added to another factors have influence on the ecology of these organisms. It could be a possible factor of speciation (Harris, 1991).
**intertidal: data presented in previous paper.*

95.

INCIDENCE OF DIFFERENT GENUS OF HALOPHILIC ARQUEOBACTERIA IN SAMPLES OF MATURED SALTED ANCHOVY (*Engraulis anchoita*)

Felix M[1], Ameztoy I[2,3], Ramirez E[3,4], Yeannes MI[2,3].
[1]UNMDP; [2]CONICET; [3]UNMDP; [4]U CLP.- Preservación y Calidad de Alimentos. Depto Química. FCEyN. UNMDP. Funes 3350. 7600 Mar del Plata. Argentina. E-mail: myeannes@mdp.edu.ar

Salted and matured anchovy is a traditional product of Argentina fishing industry. The activity of water of this product (0.75-0.82) it prevents the development of most of the flora responsible for the deterioration and it assures the inactivation of the pathogenic microorganisms. Halophilic microorganisms, find good conditions for their development, being able to cause degradation of the muscle and promoting sensorial changes. The extremely halophilic arqueobacteria grow in means with 2,5-5,2M of ClNa. The aim of this work was to determine the presence of genus of extremely halophilic bacteria and their appearance frequency in matured salted anchovy. Were analyzed 76 samples for determination of extremely halophilic bacteria by plate count. Starting from a dilution 10/90 p/v of anchovy fillets in broth salt (20% ClNa) they are carried out dilutions. These were spread in surface (0,1ml) in agar. The identifications for dye and biochemical characteristic tests were carried out. It was determined the lipolitic and proteolitic activity. On the total of analyzed samples 52 were positive for Arqueobacteria of these 16% turned out to be *Halococcus* spp; 9,6% *Haloarcula* spp.; 21% *Haloferax* spp. and 5,6% *Halobacterium* spp. Of these 30% has proteolític activity, other 30% lipolitic activity and 15% they possess activity as much proteolitic as lipolitic. The control of the growth of these bacteria improve the quality and shelf life of this product.

96.

ULTRASTRUCTURE OF THE BRANCHIAL LAMELLAE OF THE PRAWN *Palaemonetes argentinus* (CRUSTACEA, DECAPODA, CARIDEA)

Sousa LG[1], Petriella AM[1,2].
[1]Depto. Cs. Marinas, Fac. de Cs. Exactas y Naturales, Universidad Nacional de Mar del Plata. Funes 3350. B7602AYL, Mar del Plata. Argentina. [2]CONICET. E-mail: lgsou@mdp.edu.ar

This work describes the ultrastructure of the branchial lamellae of *P. argentinus* in intermolt. This species possess a phyllobranchiate gill, in which the central axis is triangular, and has one row of flattened lamellae at each side. There are one afferent channel and two efferent ones, and tegumental glands along the axis.
Gills of adult prawns, of both sexes, and at sexual rest from Sotelo stream (Argentina, 37° 45'S 57° 26'W) were dissected. They were fixed in glutaraldehyde 3% (pH= 7.2; 400mOsm Kg^{-1}) and processed following routine techniques for TEM.
In the lamellae, the principal cells consist of apical extensions with deep infoldings and a neck-like zone with bundles of microtubules. The nucleus is in a basal expansion and the basolateral membranes are interdigitated with great numbers of mitochondria within the interdigitations. Neighbouring cells abut in the middle of the lamellae bordering haemolymphatic lacunae. The cells contain characteristics of both respiratory and ion regulatory epithelium; they also have a structural role directing the haemolymph flow and supporting the lamellae. The thicker cuticle at the marginal channel increases the mechanical support. The complexity of the lamellar epithelium appears to be related to the capability of *P. argentinus* of living in freshwater and brackish water environments and adapting to daily salinity changes.

97.

PREDICTION OF NUTRITIONAL FRACTIONS OF TWO ALFALFA CULTIVARS WITH DIFFERENT WINTER REST

Cechetti S, Verdura L, Acebal MA, Calvo F, Spiller L[1], Fonseca S, Sola D, Figallo R[2].
Animal Nutrition and [1]Forages, Agricultural Sci.; [2]Biological Chemistry, Veterinarian Sci. – National University of Rosario.

Animal response depends on quality, intake and efficiency of utilization of feed. Crude Protein (CP), Neutral and Acid Detergent Fiber (NDF, ADF) are highly associated with intake and digestibility. The Alfalfa (*Medicago sativa* L.) cultivars in Argentina present different Latency Degree (LD) and there is not much information about LD/quality relation. The PEAQ method (Hintz and Albretch, 1991), based on the length of the tallest stem and maturity stage, estimates the NDF, ADF and CP content on standing crops. This work evaluated the utility of PEAQ method for estimates nutritive fractions in two alfalfa cultivars with different LD. The samples of cv. P5683 (LD6) and Siriver (LD8) in vegetative stage, ending winter period, were obtained from 0.25 m^2 of representative areas (n=16). It was measured the length of the highest stem (h, in inches) and determined (Observed Value, Ov) NDF and ADF% (Van Soest) and CP% (Kjeldahl); same fractions were estimated (Estimated Value, Ev) NDF% = 18,51 + 0,69*h; ADF%=13,15+0,53*h; CP%=28,93-0,23*h. Relation between Ev and Ov was evaluated through R^2, the RMSE (% of DM), b_0 and b_1 of regression lines. In cv with medium latency (LD6) regressions were significant only for NDF ($p<0,001$) and ADF ($p<0,05$); RMSE of NDF and ADF were acceptable. For short latency cv any regression were significant, it was taller and with a higher fiber content and a lower CP% than LD6 cv. PEAQ method appears to be a fast and simple tool to predict fiber content of standing alfalfa with medium winter rest.

99.

SCARIFIER EFFECTS IN ALFALFAR IMPLANTED IN NO-TILLAGE SOWING

Martín B, Vega MA, Montico S.
Facultad de Ciencias Agrarias, UNR.

Generally, the soils of the humid pampas are managed with no-tillage. This has a tendency to develop mechanical impedances. This is mainly for genetic causes. A possibility in order to eliminate them is the use escarifier. The objective of this study was to evaluate the effect of scarifier in the alfalfa implantation. The work was carried out in Villarino Experimental Field (33º Lat. S, 67º Long.W), Zavalla district, (UNR), on an Argiudol vertic soil, during 2001 and 2002.
Tillage of scarifier was carried out in plots from seven years of no tillage continuous. In each one of the plot the number of plants was registered/ m^2, and they were selected ten plants at random. In these were measured the air height and longitude of the roots and they registered the dry air peso and radicals. The determinations were carried out during 90 days of the emergency of the plants. Together was measured, in the 15 cm of depth, the resistance to penetration for cone penetration resistance (RPf). The results indicated year for treatment interaction. The differences in the number of plants between years were attributed to the precipitations accumulated in the experimental period (216 mm for 2001 and 289 mm for 2002). The scarifier caused a decrease in the RPf in depth, what could explain the higher radical longitude of the plants of this treatment, variables that would become independent of the climatic conditions. In the 2001 was registered a minor accumulation of precipitations concerning the climatic values of the zone, the scarifier favored the air growth and radical of the plants, probably because this technique permits one capture the useful available water in the soil in more efficient form.

98.

EVOLUTION OF MAGRARIO WEIGHT LAMBS AND THEIR CROSSES WITH IDEAL AND HAMPSHIRE DOWN BREED DURING PREWEANING PERIOD

Bulacio M, Acebal MA[1], Maiztegui LB[2], Villegas A, Picardi LA[3]
[1]Nutrición Animal; [2]Anatomía y Fisiología Animal; [3]Genética. Faculty of Cs.Agrarias. [3]CIUNR, National University of Rosario.

A breeding program to obtain a new lean meat genotype was performed since 1986. Magrario was the new ovine genotype that was obtained through several backcrosses from the Ideal Breed to Texel Breed. Magrario lambs (M) were compared with their crosses with Ideal (I) and Hampshire Down (HD) breed during the preweaning period of 2002 lambing season. The following variables were registered: Dam Weight (DW), Birth Weight (BW), First Month Weight (FMW), Weaning Weight (WW) and relative Average Daily Gain (rADG) as a measure of feed efficiency. Correlation among all variables was also estimated. Differences between M and F_1 (IxM) were significant for FMW and WW traits but no significant differences were observed between M and F_1 (MxHD). Association between DW and BW traits was nil in all genetic groups but correlation between DW-FMW and DW-WW were only significant in the F_1 (IxM) group ($r=0.82$, $R^2=0.68$; $p<0.001$ and $r=0.60$, $R^2=0.36$; $p<0.05$, respectively. Apparently in this genetic group the mother weight would affect the FMW and WW of the lambs. In all groups the FMW would be a good predictor for the WW (M: $r=0.81$, $R^2=0.65$; $p<0.0001$, F_1 (IxM): $r=0.51$, $R^2=0.26$; $p<0.05$ and F_1 MxHD: $r=0.80$, $R^2=0.64$; $p<0.05$). It was found a negative association ($p<0.05$) between FMW-rADG only in M and F_1 (IxM) lambs.

100.

PROBABILITES OF RAINFALL DAYS QUANTITY AND SEQUENCE IN ZAVALLA LOCALITY

Sacchi O, Bisaro V, Coronel A, Costanzo M.
Cátedras de Climatología Agrícola y Estadística, Facultad de Cs. Agrarias, UNR. E-mail: osacchi@.unr.edu.ar

In the pampean region, the temperature and rainfalls are restrictive factors in the production of grains and forage.
The objective of this research was to determine the empirical and theorical probabilities, for each month of the years of: a) occurrence from 0 to 10 consecutive days with rainfalls and b) the quantity of rain fallen.
Monthly rainfalls were analysed in Zavalla locality (33º 01' south latitude; 60º 53' west longitude), corresponding to 1973 – 2000 period. The distribution of frequency of sequences of rainfall days and the quantity of rain fallen for each month groups were calculated, then they were adjusted through probability models.
The quantity of consecutive days with rainfall reaches number 8. Until 4 days of rainfall were observed in all the months of the year, and the rains of longer duration occurred only in April and May.
The greater probabilities corresponded to 1 and 2 consecutive days of rainfall. The statistical model which presented the best adjustment for theses empirical probabilities was the Poisson function.
As regards the quantity of monthly rainfall, it was determined that the best model of adjustment was a Log-Normal function.

101.
THE USE OF ACTIVATORS IN THE CHEMICAL CONTROL OF BACTERIAL BLIGHT OF WALNUT

Flores P[1], Seta S[1] (ex aequo), González M[2], Coniglio R[1], Sferco S[1].
[1]Cátedra de Fruticultura; [2]Cátedra de Fitopatología. U.N.R.

Great economical looses in walnut production were caused by *Xanthomonas campestris pv juglandis.* This disease attacks vegetative and reproducive buds. The control of this disease consists in varietal resistance and chemical treatments. Different workers consider that cupper mixed with mancozeb is significantly more effective than only cupper. The purpose of this work was to evaluate cupper oxiclorure with different dosis of mancozeb as activator to control Blight Walnut. During 2002, in Zavalla, chandler, Davis, Tulare and Franquette varieties, grafted on *Juglans nigra* rootstocks, were evaluated. The experimental was conducted like a complete randomized with factorial design and analyzed by PROC. GLM. SAS 6.12. The variable was severity (affected foliar area/ total foliar area x 100). Three treatments were evaluated: T1 cupper oxiclorure in dosis of 300 g of p.c. to each 100 l of water (blank), T2 cupper oxiclorure (300 g of p.c. to each 100 l of water) + mancozeb in dosis of 100 g of p.c. to each 100 l of water, and T3 cupper oxiclorure (300 g of p.c. to each 100 l of water) + mancozeb in dosis of 50 g of p.c. to each 100 l of water. 20 plants per treatment were evaluated. The products were applied in blowing bud, ament elongation and 10% of pistiled flower and filled up fruit. The analysis of the variance showed significant differences (5%) between varieties, being Franquette the best one. No differences were observed between treatments. Franquette showed the lowest severity due to its latest blowing up date that avoid early critical inoculation, with rainny conditions that favorate infection and dispersion of the disease.

102.
PERFORMANCE OF HYBRIDS OF CORN (*ZEA MAYS* L.) TOWARDS FUNGIC PATHOGENS OF GRAINS

Incremona M, Ghio A, Gonzalez M, Papucci S, Gonzalez A, Cruciani M, Pedrol H.
Fitopatología. Fac. Cs. Agrarias. U.N.Rosario.

The objective of this work was characterized the performance of some commercial corn hybrids of different textures of endosperma as compared to pathogens of seeds in two localities of the Santa Fe south. Samples of 5 commercial hybrids included in two comparative yield trials in the year 2002/03 were obtainded in Zavalla and Oliveros. The incubation method on cultived media was used to determine the seeds pathology. A complete block randomized design with 4 repetitions was used. Two hundred seeds by hybrid were analysed. The percentage of seeds by each pathogens, the germination and the percentage total of infection were established. The considered variables were infection with ***Fusarium spp., Penicillium spp., Aspergillus spp., Diplodia spp.*** and germination. Differences between localities, hybrids and interaction were determinate. The major level the infection was in the locality of Oliveros. The major level of severity according with the hibryds were: ACA 2001 (50% ***Fusarium spp.*****),** Dk 682MG (10% ***Apergillus spp.***), Ax889 (51% ***Penicillium spp.***) and Albion MG (7% ***Diplodia spp.***). There were no differences between pathogens and grains texture. Was verified a highly significant and negative correlation (- 0,37) between germination and the infection of *Diplodia spp*. Was no relation between the grain hardness and the performance to pathogens in the evaluated genotypes. Therefore there are another characters that affect the performance evaluated.

103.
***IN VITRO* RESPONSE OF SUNFLOWER (*Helianthus annuus L.)* GENOTYPES FROM DIFFERENT TYPES OF EXPLANTS**

Traina MJ, Fauguel C, Mayor ML, Nestares G, Picardi L[1].*
*Cátedra de Genética. Fac. Cs. Agrarias UNR. [1]CIUNR. *E-mail: mariano_traina@hotmail.com*

The aim of this work was to evaluate the *in vitro* response of 12 sunflower genotypes using different types of explants. The genotypes tested were the inbred lines HA89B, HA89 (PET1), HA89 (PET2), HA89 (MAX1), HA89 (GIG1), HA89 (PEF1), HA89 (RIG), HA89 (RES1) and the commercially available hybrids MG50, GH1100, ACA872 and HELIO358. The basal medium was a MS (Murashige and Skoog, 1962) supplemented with 200 mgl^{-1}glutamine, 1 mgl^{-1} IAA, 2 mgl^{-1} KIN, pH 6.00, 9 mgl^{-1} agar. Cotyledons from mature seeds (CSM) and also cotyledons (CPT) and hypocotyls (HIP) of two-day-old seedlings were used as starting materials. Cultures were grown at 25ºC ± 2ºC with a 12-hr photoperiod for 30 days. A complete randomised block design with two repetitions of 20 explants each was used. The traits evaluated were *percentage of regeneration (R), percentage of callus (C)* and *percentage of hypertrophy (H).* Data were analysed through the non-parametric *Chi –square* test (X^2). Significant genotypic differences were observed for the regeneration capacity. Furthermore, the organogenic regeneration ability was also influenced by the type of explant. CSM was, on average, the explant with higher regeneration potencial. The inbred line HA89 (RES1) highlighted in regeneration ability when CSM was used as explant. Genotypic differences were observed for C and H *in vitro* traits. These results suggest that the *in vitro* response in this species depends on both the genotype and the type of explant.

104.
CHARACTERIZATION OF TOMATO GENOTYPES (*Lycopersicon esculentum*) BY TOTAL PROTEIN PATTERNS OF THE PERICARP TISSUE

Sequin L[1], Rodríguez G[2*], Pratta G[2], Zorzoli R[3], Picardi L[3].*
*[1]Cátedra de Genética, Facultad de Ciencias Agrarias (UNR), CC Nº 14, (S2125ZAA) Zavalla, Argentina. [2]CONICET. [3]CIUNR. *ex-aequo. E-mail: grodrig@fcagr.unr.edu.ar*

To characterize different tomato genotypes by total protein patterns of the pericarp tissue the following genotypes were used: Caimanta (C) and a variety homozygous for *nor* (N) of *L. esculentum* var. *esculentum*, the accession LA1385 of *L. esculentum* var. *cerasiforme* (Ce, the wild cherry tomato), the hybrids F_1 (C x N), F_1 (N x Ce) and F_1 (C x Ce) and two commercial hybrids "Dominic" (D, with long fruit shelf-life) and "Bambino" (B, a cherry-type germplasm). Fruits from each genotype were harvested at the breaker stage. A phenol extraction followed by methanolic ammonium acetate precipitation was made from 1.5 g of pericarp. Proteins were separated by SDS-PAGE. Seventeen bands were detected but only four (24%) were different among genotypes. A polymorphism was observed in one band corresponding to a protein of 115 kDa. It was present in N, C, D and the F_1 (N x Ce). Another protein of 32 kDa was present in Ce, B and the F_1 (C x Ce) and F_1 (C x N). For these bands the F_1 (C x N) was different to its parents, while F_1 (N x Ce) and F_1 (C x Ce) showed protein patterns similar to N and Ce respectively. The F_1 (N x Ce) was different from all genotypes because it presented a polymorphic band of 48 kDa but did not present another band of 89 kDa. The cherry genotypes (Ce and B) were different to genotypes of *L. esculentum* var. *esculentum* (C, N and D) in their protein patterns.

105.
GROWTH OF CYANOBACTERIA WITH AGITATION
Duré L, Medina M, Bourges G, Eliach J, Lara MA.
Facultad de Ciencias Agrarias, CIUNR, Univ. Nacional Rosario.

It is studied Spirulina Platensis (cyanobateria), usually considered a micro-algae for its filamentous structure. Spirulina Platensis carries out photosynthesis and it prospers naturally in alkaline lakes of warm regions. It is a natural source of proteins, with a variable concentration of high quality assimilable substances. The cultivation of this cyanobacteria is important, they are utilized as a vitamin and protein complement in the human and animal alimentation. The growth of the culture requires the control of the temperature, radiation, agitation, basicity and salinity parameters. The objective of this work is to show the influence of the culture medium agitation on the increase of the number of microorga-nisms, keeping the temperature (30ºC), light (12 hours of light daily) and nutritive medium composition parameters constant. The methodology consisted on placing transparent cylindrical bio-reactors in a controlled environment chamber that has an agitation device. Its periodicity was of 15 minutes every 2 hours during the hours of daylight with a frequency of 10 vertical displacements (of 5 cm) per minute made on the extremity of each bioreactor. The agitation allowed all the individuals to be exposed to the light, prevented the formation of lumps or coagula and aired the environment, favoring the gaseous interchange. To measure the number of microorganisms it was used the direct count technique. The evaluations were performed for 16 days, at intervals of 4 days, repeating them in 2 subsequent periods. We may conclude that agitation is a factor that influences increasing the number of microorganisms of a culture of Spirulina Platensis, keeping the temperature, light and nutritive medium composition parameters constant. Still is to discern which would be the optimum agitation.

106.
TAXONOMIC STUDY OF THE SPECIES OF *Campanulaceae* AND *Calyceraceae* OF THE SANTA FE PROVINCE
Lusardi MB, García RC, Prado DE.
Cátedra de Botánica, Facultad de Ciencias Agrarias, (UNR), C.C. Nº 14, S2125ZAA Zavalla.

The families *Campanulaceae* and *Calyceraceae* are closely related and belong in the order *Campanulales*. They can be distinguished by characters of the androecium, gynoecium and fruit. The *Campanulaceae* present free stamens or they are synantereous ovary 2-5-locular, pluriovulate; fruit a capsule, sometimes berry. The *Calyceraceae* in change present monadelphous androecium with partially fused anthers; ovary unilocular, ovule solitary; fruit achene. In the Santa Fe province there exist three native species of the *Campanulaceae*: *Triodanis perfoliata* (L.) Nieuwl. var. *biflora* (Ruiz & Pav.) T. R. Bradley; *Wahlenbergia linarioides* (Lam.) A. DC. and *Pratia hederacea* (Cham.) G. Don. The *Calyceraceae* are represented by two species and one variety: *Acicarpha tribuloides* Juss.; *Boopis anthemoides* Juss. var. *anthemoides* y *B. anthemoides* Juss. var. *subintegrifolia.* The present work contributes to the knowledge of this family providing keys to the identification of the different taxa based on morphological characters. The methodology consisted of a deep bibliographical review, consultation of national herbaria with important collections of the province (SF: Esperanza; SI: Darwinion, San Isidro; UNR: Rosario), and lab work to confirm their identity. It is here provided taxonomic information, illustrations and a distribution map.

107.
IMMUNOHISTOCHEMICAL AND MORFOMETRIC STUDY OF THE DECIDUAL CELLS IN THE DOMESTIC CAT PLACENTA
Barbeito C[1,2], Fernández P[1], Gimeno E[1], Portiansky E[1].
[1]Inst. of Pathology. Lab. of Histology and Embriology. Fac. Cs. Veterinarias. UNLP. 60 y 118. (1900). La Plata. Bs.As. Argentina.

The laberynthic zone of the cat placenta has laminar chorionic villi with a mesenchimal axis surrounded by cytotrophoblastic and syncitiotrophoblastic cells, both of foetal origin. The maternal components are blood vessels and large cells which, due to their structural and ultrastructural characteristics, were considered as decidual cells. Decidual cells derive from endometrial fibroblasts and are involved in foetal endometrium invasion and local immunoregulation. In a previous work on normal canine placenta we were not be able to demonstrate decidual cells, by the aid of immunohistochemical (IHC) techniques. The present study provides information about intermediate filament expression and morfometric characteristics of decidual cells in the cat. Samples were obtained after 40-50 days of pregnancy. Tissues were fixed in 10% neutral formaldehyde, processed and embedded in paraffin. Sections were stained with H and E and for IHC techniques monoclonal antibodies antivimentin, antidesmin and antipancytoqueratin were used. The morfometric analysis determined the major and minor axis of cells and nuclei. Decidual cells were posititve to vimentin but negative to the rest of the antibodies. 19,4% of the cells were binucleated and were excluded from the morfometrical study. Mononuclear cells showed a major axis of 14,74±0,32 µm and a minor axis of 10,95±0,29µm while the nuclei showed a major axis of 7,73±0,21µm and a minor axis of 6,36±0,16µm. The cat placental labyrinth owns decidual cells positive to vimentin. This immunoreactivity sustains the origin of the cells in the endometrial fibroblasts and determines variations between normal dog and cat placentas. This may elucidate the variations in the placental alterations of these species.

108.
MODELING OF THE CENTRAL PATTERN GENERATOR IN FOUR LEGGED ANIMALS BY USING CHAOTIC COUPLED OSCILLATORS
Castellini H[1], Cerdeira H[2], Romanelli L[3].
[1]Dpto. Física, Facultad de Cs. Exactas, Ing. y Agr., U.N.Rosario. [2]Iternational Center of Theoretical Physics, Trieste, Italia. [3]Inst de Cs, Univer Nacional de Gral Sarmiento, Buenos Aires.

The existence in the animal's nervous system of a set of specialized cells called Central Pattern Generator (CGP) is assumed by the biologists nowadays. Moreover different kinds of CGP are oriented to a specific action. The locomotion in mammals is ruled by a CGP which controls the animal gaits and recently have gained relevance in the Biomathematical studies. Collins and Steward have used permutations symmetry groups in order the characterized the animal gaits for four legged mammals. These symmetries would condition the neuronal structure of the brain. The have medelled the CGP by using a Rossler oscillator for each cell and by changing the parameters they reproduced all the known CGP animal gaits.In this work we use coupled chaotic oscillators by the Pyragas control method as a simple solution for avoiding the conjugated gaits found by Collins *et al*, since these solutions are not natural in animal gaits but with man trainee. In order to achieve this objective we use the direct synchronization of delayed time series as a function of the coupling. If varying the time delay from 0 t.u. (time units) to 40 t.u. It is possible to reproduce all the animal gaits without the conjugated solutions for four legged gaits. The algorithms were implemented by using the C/C++ language under the MPI (Message Passing Interface)in a cluster of two computers. The numerical results obtained over 10^5 cases under studied show that our objective was achieved in spite of the aparence of spureus symmetries for some delay times. These symmetries do not correspond to any animal gait, however due to the simplicity of the model make it quite useful since is better than the solution proposed by Golubisky *et al* when used a cube of eight coupled cells.

109.

TAXONOMIC STUDY OF THE *Celastraceae* OF THE SANTA FE PROVINCE

Di Sapio OA[1], García RC[2], Prado DE[2].
[1]Cátedra de Botánica, Facultad de Ciencias Bioquímicas y Farmacéuticas, (UNR), Suipacha 531, (2000) Rosario. [2]Cátedra de Botánica, Facultad de Ciencias Agrarias, (UNR), C.C. Nº 14, S2125ZAA Zavalla.

The family Celastraceae comprises 55 genera and 900 species from tropical and subtropical regions of the world. In Argentina the family Celastraceae includes three genera, but only two of them are present in the Santa Fe province: Maytenus Molina and Schaefferia Jacq. The present work contributes to the knowledge of this family providing keys to the identification of the different taxa based on morphological characters and a map of their geographical distribution. The methodology consisted of a deep bibliographical review, consultation of national herbaria with important collections of the province (SF: Esperanza; SI: Darwinion, San Isidro; UNR: Rosario), field work with some of the species in their communities and lab work to confirm their identity. As a preliminary result only one species of the genera Schaefferia Jacq. was assessed. The genera Maytenus Molina includes three species: Maytenus ilicifolia Mart. ex Reissek, Maytenus vitis-idaea Griseb. and Maytenus spinosa (Gris.) Lourt. et O'Donell. The geographical distribution of the species is concentrated mainly in the centre and northern departments of the province. It is here provided taxonomic information, illustrations and a distribution map.

110.

VASCULAR FLORA OF THE PROVINCE OF SANTA FE: FAMILY *Plantaginaceae*

McCargo J, Oakley LJ, Garcia RCA, Prado D.
Botánica, Dpto. Biología y Recursos Naturales, Fac. Cs. Agrarias, UNR, C.C. N° 14, S2125ZAA Zavalla, Argentina.

The aim of the present work is to report the taxonomic study of the family *Plantaginaceae,* and analyze its geographical and ecological distribution in our province. The family *Plantaginaceae* is composed of *Bougueria* Decne., *Littorella* Bergius and *Plantago* L. In the case of *Plantago* L., with a worldwide distribution of 215 annual herbaceous species, 34 species are cited for Argentina, three of them introduced from Europe, one from Chile and three probably from North America. As for the rest eight are local and eight endemic to our country. Up to now there exists no taxonomic review for this family in Santa Fe. Some species were observed in their habitat, but the bulk of the work was performed on collected specimens from the UNR (Rosario), Santa Fe (Esperanza) and SI (Darwinion) herbaria, together with a thorough literature review. Six species from *Plantago* L have been found in our province: *P. australis* Lam. subsp. *australis*, *P. lanceolata* L., *P. major* L., *P. myosuros* Lam. subsp. *myosuros, P. patagonica* Jacq., *P. tomentosa* Lam. subsp. *tomentosa* and *P. tomentosa* Lam. subsp. *napiformis* (Rahn) Rahn. Taxonomic information for the species is provided, as well as a geographic distribution map. A dichotomic key based on morphological characters of diagnostic value is produced.

111.

NIFEDIPINE EFFECTS ON THE RED CELL OSMOTIC FRAGILITY IN SCLERODERMA. PRELIMINARY STUDY

Parente FM[1], Leroux B[2], Svetaz MJ[3], Spengler MI[1].
[1]Física Biológica, [2]Dermatología, Fac Cs Médicas, [3]Inmunidad Celular, Depto. Bioq. Clín., Fac. Cs. Bioq. y Farm., UNR.

Red cell deformability has been reported to decrease in patients with scleroderma. This fact could contribute to the damaged microvascular flow these patients show. The aim of this work was: 1) to study the effets of nifedipine treatment –which block calcium channels- on the erythrocyte membrane osmotic fragility in patients with scleroderma and 2) to compare this fragility with normal controls. Thus, red cell osmotic fragility in patients with scleroderma treated with nifedipine (n=10) and that of the controls (n=15) was determined. The hemolysis curve has been drawn acording to hemolysis data obtained from the equation: $H=1/e^{\beta (x-x_{50})}+1$. where H is the fraction of hemolyzed cells; x is the ClNa molar concentration; x_{50} is the ClNa concentration (in moles/l) which causes a 50% hemolysis and may be considered as a measuring unit for average erythrocytic fragility; β is the measure of the breadth of the erythrocyte fragility distribution which shows the population's degree of homogeneity. Results showed that x_{50} was considerably lower ($p<0.001$) in patients with scleroderma ($x_{50}=0.361\pm0.017$ g/dl) than in normal controls ($x_{50}=0.424\pm0.015$). Nevertheless, no significant difference in the ß values was found. Therefore, we conclude that when inhibiting the intracellular accumulation of Ca^{++}, nifedipine could cause structural and functional changes in the membrane and could also be one of the factors which determines greater protection in these patients' osmotic lysis.

112.

DETECTION OF C-ERBB-2 ONCOGENE AMPLIFICATION FROM PARAFFINE EMBEDDED-FIXED TUMOURS

Zumoffen C[1], Ghersevich S[1], Capitaine Funes C[2], Nocito A[3], Cipulli G[2], Tozzini R[2].
[1]Lab. Estudios Reproductivos. Área Bioquímica Clínica y Fisiopatología. Fac. Cs. Bioq. y Farm.; [2]Cátedra de Ginecología; [3]Cátedra de Anatomopatología. Fac. de Cs. Médicas. U.N.R.

Previous studies have found that the c-erbB-2 oncogene is amplified in up to 30% of breast carcinomas. The determination of the oncogene in archival samples of tumours would allow correlating the marker expression with the disease-free interval and global survival of the patients. The aim of this work was to set up the detection of c-erbB-2 amplification from paraffin embedded-fixed tumour tissue. After tissue deparaffination, the DNA was extracted with phenol-chloroform-isoamyl alcohol and precipitated in alcohol. In order to eliminate possible interfering compounds from the deparaffination, DNA samples were preincubated in 0,01% albumin solution before being used for PCR. The c-erbB-2 amplification was investigated by differential PCR, using the gamma interferon gene as a control gene. The PCR products were analysed in 2% agarose gels and stained with ethydium bromide. The results indicated that preincubation with the albumin would yield a higher efficiency of the PCR, assessed as a better resolution of PCR product bands. The initial amount of tissue restrained the efficiency of the DNA purification, principally when an albumin preincubation step was used. Thus, the use of the albumin is not beneficial when the initial amount of tissue is low. This work was supported by a grant "Ramón Carrillo - Arturo Oñativia" from the Ministery of Health of Argentina.

113.

RBC AGGREGATION AND BLOOD VISCOSITY. DAMAGE TARGET ORGAN IN HYPERTENSIVE PATIENTS

D'Arrigo M[1], Foresto P[1], Di Tullio L[1], Filippini F[2], Gallo R[2], Barberena L[2], Rasia R[3], Valverde J[1].
[1]Dpto Bioq. Clínica, Fac. Cs. Bioq y Farm. [2]Clínica Médica, Fac. Cs. Médicas,UNR. [3]IFIR, CONICET-UNR.

The blood viscosity, RBC aggregation and plasmatic fibrinogen were study in healthy NS (n=25) and hypertensive patients with damage target organ (HDTO) (n=15). Blood viscosity was measured with a cone-plate viscometer, plasma fibrinogen concentration was determined by the Clauss method. RBC aggregate morphology was quantified using direct microscopic observation and analysis of images. ASP (Aggregation Shape Parameter) defined as the ratio of the area of the projected image to its square perimeter was calculated. RBC morphology was evaluated with the Zipursky-Forconi method which is caharacterised by the observation of the erythrocytes suspended in a viscous medium with an optical microscope. In each aliquote, 100 erythrocytes were observed and classified using the Bessis criterio. EMI (Erythrocyte Morphology Index) was calculated as relation bowls to discocytes. Comparison between these groups suggest that both whole blood viscosity at $2.30s^{-1}$ shear rate (SR) ($p< 0.001$) and the relative viscosity ($p<0.005$) are higher in the HDTO group than in the control group. Plasma viscosity values showed no difference between both groups at high SR. ASP appears significantly higher ($p<0.001$) in HDTO (0.69±0.11) than in NS (0.25±0.12). EMI results show that bowls, the most deformable RBC, decreased significantly in HDTO with EMI values of 0.88±0.69 in HDTO respect to NS (2.07±1.05; $p<0.001$. Our results suggest that ischemia decrease the deformability of RBC, which is supported by study of red cell morphology.

115.

ANTIOXIDANT RESPONSE TO OXIDATIVE DAMAGE INDUCED BY PARAQUAT ON *Danio rerio* EMBRYOS

Rucci AN, Cabrera R.
Biología, Fac Cs. Bioq. y Farm., Universidad Nacional de Rosario.
E-mail: arucci@fbioyf.unr.edu.ar

The redox cycling herbicide Paraquat PQ induce oxidative stress through oxygen derived reactive species ROS. The systems that protect aerobic organims against ROS toxicity include antioxidant enzymes and low molecular weight scavengers. The evaluation of PQ induced antioxidant enzymes response during *Danio rerio* embryonic development was the main purpose of this work. Embryos at 8-cells stage were randomly assigned to four different experimental groups: the first group constituted the control; two groups were treated at sublethal concentrations of 2 mg/L and 4 mg/L. The last group was kept at PQ lowest observed effect concentration of 6 mg/L. The treatment was interrupted when the larvae reached the hatching. Embryos were frozen to –70°C and stored prior to biochemical analysis. The Superoxide Dismutase, Catalase and DT-Diaphorase activities were measured by means Polyacrylamide Gel Electrophoresis under native conditions. Statistical analysis was performed by analysis of variance (ANOVA). All PQ concentrations assayed produced a significant increase on the Mn-Superoxide Dismutase activity. A similar result was observed in Cu/Zn Superoxide Dismutase and Catalase although significant differences were not found. PQ treated embryos showed a higher DT-Diaphorase activity. Significant correlation between PQ concentration and DT-Diaphorase were observed. These results showed increased levels in antioxidant enzymes activities in response to PQ induced oxidative stress in *Danio rerio* embryos. Mn-Superoxide Dismutase and DT-Diaphorase showed the larger responses.

114.

PATTERN OF DISTRIBUTION OF CARBOHYDRATES IN THE VAGINAL EPITHELIUM OF THE PLAIN VIZCACHA (*Lagostomus maximus maximus*)

Flamini M[1,2], Barbeito C[1,2], Gimeno E[1], Portiansky E[1].
[1]Veterinary General Pathology. [2]Histology and Embryology. School of Veterinary Sciences. UNLP.

The vagina of the plain vizcacha (*Lagostomus maximus maximus*) presents two anatomically different areas: a cranial zone, divided into two equal regions by a medial septum and a caudal region, lacking of this structure. The vaginal mucosa presents a stratified epithelium containing superficial mucous cells, according to the physiologic state of the female. Present work characterizes the distribution of carbohydrates in the vaginal epithelium of pregnant and not pregnant females, using lectinhistochemistry. The vagina of 30 mature females was fixed in buffered formaldehyde during 48 to 72 hours and included in paraffin. Slides of 5 µm thick were stained with HE, PAS and Alcian Blue at different pH. For lectinhistochemistry the following lectins were used: Con A, SBA, DBA, PNA, UEA-1 and WGA. For establishing the positivity of the reaction the glucocalix present around the cells, the apical and basal regions of the different cellular strata and the secretion present in the light of the vagina were used as targets. Mucous cells were PAS and Alcian Blue positive. The UEA-I lectin showed a great reactivity in all the animals and in both areas of the organ. RCA-I intensively stained the cranial region of the vaginal cells in all the groups. Con A and PNA lectins had weak reactions while DBA, WGA and SBA were very irregular in both vaginal areas of pregnant and not pregnant females. The marked reactivity with UEA-I indicates the presence of a great quantity of glycoconjugates with fucosa, a monosaccharide related with the reproductive function in different species.