9: PIBIC Program

9.01 Lipoic acid affects renal function and aminopeptidases in mice envenomed by *Crotalus durissus terrificus*

Alegre VS, Barone JM, Santos-Reis T, Zambotti-Villela L, Yamasaki SC, Silveira PF
Laboratório de Farmacologia, Instituto Butantan, SP, Brasil

Introduction: Hyperuricemia and alterations of renal protein and aminopeptidase (AP) activities of soluble (SF) and membrane-bound (MF) fractions have been found in the kidneys of mice envenomed by the snake *Crotalus durissus terrificus*. **Objectives:** This study analyzes the effect of lipoic acid (LA) po (0.1 mg/g bw) on renal AP and in the severity of acute renal failure induced by 80% LD50 ip of *C. d. terrificus* venom (*vCdt*) (0.0512 µg/g bw) in mice. **Methods:** Measurements of classical renal function parameters by spectrophotometry and AP assay by fluorometry. **Results and Discussion:** The lethality (33-40%, n=10-12), hypercreatinemia (>1.9 mg/dL, n=6-8) and hyperuricemia (>1.6 mg/dL, n=6-8) did not differ between *vCdt* and *vCdt*+LA. However, LA mitigated the effect of *vCdt* on protein content in MF of renal cortex (1.8±0.1, n=12, control; 7.9±0.2, n=6, *vCdt*; 2.1±0.1, n=6, *vCdt*+LA) and medulla (1.6±0.1, n=12, control; 3.6±0.7, n=6, *vCdt*; 1.5±0.1, n=6, *vCdt*+LA), on basic AP (16537±6059, n=12, control; 9582±842, n=6, *vCdt*; 22255±2161, n=5, *vCdt*+LA) and neutral AP (12959±1286, n=12, control; 22645±1476, n=6, *vCdt*; 12619±1094, n=6, *vCdt*+LA) in SF of renal cortex and neutral AP in SF of renal medulla (13302±1871, n=12, control; 45637±2416, n=6, *vCdt*; 14512±1233, n=6, *vCdt*+LA). The present data suggest that LA could be beneficial for the treatment of *vCdt* nephrotoxicity.

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9.02 Morphology and structural organization of Gené's organ in ixodid ticks

Alvim MA[1], Lima-Netto S[1], Spadacci-Morena DD[2], Barros-Battesti DM[1]
[1]Laboratório de Parasitologia e Entomologia and [2]Laboratório de Fisiopatologia, Instituto Butantan, SP, Brasil

Introduction: The process of oviposition in ixodid ticks was found to be a sequence of exactly coordinated events in which Gené's organ plays the important role of waxing the eggs with a substance capable of protecting them from desiccation and microbial attacks. There are few studies about the morphology of this organ. This study was performed in order to confirm if Gené's organ is either the only producer of the wax substance or if it is responsible for the storage. **Objective:** To study the morphology of Gené's organ of *Amblyomma cajennense* (Fabricius) using light microscopy. **Methods:** Four egg-laying females of *A. cajennense* on the third day of the oviposition period were used. Scutum was removed with a microscalpel by cutting across the dorsal shield at the most anterior part, just distal to the basis capitulum to expose Gené's organ. The organs were collected and immediately immersed in Bouin, dehydrated in 95% ethanol and embedded in historesin. Sections (3 μm) were stained with 1% methylene blue and examined in a DM LS (Leica) light microscope. **Results and Discussion:** Light microscopy revealed that Gené's organ in ixodid ticks is formed by a simple columnar epithelium and the lumen is filled with an amorphous mass. Muscle fibers can be also observed, consistent with the nearness of retractor muscles by which the cuticular sac of Gené's organ everts. It suggests that it is glandular; however, more studies are in progress to confirm it.

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9.03 Paracrine interactions between astrocytes and pinealocytes in the glutamatergic modulatory effects on melatonin synthesis

Atherino VF, Abrahão MV, Mesquita LSM, Afeche SC
Laboratório de Farmacologia, Instituto Butantan, SP, Brasil

Introduction: The pineal gland is a small endocrine gland present in all vertebrates. It synthesizes the hormone melatonin which is produced and secreted by the pinealocytes. Melatonin synthesis is controlled by cyclic environmental factors and it is almost restricted to the dark period. In this way, melatonin signals to the internal millieu if it is day time or night time and even the seasons. There are glutamatergic receptors in the astrocytes and pinealocytes membranes of the rat pineal gland. Glutamate causes an inhibitory action on melatonin synthesis, and this effect is dependent on the interaction between astrocytes and pinealocytes. **Objectives:** The objective of this work was to investigate the types of glutamate receptors, both ionotropic and metabotropic, which are involved glutamate's inhibitory action on melatonin synthesis. **Methods:** Young male Wistar rats were sacrificed by decapitation; their pineal glands were isolated and dissociated by the Papain Dissociation System kit. The pinealocytes in association with astrocytes (co-culture) were kept in culture and then were submitted to the pharmacological treatments for 5 h. Afterward, the glands were kept frozen at - 80°C until melatonin was assayed by HPLC with electrochemical detection. The cells in co-culture were stimulated with norepinephrine (1 μM), indispensable to the modulation of melatonin synthesis, in association with glutamate or specific ionotropic (NMDA, AMPA) or metabotropic receptors agonists (type I: DHPG, type II: l-CCG). **Results and Discussion:** The cell group stimulated with norepinephrine and AMPA did not show alteration of melatonin production when compared with the control group, stimulated only with norepinephrine. The cell group stimulated with NMDA showed reduced melatonin synthesis when compared with the control group. DHPG and L-CCG, agonists of type I and type II metabotropic receptors, respectively, did not display any effect on melatonin synthesis. Glutamate induced the known inhibitory effect. The data showed that only the NMDA ionotropic receptors seem to be involved in the glutamatergic inhibitory effect on melatonin synthesis.

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9.04 Effects of *Bothrops jararaca* venom and lipoic acid on renal function, oxidative stress and aminopeptidases in mice

Barone JM, Alegre VS, Almeida-Assis M, Zambotti-Villela L, Yamasaki SC, Silveira PF
Laboratório de Farmacologia, Instituto Butantan, SP, Brasil

Introduction: The nephrotoxic effect of *Bothrops jararaca* venom (*vBj*) can alter important enzymes in renal physiopathology, besides stimulating oxidative stress. Moreover, antioxidants have been proposed for the therapy of renal damage. **Objectives**: This study aimed to evaluate the relation of classical parameters in renal function, aminopeptidase activities (APs) and oxidative stress with acute renal failure (ARF) induced by *vBj* venom and treated with lipoic acid (LA). **Methods:** The LD50 of *vBj* (2.08 μg/g bw) was used to induce ARF in mice and LA (0.1 mg/g bw) was used as a treatment. APs in renal soluble (SF) and membrane-bound (MF) fractions were measured by fluorometry, and classical renal function parameters and GSSG/GSH by spectrophotometry. **Results and Discussion:** LD50 of *vBj* caused hypercreatinemia (>1.4 mg/dL), hypercreatinuria (72.2±1.6mg/dL), hyperuricemia (2.0±0.13mg/dL), decrease in hematocrit (17±1.3%), protein (51.7±0.15 mg/dL) and urea (21.7±7.3 mg/dL) in plasma, hypo- (275.6±2.8) and hyper- (2650.0±28.8) osmolality (mOsm/Kg) respectively in plasma and urine, and GSH decrease, GSSG increase and consequently increase of GSSG/GSH ratio in renal cortex and medulla. In general, *vBj*, LA or *vBj*+LA affected the APs examined in renal tissue in a pattern that suggests deleterious effect, except that normal levels of neutral AP in *vBj*+LA were restored in SF of renal medulla and MF of renal cortex. LA also restored uricemia and normal ratio of GSSG/GSH in renal cortex and medulla without effect on hypercreatinemia, but with substantial proteinuria (3.0±0.7, control; 2.5±0.07, *vBj*; 8.2±0.22, *vBj*+LA). The present data include alteration in renal AP activities among mechanisms and consequences of *vBj* nephrotoxicity and demonstrate that benefits of LA in the therapy of this envenomation could be limited by the induction of proteinuria.

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9.05 Characterization of proteolytic enzymes in saliva of chronic obstructive pulmonary disease (COPD) patients: search for inhibitors in low-molecular-weight fraction of *Bothrops jararaca* venom

Barros MS[1], Santos AA[2], Silva CA[2], Portaro FCV[1]
[1]Laboratório de Imunoquímica, Instituto Butantan, SP, Brasil; [2]Laboratório de Aplicações Moleculares e Celulares em Ciências da Reabilitação, Universidade Nove de Julho, SP, Brasil

Introduction: Chronic obstructive pulmonary disease (COPD) is an inflammatory and progressive disease characterized by a decrease in the diameter of airways which increases their resistance to airflow. Incidence, morbidity and mortality rates of COPD have increased continuously in recent decades. The major risk factors for COPD are cigarette smoking, age, passive smoking and hyper-reactive airway. A common feature of COPD disease is the chronic inflammation in the airways (influx of inflammatory cells into the lung, such as macrophages and neutrophils) and the development of extensive tissue remodeling during the course of the disease. In some cases this remodeling causes destruction of healthy lung tissue, leading to emphysema. For this reason, there is an increased interest in the role of MMPs (MMP-2 and MMP-9) and neutrophil elastase in COPD. All cited proteases probably contribute to the migration of inflammatory cells into the lung, as well as to the remodeling, and sometimes even to destruction of lung tissue. This hypothesis is supported by several studies with both bronchoalveolar lavage (BAL) fluid and sputum (produced spontaneously or induced) of COPD donors. **Objectives**: The aim of this work was to investigate the activity of metallo- and serineproteases in saliva of COPD patients in comparison with saliva obtained from healthy donors. In addition, this study also aimed at the search for inhibitors in the low molecular weight (LMW) fraction from *B. Jararaca* venom specific for MMP-2, MMP-9 and elastase. **Methods**: Saliva samples from 20 COPD and seven healthy donors were analyzed using 30-300 UA (UA= units of activity; UF/min/μg) of each sample and 5 μM of the FRET substrate, Abz-FRSSRQ-EDDnp. The measure of the total metalloproteinases and serineprotreinases activities was obtained by the use of EDTA (100 mM) and PMSF (0.5 μM), respectively. The LMW fraction (100 μg) was used to block 50-100 UA of each sample and 0.7 μM of the FRET substrate described above. **Results and Discussion**: All saliva samples used in the present study showed high specific activity values toward the Abz-FRSSRQ-EDDnp substrate, but we could not find any differences between the COPD and healthy samples. The results concerning the total activity levels of metallo- and serineproteases in the saliva samples also revealed no differences between the two groups studied here. For both groups, the use of EDTA blocked around 35% and PMSF was able to inhibit 63% of the total activity measured. The use of the LMW fraction from *B. Jararaca* venom resulted in good inhibition levels when the COPD saliva samples were analyzed (49%), but a similar inhibitory power could be found in the samples from the healthy group (57%). Taken together, the results led us to hypothesize that the saliva samples, despite their ease of collection, may not be a good source for proteolytic enzymes involved in COPD. Another explanation for our results is a possible balance between the enzymes and the antiproteases that act as their inhibitors, such as TIMP-1, TIMP-2 (MMP-9 and MMP-2 inhibitors, respectively) and α1-AT (elastase inhibitor) in the saliva samples.

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9.06 Gene polymorphism of gyroxin from *Crotalus durissus* rattlesnake venom

Basdadjian YF[1], Prieto-da-Silva ARB[2], Oguiura N[1]
[1]Laboratório Especial de Ecologia e Evolução and [2] Laboratório de Genética, Instituto Butantan, SP, Brasil

Introduction: There are a great number of biochemical compounds in snake venoms, where the proteases are in large amount and show many activities. These activities are classified into two groups: serine proteases and metalloproteinases. Regarding the serine protease genes, many cDNAs were described, but just one genomic sequence from batroxobin: a toxin from the *Bothrops atrox* venom. The gyroxin is a serine protease from *Crotalus durissus* venom, which shows many enzymatic activities such as amidasic, esterasic and fibrinogenolytic. Only gyroxin cDNAs sequences from venom glands are known. The attainment of gyroxin gene partial sequence confirmed that the amplified sequences in our work are from the gyroxin gene. It also allows a preliminary study about the evolution at the exons and introns of gyroxin genomic sequences. **Objective:** Analysis of the gyroxin gene partial sequences attained by PCR amplification. **Methods:** PCR was accomplished using the primers F4gyr/R2gyr, which hybridize with exon 3 and exon 4, respectively. The amplified sequence was cloned in the pTZ57R/T vector. The plasmids were digested with the Eco RI and Hind III to confirm the presence of inserts and to subclone the gyroxin sequence. The plasmids were quantified by spectrophotometry and sequenced using the Big Dye Terminator V 3.1 Cycle Sequencing kit and the ABI Prism 3100 Genetic Analyzer from Applied Biosystems. The sequences were analyzed at BLAST (NCBI), and aligned with the ClustalW2 program. **Results and Discussion:** Five clones were analyzed after cloning the 1.9 kbp sequence. The Eco RI and Hind III double digestion identified two distinct clones, pTZ-gyr1.9-5 and pTZ-gyr1.9-7. Both sequences showed 89.1% similarity. B1_4 cDNA (EU360952.1) was compared to gyr1.9-7 and gyr1.9-5 sequenced and showed identity of 98% and 85%, respectively. Regarding the B1_3 cDNA (EU360951.1), 90% similarity to gyr1.9-7 sequence was obtained and 85% to gyr1.9-5. Besides *Crotalus* and *Bothrops* genera, isoform 6 mRNA from *Sistrurus catenatus* snake also displayed similarity of 91% and 89 % in relation to gyr1.9-7 and gyr1.9-5 sequences, respectively. Comparison to the gyroxin B1_3 cDNA showed synonymous and nonsynonymous mutations in exons 3 and 4, with a predominance of nonsynonymous at exon 4. It was observed that the intron-exon boundaries are conserved in gyr1.9-5, gyr1.9-7 and batroxobin genes with similarity of 86% and 83%, respectively. The gyr1.9-7 sequence showed more conservation of the exon 3 sequence than of the exon 4. In this case, the mutations increase the number of nonpolar amino acids and decrease the number of charged amino acids. The analysis of *Trimeresurus flavoridis* serine protease cDNAs and *Crotalus durissus* gyroxin cDNAs showed accelerated evolution which was confirmed with our results. This fact may change the biological activity of these serine proteases. Accelerated evolution also occurs in other toxins and it was first shown for phospholipase A_2 from *Trimeresurus flavoviridis* and *T. gramineus*. The new biological activities of toxins may occur to permit adaptation to environmental changes.

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9.07 Digestive astacin-like enzyme and astacin-like inhibitor from *Nephilengys cruentata*

Lopes AR, Bastelli CM
Laboratório de Bioquímica e Biofísica, Instituto Butantan, SP, Brasil

Introduction: Astacins (EC 3.4.24.21) are metallopeptidases (family of zinc-endopeptidases) which show a consensus motif HEXXHXXGXXH and was first identified in the crayfish *Astacus astacus*. To date, more than 200 members of this family have been identified in species ranging from bacteria to humans including disintegrins and matrix metalloproteinases involved in diseases such as cancer and rheumatoid arthritis. There is no description in the literature about a natural inhibitor for this family of metallopepetidases. **Objectives:** To isolate the major astacin-like enzyme present in the hepatopancreas from the giant spider *Nephilengys cruentata* and to characterize the purified enzyme and to test hemolymph homogenate in order to identify possible inhibitory activity against astacin-like activity. **Methods:** Adult females were not fed for at least two weeks. Afterward, cannibalism among spiders was favored. Spiders were dissected, the hemolymph was collected in the presence of sodium cacodylate and phenylthiourea, and the hepatopancreas was isolated and then homogeneized in cold Milli Q water in a Potter-Elvehjem homogenizer. Homogenate samples were applied ito a Hitrap Q column equilibrated in 0.02 M Tris HCl buffer, pH 9.0, and eluted with a linear NaCl gradient. Fractions active on casein-FITC were pooled and submitted to gel filtration in a Superdex G-75 column. Active fractions were individually applied on a 15% polyacrylamide gel and submitted to electrophoresis. Hepatopancreas homogenate samples and Hitrap Q pooled active fractions were also used in zymography with gelatin as substrate. Thermal inactivation was studied by incubating the homogenate samples containing the enzymes at the specified temperatures followed by determination of residual activity after different times. Hemolymph samples were homogenized in Milli Q water and added to Hitrap Q chromatographic fractions obtained from *Nephilengys cruentata* hepatopancreas using casein-FITC as substrates. **Results and Discussion:** The astacin-like enzyme present in *Nephilengys cruentata* hepatopancreas was isolated with a yield of 100 % and a purification of at least 10 times. SDS-PAGE showed the isolation of a peptidase of 14 kDa. This peptidase was inactivated at 55°C with a half-life of 46 min, and showed a Km of 0.48% using casein-FITC as substrate and a pH optimum of 8.3. Tests of inhibition of astacin with hemolymph samples of *Nephilengys cruentata* indicated an inhibition of 70% astacin activity. Anion–exchange chromatography followed by gel filtration allowed the isolation of a peptidase present in the hepatopancreas from *Nephilengys cruentata.* The low molecular mass of this enzyme and data on inhibition with 1,10-phenanthroline and EDTA indicated that this enzyme is a metallopeptidase from the astacin family (astacin-like enzyme). The inhibition of astacin activity demonstrated a specific natural inhibitor present in hemolymph. Synthesis of specific substrates for astacin and the isolation of the inhibitor are being tested.

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9.08 Gometoxins: antimicrobial peptides from *Acanthoscurria gomesiana* venom

Brito LRS, Silva Jr. PI
Laboratório Especial de Toxinologia Aplicada - CAT/CEPID, Instituto Butantan, SP, Brasil

Introduction: Infectious diseases are one of the main causes of death in human populations. For the most part, this is due to microorganisms resistant to different antibiotics. Thus, it is important to investigate natural or synthetic substances that have an antimicrobial activity, especially concerning substances that have a different mechanism of action than those of current antibiotics. In this scope, the research of antimicrobial molecules in Brazilian fauna and flora could be valuable. The study of antimicrobial molecules of arachnids is very important because this animal family is 350 million years old. Furthermore, this kind of animals lives in places which increase the development of pathogenic microorganisms. This observation tends to indicate that this research could be prolific. In arachnid toxins, mainly from spiders and scorpions, some antimicrobial peptides were identified. Concerning spiders, toxins have been extracted from venom glands of the following spiders: *Lycosa carolinensis* and *Cupiennius salei*. Some antimicrobial peptides have been discovered in scorpions including *Hadrurus aztecus*, *Pandinus imperator* and *Opisthacanthus madagascariensis*. **Objectives:** The aims of this study were to identify and characterize antimicrobial peptides in venom of *Acanthoscurria gomesiana*. This work could be useful in the scope of a future pharmaceutical use. **Methods:** The venom was obtained from glands of three animals, which were macerated with water and centrifuged, and the soluble part was dried by vacuum centrifugation and reconstituted with 1 mL of acidified water (TFA - trifluoracetic acid 0.05%). The soluble part was applied to HPLC reversed-phase chromatography on a semi-preparative or analytical Jupiter C18 column. Elution was performed with different linear gradients of ACN/TFA 0.05%. The presence of antibacterial activity was determined by a liquid growth inhibition assay against Gram-negative bacteria *Escherichia coli SBS363*, Gram-positive bacteria *Micrococcus luteus A270* and yeast *Candida albicans* MDM8. Molecular weight and purity of the molecules were analyzed by mass spectrometry (MALDI-TOF). **Results and Discussion:** After two stages of purification, four fractions with antimicrobial activity were observed: frac21 (*C. albicans*), frac22 (*C. albicans*, *E. coli* and *M. luteus*), frac30 (*C. albicans*), and frac48 (*M. luteus*). After purification and re-purification, only fractions 22, 30 and 48 were pure on analysis by mass spectrometry. The analysis revealed monocharged ions of m/z corresponding to 5,463.4, 2,731.7 and 1,659.8. These peptides were called gometoxin-1, 2 and 3 respectively. Analysis of gometoxin-3, using reduction and alkylation, showed that this peptide is linear without disulfide bonds. The complete characterization and sequencing of gometoxins are in progress.

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9.09 Histopathological evaluation of mice injected with extracts of two toxic cyanobacteria strains

Brunetti RL[1], Campos Jr AG[1], Garcia AN[1], Dogo CR[2], Mattos LFA[2], Carvalho LR[2], Rangel M[1]

[1]Laboratório de Imunopatologia, Instituto Butantan, SP, Brasil; [2]Seção de Ficologia, Instituto de Botânica de São Paulo, SP, Brasil

Introduction: Cyanobacteria are responsible for numerous cases of human intoxications. The ingestion of endotoxins produced by cyanobacteria may cause: gastroenteritis, nausea, vomiting, fever, and eye irritation. In the laboratory, the effects of cyanobacteria toxins are studied in mice in order to classify the cyanobacteria as neurotoxin, hepatotoxin or dermatotoxin producers. **Objectives:** To perform toxicological studies of the cyanobacterial strains of the *Instituto de Botânica* algae bank, classifying the strains that produce toxins and to do further histophatological evaluation of the mice treated with the cyanobacterial extracts. **Methods:** Extract preparation: the cultured cyanobacterial cells were filtered through an AP-20 filter and freeze-dried. The resulting material was then extracted with 1) 0.1 M acetic acid (4x) by ultrasonication (4 x 10 sec., 50W) and centrifugation. The supernatant was concentrated under reduced pressure (water extract, strain SPC 422 *Pseudoanabaena galeata*); or 2) MeOH/H_2O 75:25(v/v) (5x) by ultrasonication (40 x 30 sec.; 560W) and centrifugation. The supernatant was concentrated under reduced pressure (methanol extract, strain SPC 920 *Geitlerinema unigranulatum*). The extracts were kept at -20°C until they were used. The toxicity tests (i.p.) were performed in male Swiss-Webster mice (19-21 g). The mouse symptoms were observed up to 8 days after toxin administration. After death by acute intoxication or euthanasia, necropsy was performed and tissue samples were taken from liver, kidneys and lungs, fixed and used for histopathological analysis. **Results and Discussion:** The extract of the strain SPC 920 showed acute toxicity in mice, causing deaths at 10 mg/animal, within 50 min to 2 h after i.p. injection. Histological analysis showed lung abnormalities, with hemorrhagic foci and erythrocytes within some alveoli sacs. The kidneys showed fluid accumulation in the tubules, and in the liver there was an augmented space between the hepatocytes, maybe due to fluid retention. Small hemorrhagic foci were also detected in the liver. These results do not resemble the hepatotoxic toxicity induced by microcystins, which would cause strong liver hemorrhage, or by neurotoxin, lipopolysaccharide or cylindrospermopsin envenomation. The mice treated with the extract of the strain SPC 422 (20 mg/animal) did not die of acute toxicity, but showed weight loss after 7-8 days. They were euthanized and in 50% of the mice (N=6) several tumors were visualized in the liver. Liver tissue and tumor of treated animals were fixed to perform histological evaluation. Hepatocytes were found in the veins of the liver tissue, and condensed and/or fragmented nuclei were found in both (liver and tumor) samples. Microcystins may induce neoplasias, but usually with repeated doses within 20 weeks. The extract of the SPC 422 strain caused neoplasm formation in less than 10 days, in 50% of the mice. The preliminary tests for microcystin detection in this extract were negative, indicating a different class of hepatotoxin. Isolation and characterization of the toxins of these two cyanobacterial strains are in progress at the *Instituto de Botanica.*

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9.10 Characterization of biologicaly active peptides derived from the proteolysis of fibrinogen by bothropasin

Cajado D, Paes Leme A, Oliveira, AK, Serrano SM, Portaro FV, Faria M
Laboratório Especial de Toxinologia Aplicada - CAT/CEPID, Instituto Butantan, SP, Brasil

Introduction: Angiogenesis is a biological process by which new capillaries are formed from pre-existing vessels and is influenced by integrins expressed on endothelial cells, vascular smooth muscle cells, fibroblasts and platelets. The angiogenesis process is present in many pathologies of medical interest, such as arthritis, diabetic retinopathy and cancers, and result in the search for modulators. Many of these products are formed by degradation of extracellular matrix and blood plasma components, including a variety of bothropasin substrates. The bothropasin is a SMVP belonging to class III present in the *Bothrops jararca* venom. **Objectives:** The aim of the present work was the search for and characterization of proteolytic fibrinogen products resulting from the bothropasin activity able to act on cell migration and proliferation. Also, we studied the bothropasin itself and its disintgrin-cysteine rich domain (DC) with respect to direct action in proliferation and migration assays. **Methods:** The products of the fibrinogen digestion by bothropasin were purified by HPLC, screened by cell assays and the active ones were sequenced by MALDI-TOF mass spectrometry. The cell lines used in these assays were HUVEC and VSMC. For the migration assay using bothropasin and DC domain, the plates were previously coated with fibronectin. In the wound healing assay, a scratch was made after cell confluence using a p200, simulating a wound. The bothropasin and DC domain were added and the cells were photographed at time zero and after six hours. These same cells were subjected to an immunofluorescence assay using anti-FAK and phosphorylated anti-FAK. The same procedures were made for the scratch assay, but instead of the fluorescence step the cells were counted using the ImageJ program. The proliferation assay was performed with the BrdU Cell Proliferation Assay kit, 1000 Tests (Chemicon), and the factors utilized were bothropasin, DC domain and FGF. **Results and Discussion:** Our results show that a peptide fraction, resulting from the cleavage of fibrinogen by bothropasin, displays inhibitory activity in a variety of biological assays with different cell lines. In fibroblasts these peptides inhibit up to 50% of bFGF-triggered mitogenesis. In HUVEC and smooth muscle vascular cells, these peptides have a more reduced inhibitory effect on FGF and serum triggered mitogenesis, reducing DNA synthesis by at most 30%. The next step will be the testing of their synthetic counterparts. The results concerning the study of the bothropasin show that the migration was stimulated by this SMVP. Since bothropasin degraded the subtrate fibronectin, the observed stimulation may have resulted from fibronectin proteolytic fragments binding to specific receptors on the cell membrane, likely integrin-type, as pointed out by FAK localization changes in immunofluorescence result. VSMC proliferation was inhibited in the presence of bothropasin and DC domain, which also blocked the action of FGF. On the other hand, HUVECs were not affected by the DC domain, even at higher concentrations; this result points to a DC anti-proliferative action that is specific for muscle cells and therefore needs further investigation.

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9.11 Analysis of enteropathogenic *Escherichia coli* outer membrane proteins by two-dimensional electrophoresis

Cardoso FA, Abreu PAE, Piazza RMF, Elias WP, Sonobe MH
Laboratório de Bacteriologia, Instituto Butantan, SP, Brasil

Introduction: *E. coli* is a versatile pathogen in animals and humans. EPEC has been identified as a main causative agent of acute diarrhea in developing countries. **Objectives**: The goal of this work was to verify the reproducibility of the two-dimensional (2D) electrophoresis protocol of OMPs extracts derived from one strain of EPEC (9100-83, O125:H6). **Methods**: 2D electrophoresis was performed by a two-step protocol, the first dimension by focusing of 13 cm, pH 4-7 strips (IPGphor III, GE Healthcare) and the second dimension by 15% acrylamide SDS-PAGE electrophoresis (SE 600 Ruby, GE Healthcare). **Results and Discussion**: Data from three 2D electrophoresis gels were compared by ImageMaster Platinum software (GE Healthcare). A total of 98 spots were observed in the first gel in the range of 13 kDa to 93 kDa and pI 4.03 to 6.66, 52 spots with molecular weight (mw) between 18 kDa and 90 kDa, pI 4.01 to 6.91 in the second and 44 spots (mw 17 kDa - 92 kDa, pI 4.12 – 6.63) in the third. Three high similarity regions were visualized in all gels. One between 30 kDa and 45 kDa, pI 5.11-5.24 with two main spots, region two with six major spots (24 kDa-30 kDa, pI 4.82-4.94), and region three with two spots (20 kDa, pI 5.47-6.06). One area (14 kDa-20 kDa, pI 5.00-6.91) was significantly distinct comparing the first gel (12 spots) to the other two (only 2 spots). All gels had quite similar spot ranges of mw and pI. Data from literature showed 100 spots from outer membrane extracts. The lower numbers of spots of gels 2 and 3 in relation to the first gel suggested a probable proteolysis process in the storage period of the corresponding strips at -20°C. The general profile of OMPs from EPEC (9100-83) by analysis of three gels was reproducible. However, future assays are necessary and they will determine the proteins that will be identified by mass spectrometry.

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9.12 Study of the plasticity of immature stem cells from human tooth pulp

Carvalho CK, Kerkis I
Laboratório de Genética, Instituto Butantan, SP, Brasil

Introduction: Stem cells are cells that are able to self-renew and to differentiate into a specific cell line. The stem cells may be embryonic or of adult origin. Adult stem cells are less problematic to use than embryonic stem cells, because their use does not require the destruction of embryos. The use of adult stem cells in developing research in tissue engineering and regenerative medicine is important and has advantages, since the differentiation is more controlled. Furthermore, when introduced into the organism, they hardly produce tumors. Our group was able to establish a protocol that allows the original isolation, extraction, cultivation and differentiation of immature stem cells from tooth pulp, thus holding the know-how to manipulate these cells. This project will use two specific lines of stem cells from human tooth pulp, DA1 and DL7 which respectively refers to the second molar of a patient of 15 years and the primary tooth of a child of 5 years. **Objectives:** To study the capacity of proliferation, the plasticity and the mechanism of chondrogenic and osteogenic differentiation in vitro of stem cells from tooth pulp. **Methods:** Growth curve was the method performed to assess cell proliferation. The cells were cultured under appropriate conditions, with daily change of shift. The analysis was done by immunofluorescence in immature stem cells from tooth pulp using specific antibodies to embryonic and mesenchymal stem cells. For the analysis of the differentiation process, we used various antibodies and specific staining and morphological analysis enabling a more comprehensive characterization of the differentiation of the immature stem cells from tooth pulp for osteogenic and chondrogenic lineages. For chondrogenic differentiation the cells were grown in suitable conditions and concentrations with the use of a means of inducing chondrogenic differentiation. This inducer was renewed daily for 21 days. **Results and Discussion:** On the graph, it can be observed that both strains studied (DA1 and DL7) have considerable potential for change in proliferation after the first five passages, reaching amounts unviable to continue the growth curve after transition 15.

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9.13 Effect of crotoxin on secretory activity of peritoneal macrophages during tumor progression

Costa ES, Francisco AMSC, Faiad OJ, Cury Y, Sampaio SC
Laboratório de Fisiopatologia, Instituto Butantan, SP, Brasil

Introduction: Several lines of evidence indicate that the crotalid venom modulates macrophage function, stimulating hydrogen peroxide and nitric oxide production, anti-candidacidal activity and glucose and glutamine metabolism of these cells. Crotoxin (CTX), the major toxin of *Crotalus durissus terrificus* venom induces an inhibitory effect on tumor growth and modulates, particularly, the functions of macrophages, cells essential for innate defense mechanisms. One of the functions of macrophages is to provide a defense mechanism against tumor cells. These cells can be involved in both tumor killing and stimulation of tumor development, depending on stage of the macrophage activation and the development stage of the tumor. Functional plasticity is a well-known characteristic of the mononuclear phagocyte system, and the paradigm of *M1* and *M2* polarization identify the two extremes of the whole spectrum of macrophage functional activities. In the beginning of tumor progression, *M1* macrophages are avidly phagocytic and secrete or release reactive nitrogen intermediates-RNI/ROI and cytokines TNF-α, IL-1β and IL-6. On the other hand, when the tumor is established, tumor-associated macrophages (*M2* macrophages) show these abilities to be decreased. **Objectives:** The aim of this work was to investigate the effects of CTX on peritoneal macrophages secretory activity obtained from Walker 256 tumor-bearing rats in two different protocols: 1) CTX injected during the establishment of the tumor (to investigate the action of CTX on *M1* macrophages) and 2) CTX injected on the 5^{th} day after the injection of Walker 256 tumor cells (to investigate the action of CTX on *M2* macrophages). **Methods**: Male Wistar rats were inoculated subcutaneously in the right flank with 1 mL of sterile suspension of $2x10^7$ Walker 256 tumor cells and immediately after were treated with CTX (18 μg in 300 μl per rat) or saline, in the same volume (control), s.c. administered. In other groups, rats were inoculated subcutaneously in the right flank with 1 mL of sterile suspension of $2x10^7$ Walker 256 tumor cells, and after five days of this injection, animals were treated with CTX (18 μg in 300 μl per rat) or saline, in the same volume (control), s.c. administered. Animals from control groups were inoculated subcutaneously in the right flank with 1 mL of PBS, treated with CTX or saline. Peritoneal macrophages were obtained on the 14^{th} day after tumor cell injection, and H_2O_2 release and NO production were measured and levels of IL-1β, IL-6 and TNF-α in the culture supernatants were determined by ELISA using kits from R&D Systems. **Results and Discussion**: In both protocols evaluated, CTX stimulated the H_2O_2 release and NO production and the secretion of the cytokines IL-1β and TNF-α by peritoneal macrophages obtained from both Walker 256 tumor-bearing rats and non-tumor-bearing rats. For the first time, it was demonstrated that CTX modulates, *in vivo*, secretory activity of peritoneal macrophages and this effect is induced by a single dose of CTX. These results contribute to the elucidation of mechanisms involved in the CTX antitumor effect and bring new prospects for the development of a new substance with therapeutic properties.

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9.14 Purification of factor X from the blood of the snake *Bothrops jararaca*

Couto, MC[1,2] , Morais-Zani, K[1], Tanaka-Azevedo, AM[1]
[1]Laboratório de Fisiopatologia, Instituto Butantan, SP, Brasil; [2]Escola de Artes, Ciências e Humanidades, Universidade de São Paulo, SP, Brasil

Introduction: The clotting factors II, VII, IX and X as well as the inhibitors protein C and protein S belong to the group of vitamin K-dependent proteins. This group of proteins plays a key role in blood coagulation. Coagulation factor X (FX) plays an important role in the regulation of blood coagulation by converting prothrombin into thrombin. Human FX has molecular mass about 62 kDa and consists of two polypeptide chains, light (17.5 KDa) and heavy (45 KDa). Prothrombin (FII) is the precursor of the enzyme thrombin that converts fibrinogen into fibrin and amplifies its self-generation. Human FII shows molecular mass of 72 kDa and consists of only one polypeptide chain. Protein C (PC) inhibits coagulation by selectively inactivating the active forms of factor V and factor VIII. Human PC has a molecular mass of about 62 kDa and consists of two polypeptide chains, light (21 kDa) and heavy (41 KDa). **Objectives:** The aim of this study was to purify *Bothrops jararaca* (*B. jararaca*) FX, FII and PC. **Methods:** The purification process consisted of barium chloride precipitation, two sequential steps of ammonium sulfate fractionation (40 and 67% saturation) and HiTrap DEAE Fast Flow chromatography. Along all the purification steps, protein concentration was determined by absorbance at A_{280}. Protein activity was measured using specific chromogenic substrate. The fractions were analyzed by SDS-PAGE (10%). **Results and Discussion:** The three proteins were concentrated by barium chloride precipitation and ammonium sulfate fractionation and partially purified by HiTrap DEAE Fast Flow chromatography showing that this process can be suitable to separate these proteins simultaneously. The perspectives for this work are to improve the purification process in order to get higher purity proteins and to compare them biologically and biochemically with other animals' FX, FII and PC.

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9.15 Characterization of a putative outer membrane lipoprotein of *Leptospira interrogans*

Cunha FF[1], Xavier DP[1], Monaris D[1], Gonçales AP[2], Morais ZM[2]; Vasconcellos AS[2], Barbosa AS[1], Abreu PAE[1]

[1]Laboratório de Bacteriologia, Instituto Butantan, SP, Brasil; [2]Laboratório de Zoonoses Bacterianas, Faculdade de Medicina Veterinária, Universidade de São Paulo, SP, Brasil

Introduction: Leptospirosis is a widespread zoonosis caused by pathogenic spirochetes belonging to the genus *Leptospira.* The wide distribution of *Leptospira spp* results from their ability to colonize the renal tubules of mammalian hosts, including humans, wildlife, and many domesticated animals. Transmission to humans involves either direct or indirect contact with the urine from chronically infected animals. The genome of *L. interrogans* serovar Copenhageni has been sequenced and *in silico* analysis allowed identification of predicted outer membrane lipoproteins that could be involved in pathogenesis and immune responses. The development of a vaccine and of an effective diagnostic assay is important to the treatment and control of this disease. **Objectives:** The aim of this project was the cloning, expression, purification and characterization of the probable lipoprotein encoded by the gene LIC10704 identified in *L. interrogans* serovar Copenhageni genome. **Methods:** The gene was amplified from genomic DNA by PCR and cloned into the expression vectors pAE and pLIPO, which provide expression of non-lipidated or lipidated recombinant proteins. The recombinant 6xHis-tagged proteins were purified by metal affinity chromatography, and characterized by circular dichoism spectroscopy (CD). The recombinant purified proteins were used in immunization and challenge assays, and their capacity to mediate attachment to ECM components was evaluated by binding assays. **Results and Discussion:** LIC10704 encoded proteins were expressed in *E. coli* BL21-SI (pAE) or *E. coli* BL21-C43 (pLIPO) and purified from the soluble fraction (pAE) or detergent fraction (pLIPO). The purified proteins exhibited a single major band of 23.5 kDa (pAE) or 23.7 kDa (pLIPO) in SDS-PAGE. Their structural integrity was assessed by CD spectroscopy, which revealed a predominant α-helical secondary structure. PCR analysis demonstrated that the gene is conserved among different pathogenic serovars of *Leptospira.* Mouse polyclonal antiserum against the recombinant protein (pAE) was produced with a high antibody level detected by ELISA. However, the recombinant proteins do not display extracellular matrix binding properties, and probably they are not a leptospire antigen involved in adherence to host tissues. Hamsters immunized with recombinant proteins were not protected against mortality upon challenge with a lethal inoculum of *L.interrogans* serovar Copenhageni. This result indicated that LIC10704-encoded protein is not a vaccine candidate against leptospirosis.

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9.16 Multiplex RT-PCR for typing and subtyping of influenzavirus A

Durigan MS[1], Sacramento PR[1], Vedovello D[1], Comone P[1], Giglio A[2], Vieira, SE[2], Miyaki C[1], Tenório ECN[1], Stewien KE[3], Durigon EL[3], Botosso VF[1]
[1]Serviço de Virologia, Instituto Butantan, SP, Brasil; [2]Hospital Universitário and [3]Departamento de Microbiologia, Instituto de Ciências Biomédicas, Universidade de São Paulo, SP, Brasil

Introduction: Influenza virus is a common childhood disease with the highest morbidity occurring in preschool children. The period of influenza virus circulation is associated with increased medical practitioners' consultation, hospitalizations, and excess deaths. The virus shows high genetic and antigenic variability, mainly in its surface glycoproteins, such as hemagglutinin and neuraminidase, which are responsible for the subtyping of *Influenzavirus A*. Due to the segmented nature of its genome, the virus can display rearrangements, generating new viruses, different from those in circulation, for which the population does not have immunity. The only way to prevent the flu and its possible consequences is through vaccination. Great efforts are made worldwide for the monitoring and characterization of circulating virus. It makes possible the detection of emergent viruses, such as avian influenza (H5N1) and influenza A (H1N1), as well as the choice of samples that will be part of the composition of the vaccine in the following year. The fast and specific detection of influenza viruses has significant importance in influenza monitoring and control programs. In Brazil, few data on infections of influenza in children are available, who are considered the most important vectors in transmission of the virus. **Objectives:** The aim of this work was to standardize RT-Multiplex-PCR technique, using specific primers to HA and NA genes in order to subtype *Influenzavirus A*. This method was also used to characterize the isolated virus from children hospitalized in University Hospital of the University of Sao Paulo (HU-USP), during the year of 2006. **Methods**: Nasopharyngeal aspirates from 500 patients with acute respiratory illness, ranging from infants to 5-year-olds, were collected at the HU-USP and tested using duplex RT-PCR for detection of influenza A and B. The multiplex RT-PCR was used to subtype *Influenzavirus A*. **Results and Discussion:** Twenty-three samples were positive by duplex-RT-PCR. Among these, 4 (17.4%) were positive for *Influenzavirus B*, and 19 (82.6%) for *Influenzavirus A*. During the year of the study, influenza A (H3N2) was the prevalent subtype (65%). A rapid detection and subtyping method are necessary to obtain detailed information about the prevalence of different subtypes of influenza virus and mainly to establish effective control measures.

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9.17 Skin and cutaneous glands during the development of the toad *Rhinella granulosa* (Amphibia, Anura). Ultrastructural aspects

Ferro RF, Carneiro SM, Antoniazzi MM, Jared C
Laboratório de Biologia Celular, Instituto Butantan, SP, Brasil

Introduction: Amphibian skin is a highly specialized organ with a wide spectrum of functions such as for the preservation of the whole body, defense against injury and microorganisms, sensitivity, diffusion of molecules, and it varies according to environment and life stage. **Objectives:** We aimed to describe the ultrastructural differentiation of the skin cells and cutaneous glands, during larval and juvenile stages of the toad *R. granulosa*. **Methods:** Tadpoles and newly metamorphosed toads of *R. granulosa* were collected in Angicos, RN, Brazil, during the rain season (February, 2004). Larvae were staged according to Gosner (1960). Skin samples of juvenile toads and of larval individuals were fixed in Karnovsky and osmium tetroxide, and embedded in Epon resin. Ultrathin sections (70 nm) were stained with uranyl acetate and lead citrate, and examined in a LEO 906E transmission electron microscope. Larval stages 25, 43, and newly metamorphosed toads were analyzed. **Results and Discussion:** At the early larval stage 25, the skin is composed by 2 layers of epidermal cells resting on a thick acellular basal membrane, the adepidermis. Basal cells show a well-developed system of intermediate filaments – the Eberth figures – which provide stability and flexibility to the epidermis. The superficial layer has 2 types of cells: a) cells with numeorus apical granules corresponding to the PAS positive granules observed in histological sections; and b) cells with remnants of cilia. Other types of cells are the Merckel cells with neurotransmitter granules, and vacuolated cells. At stage 43, the adepidermis is almost totally invaded by fibroblasts, capillaries, melanocytes , and projections of cutaneous glands, becoming a true dermis. Gradual cornification of the external layers is observed. Cutaneous glands are not fully developed. Granular glands are composed of a syncitial secretory epithelium with abundant, rough and smooth endoplasmic reticulum, in active stage of synthesis and storage of granules. The newly metamorphosed toads show a cornified epidermis with a sloughing external layer. Granular and mucous glands are well developed. In fully differentiated granular glands the atrophied cytoplasmic organelles are segregated to the syncitium periphery by the numerous maturing lamellar vesicles that are merged in an electron-dense cytoplasm. The amphibian skin is a dynamic system adapted to each phase of the animal life and is greatly influenced by thyroid hormones in the final larval stages. The cell ultrastructure reflects the varied functions required for the animal adaptation and survival during larval development. Cutaneous glands, important for the toad defense, are present and differentiated by the time of metamorphosis.

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9.18 Antibacterial activity of extract of marine sponge *Amphimedon viridis*

Franzolin TMP[1], Garcia AN[2], Campos Jr AG[2], Correia D[3], Sovierzoski HH[3], Carvalho LR[4], Franzolin MR[1], Rangel M[2]
[1]Laboratório de Bacteriologia and [2]Laboratório de Imunopatologia, Instituto Butantan, SP, Brasil; [3]Instituto de Ciências Biológicas e da Saúde, Universidade Federal de Alagoas, AL, Brasil; [4]Seção de Ficologia, Instituto de Botânica, SP, Brasil

Introduction: The emergence of new infectious diseases and the multiple-drug resistant strains of bacteria, besides the indiscriminate use of antibiotics, created an urgent need for the development of new strategies to treat bacterial infections and for the development of new antimicrobials. Many natural products from marine sources are endowed with promising antibacterial activities. **Objectives:** The objective of this study was to evaluate the antibacterial activity of extracts obtained from the marine sponge *Amphimedon viridis*. **Methods:** The water extract (AvA) and methanol-water extract (AvM), prepared from the marine sponge *Amphimedon viridis*, collected in the Maceió cith (Alagoas), were initially tested against *Escherichia coli* and *Staphylococcus aureus*, using the agar diffusion method for antimicrobial susceptibility, following Kirby and Bauer methodology. The extracts that showed inhibitory activity against these two bacterial strains were also tested against other bacterial species and *Candida albicans* using the same methodology. **Results and Discussion:** The AvA extract did not show an inhibition halo, whereas the AvM extract, at a concentration of 1 mg/ml and 5 mg/ml inhibited the bacterial growth of these two species. At 2 mg/ml, the AvM extract showed an inhibition halo to Gram-positive bacteria: *Enterococcus spp*, *Streptococcus agalactiae*, *Streptococcus pyogenes*, *Micrococcus luteus*, and *Staphylococcus epidermidis*, but did not inhibit the growth of *Bacillus spp*. With respect to Gram-negative bacteria, such as *Proteus mirabilis*, *Klebsiella oxytoca*, *Acinetobacter baumanii* and *Pseudomonas aeruginosa*, the AvM extract showed low capacity of inhibition and did not inhibit the growth of *Stenotrophomonas maltophilia* and *Candida albicans*. This extract may contain halitoxin (which shows diverse toxic and cytotoxic activities) or other active substances in its composition and will be purified to study its antibacterial activity.

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9.19 How can biological variables influence the incidence of *Bothrops jararaca* snakebites in São Paulo State, Brazil?

Garcia CR, Almeida-Santos SM
Laboratório Especial de Ecologia e Evolução, Instituto Butantan, SP, Brasil

Introduction: Snakes of the genus *Bothrops* are responsible for almost 90% of all accidents occurring in Brazil. *Bothrops jararaca* is responsible for almost 93% of all *Bothrops* accidents. **Objectives:** The aim of this study was to evaluate the influence of biological variables in *B. jararaca* snakes which cause snakebites in São Paulo State. **Methods:** *Bothrops jararaca* specimens that have caused accidents from 1995 to 2008 (N=3053) are preserved at "Collection Vital Brazil" at Institute Butantan. All these snakes were dissected, examined, and measured. Snake stomachs were dissected to check whether or not they had stomach contents. **Results and Discussion:** These data revealed that newborn males of *Bothrops jararaca* fed mainly in spring, whereas female newborns fed mainly in winter. *B. jararaca* male newborns had 128 identifiable items in their stomachs (86% endothermic preys and 14% exothermic preys), whereas female newborns had 110 identifiable items in their stomachs (89% endothermic preys and 11% exothermic preys). A large number of accidents occurred during the day, between 6:00 am and 6:00 pm (67%). However, many accidents occurred during the night period, between 7:00 pm and midnight (19%). Adult snakes caused more accidents during the day period. Our data show that 71% of the accidents are caused by newborns, whereas 29% are caused by adults. Adult females (53%) caused more accidents than adult males (47%). Males caused more accidents during the autumn, whereas females caused more accidents during the summer. Newborns (male and female) caused more accidents during the spring and summer. Analyzing the female reproductive status of the snakes that caused accidents, 45.6% were non reproductive females, and 16.5% were reproductive. However, pregnant females were found in late spring. Newborns are more abundant in autumn. Seasonal patterns of accidents are different between *B. jararaca* adults and newborn. However, newborn caused more accidents than adults. Preliminary analysis shows that females cause more accidents than males. Adult males cause more accidents during the autumn, whereas adult females cause more accidents during the summer. These periods coincide with this species' reproductive pattern, mainly for females which must feed heavily during this period, so they can have enough energy for vitellogenesis. The spermatogenic activity begins in the spring, with spermiogenesis occurring in spring-summer and spermiation in the autumn. During the autumn, males are searching for females (mating period), and therefore, the increase of risks of accidents is higher.

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9.20 Microvesicles in *Crotalus durissus terrificus* venom (Serpentes-Viperidae) originate from microvilli

Giannotti KG[1], Sant'Anna SS[2], Carneiro SM[1]
[1]Laboratório de Biologia Celular and [2]Laboratório de Herpetologia, Instituto Butantan, SP, Brasil

Introduction: Microvesicles 40-80 nm in diameter are consistently observed in the tubular and venom gland lumina of viperid snakes associated with secretory cell apical microvilli. They have been isolated from *Crotalus durissus terrificus* venom by ultracentrifugation and characterized morphologically. Intramembranous particles detected by freeze-fracture indicate intramembranous proteins in microvesicles. Similar vesicles, observed in *Glodyus blomhoffii blomhoffii*, were supposed to originate from multivesicular bodies, and they show dipeptidyl peptidase and ecto-5'-nucleotidase activities, which may disturb hemostasis. **Objectives:** To standardize a fractionation protocol for the recovery of microvesicles from *C. d. terrificus* venom by ultracentrifugation, and to analyze the microvilli-microvesicles relationship. **Methods:** Four milliliters of venom from 18-20 specimens of *C. d. terrificus* maintained in the Laboratory of Herpetology were used in five experiments. Venom was submitted to a first centrifugation varying from 150 *g* to 32,500 *g* at 10ºC to eliminate sloughed cells and cell debris, and afterwards the supernatants were ultracentrifuged from 100,000 to 200,000 *g* for 60 min in a refrigerated Sorvall OTD 75 B with an AH650 rotor. The resulting pellets were fixed in Karnovsky aldehydes and in osmium tetroxide, and embedded in Epon resin. Ulltrathin sections and negative stained preparations were examined in the LEO 906E transmission electron microscope. **Results and Discussion:** The best recovery of venom microvesicles was achieved with an initial centrifugation at 3,600 *g* for 15 min, followed by an ultracentrifugation at 190,000 *g* for 1 h. Clustered microvesicles and fragments of secretory cell apical cytoplasm with microvilli and associated microvesicles pelleted even at the lowest centrifugation speed. Microvilli fragmentation and microvesicle budding from microvilli are often observed in ultrathin sections of pellets. Amorphous material was observed outside the microvesicles. Negative stained images from pellets also showed clusters of microvesicles and microvesicles associated with microvilli. Negative staining of the final supernatant showed numerous electron-dense granules, sized from 0.3 to 0.5 μm, but no microvesicles. Our electron microscope images provide show that the bulk of microvesicles found in *C. d. terrificus* venom originates from microvilli, by fragmentation or membrane budding. Their release into the venom, in association with fragments of apical cytoplasm of secretory cells, likely represent a way to eliminate excess membranes, while, at the same time, they expose proteins that may have biological or biochemical effects.

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9.21 Piperacea extracts as bait to control *Achatina fulica* and *Rumina decolatta* (Gastropoda: Stylommatophora)

Giarletti OLA[1], Toledo-Piza AR[1], Kato MJ[2], Kawano T[1]
[1]Laboratório de Parasitologia, Instituto Butantan, SP, Brasil; [2]Instituto de Química, Departamento de Química Fundamental, Universidade de São Paulo, SP, Brasil

Introduction: Terrestrial mollusks are commonly known as snails and slugs. Some of them are used as food and they have great nutritional value, including vitamins and minerals. They can accomplish an important function on flower pollination and dissemination of seeds and spores through their mucus or feces. On the other hand, they cause damage to agriculture, mainly exotic species, injuring crop plantations and ornamental plants. Furthermore, they can be intermediate hosts of some parasites. The control of these animals in nature is difficult. There are two kinds of chemical substances used as bait: carbamate and metaldehyde. But, they bring risks to the environment and have a high production and application cost. In this manner, studies with plant extracts are being done to find more options in the population control of gastropods. **Objectives:** The aim of this project was to analyze the molluscicidal effect of different plant extracts from the family Piperaceae on *Achatina fulica* Bowdich, 1822 and *Rumina decollata* Férussac, 1821 to achieve a less damaging option to the environment. **Methods**: We used five young snails of each species in duplicate, exposed to 1000 ppm of extract through ingestion for 24 h and were observed for nine days. The tested extracts were: leaves of *Piper crassinervium*, *Piper diospyrifolium*, *Piper fuligineum*, *Piper gaudichaudianum*, *Piper tuberculatum* and *Photomorphe umbellatum*, besides the fruit of *P. crassinervium* and inflorescence of *P. tuberculatum*. A commercial molluscicide, Pikapau®, based on metaldehyde (3%) was used as positive control. **Results and Discussion**: Despite that *R. decollata* was more sensitive to metaldehyde (70% mortality), *A. fulica* showed higher mortality after exposure to plant extracts, so that *P. crassinervium* (leaf) was the most effective (30%). Also recorded were changes in the feeding behavior of the animals after eating the extracts. *P. crassinervium* (leaf and fruit), *P. tuberculatum* (inflorescence), *P. gaudichaudianum* (leaf) an *P. diospyrifolium* (leaf) inhibited feeding of *A. fulica*, but only *P. fuligineum* (leaf) recorded this response on *R. decollata*, while the extracts of *P. gaudichaudianum* (leaf), *P. diospyrifolium* (leaf), *P. tuberculatum* (inflorescence) and *P. crassinervium* (fruit) stimulated feeding of *R. decollata*. Therefore, the response to ingested plant extracts was different between the species, which means that their physiology and behavior were distinct and must be considered in field attempts of population control of these snails.

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9.22 Expression of iNOS and arginase mRNA in murine macrophages stimulated *in vitro* with *Bordetella pertussis*

Iamashita P, Galhardo CS, Mafra DG, Silva LB, Barbosa AS, Borges, MM
Laboratório de Bacteriologia, Instituto Butantan, SP, Brasil

Introduction: L-arginine metabolism results in the production of nitric oxide (NO) and arginase, molecules involved in the control of several infections and inflammatory process. iNOS and arginase compete for the same substrate, L-arginine and co-regulate the function of each other. The activities of both enzymes are regulated in macrophages by cytokines and microbial components while the concentration of iNOS and arginase at the site of inflammation can be responsible for modulating the level of immune response. The macrophage is one of the main defense cells in the organism and also regulates the activity of the inflammatory cell including the activity of T lymphocytes. *Bordetella pertussis* is the causative agent of whooping cough and its prevalence in the vaccinated population is increasing in many countries. The mechanisms that regulate the expression of iNOS and arginase in activated macrophages with *B. pertussis* are unknown. **Objective:** We examined the expression of iNOS and arginase I mRNA in macrophages activated *in vitro* by *B. pertussis*. **Methods:** Mouse bone marrow macrophages (BMDMϕ) from C57/BL6 mice were cultured and differentiated for 7 days in RPMI supplemented with 10% of FBS, 20% supernatant of L929 fibroblasts, 50 U/ml penicillin, 50 μg/ml streptomycin and non essential aminoacids. BMDMϕ was activated with soluble protein from *B. pertussis* (30 μg/ml) in the presence or absence of IFN-γ for 6 and 24 h. At the indicated time points, total cellular RNA was prepared with Trizol reagent (Invitrogen), according to the manufacture's instruction. Total RNA (2 μg) was converted to cDNA. PCR was performed using sense and antisense primers, respectively, for iNOS: AGGAAGAAATGCAGGAGATG and CACCTGCTCCTGGCTCAAG (253-bp); arginase: TTCTGGGAGGCCTATCTTAC and CCAAGGTTAAAGCCACTGCC (162-bp); β-actin: ACTACATTCAATTCCATCAT and CGATCCACACAGAGTACTTG (196-bp). PCR products were separated by electrophoresis on 2% agarose gels and visualized by staining with ethidium bromide. **Results and Discussion:** Relative levels of mRNA for iNOS and arginase I were determined by semi-quantitative RT-PCR at 6 and 24 h after macrophage stimulation. iNOS and arginase I mRNA was present at a very low level prior to treatment. After stimulation with *B. pertussis* antigen for 6 and 24 h, both mRNA increased markedly. These results show that components of *B. pertussis* induce the expression of mRNA for iNOS and arginase, but not the synthesis of NO. The mechanisms involved in NO regulation are under investigation.

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9.23 Inventory of ground dwelling spiders (Arachnida, Araneae) from the Parque Estoril, São Bernardo do Campo, São Paulo State, Brazil

Janini CRV [1,2], Brescovit AD[1]
[1]Laboratório de Artrópodes, Instituto Butantan, SP, Brasil; [2]Centro Universitário São Camilo, Campus Pompéia, SP, Brasil

Introduction: Brazil is responsible for 20% of the world's spider fauna. Nevertheless, knowledge of the ground-dwelling species is poor, and studies concerning this particular habitat in the state of São Paulo are rare. **Objective:** The aim of this study was to analyze the ground dwelling species of spiders from Parque Estoril, São Bernardo do Campo, during two years, and comment on their seasonality patterns. **Methods:** Twelve samplings, using pitfall traps, were carried out every two months for two years. In each sampling period, one hundred traps of 500-ml plastic cups were installed for 5 days. Each trap with spiders was considered an independent sample. The resulting material was sorted and identified to family and morphospecies level. **Results and Discussion:** This work provided an important database of soil spider fauna to compare with another sample from the Atlantic Forest. A total of 4340 spiders were collected, 3395 adults and 947 juveniles belonging to 25 families and 77 species. The seven most abundant species were *Sphecozone castanea* (Millidge, 1991), *Meioneta* sp.1, *Sphecozone* sp.2, *Vesicapalpus simplex (*Millidge, 1991*)*, Zoridae sp.1 and sp.2, Linyphiidae sp. 1. The families Theridiidae (18 species), Linyphiidae (17 species) and Araneidae (8 species) showed the most richness. The Nemesiidae were represented by only three juveniles. A total of 25 (32.5%) singletons, 13 (16.8%) doubletons, 31 uniques (40.2%) and 12 duplicates (15.6%) were found. The Bootstrap method yielded the lowest richness estimates and Jack 2 the highest, with 89 and 122 species respectively. While Chao 1 showed a stable result with 98 species.

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9.24 Convulsant effect of intraperitoneal administration of whole venom of *Tityus serrulatus* scorpion: electroencephalographic, behavioral and histopathologic aspects

Katsumata E, Prandini B, Lebrun I, Sandoval MRL
Laboratório de Farmacologia, Instituto Butantan, SP, Brasil

Introduction: Clinical data have shown that scorpion venom can induce convulsion, mainly in children. These data confirmed results obtained in our early studies, where rats received intrahippocampal injection of TsTx toxin isolated from *Tityus serrulatus* scorpion, which resulted in behavioral and electroencephalographic seizures and neuronal damage. We have shown that i.v. injection of whole venom of scorpion causes convulsions in adult and newborn rats. In view of these facts, there are some questions we want to research further: Is it possible that convulsion caused by systemic injection of whole venom of scorpion induces late epilepsy? Could treatment with anticonvulsivants given just a few hours after the beginning of convulsions prevent late epilepsy? **Objective:** The aim of this study was to study the acute convulsant effects of i.p. administration of *Tityus serrulatus* scorpion venom in male and female adult rats. An electroencephalografic, behavioral and histopathological study was performed. **Methods:** Surgery to implant electrodes in the hippocampus area to record eletroencephalografic analysis was performed. After three-four days, the whole venom of *Tityus serrulatus* scorpion in 0.75, 1.0, 1.5 and 2.0 mg/kg doses was administered (i.p.). Behavior and electroencephalographic activity of rats were observed for six hours uninterruptedly. One week later, rats were anesthetized with carbon dioxide to do a perfusion. They were then decapitated and their brain was removed and preserved in formaldehyde for one week. Brains were processed for histological analyses. The cells of CA1, CA3, hillus and dentate gyrus of hippocampal formation were counted on an area of 100 um^2. **Results and Discussion:** Electroencephalographic recordings were characterized by isolated spikes and epileptic discharges in all groups of rats that had received different doses of venom (0.75, 1.0, 1.5, 2.0mg/kg). The behavioral modifications were characterized by paralysis, "wet-dog shake," intense salivation, convulsion, and respiratory and locomotion difficulties. These behavioral modifications were shown in all groups of rats that received different doses of the venom (0.75, 1.0, 1.5, 2.0 mg/kg). A dose of 1.5 mg/kg of whole venom caused death in 100% of rats. Histopathologic analysis of brains of these rats did not show injuries in the hippocampus and in any other area of the brain. These results showed that the systemic venom injection in adult rats was able to induce central effects such as convulsion and epileptiforme activity. These data are in concordance with neurotoxic symptoms observed in severe envenomation in human.

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9.25 Exploring the proteome and the peptidome of South American viperid venoms

Kitano ES, Tashima AK, Zelanis A, Serrano SMT
Laboratório Especial de Toxinologia Aplicada – CAT/CEPID, Instituto Butantan, SP, Brasil

Introduction: Snake venoms are complex mixtures of components that have a diverse array of actions on prey. Most of these components are active proteins and peptides that work to kill or to immobilize the prey as well as to assist in their digestion. Proteomic approaches have been extensively used to study the complexity of viperid venoms and various mass spectrometric techniques allow an accurate analysis of venom proteins and peptides. These techniques have shown to be important tools in the study of the proteome and the peptidome of snake venoms. **Objectives:** The main goal of this study was to explore the proteome and peptidome of South American viperid venoms. **Methods:** Venoms were provided by the Herpetology Laboratory (Instituto Butantan, SP, Brazil). *Bothrops cotiara* venom was chromatographed (FPLC system) on a gel filtration column (Superdex 75 10/300 GL; GE Healthcare). Fractions containing proteins up to 30 kDa were further submitted to cation-exchange chromatography (MonoS HR 5/5; GE Healthcare). Absorption of column eluates were detected at 225 nm and 280 nm, and the proteins were analyzed by SDS-PAGE. Protein identification was performed by *in gel* trypsin digestion followed by liquid chromatography/mass spectrometry/mass spectrometry (LC/MS/MS) analysis in an ion trap mass spectrometer (LTQ-XL, Thermo Scientific). Mass spectra of peptides were submitted to database search (MASCOT 2.2.04, Matrix Science) restricted to Serpentes taxonomy. *Bothrops jararaca* venoms (from newborn and adult specimens) were fractionated by gel filtration (Superdex 75 10/300 GL; GE Healthcare). Fractions containing peptides were submitted to RP-HPLC (Shimadzu) on a C_{18} column (Discovery 10mm x 250mm, Supelco, particle size 5 μm), to isolate peptides. Peptide fractions were manually collected according to absorbance values at 215 nm and analyzed by MALDI-TOF mass spectrometry (MALDI-TOF, GE Healthcare). **Results and Discussion:** The complexity of Bothrops venoms has been assessed by our group using various chromatographic and mass spectrometric approaches. Previously, we had detected the presence of a proteinase of ~30 kDa with high affinity for heparin in the venom of *B. cotiara*. Here, we isolated the enzyme that was identified as a serine proteinase (SVSP) by *in gel* trypsin digestion and mass spectrometry. The functional activity of this SVSP indicates that it is active upon platelets. To compare the peptidomes of venoms from newborn and adult specimens of *B. jararaca*, the peptide fractions were analyzed by LC/MS/MS and the peptide spectra revealed similarities and differences, and some of these peptides were initially identified as bradykinin potentiating peptides; however, the presence of unknown peptides was detected and these should be further characterized.

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9.26 Effect of *Crotalus durissus terrificus* venom (CdtV) on the formation of multinucleated giant cells in chronic inflammatory process: immunohistochemical analysis of F-actin and phosphotyrosine

Kodama KK, Schnyder R, Sampaio SC, Gonçalves LRC
Laboratório de Fisiopatologia, Instituto Butantan, SP, Brasil

Introduction: In human envenoming by South American rattlesnakes there are no inflammatory reactions at the site of the bite. Experimentally, the CdtV inhibits the acute phlogistic activity induced by thioglycolate and carrageenan, showing significant anti-inflammatory action. Still, this venom shows a dual action on macrophage functions, inhibiting the ability of spreading and the phagocytic activity of this cell, and stimulating its bactericidal activity by increasing the release of hydrogen peroxide and nitric oxide. Macrophages are key cells of acute inflammation and the main cellular component of chronic inflammation. In chronic cases the macrophages fuse into multinucleated giant cells and, subsequently, differentiate into epithelioid cells, forming the granuloma. This process of cell differentiation depends on the participation and the dynamics of actin filaments (F-actin) and signaling proteins, such as phosphotyrosine which mediates this process. Previous studies showed that CdtV changes the patterns of F-actin expression and phosphotyrosine in macrophages subjected to an acute inflammatory stimulus and, when applied prior to a chronic inflammatory stimulus this venom reduces the formation of multinucleated giant cells. **Objectives:** To assess the effect of Cdt venom on the rearrangement of F-actin and phosphotyrosine in animals subjected to a chronic inflammatory stimulus. **Methods:** For the induction of chronic inflammation, round glass cover slips were implanted in the subcutaneous tissue of Swiss mice pre-treated with CdtV or with saline (control animals). Immunohistochemical assays with antibodies against F-actin and phosphotyrosine were performed on cover slips removed 4, 7 and 21 days after implantation and analyzed with a confocal microscope. **Results and Discussion:** The immunostainings for F-actin were significantly inhibited in cover slips removed 4 and 7 days after implantation in the groups pre-treated with CdtV, when compared to control groups. In cover slips removed 21 days after implantation, this difference was not observed. Regarding phosphotyrosine, differences were not observed in the staining of cells of groups treated with CdtV or with saline, in any of the times studied. These results differ from those of acute inflammation, in which an increased expression of F-actin and reduced phosphotyrosine was observed in macrophages collected from animals pre-treated with CdtV. Inhibition of F-actin expression in the implants of 4 and 7 days observed in this study were positively correlated with the inhibition of the giant cell formation observed previously in cover slips implanted in the same period and stained with hematoxylin/eosin. Taken together, these data suggest that CdtV has a significant inhibitory action on the progression of the chronic inflammatory response.

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9.27 Description of adults and redescription of the larvae of *Carios mimon* (Kohls, Clifford & Jones, 1969), a tick species currently included in the Brazilian fauna

Barros-Battesti DM[1], Landulfo GA[1], Venzal JM[2], OnofrioVC[1]
[1]Laboratório de Parasitologia, Instituto Butantan, SP, Brasil; [2]Departamento de Parasitología Veterinaria, Facultad de Veterinaria, Universidad de la República, Regional Norte-Sede Salto, Uruguay

Introduction: *Carios mimon* (Kohls, Clifford & Jones, 1969) is an argasid tick parasite of Chiroptera, originally described from larvae collected on *Mimon crenulatum* (E. Geoffroy, 1810) of Bolívia and on *Eptesicus brasiliensis* (Desmarest, 1819) of Uruguay. Later, it was recorded on other species of bats from Argentina, and recently, it was included in the Brazilian fauna. This tick is known only through the description of the larval stage, this makes its morphological separation from other closer species belonging to the genus *Carios* difficult. **Objective:** To describe males and females and to redescribe larvae of *C. mimon*. **Methods:** Females of *C. mimon*, collected together with nymphs of different instars, in the municipally of Araraquara, SP, were maintained in colony using laboratory animals as hosts. Redescription of larvae and description of males and females were based on optical and scanning electron microscopy. **Results and Discussion:** Among other characteristics, the species *C. mimon* could be separate from the others by the presence of cheeks and by the absence of humps on tarsus I.

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9.28 Melatonin's protective role against the oxidative effects of methylecgonidine and phospholipases from the venom of *Micrurus lemniscatus*

Lucena RVL[1], Garcia RCT[3], Zamboni DM[1], Dati LMM[3], Frare EO[1], Cipolla-Neto J[2], Marcourakis T[3], Sandoval MRL[1], Afeche SC[1]

[1]Laboratório de Farmacologia, Instituto Butantan, SP, Brasil; [2]Departamento de Fisiologia e Biofísica, Instituto de Ciências Biomédicas and [3]Departamento Análises Clínicas e Toxicológicas, Faculdade de Ciências Farmacêuticas, Universidade de São Paulo, SP, Brasil

Introduction: Melatonin is part of a system that generates circadian biological oscillations. This hormone is an indolamine (N-acetyl-5-metoxytryptamine) derived from the amino acid tryptophan. Its actions may occur by direct molecule-molecule interaction or through cell receptors. Acting on membrane receptors (MT1 and MT2), melatonin can induce the expression of the antioxidant enzymes superoxide dismutase and glutathione peroxidase preventing oxidative stress. The heating of cocaine base during smoking of crack produces methylecgonidine, also called methyl ester anhydroecgonine (AEME). AEME is a potent inducer of cell death. *Micrurus lemniscatus* is a snake from the family Elapidae. The predominant clinical manifestations of Elapid snake bite are related to the neurotoxic and myotoxic actions of the venom, causing blockade of the peripheral nervous transmission. Two PLA2 neurotoxins, Mlx-8 and Mlx-9, isolated from the venom of *Micrurus lemniscatus*, induced neuronal death in primary culture of embryonic hippocampal neurons. **Objectives:** We examined the protective effect of melatonin, which is reported to be a powerful antioxidant, on the actions of the phospholipase toxins (Mlx-8, Mlx-9) and the cocaine metabolite AEME, studying cell viability. **Methods:** Hippocampal neurons were isolated and maintained in culture for 24 h in Neurobasal medium. The cells were incubated with different concentrations of AEME (0.1 mM, 1 mM and 10 mM), Mlx-8 (10 ng/mL), Mlx-9 (100 ng/mL and 1000 ng/mL) and melatonin (10^{-7} M and 10^{-9} M). Viability was measured by the MTT test. **Results and Discussion:** AEME (0.1, 1 and 10 mM) induced a decrease in cell viability. The toxin Mlx-8 (10 ng / ml) induced only a small reduction in the cell viability. The cells incubated with Mlx-9 (100 and 1000 ng / mL) showed significant reduction in cell viability. Melatonin at 10^{-9} M reversed the induction of neuronal death by AEME (0.1 and 1 mM) which was not observed in relation to Mlx-8 (10 ng / mL) and Mlx-9 (100 and 1000 ng / mL). Melatonin at 10^{-7} M did not show any protective effect. Thus, melatonin showed protection at the physiological concentration (10^{-9} M) against the neurotoxic effects of AEME, but did not against the Micrurus venom toxins. It is possible that the mechanisms of cell death induction of both toxins and AEME are different.

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9.29 Collagen type I induces apoptosis by caspase-3 pathway in mice bearing melanoma and breast adenocarcinoma

Martins V, Inada DFO, Kleber A, Azevedo R, Maria DA
Laboratório de Bioquímica e Biofísica, Instituto Butantan, SP, Brasil

Introduction: Tissue microenvironments play an important role in maintaining normal cell behavior. Moreover, type I collagen is a prevalent component of the stromal extracellular matrix; its expression is spatially and temporally regulated during mammary ductal formation and dermal differentiation, suggesting that it plays important roles in development. The primary function of collagen fibers is to add strength to the connective tissue and can have a stimulatory or inhibitory effect on cell proliferation, and organized fibrillar structure inhibits normal and malignant cell proliferation. **Objective:** To evaluate the inhibitory effects of type I collagen on cell proliferation and apoptosis in murine B16F10 melanoma and Ehrlich breast adenocarcinoma in C57BL/6J and Balb-c mice. **Methods:** We determined the inhibitory concentrations (IC50%) of collagen type I in B16F10 melanoma, Ehrlich breast adenocarcinoma and normal fibroblasts by the colorimetric method - MTT. Activity of collagen in induced cell death pathway caspase-3 was tested by an enzymatic-fluorogenic method. C57BL/6J and Balb-c mice were implanted with $5x10^4$ B16F10 melanoma cells and $1x10^4$ breast adenocarcinoma of Ehrlich. The treatment was performed with collagen at different concentrations, administered by intraperitoneal route for 30 days. **Results and Discussion:** The interaction of collagen in the stroma as an intrinsic defense mechanism to establish a breast tumor is likely to produce morphological changes in epithelial and stromal cells of breast. The inhibitory concentrations (IC50%) were 18.6 mg/ml for B16F10 melanoma and 49.9 mg/ml for breast adenocarcinoma. Microscopic analysis of tumor cells showed multicellular aggregation and loss of adhesion in extracellular matrix. The treatment by collagen showed cytotoxic effects and morphologic changes but cell proliferation and sites of collagen by dermal fibroblasts. Collagen had significant activity as a substrate for active site specific of proteolytic cleavage of caspase 3 in mitochondrial intrinsic pathway. It is an inducing agent of apoptosis, where activity proved to be selective for cell tumor effects and no toxicity in normal cells such as fibroblasts. Animals bearing the B16F10 melanoma and Ehrlich adenocarcinoma treated with collagen showed significant reduction of tumor mass, respectively 79% and 68%. The survival rate calculated by the Kaplan-Meier test showed that treatment with the samples of collagen induced a significant reduction in mortality rate and reduction in number internal metastases. Collagen significantly reduced tumor growth, active caspase-3 and formation of metastases in experimental models of melanoma and breast adenocarcinoma. Collagen type I and hydrolysate peptide products are a promising new weapon for cancer treatment.

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9.30 Hippocampal effects of toxins isolated from pool 5 of *Tityus bahiensis* scorpion venom in rats

Martins AN[1]; Ossanai LTT[1], Lourenço GA[1], Nencioni ALA[1], Lebrun I[2], Dorce VAC[1]
[1]Laboratório de Farmacologia and [2]Laboratório de Bioquímica e Biofísica, Instituto Butantan, SP, Brasil

Introduction: In Brazil, one of the most dangerous scorpions is *Tityus bahiensis*. Nevertheless, there are few studies about the effects of this venom, mainly in the central nervous system. Scorpion venoms are composed of neurotoxins, which are polypeptides of low molecular weight. In previous studies performed in our laboratory, it was demonstrated that the venom of *T. bahiensis* is very convulsive, with different characteristics from *T. serrulatus*. Six fractions of this venom were studied and four of them showed interesting results in the observed parameters (behavior and electroencephalographics alteration and neuronal loss). Fraction P5 was chosen to develop this work due to its ability to cause behavioral and electroencephalographic convulsion and large neuronal loss. **Objectives**: Evaluation of the histological effects after intrahippocampal injection of toxin obtained from pool 5 of *T. bahiensis* venoms. **Methods**: The crude venom, with adequate treatment, was applied in a Sephadex G50 column. Chromatographic profile showed six distinct fractions. Toxic fraction P5 was separated on reverse-phase column by HPLC. The profile revealed five peaks. Three of them were used in this study. Four groups of Wistar male rats (n=6) weighing 250 g were submitted to stereotaxic surgery to implant cannulas in the hippocampus. One day after the surgery, the animals were injected with 1 µl of Ringer solution (control group) or with solution of toxin I-V, IV-V or V-V at a concentration of 1 µg/µl. Seven days after the injections, the animals were sacrificed and perfused. The brains were removed and prepared for histological analysis. Statistical analysis was done by ANOVA test followed by Tukey Kramer, with $p < 0.05$. **Results and Discussion**: When animals were injected with toxins I-V and IV-V, there was a significant decrease in cell number only in the CA1 area contralateral to injection site. No significant differences were found in other hippocampal areas; however, toxin IV-V caused neuronal loss in CA1 ipsilateral area in half of observed animals without effect in the others. In animals injected with toxin V-V no neuronal loss was observed in any area. The toxins of pool 5 of *T. bahiensis* scorpion act on the central nervous system causing neuronal loss in the hippocampus on the opposite side of injection. Thus, these toxins may be useful tools for the study of hippocampal ways.

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9.31 Secreted autotransporter toxin (Sat) in atypical enteropathogenic *Escherichia coli*

Mazur TR, Melo KCM, Soares ES, Piazza RMF, Ruiz RC
Laboratório de Bacteriologia, Instituto Butantan, SP, Brasil

Introduction: *Escherichia coli* is an incredibly diverse bacterial species with the ability to colonize and persist in numerous niches both in the environment and within the host. *E. coli* strains associated with the human host are classified as commensals, enteric pathogens or extraintestinal pathogens, more frequently including the strains associated with urinary tract infection. These differences depend on the set of virulence genes and clinical properties of each pathotype. Enteropathogenic *Escherichia coli* (EPEC) constitutes one of the six diarrheagenic *E. coli* categories, which was divided into two groups, typical EPEC and atypical EPEC, whose basic difference (as compared to typical EPEC) is the absence of the EPEC adherence factor plasmid (pEAF), and wider heterogeneity of virulence factors. The diffusely adhering *E. coli* (DAEC) is one of the human pathogenic *E. coli* strains responsible for recurrent urinary tract and gastrointestinal infections. Studies have shown that some DAEC strains harbor virulence factors found in uropathogenic *E.coli* (UPEC) strains, such as the secreted autotransporter toxin, Sat. Sat belongs to the subfamily of serine protease autotransporters of *Enterobacteriaceae*. This family, which has a specific and distinct function, has been indentified only in pathogenic bacteria, and includes a variety of other virulence toxins. **Objective:** To investigate Sat toxin expression in isolates of aEPEC, whose gene *sat* was amplified by multiplex PCP. **Methods:** Cytotoxicity assays in HEp-2 cells were performed with the bacterial culture or supernadant of the bacterial culture from isolates 589 (O5:H2) *sat/pic/east*; 1887 (O111:H38) *sat/hly*; 2294 (O9:H33) *east/sat* and 3170 (O145:H2) PCR negative. DAEC strain C1845 was used as a prototype strain to study the expression of Sat. **Results and Discussion**: Our results show that isolates positive for the *sat* gene were unable to produce cellular alterations in HEp-2 cell cultures. On the other hand, cells incubated with the concentrated supernatant from bacterial cultures (50 and 100 μg/mL), containing only molecules over 50 kDa, showed high levels of vacuolization, after 5 h of incubation. A partial cell detachment was only detected in cultures incubated with concentration of 250 μg/mL, for 24 h. These results suggest for the first time that aEPEC may express the Sat toxin, important for infection by both UPEC and DAEC. The study of the expression and activity of bacterial toxins is of essential importance for the understanding of the pathogenesis of the bacterial infections. In particular, the finding of the expression of the Sat toxin in aEPEC will certainly contribute to the understanding of the pathogenesis of infections by this class of *E. coli*, still very poorly known.

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9.32 Hematological parameters of the snakes *Oxyrhopus guibei* and *Xenodon neuwiedii* (Ophidia:Colubridae)

Ozzetti PA[1], Cavlac CL[1,2], Sano-Martins IS[1]
[1]Laboratório de Fisiopatologia, Instituto Butantan, SP, Brasil; [2]Departamento de Fisiologia, Instituto de Biociências, Universidade de São Paulo, SP, Brasil

Introduction: Hematological values usually express the physiological conditions of the animal. Hematological changes are often early indicators of some pathology and aid in disease diagnosis. In snakes, the hematological studies are more frequent in venomous species than in non-venomous ones, probably due to their medical importance, and usually, venomous snakes are kept in captivity to supply venoms for anti-venom production. **Objectives:** The aim of this study was to establish the reference range of hematological data of two species of Brazilian non-poisonous snakes, recently wild-caught and sent to Instituto Butantan. **Methods**: One milliliter of blood was collected in 0.1% EDTA from ventral caudal vein and 1 ml from the abdominal aorta of *Xenodon neuwiedii* (n=19) and *Oxyrhopus guibei* (n =26) snakes. The total red blood cells (RBC), white blood cells (WBC) and thrombocytes (TBC) were determined manually with the improved Neubauer counting chamber, using Natt and Herrick solution (1:200). The packed cell volume (PCV) was determined by the microhematocrit method (Ht) and hemoglobin(Hb) concentration was determined by the cyanomethemoglobin method. The differential white blood cell count was done in blood smears stained with May-Grunwald-Giemsa and pro-erythrocytes (PE) were counted in smears stained by New methylene Blue. **Results and Discussion:** The mean values (X±SD) for *X. neuwiedii* and *O. guibei* were respectively: RBC $x10^9$/L (455.4±125.9 and 476.6±138.8); WBC $x10^9$/L (4.4±1.9 and 4.8±2.1); TBC $x10^9$/L (7.6±2.8 and 7.3±2.7), mean corpuscular volume fl. (523.9±60.5 and 524.1±98.4), mean corpuscular hemoglobin pg. (138.5±27.6 and 147.9±50.4), mean corpuscular hemoglobin concentration % (25.6±5.3 and 27.8±6.7), Ht % (23.8±6,7 and 24.3±6.0); Hb g/dl (6.3±2.0 and 6.8±2.3); PE $x10^9$/L (23.5±18.6 and 21.5±25.3). The following types of white blood cells were described respectively for Xn and Og respectively ($x10^9$/L): lymphocytes (1.7±1.0 and 1.8±0.7); azurophils (1.9±0.8 and 1.9±1.0); heterophils (0.6±0.4 and 0.8±0.9) and basophils (0.4±0.3 and 0.4±0.2). There were no significant differences in caudal vein hematotological parameters between the two species of snakes and also, between males and females in *X.neuwiedii* snakes. However, hematocrit and hemoglobin values were significantly higher in *O. guibei* males. In the comparison between the two forms of collection, tail vein and abdominal aorta, there were significant differences in hematocrit and CGE in the snake *O. guibei* ($p < 0.05$). Intracellular parasites were not found in any of the specimens studied. Our results are similar to reference ranges of hematological data obtained for other snakes, such as the families Viperidae and Boidae. In some *X. neuwiedii* snakes, there were morphological changes of heterophils such as cytoplasmic extension, not yet described in literature, but its function unknown. The hematological changes observed in both species are probably associated with factors that influence their physiological state such as stress of capture, seasonality, and food, among others.

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9.33 *Tityus serrulatus* venom modulates the immune response of mouse spleen cells

Papotto PH; Lara PG; Magnoli FC; Portaro F; Sant'Anna OA; Tambourgi DV; Spadafora-Ferreira M
Laboratório de Imunoquímica, Instituto Butantan, SP, Brasil

Introduction: Human accidents caused by scorpion venom represent a significant public health problem in Brazil, according to the Health Department. *Tityus serrulatus* venom (TsV) is responsible for most of scorpion envenomations in Brazil. This venom causes various neurotoxic, circulatory and muscular effects. Inflammatory properties of this venom have been described in humans and mice. As lymphocytes play an important role in the inflammatory response, it is important to understand if TsV can affect these cells. **Objectives:** The aim of this study was to evaluate the activity, *in vitro,* of TsV and its components on the proliferation and cytokine production of mouse spleen cells. **Methods:** Whole TsV and 11 fractions obtained by gel filtration chromatography were used in this study. For analysis of TsV activity on proliferation, spleen cells from naive mice were labeled with CFSE and incubated with TsV and the different fractions, in the presence or absence of ConA. After 72 h at 37°C, cells were labeled with antibodies and analyzed by flow cytometry. For the analysis of cytokines, spleen cells were stimulated with phorbol myristate acetate plus ionomycin or ConA and incubated with TsV and each fraction. $IFN\gamma^+$ cells were detected by FACS analysis of intracellular staining. Cytokines in cell supernatants were detected by ELISA or CBA analysis. **Results and Discussion:** TsV induced (10.9%) spleen cell proliferation ($CFSE^{low}$ cells) when compared to untreated control cells (2.2%). Proliferation was mainly detected in B220+ (8.5%), compared to CD3+ (0.7%) cells. Moreover, some TsV fractions also induced CD3+ and CD19+ cells, and others were able to inhibit proliferation induced by conA. TsV inhibited the production of intracellular IFNγ (0.6%) compared to untreated PMA/ionomycin 24h stimulated cells (7.4%). The analysis of the activity of TsV on kinetic production of cytokines showed that TsV induced the production of TNFα (48 h), but inhibited IL-10 production induced by conA. The analysis of the activity of each fraction on the kinetic production of cytokines showed that all induced TNFα, but most of them inhibited IL-2. Moreover, four fractions of low molecular weight inhibited IFNγ production induced by conA after 24h incubation. TsV and its components did not affect the production of IL-4 and IL-5. In this study, we demonstrated that TsV and its components are able to modulate immunocompetent cells, *in vitro*, especially T and B cells either by induction or inhibition of proliferation and cytokine production. The identification of the TsV components responsible for these activities is currently being investigated.

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9.34 On the genus *Pycnothele* Chamberlin (Araneae: Mygalomorphae, Nemesiidae)

Passanha V[1,2], Indicatti RP[1,3], Lucas SM[1]
[1]Laboratório de Artrópodes, Instituto Butantan, SP, Brasil; [2]Centro Universitário São Camilo, Campus Pompéia, SP, Brasil; [3]Universidade Federal Rural do Rio de Janeiro, RJ, Brasil

Introduction: The genus *Pycnothele* was described by Chamberlin based on the type species, *P. perdita* Chamberlin, male and female from Mendes, Rio de Janeiro, Brazil. Currently, the genus comprises five species: *P. perdita*, *P. singularis* (Mello-Leitão) and *P. piracicabensis* (Piza) from Brazil; *P. auronitens* (Keyserling) from Brazil and Uruguay and *P. modesta* (Schiapelli & Gerschman) from Argentina and Uruguay. Pérez-Milles & Capocasale in 1988 and Goloboff in 1995, made revisions of the genus but did not include all known species. **Objectives:** To revise the genus *Pycnothele*, with emphasis on the Brazilian species. To enhance the distribution range of all species of the genus. To describe two new species from Brazil and transfer *P. piracicabensis* to the genus *Rachias* Simon. **Methods:** The examined material is deposited in several Brazilian and two foreign collections. Female spermathecae were dissected and cleared in clove oil for observation of internal structures. The drawings were made on a Leica MZ 12.5, with a camera lucida. **Results and Discussion:** The examined material enabled us to redescribe the Brazilian species, enhance the distribution range of *P. perdita*, *P. singularis* and *P. auronitens* and record the occurrence of *P. modesta* in Brazil for the first time. In addition, two new species are described, *P.* sp. 1 from Araraquara, São Paulo and *P.* sp. 2 from Arapongas, Paraná and Ribeirão Grande, São Paulo, both in Brazil. Based on the study of the characters of the genus, *P. piracicabensis* is transferred from *Pycnothele* to the genus *Rachias*.

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9.35 Comparative study of rDNA 28S of diploid and tetraploid *Odontophrynus americanus* (Amphibia, Anura)

Paulikevis G[1] , Souza AF[1], Kobashi LS[2], Ruiz IRG[1]
[1]Laboratório de Genética and [2]Laboratório de Biotecnologia, Instituto Butantan, SP, Brasil

Introduction: Ribosomal cistrons are represented by hundreds of copies of rDNA units per haploid genome in eukaryotes. These multiple copies have essential role in the cells to provide appropriate supplies of rRNAs 18S, 5.8 S and 28S for ribosome assembly. Previous study on the 18S rRNA of *Odontophrynus americanus* provided primary sequence and secondary structure comparisons between diploid (2n) and tetraploid (4n) specimens, confirming their close evolutionary relationship. The intergenic spacers (IGS) of *O. americanus* 2n and 4n were also cloned and sequenced. Although the same structural elements were present in both IGSs, the highest level of repeated elements in IGSs 4n sequences suggested that the ancestor IGSs must have been subjected to several rounds of internal duplication. **Objective:** This work aimed to complement previous studies on the 28S rDNA 2n and 4n sequencing in order to establish comparisons concerning the molecular evolution of these polyploid amphibians. **Methods:** Clones previously constructed (POA EK 211, EK 417 POA, POA 211 KB, 417 KB POA, POA 211 BB, 417 BB POA, POA BH 211, HK 211 and POA POA 417 BK) were plated in LB agar medium containing ampicillin, and the recombinant plasmids were obtained through mini preparations. The inserts were subsequently sequenced (*Big Dye Terminator Kit* and *ABI Prisma 3100, Applied Biosystems*). **Results and Discussion**: The sequences were compared with, and complemented most data previously obtained. As compared to *O. americanus* 2n, the conserved region of the 28S rDNA region showed 62 deletions, 46 insertions and 33 substitutions in *O. americanus* 4n and 29 deletions, 11 insertions and 11 substitutions in *Xenopus laevis*. So far, the divergent domains of *O. americanus* 4n showed 6 deletions, 2 insertions and 5 substitutions, and *X. laevis* showed 136 deletions, 65 insertions and 220 substitutions, as compared to *O. americanus* 2n. The final confirmation of the results depends on further sequencing experiments. The complete sequence of the 28S rDNA would help to understand the mechanisms involved in the molecular evolution of ribosomal cistrons and would also contribute to the understanding of the control of gene expression in polyploid amphibians.

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9.36 Comparison of the litter spider fauna (ARANEAE) from sixteen areas, sampled with soil traps in the Atlantic Forest

Godoy T[1,2], Brescovit AD[1]
[1]Laboratório de Artrópodes, Instituto Butantan, SP, Brasil; [2]Centro Universitário São Camilo, Campus Pompéia, SP, Brasil

Introduction: Of a total of 109 known spider families, 67 occur in Brazil, and approximately 70% include species that live exclusively in the litter layer. The study of the composition of the spider community aims to compile taxonomical and statistical data that will characterize this community in the environment. The information obtained from these studies constitutes the basis of more complex ecological studies. In this study we analyzed the material collected with pitfall traps, during the development of the BIOTA/FAPESP project, and deposited in the collection of the Butantan Institute. The resulting data will be analyzed taxonomically and ecologically in order to obtain information on the still poorly studied soil spider fauna. **Objectives:** To examine the material from the areas sampled during the BIOTA/FAPESP project and to compare them in order to enhance our knowledge of soil-dwelling spiders. **Methods:** The material was collected with pitfall traps made out of 500-ml plastic cups with 7-cm opening and filled up to 1/3 with 80% alcohol. Approximately 170 traps were placed in each area and left for 5 days. The identification data were copied into Excell worksheets for future analysis. **Results and Discussion:** From a total of 16 areas, 10,255 spiders were collected, belonging to 45 families. Twenty-two families have been sorted to morphospecies but we will emphasize Linyphiidae, Oonopidae and Amaurobiidae, since the number of specimens is high (n>36). Linyhiidae was the most abundant family with 62 species. Of these, 49 are new to science and 11 could not be placed in any known genera. The Floresta da Cicuta yielded the highest number of adult Linyphiidae (385 specimens), followed by Caraça (364 specimens). The genus *Sphecozone*, with 737 adult specimens, was represented by *S. castanea*, *S. novaeteutoniae*, *S. labiata* and 8 new species. The genus *Moyosi,* with 122 adult specimens, was represented by *Moyosi prativaga* and 7 new species. From the family Oonopidae, 561 specimens were collected, belonging to 29 species: *Ichnothyreus peltifer, Oonops reticulatus, Triaeris stenapis* and 26 new species. The areas with the highest abundance of Oonopidae were Cicuta, with 152 adult specimens, and Caparaó, with 97 adult specimens. From the family Amaurobiidae, 218 specimens were collected, distributed in 7 morphospecies of which one belongs to the subfamily Macrobuninae, and 6 could not be placed in any known genera. The area with the highest number of species was Caraça, with 98 specimens, all belonging to Amaurobiidae sp. 6, and the area with the highest diversity was Foz do Iguaçu, with 7 specimens belonging to 3 morphospecies.

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9.37 Proteomic analysis of the effects of the platelet-aggregating enzyme PA-BJ, a serine proteinase from *B. jararaca* venom, on platelets

Preto MC[1], Yamashiro ET[1], Paes Leme AF[2], Tashima AK[1], Serrano SMT[1]
[1]Laboratório Especial de Toxinologia Aplicada - CAT/CEPID, Instituto Butantan, SP, Brasil;
[2]Laboratório Nacional de Luz Síncrotron, SP, Brasil

Introduction: PA-BJ is a serine proteinase isolated from *Bothrops jararaca* venom that induces platelet aggregation on platelet rich plasma and on washed platelet suspensions. It is a 30-kDa protein that contains one N- and one O-glycosidically linked carbohydrate moiety. The effect of PA-BJ on platelets is mediated by the thrombin receptors PAR1 and PAR4. It induces calcium mobilization in platelets and desensitizes platelets to the action of thrombin and the SFLLRN peptide. In vitro, PA-BJ cleaves the recombinant exodomain of the receptor PAR1 at peptide bonds Arg41-Ser42 and Arg46-Asn47 resulting in the inactivation of the tethered ligand. **Objectives:** This study had two main objectives, namely to achieve an improved method to isolate PA-BJ using less chromatographic steps than the previously published method and to analyze the global effects of PA-BJ on platelets using proteomic approaches. **Methods**: For the isolation of PA-BJ, the venom was submitted to cation-exchange chromatography on a HiPrep 16/10 SP XL column using an Äkta purifier FPLC system, and bound proteins were eluted with an increasing gradient of NaCl. Fractions that contained PA-BJ as analyzed by SDS-PAGE and activity on platelets were further chromatographed on a Mono S HR 5/5 column resulting in the elution of two groups of chromatographically distinct forms of PA-BJ which were re-chromatographed on the Mono S column. To test the effects of PA-BJ, platelets were washed with Tyrode solution, and the suspensions of washed platelets were incubated with 10 nM thrombin, 127 nM PA-BJ or without any agonist (control sample) at 37°C for 8 min in cuvettes, and the aggregation was recorded using an aggregometer (490 Four Channels, Chronolog). The effects of agonists on platelet proteomes were evaluated by two-dimensional gel electrophoresis (2DE). Platelet proteins (250 μg) were separated in the first dimension by isoelectric focusing using a pH gradient of 4-7 and in the second dimension on 12% SDS-polyacrylamide gels. The gels were stained with colloidal Coomassie blue, analyzed by the software Image Master 2D Platinum (GE Healthcare), and some spots were identified by mass spectrometry and database search. **Results and Discussion:** The previously published method for the isolation of PA-BJ involved five isolation steps and resulted in the isolation of one PA-BJ form. Here, using three chromatographic steps we isolated six chromatographically distinct forms of PA-BJ differing slightly in isoelectric points as observed by their elution profiles. The presence of forms of this serine proteinase in the venom is likely due to different degrees of glycosylation that can be detected by their slightly distinct mobilities in SDS-PAGE indicating different molecular masses. PA-BJ had significant effects on washed platelets, inducing up to 70% aggregation at a concentration of 127 nM. Differential spots were observed on the 2D-gel of PA-BJ-activated platelets which were not present on the control and thrombin 2D-gels. These spots were identified as cytoplasmic beta-actin. Other protein spots common to the three 2D-gels were also identified.

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9.38 Evaluation of the adjuvant activity of dioctadecydimethyl ammonium bromide (DODAB) on mouse humoral immune response to crotoxin isolated from *Crotalus durissus terrificus* venom

Ranéia PA[1], Silva SR[2], Lincopan N[3], Carmona-Ribeiro AM[3], Faquim-Mauro EL[1]
[1]Laboratório de Imunopatologia, Instituto Butantan, SP, Brasil; [2]Departamento de Imunologia, Instituto de Ciências Biomédicas and [3] Instituto de Química, Universidade de São Paulo, SP, Brasil

Introduction: Crotoxin (CTX) is the main toxic component of the *Crotalus durissus terrificus* venom (C.d.t.), a rattlesnake of South America. Furthermore, the CTX exerts an inhibitory effect on the immune system. In contrast, substances called adjuvant have the ability to stimulate the innate immunity and consequently to promote robust adaptive immunity to distinct antigens. Dioctadecydimethl ammonium bromide (DODAB) has been reported as a novel and potent adjuvant. **Objective:** We evaluated in mice the adjuvant activity of DODAB on humoral immune response against CTX isolated from C.d.t. venom. **Methods:** CTX was purified from C.d.t. venom by anion-exchange chromatography in FPLC system. Afterward, CTX adsorption onto DODAB was quantitatively determined for establishing ranges of maximal adsorption by ZetaPlus-ZetaPotential Analyzer. Next, BAB/c mice were immunized s.c. with CTX (5 μg/animal)/DODAB (2 mM) or CTX (5 μg/animal) adsorbed onto alum (1 mg/animal). After 14, 21 and 28 days, the mice were bled and on day 21 received the antigenic booster of CTX (5 μg). Anti-CTX antibody production was measured by ELISA. **Results and Discussion:** On days 14 and 21 after immunization, higher anti-CTX IgG1 and IgG2a antibody production in serum from mice immunized with CTX/DODAB was demonstrated compared with the CTX-alum immunized mice. In the secondary antibody response (28 days after immunization), the specific anti-CTX IgG1 and IgG2a antibody production was 7 and 2 times higher in serum from CXT/DODAB immunized mice compared to CTX/alum immunized mice, respectively. The adsorption of CTX onto DODAB resulted in a stable complex, which induced the activation of the immune system resulting in high levels of specific antibodies. Furthermore, DODAB was more efficient in promoting anti-CTX antibody response compared with alum adjuvant.

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9.39 Genotoxicity of textile dye Remazol Orange 3R in snail *Biomphalaria glabrata* (Say 1818) using the alkaline comet assay

Rossi RKE[1], Pinheiro AS[1,2], Grazeffe V[1], Kawano T[1], Nakano E[1]
[1]Laboratório de Parasitologia, Instituto Butantan, SP, Brasil; [2]Centro de Tecnologia das Radiações, Instituto de Pesquisas Energéticas e Nucleares, Comissão Nacional de Energia Nuclear, SP, Brasil

Introduction: Synthetic dyes represent an important group of xenobiotic chemicals. Among textile dyes, approximately 10-15 % of world production is lost to the environment during synthesis, processing and application. The presence of these compounds in aqueous ecosystems may cause negative impacts, deteriorating water quality. Furthermore, these dyes and/or their degradation products may be toxic and mutagenic. The single cell gel eletrophoresis, or comet assay, has been the major tool in the evaluation of genotoxic effects in genetic toxicology. **Objective:** The aim of this work was to evaluate the genotoxic effects of synthetic textile dye Remazol Orange 3R in hemocytes of *B. glabrata* in the alkaline single gel eletrophoresis assay. **Methods:** Snails were exposed for 7 days to four different dye concentrations (100 mg/L, 500 mg/L, 1000 mg/L and 2000 mg/L) and the solutions were renewed every 48 h. To perform the comet assay, about 100 µL of hemolymph containing hemocytes of each animal were collected by pedal stimulus, and then, added to 500 µL of LMP agarose 0.5% (w/v), mixed, and placed on two microscope slides pre-coated with NMP agarose 1.5% (w/v). The slides were immersed in a lysis solution (2.5 M NaCl, 100 mM EDTA, 10 mM Tris, 1% Triton X-100 and 20% DMSO, pH 10.0), kept at 4 °C and protected from the light for at least 12 h. They were subsequently incubated in freshly prepared alkaline buffer (300 mM NaOH and 200 mM EDTA, pH>13) for 10 min for DNA unwinding. Electrophoresis (30 min at 300 mA and 23 V (0.74 V/cm) was performed in the same buffer. After electrophoresis, the slides were neutralized in 400 mM Tris-HCl (pH 7.5) and fixed for 10 min in alcohol. Prior to examination, the slides were stained with 20 µg/ml ethidium bromide, and 100 cells per slide (200 per animal) were analysed using a Zeiss Axioplan epifluorescence microscope. The extent of the DNA damage was determined by visual anaysis. **Results and Discussion:** DNA damage was measured as percent number of comets (classes 1, 2 and 3) and normal cells (class 0). There was no increase in DNA migration with any of the concentrations. The trypan blue exclusion test showed no cytotoxicity for all the concentrations tested. Further studies with the hydrolysed form of the dye, the form found in industrial effluents, will give important information on the risks of environmental exposure.

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9.40 Efficacy of Vaxcine as an oral adjuvant for a conjugated vaccine against O111 polysaccharide

Ruela AC[1], New RRC[2], Andrade GR[1], Domingos MO[1]
[1]Laboratório de Bacteriologia, Instituto Butantan, SP, Brasil; [2]Proxima Concepts, London, United Kingdom

Introduction: In order for an oral vaccine to be effective, it is very important that the antigens employed be delivered in a highly efficient carrier system. Vaxcine is an oil-based formulation which has been used successfully as an oral antigen carrier, and whose components are all approved for human use. However, its ability to generate an immune response against polysaccharide antigens via the oral route has never been tested. **Objective**: The objective of this work was to determine whether an O111 polysaccharide conjugated vaccine incorporated in Vaxcine is able to induce an IgA response in the mucosa and in the blood against the polysaccharide. **Methods**: Balb/c female mice (6-8 years old) were immunized orally twice with an O111-LTB conjugated vaccine incorporated in Vaxcine as an adjuvant and delivery system. One day before, and 7 and 21 days after immunization, stool and blood samples were collected to determine by ELISA technique the presence of IgA and IgG antibodies against O111 polysaccharide. **Results and Discussion**: The results showed that incorporation of the conjugated vaccine in Vaxcine protected the conjugate against degradation during its passage through the digestive tract and also increased the antibody responses against the polysaccharide. The results obtained in this work indicate that the Vaxcine formulation has the potential to be used as an oral delivery system and as an adjuvant for conjugated vaccines containing polysaccharide antigens.

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9.41 Distribution of mygalomorph spiders at different altitudes in the Serra do Japi, São Paulo State, Brazil

Sanfilippo RA[1], Nagahama RH[1], Fukushima CS[1,2] , Bertani R[1]
[1]Diretoria Técnica, Instituto Butantan, SP, Brasil; [2]Departamento de Zoologia, Instituto de Biociências, Universidade de São Paulo, SP, Brasil

Introduction: Mygalomorph spiders, known in Brazil as "aranhas-caranguejeiras," is a diverse taxon having 2,628 species distributed in all continents, except Antarctica. Fifteen families are recognized; 11 have representatives in Brazil and 8 in the state of São Paulo: Actinopodidae, Barychelidae, Ctenizidae, Cyrtaucheniidae, Dipluridae, Idiopidae, Nemesiidae and Theraphosidae. Little is known of their biology and ecology. Habits are rarely recorded and almost nothing is known about the influence of altitude in the distribution of mygalomorphs. **Objectives**: We are making an inventory of mygalomorph species that occur at three different altitudes in the Serra do Japi, Jundiaí, state of São Paulo. The faunal composition will be compared between the sampled areas. **Methods:** The collections were carried out for a week in each season for a period of one year. To date, two expeditions were conducted: in the summer and the autumn. The specimens were sampled in two areas at each altitude of roughtly 800 m, 1,000m and 1,200m, totaling 6 areas. Four collecting methods were used in all areas: 50 pitfall traps with preservative medium; four diurnal one hour samplings; nocturnal collection in four transects of 60 m^2 and one 1 m^2 of litter sample. **Results and Discussion:** After two expeditions, a total of 147 mygalomorph individuals consisting of 49 adult males and 98 females or immatures were collected. Four families were recorded and nine species belonging to eight genera were identified: Dipluridae, 1 species, 7 individuals; Idiopidae, 2 species, 32 individuals; Nemesiidae, 4 species, 104 individuals; Theraphosidae, 2 species, 4 individuals. The majority of the individuals sampled belong to the Nemesiidae (70.14%) and the Idiopidae (22.22%). The collecting method that resulted in the capture of the highest number of individuals was the pitfall traps (135 specimens collected or 92.2%). Comparions among the samples of each altitude will be made after the conclusion of the expeditions.

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9.42 Immunohistochemical expression of collagen IV, fibronectin and laminin in actinic cheilitis and human lip squamous cell carcinoma

Silva TJ[1], Martins MT[2], Lourenço SV[2], Menta MS[3], Neves AC[1]
[1]Laboratório de Biologia Celular, Instituto Butantan, SP, Brasil; [2] Faculdade de Odontologia and [3] Faculdade de Medicina, Universidade de São Paulo, SP, Brasil

Introduction: Actinic cheilitis is a pathologic condition affecting mainly the lower lip and it is caused by chronic and excessive exposure to the ultraviolet radiation of sunlight. Actinic cheilitis can also develop into squamous cell carcinoma of the lip. The basement membrane (BM) is a ubiquitous extracelular matrix that separates organ parenchymal cells from the interstitial stroma. Profound changes occur in the distribution and quantity of the epithelial BM during the transition from benign to invasive carcinoma. **Objectives**: To evaluate by immunohistochemical technique the expression and distribution of collagen IV, fibronectin and laminin in actinic cheilits and human lip squamous cell carcinoma at different histological grades. **Methods:** Paraffin-embedded tissues of actinic cheilitis and lip squamous cell carcinoma provided by Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo were submitted to immunohistochemistry for collagen IV (Clone CIV 22, Dako:), fibronectin (Clone: A0245, Dako) and laminin (polyclonal, Dako). Slides were examined by light microscopy. **Results and Discussion**: The majority of cases of actinic cheilitis showed no expression of collagen IV, fibronectin and laminin in epithelial BM, and immunoexpression of these proteins was seen only in endothelial BM. Slides of superficially and invasive squamous cell carcinoma showed immunostaining for these proteins in epithelial and endothelial BM. However, in some cases there was a lack of immunoexpression in small areas of tumor islands and strands, but it was not possible to define a pattern of staining for these proteins. The lack of immunostaining observed in actinic cheilitis is probably due to abnormal elastotic material seen in the dermis, a process called solar elastosis, which is caused by sun exposure.

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9.43 Monosodium glutamate effects on melatonin synthesis

Teixeira JP[1], Buonfiglio DC[2], Amaral FG[2], Cipolla-Neto J[2], Afeche SC[1]
[1]Laboratório de Farmacologia, Instituto Butantan, SP, Brasil; [2]Departmento de Fisiologia e Biofísica, Instituto de Ciências Biomédicas, Universidade de São Paulo, SP, Brasil

Introduction: The pineal gland synthesizes melatonin during the night, which participates in the internal temporal organization. Melatonin has a signaling role, indicating night and day by its presence and absence, respectively, in body fluids. There are other sites of melatonin synthesis, such as the retina and gastrointestinal tract. Monosodium glutamate (MSG), when administered neonatally, induces a metabolic syndrome due to a hypothalamic lesion, in particular the arcuate nucleus, characterized by obesity, growth reduction, insulin resistance and type 2 diabetes. Moreover, hippocampal neurons from CA1 region are lesioned and the amacrine, ganglion and bipolar cells of the retina are degenerated. **Objectives:** The aim of the present work was to analyze the nocturnal melatonin profile in the pineal glands and retinas of male adult Wistar rats injected with monosodium glutamate (MSG) during the neonatal period. **Methods:** MSG was administered subcutaneously at a dose of 4 mg/g of body weight in Wistar rats just after birth for 8 days. Animals were sacrificed at the age of 3 months, during the dark period. **Results and Discussion:** The results showed that with 3 months of age, the MSG rats were obese and, according to the literature data, probably with insulin resistance. In fact, glycemia was greater in MSG rats than in controls, suggesting the presence of type 2 diabetes. Melatonin synthesis in the pineal gland was elevated in MSG rats only in the ZT=14.5 and did not differ in the retina. The alterations observed may be a consequence of central lesions or peripheral disturbances that result from them. The elevation of pineal melatonin synthesis may be due to a hyperinsulinemia or elevated corticosterone levels. In the retina, it is possible that there was no lesion in the photoreceptor cells responsible for melatonin synthesis.

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9.44 The suppression of acute inflammation is mediated by A*scaris suum*-primed regulatory T cells

Titz TO, Araujo CAA, Macedo-Soares MF
Laboratório de Imunopatologia, Instituto Butantan, SP, Brasil

Introduction: Helminth parasites stimulate regulatory mechanisms that are associated with suppression of host immune responses. We have recently demonstrated that *Ascaris suum* experimental infection downregulates pulmonary allergic inflammation and LPS-induced acute inflammation. **Objective:** We investigated the effect of *Ascaris suum*-primed leukocytes, $TCD4^+CD25^-$ and $TCD4^+CD25^+$ cells, from mouse lymph nodes and the immunomodulatory cytokine IL-10 on the LPS-induced inflammation. **Methods:** C57BL/6 wild type and IL-10 knockout mice were infected by intragastric route with 2,500 *Ascaris suum* embryonated eggs. On day 8 after the infection, the mesenteric lymph nodes were removed in order to isolate $TCD4^+CD25^-$ and $TCD4^+CD25^+$ cells by magnetic activated-cell sorter (MACS). The cells were adoptively transferred to recipient mice by intravenous route, and on day 12 the air pouches made with sterile air on the back of the recipient mice were LPS-injected. Control group received only PBS in their pouches. Three hours after the stimulation, the air pouch exsudates were recovered for the measurement of inflammatory cytokine levels by ELISA and quantification of cellular migration. **Results and Discussion:** Our results demonstrated that adoptive transfer of $CD4^+CD25^+$ Treg cells from WT infected mice, but not from IL-10-deficient donor mice, resulted in a decrease of leukocyte recruitment and inflammatory cytokine levels (IL-1β, IL-6 and TNF-β), but an increase in IL-10 and TGF-β cytokines. On the contrary, transfer of $TCD4^+CD25^-$ cells did not show suppression of leukocyte influx or cytokine levels into the air pouches. We showed that the suppression of the LPS-induced inflammation was only observed after adoptive transfer of $CD4^+CD25^+$ Treg cells from *Ascaris suum*–infected WT mice, suggesting that primed-Tcells producing IL-10 are crucial for the suppressive effect. Cells from knockout mice or non-infected mice had no effect on LPS-induced inflammation. In conclusion, our results demonstrated that *Ascaris suum* infection primes $CD4^+CD25^+$Treg cells which play an important role in the suppression of LPS-induced inflammation. Furthermore, IL-10 was implicated as an effector molecule of Treg cell-mediated suppression.

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9.45 E6 gene cloning of bovine papillomavirus type 2 in *Escherichia coli*

Tofanello WS[1], Mazzuchelli-de-Souza J[1], Ruiz RM[1,2], Sircili MP[2], Beçak W[1], Stocco RC[1], Carvalho RF[1]

[1]Laboratório de Genética and [2]Laboratório de Bacteriologia, Instituto Butantan, SP, Brasil

Introduction: Bovine papillomatosis is an infectious disease showing lesions in skin and/or mucosa in mamals, including humans. These lesions cause dramatic hazards for milk and meat production. Papillomaviruses are double-strand DNA viruses and the related lesions can regress or progress to cancer. In cattle, BPV-2 causes cutaneous fibropapillomas, bladder cancer and its clinical manifestation, enzootic hematuria. **Objectives:** Cloning of E6 BPV-2 gene in a bacterial system where expression and purification of E6 protein could allow the production of biotechnological inputs. **Methods:** In this work, specific primers were designed and the polymerase chain reaction (PCR) was used to amplify the E6 BPV-2 gene, using the genomic DNA of BPV-2 previously cloned in pAT153 vector as template. The PCR product was sequenced, analyzed to confirm E6 BPV-2 sequence and cloned into pCR4-TOPO vector. The recombinant plasmid was transformed into *E. coli* JM 109 by heat shock method. Colonies with recombinant plasmid were selected for ampicillin resistance and cultured for plasmid purification. Recombinant plasmid preparations were used for subcloning into pET 28a expression vector for *E. coli* BL21 strain transformation. **Results and Discussion:** E6 BPV-2 gene was successfully cloned in transformed *E. coli* cells, allowing viral protein expression and its purification by chromatographic affinity columns. Therefore, papillomavirus antigen production in prokaryotic system such as *E. coli* could be useful for further developments in diagnostic and vaccine research.

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9.46 Mutation spectra induced by 5-aminolevulinic acid in bacterial system

Yoneta SS[1,2], Onuki J[2]
[1]Faculdade de Saúde Pública, Universidade de São Paulo, SP, Brasil; [2]Laboratório de Bioquímica e Biofísica, Instituto Butantan, SP, Brasil

Introduction: 5-Aminolevulinic acid (ALA) is a heme precursor accumulated both in inborn and acquired hepatic porphyria, such as acute intermittent porphyria (AIP), tyrosinosis and lead overload. Increased hepatocellular carcinoma (HCC) incidence in patients with AIP has been reported by several authors and has been hypothesized to be related to ALA and its derivatives. *In vitro*, ALA undergoes enolization and subsequent metal-catalyzed aerobic oxidation yielding reactive oxygen species, which can cause oxidative damage to DNA and proteins which could be involved in the initiation and promotion of cancer. We demonstrated that ALA is able to cause single strand breaks in plasmid and calf thymus DNA *in vitro,* and to increase the steady state level of 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodGuo) in liver DNA of ALA-treated rats. Besides, ALA was also shown to promote the formation of 2,6-diamino-4-hydroxy-5-formamidopyrimidine, 8-oxo-7,8-dihydro-2'-deoxyadenosine, 5-hydroxy-2'-deoxycytidine (5-OHdCyd), 5-hydroxy-2'-deoxyuridine, cytosine glycols, 5-(hydroxymethyl)uracil and 5-formyluracil in isolated calf thymus DNA. The level of 5-HdCyd, which was also measured in liver DNA, was found to increase upon treatment of rats with ALA. Another mechanism that could be involved in the triggering of cancer is the alkylating property of the final oxidation product of ALA, 4,5-dioxovaleric acid (DOVA). We established that DOVA is an efficient alkylating agent of the guanine moieties within nucleoside and isolated DNA. Diastereoisomeric adducts were produced through the formation of a Schiff's base involving the N^2 -amino group of 2'-deoxyguanosine and the ketone function of DOVA. ALA and DOVA were shown to be mutagenic in Salmonella/microsome mutagenicity assay and Chromotest. **Objectives:** The main objective of this work was to determine the mutation spectra promoted by ALA in a bacterial system, contributing to the elucidation of the mechanism involved in DNA damage promoted by ALA. **Methods:** Competent *Escherichia coli* DH10b strain was transformed with plasmid pAC189, which contains the *supF* gene. The plasmid DNA was extracted and treated with different concentrations of ALA. MBL50 strain was then transformed with ALA-treated plasmid DNA and plated in selective medium containing X-gal, IPTG or *L*-ara. Survival rate of transformed bacterial colonies was calculated. Mutants were selected, and *supF* gene was sequenced to obtain the mutation spectra. **Results and Discussion:** An ALA dose-dependent decreased colony survival rate and an increased mutation rate were observed. Preliminary results showed that the predominant mutagenic event was base substitution involving G:C pair (71.4 %), followed by G:C-A:T transition (33.3%), G:C-C:G (28.6 %) transversion and A:T-G:C (14.3 %) transition. Further analysis is under investigation to obtain more mutants and determine wide mutation spectra and possible hot-spots. These results showed the possible mutagenic events involved in the mechanism of DNA damage induced by ALA and its derivatives, which could act as endogenous weapons, and are consistent with the hypothesis that these compounds could be associated with deleterious processes involved in the development of HCC in symptomatic AIP patients.

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