11. PIBIC Program

XII Reunião Científica Anual do Instituto Butantan
01 a 03 de Dezembro de 2010, São Paulo, SP

11.01 Maternal exposure of Wistar rats to the venom of the *Tityus bahiensis* during lactation and its effects on the offspring.

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Introduction: Previous studies with the venom of the scorpion *Tityus bahiensis* demonstrated that it causes deleterious effects in the offspring when injected into rats during pregnancy. There are no studies in the literature demonstrating if there are some effects on the offspring when the venom is inoculated during lactation. **Objectives:** The aim of this work was to evaluate the effects of the injection of *T. bahiensis* venom during lactation on the offspring. **Methods:** The dose of venom was 2.5 mg/kg (sc). Pregnant females were separated into control groups injected with saline on the 10th (10C) or 16th postnatal days (16C) and into experimental groups injected with venom on the 10th (10E) or 16th postnatal day (16E). Pups were evaluated for their behavioral development. The parameters measured were: forced swimming, box activity and enriched environment. **Results and Discussion:** The following results were obtained for animals injected on the 10th day and assessed for forced swimming: latency to stop swimming (females: C 3.4±2.9, E 17.4±14.6; males: C 8.3±8.3, E 17.7±22.5) and immobility (females: C 107.8±53.9, E 64.1±12.6; males: C 99.0±47.2, E 69.9±26.4) and For the injection on the 16th day the results were: latency (females: C 13.1±11.6, E 13.7±11.6; males: C 11.9±7.8, E 21.5±8.7) and immobility (females: C 64.9±46.5, E 68.6±57.6, males: C 71.9± 9.6, E 46.1±35.1). The animals injected on the 10th day assessed for box activity gave the following results: total activity (females: C 453.6±61.0, E 586.7±93.3; males: C 232.5±123.7, E 470.0±229.1) and ambulation (females: C 322.3±79.3, E 440.0±81.0; males: C 145.0±59.4, E 328.5±183.1). For the injection on the 16th day the results were: total activity (females: C 554.6±245.5, E 594.3±147.1; males: C 474.5±90.4, E 482.7±30.9) and ambulation (females: C 421.6±189.2, E 442.6±118.9; males: C 330.0±70.2, E 333.0±41.4). The animals injected on the 10th day assessed for enriched environment showed the following results: total activity (females: C 719.0±342.7, E 720.2±28.1; males: C 408.5±123.7, E 556.0±25.4), ambulation (females: C 536.6±315.3, E 549.2±20.7; males: C 309.0±124.4, E 403.0±12.7), and exploratory activity (females: C 130.2±21.4, E 162.6±60.8; males: C 213.00±5.5, E 191.0±25.4). For the injection on the 16th day the results were: total activity (females: C 686.7±116.6, E 671.7±210.9; males: C 579.5±180.0, E 408.5±279.1), ambulation (females: C 524.7±103.6, E 440.2±228.5; males:C 400.2±157.8, E 201.7±170.5), and exploratory activity (females:C 172.8±35.4, E 170.6±32.3; males:C 206.0±24.7, E 165.2±65.6). There were no significant changes in all parameters studied. Thus, no deleterious effect was observed when the venom was injected during lactation, in contrast to the findings obtained when venom inoculation occurs during pregnancy.

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11.02 Antimicrobial susceptibility pattern of enterotoxigenic *Escherichia coli* (ETEC) and enteroaggregative *Escherichia coli* (EAEC) strains.

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Introduction: *Escherichia coli* are facultative anaerobes in the normal intestinal flora; however, pathogenic strains of these bacteria are an important cause of infectious diarrhea. The diarrheagenic *E. coli* pathotypes include *E. coli* enterotoxigenic (ETEC) and *E. coli* enteroaggregative (EAEC). The emergence and spread of antimicrobial resistance in *E. coli* have been well documented as a serious public health problem worldwide, making it necessary to perform tests to assess drug sensitivity. For the treatment of some bacterial infections, antibiotics that are used such as the β-lactams can be degraded by the extended-spectrum beta-lactamases (ESBLs). **Objectives:** This study was carried out to determine the antimicrobial susceptibility patterns of ETEC and EAEC strains isolated from infantile diarrhea cases in Salvador, Bahia and presumptively to detect ESBL production. **Methods:** The ETEC and EAEC strains (20 strains each) were tested for susceptibility with the Kirby-Bauer disc diffusion method, utilizing Muller-Hinton agar and the following antibiotics: amoxicillin + clavulanic acid (AMC), nalidixic acid (NAL), ampicillin (AMP), cephalothin (CFL), ciprofloxacin (CIP), chloramphenicol (CLO), streptomycin (EST), gentamicin (GEN), sulfamethoxazole + trimethoprim (SUT) and tetracycline (TET). The following antibiotics were used for the presumptive detection of ESBL: cefotaxime (CTX), aztreonam (ATM), ceftazidime (CAZ) and ceftriaxone (CRO). **Results and Discussion:** The ETEC strains showed 100% sensitivity to NAL, AMC, CIP and CLO; resistance to SUT (45%), AMP (40%), TET (25%), EST (20%) and GEN (5%); and intermediate pattern to EST (15%), CFL (10%) and AMP (5%). The EAEC strains displayed 100% sensitivity to NAL, CIP and GEN; resistance to AMP and SUT (55% each), TET (40%), EST (15%), CLO and AMC (10% each), and CFL (5%); and intermediate pattern to EST (25%) and CFL (5%). We found an elevated percentage of strains (45% of ETEC and 50% of EAEC strains) that showed multiple drug resistance, mainly to AMP, EST, SUT and TET. The antibiotics used for detection of ESBL did not detect any strain producing the enzyme. Research on the antimicrobial susceptibility profile of ETEC and EAEC strains has been shown to be lacking. Although *E. coli* strains usually show sensitivity to a broad spectrum of antibiotics, exposure to these substances tends to select resistant organisms. Plasmids encoding β-lactamases in *E. coli* strains are the main form of resistance against β-lactam antibiotics, and its detection is very important, especially in the pediatric area. Routine monitoring of antibiotic resistance provides data for antibiotic therapy and resistance control among the main etiological agents of diarrhea.

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11.03 Effects of trypsin/chymotrypsin inhibitors from *Nephilengys cruentata* on the development of the dengue fever vector *Aedes aegypti*

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Introduction: *Aedes aegypti* is the urban vector of dengue viruses worldwide causing significant morbidity and mortality. *Ae. aegypti* is a mosquito that exploits peridomestic water containers as its larval habitats, and human reservoir hosts that are preferred for blood feeding. Efficient alternative control strategies are still required. Spiders are carnivorous arthropods and insects are their major source of prey. Predators ingest a large quantity of peptidases from their prey which have to be controlled. There are some suggestions in the literature of the presence of peptidase inhibitors in the digestive system of some spider species. Our group has shown that the spider *Nephilengys cruentata* contains trypsin/chymotrypsin inhibitors (TCI) in their digestive juice and their hepatopancreas. These inhibitors are very efficient in inhibiting digestive trypsins and chymotrypsins from insects. **Objectives:** The aims of this study were to isolate TCI from *N. cruentata* hepatopancreas, to determine the dissociation constant of inhibitor/chymotrypsin complex, and to observe *in vivo* effects of these inhibitors on *Ae. aegypti* larval development. **Methods:** Hepatopancreas from adult females from *Nephilengys cruentata* were isolated by dissection and were homogenized in 0.1 M acetate buffer, pH 3.5, and centrifuged at 13,000 rpm for 30 min. The soluble portion was then boiled for 5 min. Samples were centrifuged again and the soluble portion was used as inhibitor sample to be applied onto a Superdex G75 column (gel filtration). Fractions able to inhibit insect digestive trypsins were pooled and submitted to anion-exchange chromatography (ResourceQ column). Inhibitory fractions were pooled, lyophilized and applied to a 15% polyacrylamide gel stained with Comassie Blue R. Different concentrations of isolated inhibitor were tested against bovine chymotrypsin, resulting in the determination of the dissociation constant of the inhibitor/chymotrypsin complex. Enriched inhibitory samples were added to the diet of *Ae. aegypti* larvae in order to test the effect of the presence of these inhibitors on larval development. **Results and Discussion:** We observed that *N. cruentata* has at least two important TCIs which are very efficient against insect digestive trypsins and chymotrypsins. We isolated the major TCI form from *N. cruentata* hepatopancreas which is thermal and pH stable and has a molecular mass of 13 kDa. This inhibitor showed a K_D of 67 nM against bovine chymotrypsin. These inhibitors in *Ae. aegypti* larval diet caused a delay in larval development indicating that these molecules may be good candidates in dengue vector control.

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11.04 Allergy to *Loxosceles* spider venom as occupational disease in arachnologists

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Introduction: It is sometimes suggested that spider-bites can cause hypersensitivity reactions. Despite this common notion, reports on allergic reactions from spiders are rare. However, contact with spiders has been reported to cause urticarial reactions, almost exclusively from large spiders from the family *Theraphosidae*. These spiders have large numbers of urticating hairs on their abdomen that can be ejected from their body when they are disturbed. To our knowledge, there are no reports of allergic type reactions occuring following contact with other groups of spiders. Moreover, several workers of the Laboratory of Arthropods of the Butantan Institute have complained of allergic symptoms related to contact with spiders (or its venom) from the genus *Loxosceles*. **Objectives:** The aim of this work was to develop methods to investigate the prevalence and predictors of venom allergy among workers exposed to spiders from the genus *Loxosceles* and to confirm the involvement of IgE-mediated mechanisms in this condition. **Methods:** Initially there will be a detailed study of the work environment to identify all tasks that involve exposure to spiders from the genus *Loxosceles*. Workers will be assessed for venom allergy using questionnaires and immunological tests. The presence of venom sensitization will be determined through quantification of specific IgE (ELISA). Allergens will be studied using the Western blots and inhibition assays. **Results and Discussion:** Based on the study of the work environment, we developed a questionnaire containing questions regarding personal history of allergy, spider stings, and contact (oral or ocular) with spider venom, as well as work history (length of employment and specific work tasks) and work-related symptoms. We observed that the job of arachnologists entails specific tasks, including spider cage cleaning, spider feeding, spider venom extraction, the handling of spider venom, spider room cleaning and the handling of spiders for identification. Estimates of the exposure times to spiders or spider venom will be derived from the following questions: ''How many days per week do you perform [the specific task]?''; and ''For how many years have you been performing [the specific task]?'' The frequency of exposure to each specific task will be reported as a continuous variable, in days per year. To assess the influence of each task at different frequency levels, an exposure time index will be calculated by multiplying the years of exposure by the frequency of exposure to each specific task. Our next step is to apply the questionnaires, collect blood samples and perform laboratory tests.

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11.05 *Nephilengys cruentata* hemolymph as source of metallopeptidase inhibitors

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Introduction: Notwithstanding the fact that peptidases are present in all biological processes and correspond to 2% of all genes, these enzymes must be strictly controlled. During evolution, two strategies were selected to diminish proteolytic activity: the synthesis of peptidases as zymogens and specific inhibitors. The discovery of new inhibitors is important to control undesired peptidases as observed in diseases, such as cancer and rheumatism. *N. cruentata* showed two classes of proteolytic enzymes involved in protein digestion: a cathepsin L-like enzyme and a metallopeptidase: an astacin-like enzyme (NcAst) already isolated by our group. There is some evidence in the literature of the presence of metallopeptidase inhibitors in the hemolymph of crustaceans and spiders. The isolated astacin from *N.cruentata* was efficiently inhibited by *N.cruentata* hemolymph samples. However, in order to isolate this inhibitor we needed to establish sensitive assays to NcAst. **Objectives:** The aim of this study was to establish a sensitive method to determine NcAst, and to isolate a NcAst inhibitor from *N.cruentata* inhibitor. **Methods:** *N.cruentata* adult females were collected, immobilized and dissected. Hemolymph was recovered with the help of a micropipette and added to a 10 mM sodium cacodylate and calcium chloride solution. Samples were homogenized with a pellet pestle and submitted to gel filtration on a Superdex G75 column. Hepatopancreas homogenate samples and isolated astacin were used as astacin activity source, and the synthesized Abz-GPKRAPWV-K(Dn)-OH (ASub) was used as substrate. Inhibitory activity was pooled and samples were applied to a 7.5% polyacrylamide gel and submitted to electrophoresis. Gels were silver stained. The kinetic parameters of the isolated astacin to the new substrate were determined using different substrate concentrations. **Results and Discussion:** Previously isolated NcAst was characterized using ASub as substrate. The purification steps used in astacin isolation were repeated and followed with casein-FITC (the former substrate) and with the new one. There was no alteration of astacin isolation indicating that this substrate is a specific substrate to this enzyme. The isolated astacin showed a Km of 45 μM ± 5.5 μM for ASub. The presence of hemolymph sample in astacin activity assay indicated an inhibition of approximately 70%. Hemolymph samples were applied to a SuperdexG75 column. The estimated molecular mass of this inhibitor in gel filtration was 140 kDa. SDS-PAGE of the inhibitory pooled fractions indicated an enriched protein band of 70 kDa suggesting that, under native conditions, this inhibitor dimerizes. This inhibitor will be tested with astacin from *Loxosceles gaucha* venom and with the recombinant astacin from *Astacus astacus*.

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

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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. 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 in the 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:** We observed an ALA dose-dependent decrease in colony survival rate and an increased mutation rate. The results showed deletions of bases, transitions and transversions. Further analysis is under investigation to obtain more mutants and determine the 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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11.07 Search for new human peptidase inhibitors present in the low molecular weight fraction from the venom of *Tityus serrulatus*

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Introduction: Scorpions are chelicerates that had a great evolutionary success and are therefore widely distributed throughout the world. Because of this, it is of medical importance as the major cause of human animal poisoning. In Brazil, the main species is *Tityus serrulatus*, the yellow scorpion, and, until now, little information about both venom's components and their mechanism of action are available. **Objectives:** The aim of this work was to investigate the action of the peptide fractions present in *T. serrulatus* venom (TsV) on human peptidase activities, such as elastase, thrombin and neurolysin. **Methods:** In the first step, TsV (2 mg/mL) was fractionated by a gel-filtration chromatography using a Superose 12 column, which resulted in 11 new fractions. These fractions (F0-F10) were studied with the cited proteases through the use of FRET substrates in a spectrofluorimeter. The fractions that showed inhibitory or activator activities (F4, F6 and F10) were subjected to HPLC purification steps and the peaks were collected manually for later proteolytic assays **Results and Discussion:** It was observed that certain fractions obtained from the first purification step were able to reduce the activity of proteases, such as the F4's inhibitory action on thrombin (40%) and neurolysin (75%), or even increase proteolysis, as with the effect of F10 on elastase (220%). At this moment, we focused on the analysis involving neurolysin, considering its importance for CNS and good inhibitory potential by TsV fractions. F4 was selected and submitted to the HPLC-RP purification, obtaining 14 new peaks, which were tested with neurolysin. Of this total, 3 fractions showed a good inhibitory potential and the analyses of primary sequences of these peptides revealed some sequences already described in literature and other new peptides. The methodology used here led us to uncover a new fragment from the known peptide named hypotensin I (AEIDFSGIPEDIIKQIKET) and the PAPE fragment (AEPAAPAAAAEPEP). In addition, the results showed new interesting molecules similar to sequences in anemone venom proteins, which could suggest a positive Darwinian selection in the evolution of scorpion venom. The observation of neurolysin activity inhibition and the presence of hypotensins are possibly related to the strong pressure drop observed in the post-envenomation symptoms and it may help to further studies of the venom of *T. serrulatus* and its mechanism of action.

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11.08 Taxonomic characterization of *Oxyrhopus guibei* (Serpentes, Dipsadidae, Xenodontinae)

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Introduction: Snakes of the genus *Oxyrhopus* belong to the tribe Pseudoboini, subfamily Xenodontinae. The tribe Pseudoboini comprises nine genera, whose representatives are found from southern Mexico to Argentina. The genus *Oxyrhopus* is currently composed of 14 species distributed over almost all Latin America regions. Snakes of this genus are opistoglyphous and oviparous and have elliptical pupils, strong sexual dimorphism, a pair of apical pits on dorsal scales, single cloacal plate, and divided subcaldals. Its species show predominantly nocturnal activity and their diet is composed basically of lizards and rodents. *Oxyrhopus guibei* is a common species that occurs in western to southeastern Brazil, including areas from the Brazilian state of Paraná, Paraguay, Bolivia, and Argentina. *Oxyrhopus trigeminus guibei* was described based on 13 specimens. The holotype is an adult female from Londrina, Paraná. To date, it is possible that the type series has been lost during the recent fire accident that destroyed most of the Herpetological Collection of the Institute Butantan. The authors that described *O. trigeminus guibei* differentiated it from *O. t. trigeminus* on the basis of the number of subcaudals 59-91 (vs. 53-81 in *O. t. trigeminus*), black snout (vs. snout scattered with white dots), and triads overlapping the belly (vs. belly immaculate or with black cross lines). It was later elevated to specific rank based on hemipenial morphology, color pattern, and pholidosis characters. Currently, the species is still insufficiently diagnosed due to overlapping nature of meristic and color pattern characters. **Objectives:** The main aim of this study was to provide a robust diagnosis for *Oxyrhopus guibei* with respect to other congeners, accurately delimiting their area of distribution. Another objective was to identify the areas where it occurs parapatric or sympatric with *O. trigeminus*. **Methods:** Terminology and nomenclature employed in the study followed the traditional use in snake systematics. We examined specimens of the Instituto Butantan, but additional samples from other institutions should be analyzed in order to finish the study. We took data from external morphology (meristic, morphometric, and color pattern characters) along most of the range of distribution of the species. **Results and Discussion:** To date, we have analyzed a hundred specimens of *Oxyrhopus guibei*, as well as additional specimens of *O. trigeminus* and *O. melanogenys* for comparative purposes. *Oxyrhopus guibei* is distinguished from the other species of the genus by the following combination of characters: 19/19/17 dorsal scale rows; ventral scales 197-217 ($\bar{x}$=207) for females and 186-199 ($\bar{x}$=192) for males; subcaudals 59-82 ($\bar{x}$=73) for females and 67-91 ($\bar{x}$=78) for males; eight supralabials; ten infralabials; 2+3 temporals; single preocular; two postoculars; divided nasal; black snout; and triads overlapping the belly.

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11.09 Isolation of prothrombin from *Bothrops jararaca* plasma: preliminary results

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Introduction: Prothrombin is the most abundant of the vitamin K-dependent blood clotting proteins, circulating at plasma concentrations between 1 and 2 μM. This factor is a plasma zymogen that is converted to thrombin – an essential enzyme that converts fibrinogen to fibrin, the main structural component of the clot. **Objectives:** The aim of this study was to purify *Bothrops jararaca* (*B. jararaca*) prothrombin and to compare it with prothrombin of humans and other animals. **Methods:** Prothrombin was partially purified from snake plasma through HiTrap DEAE Fast Flow chromatography, followed by affinity cromatography on HiTrap Cu^{2+}Chelating HP and HiTrap Blue HP columns. Along all the purification steps, protein concentration was determined by absorbance at A_{280}. Purification steps were analyzed by SDS-PAGE. Amidolytic thrombin activity was measured using chromogenic substrate (S-2238) after prothrombin activation by *Oxyuranus scutellatus scutellatus* venom. **Results and Discussion:** Our results indicate the presence of prothrombin after HiTrap DEAE Fast Flow and HiTrap Cu^{2+}Chelating HP chromatographies. However, after these purification steps, the protein was not pure and the activity was low. The perspectives for this work are to improve the purification process in order to get higher purity protein and to compare it biologically and biochemically to prothrombin of other animals.

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11.10 Effect of crotoxin on secretory activity of peritoneal macrophages co-cultivated with tumor cells. Involvement of formyl peptide receptors

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Introduction: Crotoxin (CTX) inhibits tumor growth and modulates the function of macrophages. Despite this evidence, the contribution of macrophage inhibition to the decrease in tumor growth, caused by CTX, was not determined yet. Macrophages provide a defense mechanism against tumor cells and two distinct polarization states, M1 and M2, have been described for these cells. In the beginning of tumor progression, M1 macrophages release reactive nitrogen/oxygen intermediates and the cytokines TNF-α, IL-1β and IL-6. In contrast, during tumor development, the release of these mediators by tumor-associated macrophages (M2 cells) is inhibited, contributing to tumor development. **Objectives:** In the present study, the effect of CTX on the activity (nitric oxide-NO) of macrophages co-cultivated with LLC WRC 256 tumor cells (Ethical Committee For Animal Research of Butantan Institute, No. 631/09) was evaluated. **Methods:** In *in vitro* assays, the effect of CTX on nitric oxide-NO production by macrophages co-cultivated with LLC WRC 256 tumor cells was investigated. Macrophages were obtained from peritoneal cavity and cells ($2x10^5$) were incubated with CTX (0.3 μg/mL) for 2 h at 37°C. After this time, the macrophages were co-cultivated in the presence of LLC WRC 256 tumor cells ($2x10^4$), previously plated in 96-well culture plates. After 48 h, at 37°C, in a humidified atmosphere of 5% CO_2 in air, the effect of CTX on the production nitric oxide-NO was evaluated. After this period, cell proliferation was measured by MTT assay. The involvement of formyl peptide receptors with the stimulatory effect of CTX on the production nitric oxide-NO by macrophages was evaluated using Boc2, a selective antagonist of formyl peptide receptors. **Results and Discussion:** The results showed that macrophages previously incubated in the presence of CTX and co-cultivated with tumor cells generated a greater quantity of NO (35%) than control cells. Tumor cells co-cultivated with macrophages pre-incubated with CTX showed reduction (25%) of proliferation. Boc-2 reversed the stimulatory effect of CTX on secretory activity of macrophages and the inhibitory effect of these macrophages on tumor cell proliferation. Taken together, the results indicate that CTX modifies the secretory activity of M2 cells, which may contribute to the inhibitory action of the toxin on tumor growth, and activation of formyl peptide receptors seems to play a major role in this effect. These data reinforce the actions of CTX on defence mechanisms and open new perspectives for the development of a new substance with therapeutic properties.

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11.11 Isolation of toxins with high affinity for heparin from *Bothrops cotiara* venom

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Introduction: Heparin is a highly-sulfated glycosaminoglycan released from mast cells and is involved in anti-coagulant and anti-inflammatory processes. We had previously shown that *Bothrops* venoms contain some toxins with high affinity for heparin *in vitro*. **Objectives:** The aim of this study was to isolate toxins with high affinity for heparin from *B. cotiara* venom. **Methods:** *B. cotiara* venom was chromatographed on a heparin-Sepharose column previously equilibrated with 0.1 M ammonium acetate, and proteins were eluted with increasing ammonium acetate concentration and analyzed by SDS-PAGE. The fractions with high affinity for heparin were submitted to reversed-phase HPLC, using a Shim-Pack CLC-C_8 column (250 mm×4.6 mm, particle size 5 µm, Shimadzu). Alternatively, *B. cotiara* venom was chromatographed (FPLC system) on a gel filtration column (Superdex 75 10/300 GL; GE Healthcare). Fractions containing proteins of ~ 30 kDa were further submitted to cation-exchange chromatography (MonoS HR 5/5; GE Healthcare). Protein fractions 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 on a 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. The isolated serine proteinase with affinity for heparin was submitted to the following assays: platelet-aggregating, hemorrhagic, fibrinogenolytic, coagulant, and amidolytic activities. Moreover, the enzyme was tested for its ability to activate coagulation factors II and X. **Results and Discussion:** Visual inspection of the gels indicated few proteins with high affinity for heparin in *B. cotiara* venom (eluted with 2.0 M ammonium acetate from the heparin-Sepharose column), and these did not show gelatinolytic and hemorrhagic activities. Moreover, immunostaining with specific antibodies showed the presence of metalloproteinases and serine proteinases among the proteins with high affinity for heparin. The gel-filtration chromatography of the crude venom followed by cation-exchange chromatography resulted in the isolation of a novel serine proteinase of ~30 kDa with high affinity for heparin. The enzyme showed amidolytic activity on peptide p-nitroanilide substrates, and it is devoid of hemorrhagic, coagulant, platelet-aggregating and fibrinogenolytic activities. Interestingly, the enzyme was able to directly activate coagulation factor II (prothrombin) but not factor X. This is an unusual activity for a snake venom serine proteinase, and we are currently investigating the activity of the enzyme on plasma recalcification time.

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11.12 Neurobehavioral effects of dantrolene in rats

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Introduction: In previous studies in our laboratory, dantrolene, a drug that inhibits the release of intracellular calcium, was used to reverse the effects of a scorpion toxin. In this experiment dantrolene significantly reduced the hippocampal damage caused by the toxin, but when used individually, lesions were observed in the hippocampus of the animals. **Objectives:** Based on this study, we aimed to investigate whether the administration of dantrolene could cause neurobehavioral changes in rats. **Methods:** Forty male Wistar rats (240-260g) were divided into four groups: control group (C) treated with saline (0.9%), experimental group treated with 5.0 mg/kg of dantrolene (D5), experimental group treated with 10.0 mg/kg of dantrolene (D10), and experimental group treated with 15.0 mg/kg of dantrolene (D15). Tests included: box activity, enriched environment, forced swimming and social interaction. **Results and Discussion:** In animals observed in the box activity, there was a reduction in locomotion with the dose of 10.0 mg/kg and 15.0 mg/kg (C: 594.8±61.02; D5: 456.2±47.34; D10: 268.0±46.69*; D15: 288.6±47.55*) and proportionately in general activity (C: 428.8±33.93; D5: 376.9±39.26; D10: 173.0±18.75*; D15: 195.0±38.16*). In the enriched environment, it was observed that locomotion (C: 482.2±53.61; D5: 226.6±29.69*; D10: 191.0±34.38*; D15: 234.2±49.33*) and general activity (C: 638.6±56.61; D5: 341.6±43.07*; D10: 288.0±44.38*; D15: 362.4±75.94*) were also reduced for all the doses, despite that the time (in seconds) spent in the exploratory activity did not change (C: 149.4±21.97; D5: 129.6±45.84; D10: 41.40±17.23; D15: 112.2±26.60). There was no change in the forced swimming, evaluated as latency to stop swimming (C: 6.600±1.030; D5: 8.400±1.288; D10: 6.200±2.107; D15: 8.000±4.171), and time of immobility (C: 163.6±51.07; D5: 223.6±55.47; D10:78.40±23.80; D15: 41.20±11.65). The dose of 5.0 mg/kg caused a reduction in the time spent in social interaction of the animals (C: 128.4±3.745; D5: 43.60±10.46*; D10: 140.6±4.754; D15: 127.2±8.696). We can conclude that the three doses of dantrolene cause behavioral changes in animals, which can be associated with lesions previously observed.

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11.13 *Bothrops jararaca* snakebites in São Paulo State, Brazil: the influence of biological variables

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Introduction: In Brazil, there are about 20,000 accidents with snakes annually, mostly with the *Bothrops* genus (about 90% of all accidents). *Bothrops jararaca* is responsible for almost 93% of all *Bothrops* accidents. **Objectives:** The present work aimed to determine the epidemiological profile of accidents caused by *B. jararaca* and evaluate the interference of biological variables which caused snakebites in São Paulo State. **Methods:** *Bothrops jararaca* specimens that have caused accidents from 1995 to 2010 are preserved at "Coleção Vital Brazil" at Instituto Butantan. All these snakes were dissected and examined. Data related to seasonal activity, mating season, sexual maturity and diet were collected, analyzed statistically and discussed. **Results and Discussion:** Snake stomachs were dissected to check whether or not they had stomach contents. These data revealed that *Bothrops jararaca* male juveniles fed mainly in spring, whereas female juveniles fed mainly in winter. *B. jararaca* male juveniles had 128 identifiable items in their stomachs (110 endothermic and 18 ectothermic prey, 86% and 14%, respectively), whereas female juveniles had 110 identifiable items in their stomachs (98 endothermic and 12 ectothermic prey, 89% and 11%, respectively). Most accidents occurred during the day, between 6 am and 6 pm. However, many accidents occurred during the night period, between 7 pm and midnight. Adult snakes caused more accidents during the day period. Our data show that 71% of the accidents were caused by juveniles, whereas 29% were caused by adults. Adult females caused more accidents than adult males. Males caused more accidents during the fall, whereas females caused more accidents during the summer. The juveniles (male and female) caused more accidents during the spring and summer. Analyzing the female reproductive status of the snakes that caused accidents, reproductive females were found during the summer and pregnant females were found starting at the end of spring. The juveniles were born in the fall. Accident seasonal patterns are different between *B.jararaca* adults and juveniles. However, juveniles caused more accidents than adults. Preliminary analysis shows that juveniles and adult females cause more accidents than juveniles and adult males. Adult males caused more accidents during the fall, whereas adult females caused 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. During the fall, males are looking for females (mating period).

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11.14 Purification and characterization of antimicrobial peptides present in the venom of *Nephilengys cruentata*

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Introduction: The first description of antimicrobial activity in spiders was published in 1989, with one species in China. Since then, many studies involving antimicrobial peptides were performed. More than 1200 antimicrobial peptides have been identified in all living species. In recent years, a large number of these molecules were isolated from insects. Examples of antimicrobial peptides from spiders may be cited: two peptides (lycotoxins I and II) of the venom of the spider *Lycosa carolinensis*, a family of peptides (cupienins) of the spider *Cupiennius salei* and one peptide (gomesin) of hemocytes of *Acanthoscurria gomesiana*. Currently, there is a big problem with respect to the emergence of bacterial strains resistant to conventional treatment with antibiotics. The main reason is due to overuse and inappropriate use for human beings. Increased migration of the population contributes to the spread of these resistant organisms emerging in the world, so there is much interest on the pharmacological application of antimicrobial peptides (AMPs) in the treatment of infections. **Objectives:** The aim of this study was to separate and characterize bioactive molecules with antimicrobial function, previously obtained from the venom of the spider *Nephilengys cruentata* (Araneomorphae, Nephilidae). **Methods:** To obtain the poison, a low-voltage electrical stimulator was used, where the poison was harvested with a pipette and subjected to centrifugation at 14,000x 3 min. The material obtained was concentrated in a vacuum centrifuge (Savant Instruments, Inc.). After centrifugation the material was dissolved in 0.05% trifluoroacetic acid (TFA) and applied in two disposable SEP-PAK C18 columns connected in series, in order to pre-purify antimicrobial peptides. There were three stages of successive elution using different concentrations of acetonitrile (5%, 40% and 80%) in acidified water. The resulting fractions were concentrated in a vacuum centrifuge, resuspended in acidified water and subjected to liquid chromatography (HPLC). The organisms used for testing the presence of antimicrobial activity were the Gram-positive *Micrococcus luteus* A270, Gram-negative *Escherichia coli* SBS363 and yeast *Candida albicans* SBS363 MDM8. **Results and Discussion:** Two fractions eluted at 40% obtained by high performance liquid chromatography showed antimicrobial activity against bacteria *E. coli* and *M. luteus*. When subjected to mass spectrometry ESI-MS type, it was not possible to obtain the masses of the two samples, since they were not pure. A second purification of two samples was then carried out by high performance liquid chromatography. One sample had six fractions, but their antimicrobial activity was lost, probably due to the low concentration of molecules, and the second sample also showed several fractions, where two of them continued to show antimicrobial activity.

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11.15 Studies on the growth of *Neisseria lactamica*

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Introduction: *Neisseria lactamica* and *Neisseria meningitidis* are Gram-negative, diplococcal bacteria which occur in the nasopharynx of humans. *Neisseria meningitidis*, can cause septicemia or meningococcal disease, especially in young infants. Bacterial meningitis remains a serious threat to global health, reaching 500,000 cases a year around the world, with at least 50,000 deaths and at least the equivalent of cases with neurological damage. Serogroup B polysaccharide vaccines fail to induce bactericidal antibodies. An alternative approach to producing a vaccine for serogroup B is the development of an outer membrane vesicle, OMV, vaccine based on the commensal bacteria *Neisseria lactamica,* a closely related species of *N. meningitidis*. During bacterial growth, OMV are constantly being discharged from the surface of the cell. Immunological and epidemiological evidence suggests that carriage of *N. lactamica* contributes to natural immunity against *Neisseria meningitidis*. **Objectives:** The aim of this work was to study the growth kinetics of *Neisseria lactamica* in shaker culture in different growth media and to analyze and compare the yield of OMV and the electrophoretic pattern of major proteins. **Methods:** *N. lactamica* was cultivated on a shaker, at 200 rpm, 36°C for 8-17 h. The culture media tested were: defined culture media MC2LAA and MC with or without the addition of ultrafiltrate yeast extract or enzymatic digested soybean. Biomass was measured by reading optical density at a wavelength of 540 nm, and the yield of OMV was determined by Lowry's method. **Results and Discussion:** Growth was approximately OD_{540} 0.5, 1.7, 2.2 and OMV yield was 15 mg/L; 31 mg/L, 54 mg/L in defined culture media MC2LAA, defined medium with enzymatically digested soybean and defined medium with ultrafiltrate yeast extract, respectively. The electrophoretic pattern was similar in all media. Our results suggest that limiting growth factor of *N. lactamica* is not only due to carbon or nitrogen sources but may be related to the limitation of vitamins present in the yeast extract.

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11.16 Description of the male of *Acanthoscurria rondoniae* Mello-Leitão 1923 and the female of *Acanthoscurria insubtilis* Simon 1892, and new distribution records (Araneae: Mygalomorphae, Theraphosidae)

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Introduction: The genus *Acanthoscurria* Ausserer is represented by 40 species, but several are only known by the holotype male or female. Simon described in 1892 *Acanthoscurria insubtilis* based on a male from San Mateo, Bolívia. The species *Acanthoscurria rondoniae* Mello Leitão, 1923 was described based on a female from Mato Grosso, Brazil. **Objectives:** The aim of this study was to describe the female of *A. insubtilis* and the male of *A. rondoniae*. **Methods:** The material examined is deposited in the collection of the Instituto Butantan, São Paulo, Brazil. Female epigynum was dissected and cleared in lactic acid for observation of internal structures. The drawings were made on a Leica MZ 12.5, with a camera lucida. **Results and Discussion:** Females of *A. insubtilis* resemble *A. theraphosoides* (Dol. in Ausserer, 1871) and *A. rondoniae* by the morphology of the seminal receptacles, with a smaller base involving the seminal receptacles, but can be distinguished by the bulged seminal receptacle without basal constriction and distant one from the other. The male of *Acanthoscurria rondoniae* is close to *A. paulensis* and *A. chacoana* due to the appearance of the embolus with two prolateral keels, one superior and other inferior and presence of a blunt tubercle on the palpal tibia, but can be distinguished by the less developed keels with a well-marked anterior projection of the inferior. The study contributed to the better knowledge of both species, until known only by the holotypes. The distribution range of *A. insubtilis* is enhanced for Brazil: states of Rondônia, Acre, Mato Grosso and Mato Grosso do Sul, and *A. rondoniae* for states of Tocantins, Mato Grosso, Rondônia, Goiás, Minas Gerais and Mato Grosso do Sul (Brazil).

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11.17 Expression of laminin-5 and integrins in actinic cheilitis and lip squamous cell carcinomas

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Introduction: Actinic cheilitis is the initial and incipient stage of squamous cell carcinoma, resulting from excessive and long-term exposure to solar ultraviolet radiation. Malignant tumors are characterized by unrestrained cell growth, invasion in adjacent tissue and their ability to metastasize. Laminin-5 is a heterotrimer containing α3, β3, γ2 chains, a playing important role in development of squamous cell carcinoma and its invasive properties. One of the functions of laminin-5 in epidermal cells is its ability to interact with two major epithelial integrin receptors, α3β1 and α6β4. The expression of laminin receptors, in particular α6β4 integrin, has also been shown to have an important role in squamous cell carcinoma progression. The integrin α6β4 is concentrated in hemidesmosomes during the migration of keratinocytes. The development of carcinomas is associated with disassembly of hemidesmosomes. The domain III γ2 chain of laminin 5 interacts with EGFR to induce phosphorylation of tyrosine in the cytoplasmic domain of integrin β4, leading to disassembly of hemidesmosomes and stimulating cell migration. **Objectives:** The aim of this study was to analyze and evaluate through immunohistochemical techniques the expression and distribution of laminin-5 and integrins β1, β4 and α3 in actinic cheilitis and in lip squamous cell carcinomas. **Methods:** Paraffin blocks of actinic cheilitis, superficially invasive squamous cell carcinoma and invasive squamous cell carcinoma, from Hospital das Clínicas da Faculdade de Medicina da USP, were sectioned. Immunohistochemical reactions to laminin-5 gamma-2 chain, β1, β4 and α3 were carried out, and the slides were examined by light microscopy. **Results and Discussion:** The majority of cases of actinic cheilitis and superficially invasive squamous cell carcinoma showed lack of expression of β1, β4 and α3 integrins in basal and parabasal layers of epithelium. In areas of dysplastic epithelium, loss of expression was also observed in cells of granular and spinous layers. Slides of invasive squamous cell carcinomas showed loss of β1, β4 and α3 immunoexpression in peripheral layers of tumor islands and strands. Cytoplasmic staining for laminin-5 gamma 2 chain was absent in actiic cheilitis cases. All the cases of superficially invasive carcinoma and invasive carcinoma showed laminin 5 gamma 2 chain positivity located in the extracellular matrix and in the peripheral cells of tumor invasive front, but expression was not homogeneous. No cancerous tissues close to invasive areas showed cytoplasmic expression in the epithelial basal layer.

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11.18 Separation and identification of components present in egg wax of *Rhipicephalus sanguineus* and *Amblyomma cajennense* (Acari: Ixodidae)

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Introduction: Most species of ixodid ticks lay between 2,000 to 5,000 eggs, which are viable in the natural environment because of a substance surrounding the eggs which is capable of protecting them from desiccation and microbial attacks, mainly because of its lipid components. **Objectives:** The objective of this study was to evaluate the lipid composition of the wax that surround the eggs of *A. cajennense* and *R. sanguineus* species. **Methods:** Eggs were obtained from female ticks fed on New Zealand rabbits. The females were kept at 27ºC ± 1ºC and 90% ± 5% relative humidity to complete the oviposition period. The egg surface material was extracted based on two previously described protocols from the literature. The crude extracts were analyzed by two different chromatographic methods, TLC (thin layer chromatography) and HPLC (high performance liquid chromatography). **Results and Discussion:** TLC allowed a visual determination of qualitative lipid content of the egg wax, suggesting the presence of cholesterol esters, free fatty acids, cholesterol, monoglycerides and diglycerides for both species. Triglycerides seem to be present in the crude wax of *R. sanguineus*. The identification of these compounds may help to develop a substance to be used for therapeutic purposes, since there is evidence of that the wax shows antimicrobial resistance.

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11.19 Evaluation of the accidents caused by spiders, scorpions and lepidopteran larvae (caterpillars) in patients seen at Vital Brazil Hospital, Butantan Institute

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Introduction: In Brazil, in 2006, 37,632 accidents by scorpions were reported, 2,658 by *Phoneutria* and 1,215 caused by larvae of Lepidoptera. The most frequent and key characteristic of these three accidents is local pain, which usually is acute and intense and with variable frequency of other phenomenons of local inflammation. **Objectives:** The objective of this study was to compare the epidemiological, clinical and therapeutic characteristics of accidents caused by spiders, scorpions and caterpillars admitted to the Hospital Vital Brazil. **Methods:** This is a prospective observational study, which collected information about the animals that caused the accident (sex, sexual maturity, identification of genus and species), the patient and also the circumstances in which the accident occurred and the characteristics of the painful phenomenon (intensity, irradiation , temporality, frequency, rhythm, factors of improvement and worsening, duration and the kind of pain sensation), the treatment applied and outcome. **Results and Discussion:** The period of data collection began in November. By June 2010, 108 records were filled and will be submitted later. The inclusion criteria are: patients bitten by scorpions and spiders and contact with caterpillars in less than 24 h, without previous treatment and only mild and moderate cases. In this summary, 61 records were analyzed, 32 of which brought the animal and conisdered the first inclusion criteria. Twenty-one (65%) were men, and 5 accidents occurred in rural and 27 in urban areas. Eighteen spiders were brought (15 specimens of *Phoneutria nigriventer*, a genus of *Corinna*, a copy of *Cterus ornatus* and one genus *Lycosa sp*), 13 scorpions (9 *Tityus serrulatus*, 3 *Tityus bahiensis* and 1 *Tityus costatus*) and 3 caterpillars (1 *Megalopygidae* , 1 *Automeris* and 1 without identification). There was an accident caused by *Centruroides sp*, which was not included because it received analgesia prior to Hospital Vital Brazil admission. With respect to the intensity of pain on a 0-10 scale, there was an average intensity of 7 in accidents caused by spiders, 6.5 by scorpions and 6.0 by caterpillars. Pain intensity in 12 cases (37%) was classified as being of intensity less than 5, in 19 cases (59%) above 5, and in 1 case (3%) the intensity of pain was not recorded. Of the patients whose pain was classified as less than 5, 6 were initially treated with hot water, 7 initially received oral analgesic, and 2 local anesthetic, and in two accidents there was no record of treatment. Of the patients whose pain was greater than 5, 15 were first submitted to hot water, 9 received oral analgesic and 11 were treated with local anesthetic. Some patients received at the time of admission, more than one treatment. There are important epidemiological and clinical differences between these accidents that after the conclusion of this study will allow us to distinguish their characteristics and choose better treatments.

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11.20 Antifungal secondary metabolites produced by endophytic fungi

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Introduction: Fungal infections affect millions of people worldwide and their pathogens have been developing resistance to the antifungal chemotherapy available. Therefore, the development of new antifungal agents is urgently needed, and secondary metabolites produced by microorganisms, such as endophytic fungi, can be a huge source of new pharmacological agents. **Objectives:** The aim of the present study was to evaluate the potential of 66 endophytic fungi isolated from coffee tree on producing secondary metabolites with antifungal activity. **Methods:** Antagonism assay was used to evaluate 66 endophytic fungi strains against *Trychophyton rubrum* IOC 4527, *Candida albicans* ATCC 36802/ IOC 3704, *Cryptococcus neoformans* ATCC 90112 and *Aspergillus fumigatus* IOC 4526. The inhibition halo of the pathogen growth was measured in millimeters (mm) and the strains that inhibited the growth of at least 2 pathogens were selected to produce crude extracts. Thus, 15 strains were inoculated into potato dextrose broth and incubated at 28ºC and 150 rpm for 7 days, and the crude extracts were obtained through the supernatant extraction with hexane (HEX) and ethyl acetate (AE) consecutively. The minimal inhibitory concentration (MIC) was determined for each organic extract against the same above pathogens in the range of 8 to 1,000 µg/mL. The crude extract BG9-IId3 was purified by HPLC in 2 steps using CN and PFP column as stationary phase, and acetonitrile as mobile phase. MIC was determined for each fraction obtained against *C. neoformans* ATCC 90112 and *C. albicans* ATCC 36802. **Results and Discussion:** Fifteen fungal strains out of 66 inhibited the growth of at least two pathogens by the antagonism assay with an inhibition halo larger than 5 mm, and organic extracts were produced by these strains. Among 29 extracts, 16 showed MIC ≥ 1,000 µg/mL for all the pathogens, and 11 extracts showed a MIC lower than 1,000 µg/mL against at least one pathogen. The extracts BII-01 HEX, BG1-IIIf HEX and BG9-IId3 HEX were the most effective with MICs lower than 500 µg/mL against *C. albicans* ATCC 36802/ IOC 3704, *C. neoformans* ATCC 90112 and *T. rubrum* IOC 4527. The extract BG9-IId3 HEX showed MICs of 162.5 µg/mL against *C. albicans* and *C. neoformans,* and its purification by HPLC yielded the fractions F1 and F2 in the first step. Fraction F2 with a MIC of 62.5 µg/mL against *C. albicans* was re-purified, yielding 3 isolated compounds named F2a, F2b and F2c, which are being characterized by physical and chemical methods (^{1}H NMR, ^{13}C NMR, and mass spectrometry). These results show that endophytic fungi isolated from the coffee tree are promising sources of bioactive antifungal secondary metabolites that can also be hits for new antimicrobial compounds.

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11.21 Inhibitory effect of rattlesnake (*Crotalus durissus terrificus*) venom on the formation of multinucleated giant cells in an experimental model of chronic inflammation

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Introduction: The venom of the *Crotalus durissus terrificus* (*Cdt*V) alters some functions of macrophages, cells that in chronic inflammatory processes are fused to form multinucleated giant cells (MGC). This process depends on the participation of actin filaments (F-actin) and signaling proteins, such as phosphotyrosine (PTy). Previous studies showed that *Cdt*V changes the patterns of F-actin expression and PTy 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. Yet, it is known that, after an ion exchange chromatography of *Cdt*V, 3 different fractions are obtained: PI, PII (corresponding to the crotoxin- CTX) and PIII. **Objectives:** Our objective was to evaluate qualitatively and quantitatively the effect of the *Cdt*V on the rearrangement of F-actin and PTy in mice subjected to a chronic inflammatory stimulus and to assess the fraction of the venom responsible for the inhibitory effect on the formation of MGC. **Methods:** Glass coverslips were implanted s.c in mice pretreated with *Cdt*V, fractions PI, PII (CTX) or PIII or saline. After 7 days, cover slips were removed and stained with H & E and counting of CGM in different groups was done. Preparations for immunohistochemical identification of F-actin and PTy were made in cover slips removed 4, 7, 14 and 21 days after implantation and analyzed in a confocal microscope, where fluorescence intensity was evaluated with the aid of Image-J software. **Results and Discussion:** The immunostainings for F-actin were significantly inhibited in cover slips removed 4, 7, 14 and 21 days after implantation, when compared to control groups. Regarding PTy, differences were not observed in any of the times studied. The 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 cells formation observed previously in cover slips implanted in the same period and stained with hematoxylin/eosin. Related to the fraction responsible for such inhibition, results indicate CTX as the inhibitor since after the total count of MGC, it was observed that the number of fused MGC in Cdt pre-treated groups was similar to the PII pre-treated ones, and in groups treated with PI or PIII, the results were similar to the control group treated with saline. The compiled data show a significant inhibitory action of *Cdt*V on the progression of the chronic inflammatory response.

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11.22 Characterization of enteropathogenic *Escherichia coli* (EPEC) outer-membrane proteins (OMPs) by two-dimensional electrophoresis and mass spectrometry

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Introduction: *E. coli* is a versatile pathogen in animals and humans. Enteropathogenic E. coli (EPEC) has been identified as the main causative agent of acute diarrhea in populations of developing countries. Diarrhea is still one of the most significant causes of global child mortality. **Objectives:** The goal of this work was to characterize and identify the outer-membrane proteins (OMPs) separated by two-dimensional electrophoresis (2-DE) of extracts derived from one strain of EPEC (strain 9100-83, serotype O125:H6). **Methods:** 2-DE was performed by a two-step protocol: the first dimension by focusing on 13-cm pH 4-7 strips (IPGphor III, GE Healthcare) and the second dimension by SDS-PAGE using 15% SDS-polyacrylamide gels (SE 600 Ruby, GE Healthcare). Characterization of the proteins was performed by in-gel trypsin digestion followed by mass spectrometric analysis (ETTAN MALDI-TOF/PRO – Amersham Biosciences and ESI QTOF Ultima – Waters). The resulting spectra were searched against non-redundant protein database (NCBInr) using MASCOT v2.0 engine (Matrix Science, www.matrixscience.com). **Results and Discussion:** Twenty-two spots were identified with high scores allowing the characterization of eleven distinct proteins. All proteins have membrane localization, a fact that indicates the efficiency of the extraction method. Five proteins were OMPs or porins (OMP A, OMP X, outer membrane channel – specific tolerance to colicin E1, outer membrane Tol C and maltoporin). Two transporters were found (long-chain fatty acid and ferrichrome outer membrane transporters). Moreover, one enzyme (glutamate decarboxylase alpha), a receptor (vitamin B12), an elongation factor (EFTu) and a protection protein (DNA protection during starvation) were detected. OMP A was one of the most abundant gel components. The biological function of OMP X is unknown, although it has been suggested that it binds foreign proteins on the *E. coli* cell surface, possibly as part of a cellular defense mechanism, and that this binding affinity is used to achieve cell adhesion and invasion. These preliminary data indicate that the majority of proteins identified have important roles in membrane permeability and at least two of them, OMP A and OMP X, are involved in the adhesion of the pathogen to host cells.

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11.23 Cytochemical characterization of blood cells of the snakes *Oxyrhopus guibei* and *Xenodon neuwiedii*

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Introduction: Circulating blood cells of reptiles can be classified as erythrocytes, thrombocytes and leukocytes. Four types of leukocytes were identified in the blood of snakes: lymphocytes, azurophils or monocytes, heterophils and basophils. General morphologic characteristics of erythrocytes and thrombocytes from the Reptilia blood are similar, showing little variation among groups while the characteristics of leukocytes, mainly the granulocytes are limited and inconsistent. Furthermore, considerable controversy regarding heterogeneous nomenclature of leukocytes remains. Even the presence of eosinophils in the Squamata remains controversial since criteria have not been well defined to distinguish eosinophils and heterophils in snakes. Thus, cytochemical staining is used to evaluate morphological characteristics of blood cells. **Objectives:** The aim of this study was to provide a morphologic description of blood cells of *Oxyrhopus guibei* and *Xenodon neuwiedii* snakes using cytochemical staining. **Methods:** Three *Oxyrhopus guibei* and four *Xenodon neuwiedii* snakes were anesthetized with thiopental sodium and the blood was withdrawn from the abdominal artery. Blood smears without anticoagulant were prepared immediately after blood collection. Enriched leukocytes were also prepared from at least 2 ml of whole peripheral blood, fixed in 2% glutaraldehyde and 4% paraformaldehyde in Tyrode buffer and embedded in historesin. The cytochemical reactions (benzidine peroxidase, sudan black B(SBB), periodic acid Schiff(PAS) and toluidine blue) were carried out both in blood smears and historesin sections. **Results and Discussion:** Most lymphocytes found were small and round but they often had irregular cell outlines. Lymphocytes did not stain with any of the cytochemical stains used. Azurophils are round or amoeboid cells with the eccentrically placed nuclei. The cytoplasm of azurophils was strongly positive with peroxidase, SBB and moderately stained or negative with PAS. Heterophils were the largest peripheral blood cells with a round to oval with eccentric nucleus. The cytoplasm is filled with numerous eosinophilic granules which were strongly positive for peroxidase and SBB while for PAS the reaction was weak or negative. Basophils are round to oval cells and contained a nucleus that was masked with numerous basophilic granules in the cytoplasm which strongly stained with toluidin blue and PAS. The positive reaction of the granules was better demonstrated in the histological section than in blood smears. Thrombocytes in general were oval or round-shaped cells; the nucleus was centrally located with hyaline cytoplasm which was strongly positive for PAS. There was no evidence of the presence of eosinophils in the blood of these two species.

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11.24 *Ornithodoros mimon* (Acari: Argasidae): third generation under laboratory conditions

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Introduction: The biological cycle of the argasid tick comprises embryonic eggs, larvae, nymphs (many instars), and adults. Adults feed many times, in general, before mating and ovipositions. *Carios mimon* is a tick common on bats, described from Bolivia and Uruguay. It was recently found in Argentina and Brazil where it is very aggressive to human and domestic animals. **Objectives:** Our aim was to study the 3rd generation of *C. mimon* in the laboratory, by observing pre-feeding and feeding periods as well as pre-molting and molting of all stages and instars and number of gonotrophic cycles per females. In addition, pre-oviposition and oviposition periods as well as the eclosion period of larvae of the 4th generation were also observed. **Methods:** The colony of *C. mimon* started from ticks collected in a household in Araraquara municipality, São Paulo State. Ticks were allowed to feed on rabbits in the laboratory of Parasitology of the Instituto Butantan, following the Protocol on Ethical Principles in Animal Research adopted by the Brazilian College of Animal Experimentation. Engorged specimens were left in an incubator at 27°C ±1 and 90% relative humidity, in order to obtain molts to the adult stage or larvae from female ovipositions. Larvae of the 3rd generation (N=78) were placed in a cotton chamber fixed on the dorsum of rabbits. Nymphs from each instar (N=40) and adults (9 females, 9 males) fed directly on animals. All data about the biology of the 3rd generation of this species were monitored. **Results and Discussion:** After feeding, the larvae of the 3rd generation showed different results from those of prior generations. Periods of larval feeding and pre-molts to nymphs of first instar (N1) were shorter (3 to 7 days) than those observed in previous generations (5 to 8 days). Pre-feeding and feeding periods of these N1 were longer (15-50 min, respectively). Although most of N1 from previous generations molted to 2nd instar (N2) after feeding, some of them molted without feeding. However, most of the nymphs N1 from the 3rd generation needed a meal before molting to N2 and they molted 9 days after. Those N1 that did not feed were in the same instar. The N2 fixed and fed quickly. Most of N2 that fed between 30 to 35 min molted to males, while the majority of N2 that spent 45 to 50 min molted to N3 (N=37). Of the N2 that molted to adults, 4 males and 2 females emerged after 9 days. The remainder of the N2 molted to N3. After 11 to 18 days, the engorged N3 molted to adults, and most of them molted to females. After 8-10 days each mated female laid 80-100 eggs in only one gonotrophic cycle. Larvae of the 4th generation (N=280) hatched after 10 days. From the larvae of the third generation to larvae of the 4th generation, the life cycle of *C. mimon* lasted 80 to 120 days.

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11.25 Characterization of antimicrobial molecules found in the venom of the spider *Acanthoscurria gomesiana*

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Introduction: There has been indiscriminate antibiotic use among the public in recent years, causing the selection of resistant microorganisms, making it difficult to treat illnesses. Research has attracted interest in the possibility of new drugs, natural or synthetic, that are efficient against these resistant strains. Peptides have been found to be molecules of innate defense in invertebrates, through the cleavage of its proteins. In the venom of the spider *Acanthoscurria gomesian,* three toxins (gometoxins 1, 2 and 3) with antimicrobial activity had been identified. **Objectives:** This study aimed to identify and characterize new molecules with antimicrobial activity present in the venom of the spider *A. gomesiana*. **Methods:** The purification of the crude venom of the spider *A. gomesiana* was performed by reversed-phase liquid chromatography using a semi preparative Jupiter C18 column. 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). The molecular weights were analyzed by mass spectrometry MALDI – TOF. Fractions with more than one mass were repurified by reverse phase liquid chromatography, using a Jupiter C18 analytical column. Two new fractions that showed antimicrobial activity were analyzed by mass spectrometry. **Results and Discussion:** Two new molecules with antimicrobial activity were isolated, named gometoxin 4 and 5. From the analysis in the mass spectrometer, the molecular weight was obtained for gometoxin 4, and the result was 878.3Da, which showed antimicrobial activity against *M. luteus*. No molecular weight was determined for gometoxin 5, which has antimicrobial activity against *C. albicans*. In future studies, the molecular weight of gometoxin 5 will be determined, and the two substances, gometoxins 4 and 5, will be sequenced.

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11.26 Purification of factor X and protein C from *Bothrops jararaca* snake plasma

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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 a molecular mass of about 62 kDa and consists of two polypeptide chains, light (17.5 KDa) and heavy (45 kDa) chains. The protein C (PC) inhibits coagulation by selective inactivation of 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) chains. **Objectives:** The aim of this study was to purify *Bothrops jararaca* (*B.jararaca*) FX and PC **Methods:** The purification process consisted of tandem steps on different chromatography columns. Briefly, plasma was applied on HiTrap DEAE FF column. The vitamin K-dependent protein fractions were applied on HiTrap Cu^{2+}Chelating HP, followed by Q-HiTrap FF, and finally on HiTrap Heparin HP. 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:** FX and PC were partially purified by this developed process, showing that additional purification steps must be included. 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 FX and PC of other animals.

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11.27 Convulsant effect of intravenous administration of *Tityus serrulatus* scorpion whole venom in 21-day-old rats: electroencephalographic, behavioral and histopathologic aspects

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Introduction: Clinical data have shown that scorpion venom can induce convulsion, mainly in children. Also, late epilepsy has been described in some of these patients. 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 were some questions we wanted to investigate: Is it possible that convulsion caused by systemic injection of whole venom of scorpion induces late epilepsy in rats? Could treatment with anticonvulsivants just few hours after the beginning of convulsions prevent late epilepsy? **Objectives:** The aim of this study was to investigate the acute and long-term convulsivant effects of i.v. administration of *Tityus serrulatus* scorpion venom in male and female rats aged 21 days. An electroencephalographic, behavioral and histopathological study was performed. **Methods:** Surgery to implant electrodes in the hippocampus area for electroencephalographic analysis was done. After two days, the whole venom of *Tityus serrulatus* scorpion in a 0.2 mg/kg dose was administered (i.v.). Behavior and electroencephalographic activity of rats were observed for six hours uninterruptedly. Seven or 90 days after venom administration, rats were anesthetized with carbon dioxide to do a perfusion, and the brain was processed to histological analysis. The cells of CA1, CA3, hillus and dentate gyrus of hippocampal formation were counted in an area of 100 um^2 and a search for mossy fiber sprouting was performed. **Results and Discussion:** During the acute period of observation (0-24h after venom injection), electroencephalographic recordings were characterized by isolated spikes and epileptic discharges in rats that had received 0.2 mg/kg of crude venom. The behavioral modifications were characterized by paralysis, "wet-dog shakes," intense salivation, convulsion, and respiratory and locomotion difficulties. Long-term effects (1-90 days after venom injection). However, late epilepsy was not observed in this study. Histopathologic analyses of brains of these rats performed 90 days after venom injection did not show mossy fiber sprouting, a phenomenon that is present in epileptic patients. These results showed that the systemic venom injection in rats was able to induce central effects such as convulsion and epileptiform activity only on the day of venom administration. However, spontaneous and recurrent seizures were not observed in a period of 90 days after venom injection. This experimental study was not able to induce late epilepsy as observed in some patients who present with severe neurologic symptoms of scorpion envenomation.

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11.28 Primary culture of B type synoviocytes and effects of a metalloproteinase isolated from *Bothrops* asper venom on these cells

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Introduction: Snake venom metalloproteinases show homology with matrix metalloproteinases (MMPs), which are increased in inflamed articular joints during arthritis. Recently, we demonstrated that the metalloproteinase BaP1 was able to induce inflammatory events in rat articular joints, including the release of PGE_2 and TNF-α, which are the major mediators of pain in inflamed joints. However, the cell sources of these mediators were not identified. During inflammatory processes in joints, the synovial fibroblasts (B-type) are central cells for production and release of inflammatory mediators. **Objectives:** The aim of this study was to establish a primary culture of B-type synoviocytes and to evaluate the action of BaP1 on these cells, focusing on: 1) cell viability, 2) release of PGE_2 and 3) the protein expression of cyclooxygenase-1 and-2 (COX-1 and -2). **Methods:** B-type synoviocytes were isolated from synovial membranes of male Wistar rats (CEUIAB 576/09) and cultivated in culture flasks with complete RPMI medium at 37°C and 5% CO_2. Presence of the membrane protein Thy-1 was evaluated by immunocytochemistry as a marker of B type synoviocytes. To standardize the number of cells to form a monolayer on microplates, $1x10^3$, $1x10^4$, $1x10^5$ and $1x10^6$ were seeded into 6-, 12- and 96-well microplates and incubated with RPMI for 24 or 48 h, and observed the formation and confluence of the monolayers under light microscopy. The synoviocytes were then incubated with BaP1 (6.25, 12.5 and 25 μg/mL) or RPMI (control) for 30 min, 1, 3 or 6 h, followed by evaluation of cell viability by LDH activity and MTT assay, PGE_2 concentration by enzyme immunoassay and protein expression of COX-1 and -2 by Western blotting. **Results and Discussion:** Cell concentrations suitable for experimental assays were $1x10^4$, $1x10^5$ and $1x10^6$ in 96-, 12- and 6-well microplates, respectively. The protein Thy-1 was present in 100% of cells in culture obtained from the fourth passage, indicating the homogeneity of B-type synoviocytes. BaP1 was non toxic to isolated synoviocytes and induced the release of PGE_2 from these cells after 3 and 6 h incubation. In addition, BaP1 induced protein expression of COX-2 at 30 min and 3 h, but did not affect COX-1 expresssion. BaP1 is able to directly stimulate B-type synoviocytes to produce and release PGE_2. Upregulation of COX-2 protein expression may be the primary mechanism for production of these mediators induced by BaP1. Moreover, the B-type synoviocyte is a target for BaP1 and a relevant cell source for production of inflammatory mediators during joint inflammation induced by this metalloproteinase.

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11.29 Identification of the endoparasites affecting colubrids offered to coral snakes (*Micrurus* sp)

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Introduction: In Brazil, there are 321 known species of snakes belonging to nine families and 75 genera, of which 70 are venomous. Among the venomous snakes, the coral snakes were always the most difficult to keep in captivity. Due to the drastic decrease in the receipt of these animals at Instituto Butantan, it has become increasingly necessary to be self-sufficient in the maintenance and breeding of these snakes in captivity for the production of anti-elapidic serum and immunobiologic research. In captivity, colubrids of various genera are offered as prey items for the coral snakes, especially: *Liophis* sp, *Oxyrhopus* sp, *Phylodrias* sp, *Sybinomorphus* sp and *Tomodon* sp. When free-ranging snakes are offered to ophiophagous ones, there is the risk of infecting the latter with endoparasites, as most snakes from nature can harbor a wide variety of parasites. Therefore, it is necessary to establish an effective prophylactic management of the colubrids offered to coral snakes in captivity in order to avoid parasitic infection that, among other consequences, can lead to the death of the animals. **Objectives:** The aim of this study was to identify the endoparasites that affect colubrids commonly offered as prey items for the coral snakes, for the effective prophylactic management of the prey before being offered to coral snakes. **Methods:** During twelve months (August 2009 – July 2010), 50 adult colubrids from São Paulo State: 10 *Liophis* sp, 10 *Oxyrhopus* sp, 10 *Phylodrias* sp, 10 *Sybinomorphus* sp and 10 *Tomodon* sp were euthanized and necropsied. Organs and tissues were analyzed and all the parasites were collected and properly fixed. Fragments of organs were fixed in 10% formalin for histopathological analysis and fecal samples were taken for coprologic examinations. The feces collected were subjected to the Willis method and centrifugation technique for the detection of eggs and/or larvae of parasites. The eggs and larvae of helminths were classified according to their class and, when possible, according to their order; the counting of eggs and/or larvae was made subjectively. The adult parasites were classified according to morphological analysis, identification keys and measurements performed with Image-Pro Express program. **Results and Discussion:** This study showed that 36% of the animals necropsied were parasitized, whereas 84% of the parasites encountered were nematodes, 12% trematodes and 4% cestodes. Most of the parasites were found in the respiratory tract and belonged to the genus *Rhabdias* sp. In the coprologic examinations 58% of the animals necropsied were positive for at least one developmental phase of the helminth. These data demonstrate the importance of identifying these endoparasites for the establishment of appropriate and specific anti-parasitic methods to prevent parasitic infection in coral snakes.

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11.30 Incidence of trematodes in the oral cavity of *Bothropoides jararaca* (Viperidae, Ophidia) of São Paulo State

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Introduction: The species *Bothropoides jararaca* can be infected by a variety of endoparasites, including trematodes. There are 44 species of trematodes affecting Brazilian snakes, belonging to four orders, 12 families and 23 genera. Trematodes are found in several organs of the snakes and affect mainly the digestive system. Clinical signs in most cases are not typical, and disease only occurs when the animal has a large parasite load. Parasitic infections are one of the most important causes of death in snakes. **Objectives:** The aim of this study was to dentify the species of trematodes found in the oral cavity of *Bothropoides jararaca* in the state of Sao Paulo. **Methods:** During the period of May 2005 to May 2010, all the *Bothropoides jararaca* from various cities of São Paulo, donated to the Instituto Butantan (IB), were examined for trematodes. Within five years, Instituto Butantan received 3717 jararacas that had their biometric data recorded (snout-vent length (SVL), total length (TL) and mass), the sex determined by the presence or absence of hemipenis and the oral cavity examined for the presence of trematodes. Whenever present, the trematodes were placed in distilled water for several hours to expel the eggs and afterward were gently compressed between two slides and immersed in a solution of alcohol, formaldehyde and acetic acid (AFA - 93 parts 70% alcohol + 5 parts 10% formaldehyde + 2 parts glacial acetic acid) for a few minutes and preserved in 10% formalin. The parasites were stained with carmine and their main structures were morphologically studied and measured using a light microscope equipped with a camera and attached to a computer with Image-ProExpress program. The genus and species of trematodes were obtained with the aid of identification keys. **Results and Discussion:** From all the jararacas donated to Instituto Butantan in the period studied, 62 (1.6%) showed trematodes in their oral cavity. Males had a higher incidence than females (1.9% and 1.5%, respectively). Young specimens (less than 40 cm SVL) were not affected by this parasite, only the adults and subadults. The incidence of infected animals is higher in summer (1.9%) and lower in winter (1.2%), while in autumn and spring the rate is 1.6%.

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11.31 On the genus Pycnothele Chamberlin: description of the female of *Pycnothele singularis* Mello-Leitão (Araneae: Mygalomorphae, Nemesiidae)

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Introduction: The genus *Pycnothele* Chamberlin was described based on the type species, *P. perdita* Chamberlin 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. Of these five, only *P. singularis* does not have a female description. The species and the genus *Androthelopsis* Mello-Leitão, in which it was originally described, underwent some taxonomic modifications during the last decades. These are shown below. *Androthelopsis singularis* was described based on a male specimen from Serrana, São Paulo, Brazil. In 1973, Lucas & Bucherl reviewed the holotype of *A. singularis* and transferred the species from the family Barychelidae and included it in the Pycnothelidae. Perez-Milles & Capocasale in 1988 synonymized *Androthelopsis* with the genus *Pycnothele* Chamberlin, resulting in a new combination, *Pycnothele singularis*. **Objectives:** The aim of this study was to continue the project "On the genus *Pycnothele*" initiated by the scholar Victor Passanha in 2006, and to describe the female of the species *P. singularis*. **Methods:** The examined material was deposited in the Arachnida collection of the Instituto Butantan. The female spermathecae were dissected and submerged in clove oil to study internal structures. The illustrations and morphological observations were made using a Leica MZ12.5 stereomicroscope with a camera lucida. **Results and Discussion:** During the study of part of the material of the Arachnida collection from Instituto Butantan, it was possible to find the female of *P. singularis*, which was unknown until the present moment. The female of *P. singularis* resembles that of *P. auronitens*, due to the less developed supraspermathecal chamber, and differs by the less twisted ducts. This discovery allowed is to enhance the distribution range of the species to Vargem Grande do Sul, São Paulo and also to complete the review of the described species of the genus *Pycnothele*.

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11.32 Recombinant expression and characterization of the metallopeptidase neprilysin (EC3.4.24.11, NEP)

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Introduction: Neprilysin, also known as neutral endopeptidase, is a membrane protein that activates or inactivates oligopeptides such as natriuretic hormones (BNP, ANP, CNP), endothelins, and bradykinin. This enzyme is also related to tumor progression and its metastatic capacity. Moreover, recent studies have shown the involvement of neprilysin in the physiopathology of Alzheimer's disease by its activity in the clearance of brain amyloid substance. **Objectives:** The aim of this study was to obtain recombinant neprilysin using a bacterial expression system and to perform site-directed mutagenensis. **Methods:** The NEP cDNA sequence was amplified by PCR from the vector pCR4-TOPO (Invitrogen) using oligonucleotide primers containing recognition sequences for the restriction enzymes *Sal* I and *Not* I, and subcloned into the vector pGEX4T-2, which had been previously digested with *Sal* I and *Not* I. Subsequently, the plasmid was used to transform *E. coli* XL1-Blue, and the clones that contained the insert were submitted to DNA sequencing on both strands to ensure that the coding sequence was correct. The cDNA cloned in the pGEX-4T2 vector, which allows the expression of soluble recombinant proteins in fusion with glutathione S-transferase (GST), was transformed into *E. coli* BL21DE3. Protein expression was induced with 1 mM isopropyl thio-b-D-galactopyranoside for 24 h. Cells were collected by centrifugation at 3500 rpm, 4C for 15 min and suspended in lysis buffer (50 mM Tris HCl pH7,5, 2 mM $MgCl_2$, 0/25 mg/mL lysozyme and 25 U/uL benzonase nuclease). After cell lysis by sonication, the cell lysate was centrifuged and the pellet containing inclusion bodies was submitted to a solubilization/refolding protocol. Purification was finally performed by affinity chromatography on a glutathione-Sepharose column. The eluate containing GST-NEP was digested with thrombin followed by filtration using a Centricon 50 membrane. The expression of NEP was analyzed by SDS-PAGE, Western-blotting, and proteolytic assays using peptide fluorogenic substrates. **Results and Discussion:** Despite its insoluble form, NEP was successfully obtained in fusion with GST in *E. coli* BL21DE3. The SDS-PAGE analysis and Western-blot using an anti-NEP antibody confirmed the expression of NEP. The yield of recNEP was estimated at 2.3 mg/L culture medium. However, no enzyme activity was detected in the purified NEP sample, indicating that the protein was solubilized from inclusion bodies but not properly folded to express its catalytic activity. We are currently working on an improved protocol to obtain the fully active wild-type recombinant enzyme as a basis for our mutagenesis studies.

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11.33 Mygalomorph spiders in the Serra do Japi, State of São Paulo, Brazil: species richness and composition at three different altitudes

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Introduction: Fifteen mygalomorph families are known from Brazil; eight of them are found in the state of São Paulo. Little is known of their taxonomy, biology and ecology. Habits are rarely recorded and nothing is known about the influence of altitude on the distribution of mygalomorphs. **Objectives:** The aim of this study was to make an inventory of mygalomorph spider species of the Serra do Japi and to compare the taxa collected at 3 different altitudes in order to determine differences in the richness and composition among the sampled areas. **Methods:** The collections were made once in each season during one year. Two areas were sampled at 880 m, 1000 m and 1200 m, totaling 6 areas. Three collecting methods were used: 50 pitfall traps in each area; diurnal hand collecting limited by time of one hour and nocturnal direct hand collecting in transects of 60 m2. **Results and Discussion:** After four excursions, a total of 294 mygalomorph spiders were obtained. Four families and 12 species belonging to 9 genera were recorded: Nemesiidae (4 genera, 6 species), Idiopidae (1 genus, 2 species), Dipluridae (1 genus, 1 species) and Theraphosidae (3 genera, 3 species). Considering only the adults, 9 species were found at the lower site, 7 species at the intermediate site and 9 species at the higher site. The frequences are respectively: Nemesiidae - *Rachias* sp.1, 3.2%; *Prorachias* sp., 3.9%; *Stenoterommata* sp.1, 26.9%; *Stenoterommata* sp.2, 7.8%; Gen. sp.1, 14.4%; Gen. sp.2, 5.9%; Idiopidae - *Idiops* sp.1, 17.7%; *Idiops* sp.2, 13.8%; Dipluridae - *Diplura* sp.1, 1.9%; Theraphosidae - *Magulla obesa*, 1.3%; *Homeoma montanum*, 1.3%; *A. gomesiana*, 1.3%. Specimens of *Stenoterommata* sp.1, Gen. sp.1 and *Idiops* sp.1, were found at all altitudes; *Stenoterommata* sp.2 at 880 m to 1000 m; *Prorachias* sp., *Homoeomma montanum* and *Idiops* sp.2, from 1000 m to 1200 m; Gen. sp.2, *Magulla obesa* and *Acanthoscurria gomesiana*, were found only at 880 m. *Rachias* sp. and *Diplura* sp. were found at 880 m and 1200 m, and probably can also be found at 1000 m. All species are being illustrated, and a key to the mygalomorph genera and species of Serra do Japi is being prepared.

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11.34 Establishment of a method for evaluation of molluscicidal activity against *Achatina fulica* (Stylommatophora: Achatinidae)

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Introduction: The giant African snail, *Achatina fulica*, originating from east Africa, can also be found in several countries, including Brazil, where it was introduced for food as an alternative to traditional "escargot" of the *Helix* genus. This snail has a high population growth rate that has drawn the attention of authorities, researchers and local people. This species has been considered a competitor with native species leading some of them to be at greater risk of extinction; it destroys crops, representing an agricultural pest, and assumes a role as an intermediate host of certain human parasites. Among the methods of *A. fulica*'s control, there is the use of chemicals, such as metaldehyde and carbamates. However, these agents showed high toxicity, affecting the environment and requiring a high cost of production and commercialization. Thus, plant extracts have been studied as an alternative for *A.fulica* control. **Objectives:** The aim of this study was to evaluate the molluscicidal effect of a crude extract of *P. gaudichaudianum* (Piperaceae family) on *Achatina fulica*. **Methods:** Thirteen young animals (3 to 5 cm) were used, which were injected with a dose of 200 mg/kg of crude extract of *P. gaudichaudianum* leaf. Besides that, diethyl ether was used to anesthetize the snails before the treatment. Positive control was done using a dose of 30 mg/kg of niclosamide (Baylucide WP70 ®). The negative control was 3%DMSO. **Results and Discussion:** After 24 h, 100% mortality was observed in those treated with niclosamide and 40% for those treated with the crude extract, and there was no mortality in those treated with 3% DMSO. The new method using extract injection was effective, because the amount of extract into the animal and the exact dose to which it was exposed are known. Unlike the ingestion method, mortality and potential molluscicide were observed in *A.fulica* injected with crude extract of *P. gaudichaudianum* leaf. Previously, methods (ingestion) using 1000 ppm intake showed no effect and the increase in concentration resulted in an increase of DMSO, inhibiting snails' appetite. Therefore, an applicable methodology was established for the evaluation of plant extracts, and the crude extract of *P. gaudichaudianum* showed potential molluscicide effect, which should be evaluated in further studies.

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11.35 Polymorphism of gyroxin in *Crotalus durissus* venom by Western blot analysis

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Introduction: Snake venom variability is related to exogenous and endogenous factors occurring at several levels such as interspecies, geographic and ontogenetic variation, resulting in differences in venom composition. Gyroxin is a toxin from rattlesnake venom, with a molecular weight of 33 kDa. It is a serine protease that has fibrinogenolytic activity and when inoculated intravenously causes an effect known as barrel rotation syndrome. The batroxobin is a serine-protease from *Bothrops atrox* venom. It is a single toxin whose genomic sequence consists of five exons and four introns. As homologous toxins, gyroxin and batroxobin show similarities between the gene structures. Data from our laboratory indicate the absence of exon 4 of the gyroxin gene in some rattlesnake genomes. **Objectives:** Our aim was to analyze the polymorphism of gyroxin in *Crotalus durissus* venoms. **Methods:** Forty venoms from São Paulo, Paraná and Goiás were fractionated by denaturing 12% SDS-PAGE and transferred to nitrocellulose membrane. Gyroxin was detected using anti-MSP1 and MSP2 (against serine proteases from *Bothrops moojeni*) as primary antibodies and anti-rabbit IgG conjugated with peroxidase as secondary one, followed by colorimetric development with 4-chloro-1-naphthol. **Results and Discussion:** Western blotting revealed a single band of gyroxin with apparent MW of 32.5 kDa. We also observed a second band with a size close to 35.5 kDa in some samples. Nine of 16 samples from São Paulo and seven out of nine from Paraná showed the larger band, while it was present in only two of 12 samples from Goiás. We did not observe any relationship between the low intensity of the gyroxin band and the absence of exon 4. All samples of rattlesnake venoms tested displayed the band corresponding to gyroxin. We identified a polymorphism of the 35.5 kDa serine protease. Despite that the majority of the samples from Goiás do not show the 35.5kDa band, we cannot establish a geographic variation in relation to this band without an increase in sample size.

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11.36 Evaluation of toxicity of two cyanobacterial strains in mice

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Introduction: Cyanobacteria are known to produce hepatotoxins, dermatotoxins, cytotoxins and neurotoxins. When cyanobacteria die in water reservoirs, such toxins can be released, causing poisoning and even deaths to humans and animals. Since 2006, our group has been investigating the cyanobacteria strains from the Algae Bank of the Botanical Institute through acute toxicity experiments in mice. The symptoms induced in mice are observed, and when the toxic effects are not related to the typical cyanotoxins poisoning, we perform further histophatological analysis of the organs. **Objectives:** The aim of this study was to characterize the toxicity of two cyanobacterial strains, SPC 1044 (*Geitlerinema amphibium*) and SPC 1049 (*Phormidium sp.*), in mice (i.p.), observing the effects on the animals after injection, and performing postmortem examinations (including histopathological analysis). **Methods:** Extract preparation: the cultured cyanobacteria cells were filtered through an AP-20 filter and freeze-dried. The resulting material was then extracted with 0.1M acetic acid (4x) or MeOH/H_2O 75:25(v/v) (5x) with ultrasonication (4 x 10 sec., 50 W) and centrifuged. The supernatant was concentrated under reduced pressure, and the extracts were maintained at -20°C until they were used. The toxicity tests (i.p.) were performed in male Swiss-Webster mice (19-21g). The symptoms of the mice were observed up to 8 days after administration. After euthanasia, necropsy was performed and tissue samples were taken from the liver, kidneys and lungs, fixed and used for histopathological analysis. **Results and Discussion:** Neither cyanobacterial extract caused death of the mice. However, the animals showed some symptoms of intoxication (SPC 1044, methanolic: dyspnea; SPC 1049, methanolic: paralysis, abdominal pain and contractions). After necropsy, the mice injected with SPC 1044 (methanolic) showed altered color of intestine and pancreas, and darkened testicles. Histological analysis showed lung abnormalities, such as augmented inter-alveolar walls. The liver showed a great quantity of cells with pyknotic nuclei which may indicate necrosis or apoptosis. The mice injected with SPC 1049 (methanolic) displayed, after necropsy, dark stains in the bile vesicle. The histopathological analysis of the organs is still ongoing. These results indicate two new toxic cyanobacterial strains. Although the extracts did not cause death in any mice, the animals showed signs of poisoning unlike those of typical cyanotoxins.

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11.37 Comparison between male and female pups of Wistar rats during their reflexological and behavioral development

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Introduction: Previous studies with *Tityus serrulatus* scorpion venom showed deleterious effects on offspring when injected into rats during pregnancy. There are no studies in the literature showing if there are effects on the offspring, when the venom is inoculated during lactation and no comparison was made between males and females regarding their behavioral and reflexological development. **Objectives:** Our aim was to study and to compare the behavioral and reflexological effects between males and females in offspring of rats in the postnatal period and adulthood after maternal administration of saline on day 10 of lactation. **Methods:** Lactating females were injected with saline on the 10th postnatal day. Their offspring were assessed for their reflex development. The parameters observed were: palmar grasp, surface righting and negative geotaxis. The same pups were evaluated for their behavioral development in adulthood (2 months) and the tests utilized were: activity box (where locomotion and total activity were determined), forced swim (where the time was spent until the animal stopped swimming and the time of immobility were determined in training and test sessions), enriched environment (where the total motor activity and the time of exploration were determined) and social interaction (where the time spent in interaction between animals were determined). **Results and Discussion:** Comparisons were made between males and females, but there was no significant difference between them. These results indicate that both, young males and females could be used for future comparisons with animals treated with venom because there is no difference between them.

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11.38 Dual effect of *Crotalus durissus terrificus* venom on neutrophil functions

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Introduction: Previous works showed that *Crotalus durissus terrificus* snake venom (CdtV) modulates macrophage function, inhibiting the spreading and phagocytic activity but increasing the microbicidal activity and the oxidative burst of these cells. In addition, crotoxin (CTX), the main component of the venom, was reported to inhibit this phagocytic activity. Recently, CdtV was shown to inhibit phagocytosis by neutrophils, via CR3 receptors. Moreover, CTX was characterized as the CdtV component responsible for the inhibitory effect on phagocytosis by neutrophils. However, phagocytosis and microbicidal activity of neutrophils obtained from rats treated with CdtV and CTX have not yet been investigated. **Objectives:** The aim of this study was to investigate phagocytosis activity, via CR3 receptors, and microbicidal activity by neutrophils obtained by carrageenan-induced peritonitis from rats treated with CdtV or CTX. **Methods:** In an *in vivo* study, male Wistar rats were treated with CTX (18 μg/300μl/rat, s.c.) or saline (control) (CEUAIB 734/10) administered subcutaneously to rats at different time periods: 2 h, or 1, 4 or 14 days before or 1 h after inoculation with carrageenan (cg, 4.5 mg/kg). Neutrophils were obtained 4 h after the intraperitoneal administration of cg. *In vitro* assay. Neutrophils were obtained 4 h after the intraperitoneal administration of cg and incubated with CdtV (0.125, 0.25, 0.5, 1.0 and 2.0 μg/mL) or CTX (0.02, 0.04, 0.08, 0.16 and 0.32 μg/mL) for 1 h at 37°C. Phagocytosis and microbicidal activities of neutrophils were evaluated after in vivo or in vitro treatment with CdtV or CTX. **Results and Discussion:** *In vivo*, the injection of a single dose of CTX reduced the percentage of phagocytosis by peritoneal neutrophils at all times of treatment: 2 h: 24%, 1 day: 31%, 4 days: 25%, 14 days: 18% and 1 h after cg: 35%. These data confirm the results obtained previously *in vitro* which indicate CTX as the CdtV component responsible for phagocytosis inhibition. Moreover, the results show that the treatment with CTX induces a long-lasting inhibitory effect on phagocytosis by neutrophils. However, CdtV and CTX *in vitro* and *in vivo* did not alter the microbicidal activity of neutrophils, unlike macrophages. Thus, the results demonstrate a dual effect of CdtV on neutrophils, since it inhibits phagocytosis but did not modify microbicidal activity. Considering the difference between our results and those reported in the literature for macrophages, these data may indicate differences in the mechanisms of microbicidal activity between these cells.

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11.39 Survey of Linyphiidae (Arachnida, Araneae) of litter in fourteen areas of the Atlantic Forest

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Introduction: The order Araneae is the second largest among arachnids, with 40,462 described species and distributed among 109 families. This number probably represents only a portion of the actual number, since recent estimates suggest the existence of more than 70,000 species worldwide. Of these families, about 70% have representatives that live exclusively in leaf litter. The family with the highest incidence in leaf litter is Linyphiidae, with about 4000 described species in 585 genera. However, the soil spiders, despite their high diversity in the neotropics, are still poorly studied. It is estimated that over 50% of material deposited in the South American collections consists of undescribed species. This project seeks to use the material collected and deposited in the collection of Instituto Butantan, through the thematic project BIOTA-FAPESP program, which also allows the ecological investigation of the soil araneofauna. **Objectives:** This study aimed to learn more about the spiders of the Linyphiidae, to survey and compare the diversity of areas, and to assess the composition of arachnids and to deposit the material in the collection of the Institute Butantan. **Methods:** The material was collected with pitfall traps and then sorted according to morphospecies and deposited. We used the collector's curve to examine the quality of sampling, correspondence analysis where it was possible to identify the characteristics of arachnids of sites, and their similarities and differences, and finally to perform comparisons with the literature that used pitfall traps as sampling methodology. **Results and Discussion:** Spiders were identified in 2132, divided into 62 morphospecies of which 49 the species could not be determined since we found in the literature, suggesting a more comprehensive taxonomy of this family because of the amount of richness found and not yet known. The area was the richest in Caraça, with 19 morphospecies and the largest number of copies in the samples. The species mostly occurring in the areas was *Meioneta* sp. 1, found in eight areas, followed by the *Vesicapalpus simplex* occurring in seven areas, allowing the conclusion that these species are generalists. The collectors' curves showed an increasing pattern, suggesting that most of the areas sampled could have been better, except in areas of Maquiné and Órgãos, which plateaued. In CA, we observed a pattern north and south; only the area is nonstardard because of generalist species and similarities with the area and Copasa Órgãos. In comparison with studies using pitfall traps, it was observed that the interior forests have an abundance of Linyphiidae in comparison with coastal forests, as in comparisons between the areas sampled in Brazil. However, we note that studies of soil spiders in the country are still preliminary and that collections of spiders in the soil should be conducted more frequently, so we can better know the litter spiders.

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11.40 Diversity of Oonopidae (Arachnida, Araneae) of litter in fifteen areas of the Atlantic Forest

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Introduction: Spiders are the dominant invertebrate predators in most terrestrial habitats. It is the second order in richness among the Arachnida, behind only the Acari, with 41,253 described species, distributed among 109 families. The family Oonopidae contains 543 described species, distributed among 74 genera. Due to their small size (1-3 mm), they are abundant in any area in the Neotropical region. **Objectives:** The objectives of this study were to evaluate the structure and estimate the diversity of soil oonopids in fifteen areas of the Atlantic Forest, and compare the data with information from other samples reported in the literature. **Methods:** The material was collected during the project BIOTA/FAPESP conducted between 2001-2003, with pitfall and Winkler traps in 15 areas of the Atlantic Forest. For analysis, we used the the collector's curve, correspondence analysis (CA) and simple linear regression. **Results and Discussion:** The total number of individuals collected in 15 areas sampled was 1326, of which 882 were adults and divided into 33 morphospecies. Of these, only three were determined to the species, suggesting that most species are still undescribed. Returned 801 specimens distributed in 31 species in the pitfall traps, 20 of which were unique to that method. Winkler obtained 81 specimens, belonging to 14 species, of which three were captured exclusively by this method. The layout of the areas revealed by the CA showed a pattern of north/south fauna, although there are some exceptions to this pattern, which presents some generalist species. Works that sampled the fauna of the canopy record a richness and diversity in general lower than that observed in the litter. The collector's curves plateaued, in general, with only those areas that had a large sampling effort, such as Caucaia Caparaó Caraça and Órgãos. Pitfall traps were more effective than Winkler, obtaining a larger number of species and individuals, while Winkler contributed some unique species. In reviewing the literature, we found that the richness of the areas sampled was similar to or greater than that recorded in the references to Atlantic Forest areas, but was less than that observed in Amazon areas. However, one must take into account that there is a wide variation in collection methods employed, sampling effort and sampling period in these surveys. The family's richness tends to grow toward the equator, which is consistent with the latitudinal gradient of species richness, a known ecological pattern. In the collections made by the project BIOTA, many potentially new species were obtained, which will broaden the diversity of species in this family.

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11.41 Antibacterial activity of fractions from the marine sponge *Amphimedon viridis*

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Introduction: The emergence of bacterial strains with multidrug resistance, besides the indiscriminate use of antibiotics, has created an urgent need for the development of new antimicrobial compounds and new strategies to treat bacterial infections. The potential contribution of marine organisms to the discovery of new bioactive molecules, mainly with antibacterial activity is very promising. The substances isolated from the sponge of genus *Amphimedon* have shown antimicrobial activity against Gram-positive and Gram-negative bacteria. **Objectives:** The aim of this study was to evaluate the antibacterial activity of the fractions obtained from the marine sponge *Amphimedon viridis*. **Methods:** The sponge *A. viridis* (Niphatidae, Haplosderida) was collected in the urban coast of Maceió, Alagoas, Brazil. The aqueous extract was desalted and the concentrated aqueous layer was submitted to chromatography on Sephadex G100 column with deionized water as mobile phase. The last fraction (LC) showed antimicrobial activity and was re-chromatographed. All fractions were submitted to microdilution antimicrobial susceptibility testing against ATCC strains of *Escherichia coli* and *Staphylococcus aureus*. The fractions were analyzed in 96-well plates, using Poor broth and bacteria at 10^4 CFU/well. The microtiter plates were incubated at 37°C for 18 h, and culture turbidity was then measured in an ELISA reader at 595 nm to assess bacterial growth. The fractions with small quantities and similar content were combined. **Results and Discussion:** The LC fraction showed a minimal inhibitory concentration of 12.5 µg/mL against *S. aureus* and 25 µg/mL against *E. coli*. The antimicrobial activity against both *S. aureus* and *E. coli* was detected in the fractions obtained from the LC fraction: 1, J (these two fractions showed the highest antimicrobial activity and also hemolytic activity), 29, H, L, N, T, U, V and AL (21.9% to 46.6% - *S. aureus* and 14.7 to 29.7 – *E. coli*). Some fractions inhibited mainly the growth of 37% to 48.6% of *S. aureus* growth: 7, 13, 15, 16, 19, 20, E, P, R and S; while others inhibited mainly *E. coli* (27.7% to 39.1%): 10, 11, M and Z. Alkylpyridine polymers called halitoxin show toxic and cytotoxic activities and have been identified previously in sponges of the same genus. These results confirm the potential antimicrobial activity of fractions obtained from *Amphimedon viridis* extract, which may contain halitoxin or other active substances in its composition.

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11.42 Characterization of glycine release from striatal tissue in superfusion: understanding Parkinson's disease

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Introduction: Parkinson disease is a neurodegenerative disorder affecting substatia nigra dopaminergic cells. Striatal tissue is largely involved in motor control under dopaminergic afferences. Although largely studied, many striatal circuits are still poorly understood. Previous investigations have shown that glycine interferes with striatal acetylcholine release, suggesting the importance of glycine as a neurotransmitter in this brain area. **Objectives:** We aimed at investigating whether glycine acts as a neurotransmitter or a co-transmitter, together with GABA, in striatal tissue. This report describes the initial characterization of glycine release. **Methods:** Male Wistar rats were used. After decapitation, brains were removed and striatal tissue was dissected and kept in ice-cold Krebs Ringer-bicarbonate (KRB) previously gased with carbogen. Tissue was cut into prisms using a McIlwain tissue chopper, suspended and pre-incubated at 37°C for 5 min. An aliquot of 20 μL of 3H-glycine was added to the incubation that lasted 20 min. Tissue was then filtered and washed twice with ice-cold KRB, transferred to a beaker and suspended for distribution into 18 parallel superfusion chambers (Brandel SF2500 – USA). A stabilization superfusion lasted 45 min and then, eighteen 3 min aliquots of effluent were taken from each chamber for radiometric quantitation. Drugs were included in the superfusion medium according to the experimental protocol. Results are expressed as fractional release, i.e., the percent of radioactivity released in a given moment of the perfusion. **Results and Discussion:** Glycine was released at a steady baseline of about 4.5 – 5% of the total loaded amount. Depolarization effected by 35 mM KCl induced a further 6% release that was partially calcium-dependent. Calcium channel blockers (L-type) calciceptine, (N-type) w-ConoMVIIc had no effect on stimulated release. Drugs that were ineffective in changing stimulated release included nicotine (cholinergic), muscimol (GABAa agonist), NMDA (glutamate agonist), glutamate itself and tetrodotoxin. A complete characterization of glycine release in this tissue including second messenger signaling and neurotransmitter interactions should add to the proposal of new therapeutic strategies to fight Parkinson disease.

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11.43 Glutamatergic inhibitory effect on melatonin synthesis and secretion involves interactions between pinealocytes and astrocytes via a soluble factor

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Introduction: The glutamatergic modulation of melatonin synthesis is well established in the literature as well as the importance of astrocytes in mediating glutamatergic signaling in the central nervous system. In our laboratory we demonstrated that the inhibitory glutamate effect on melatonin synthesis is dependent on paracrine interactions between pinealocytes (the secretory cells that synthesize melatonin) and astrocytes (the main glial cell in the pineal gland). **Objectives:** The objective of this work was to investigate the glutamate receptors involved in this glutamate inhibitory effect and the nature of the interactions between astrocytes and pinealocytes. **Methods**: Young male Wistar rats were sacrificed by decapitation and their pineal glands were isolated and dissociated using the Papain Dissociation System kit. The pinealocytes in association with astrocytes (co-culture) were kept in culture (DMEM medium + 10% BSF) and then were submitted to the pharmacological treatments for 5 h. The cells were stimulated with norepinephrine (1 μM) associated with glutamate (600 μM) or with the defined agonists to AMPA (AMPA – 50 μM) or NMDA (NMDA – 100 μM) ionotropic receptors, to type I metabotropic receptors (DHPG – 50 μM), or to type II metabotropic receptors (L-CCG – 10 and 100 μM). Moreover, the cells in co-culture were physically isolated using inserts and were stimulated with norepinephrine (1 μM) and glutamate (600 μM). The cells were also stimulated with norepinephrine (1 μM) combined with glutamate (600 μM) and with BB1101 (10 μM) which is the TNF-α inhibitor. Melatonin was quantified by HPLC with electrochemical detection. **Results and Discussion**: The inhibitory effect caused by glutamate on melatonin synthesis and secretion was also observed when NMDA agonist was used. The other agonists (AMPA, DHPG, L-CCG) did not modify melatonin synthesis. When the cells were separately cultured using inserts, the glutamate inhibitory effect also occurred. The inhibitory effect caused by glutamate on melatonin secretion could not be observed when the TNF- α inhibitor was used. The evidence obtained supports the idea that glutamate modulation of melatonin synthesis involves paracrine interactions between pinealocytes and astrocytes through the stimulation of NMDA receptor and the diffusion of a soluble factor, probably TNF-α, which inhibits melatonin synthesis.

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11.44 Study of the action of melatonin on the injury induced by toxins from the venom of *Micrurus lemniscatus* in cultured rat hippocampal neurons

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Introduction: In elapid venom of *Micrurus lemniscatus,* there are neurotoxins present with presynaptic and postsynaptic actions. Studies show that different neurotoxins in this venom are powerful pharmacological tools for studying neurotoxic processes and discovering novel neuroprotective agents. Melatonin, secreted by the pineal gland, has antioxidant function and studies show that melatonin enhances GABA receptors, hyperpolarizing the cells, and then decreases neuronal excitability, which may contribute to the neuroprotective effects of melatonin. **Objectives:** The aim of this study was to investigate the possible neuroprotective action of melatonin on the neurotoxic effects of toxins Mlx-11 and Mlx-12 isolated from the venom of the snake *Micrurus lemniscatus* in cultured primary rat hippocampal neurons. **Methods:** The neuronal culture was prepared from hippocampus taken from fetuses of 18-19 days. The tissue was dissociated with trypsin and with a Pasteur pipette. The cells in culture were placed in microplates pretreated with poly-L-lysine. These are incubated in 5% CO2. After 6-7 days in culture, hippocampal cells were exposed to 6 or 24 h to toxins Mlx-11 and Mlx-12 at concentrations of 10, 100 or 1000 ng/mL and to KCl (250 mM) as a positive control. To test the neuroprotection of melatonin, this was incubated at concentrations of 10^{-7} M and 10^{-9} M, about 30 min before the toxins. The experiment was carried out using the MTT colorimetric assay, which measures cell viability. **Results and Discussion:** The group submitted to Mlx-11 for 6 h showed statistically reduced cell viability when compared to the control group just in the group treated with 1000 ng/mL. When incubated for 24 h, the group with Mlx-11 at a concentration of 10 ng/ml was the only one that showed no statistically significant difference in the control group. In both periods, there were no statistically significant differences between the groups that received only the toxin and those who received it with melatonin. In the groups incubated with Mlx-12 for 6 h, there were no statistically significant differences between the groups treated with toxins and control. By incubating Mlx-12 for 24 h at different concentrations, all showed differences compared to the control. At a concentration of 10 ng/mL, there were no statistically significant differences between the groups that received only the toxin and those who received it with melatonin. For groups that received Mlx-12 at concentrations of 100 ng/ml to 1000 ng/ml, there were significant differences between the groups incubated with melatonin. The results show that different concentrations of Mlx-11 and Mlx-12, used in this study, significantly reduced the viability of hippocampal neurons maintained in culture. Melatonin showed a neuroprotective role against cell death caused by the toxin Mlx-12 at concentrations of 100 and 1000 ng/ml, when cells were exposed for 24 h. Melatonin at a concentration of 10^{-9} M was shown to have a higher profile than with the neuroprotective concentration 10^{-7} M.

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11.45 DNA damage in hemocytes of *Biomphalaria glabrata* (Say 1818) measured by the comet assay after treatment with different classes of genotoxins

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Introduction: The comet assay or single cell gel electrophoresis (SCGE) assay is a simple, sensitive and rapid technique for the detection of DNA damage in individual cells. This technique has been the major tool in the evaluation of genotoxic effects in genetic toxicology. In this work, two chemicals from different classes of mutagens (ethyl methanesulfonate - EMS and cyclophosphamide - CP) and hydrogen peroxide - HP were tested to evaluate the expression of DNA damage in hemocytes of *B. glabrata* in the SCGE assay. **Objective:** The aim of this study was to evaluate genotoxic activity of direct and indirect genotoxins in hemocytes of *B. glabrata* using the SCGE assay. **Methods:** Snails were exposed for 3 days to different concentrations of EMS (1, 10 and 50 mg/L) and 7 days for CP (10, 100 and 500 mg/L) with the solutions renewed every 48 h. Isolated hemocytes were exposed to hydrogen peroxide (10 30 and 50 μM) for 5 min. 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). 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 2 h. They were subsequently incubated in freshly prepared alkaline buffer (300 mM NaOH and 200 mM EDTA, pH>13) for 20 min for DNA unwinding. Electrophoresis (20 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 (pH 7.5) and fixed for 10 min in ethanol. Prior to the examination, the slides were stained with 20 μg/ml ethidium bromide and 100 cells per slide (200 per each animal) were analyzed 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 CP concentrations. The lack of genotoxic effect in the comet assay with CP was observed by other authors and was attributed to the induction of inter-strand cross-linking. The direct genotoxins EMS and HP induced a significant increase in DNA migration. The trypan blue exclusion test showed no cytotoxicity for all agents.

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11.46 Secretion of a toxin by atypical enteropathogenic *Escherichia coli* depends on the culture medium used

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Introduction: EPEC is associated with diarrhea in many developing countries. It is subdivided into typical (tEPEC) and atypical (aEPEC). aEPEC differs from tEPEC in that it does not possess the EAF plasmid and in that it shows a greater heterogeneity of virulence factors, which explains the rise in the prevalence of this group. It is known that the expression of toxins is an important factor in diseases caused by different pathogens. Still, the expression of toxins by aEPEC remains very poorly studied. Our group has been searching for toxins described in other *E. coli* categories in aEPEC. These studies led to the identification of aEPEC isolates which express the toxin Pet (plasmid encoded toxin), classically known as enteroaggregative *E. coli*. On the other hand, although there are aEPEC isolates positive for the ***sat*** gene, which encodes the toxin Sat (secreted autotransporter toxin) and described in diffusely adhering *E. coli* (DAEC) and uropathogenic *E. coli* (UPEC) strains, the expression of this important toxin is still not clear. **Objectives:** The aim of this work was to establish better culture conditions for aEPEC in order to improve studies on the expression of the toxin Sat in *sat*+ isolates. **Methods:** Cytotoxicity assays in HEp-2 cells were performed with the bacterial culture or supernatant of the culture from isolates 589 (O5:H2) *sat/pic/east*; 1887 (O111:H38) *sat/hly*; 2294 (O9:H33) *east/Sat*, DAEC (C1845 and 114) and C600 (non pathogenic) grown in DMEM or DMEM + 1% tryptone. The cell parameters used in the study of toxin expression were: morphology, detachment and cell viability, evaluated by staining with Giemsa, crystal violet/Giemsa or trypan blue, respectively. **Results and Discussion:** Our results show that isolates positive for the *sat* gene were unable to produce alterations in cells grown in DMEM. On the other hand, when 1% tryptone was added, vacuolization, cell detachment and a significant reduction in cell viability were observed. These effects were more intense than those we described previously when concentrated supernatant from bacterial cultures, containing only substances over 50 kDa, were used. Comparative cytotoxicity assays also performed with aEPEC isolates pet + showed that the cytotoxic effects, observed only after 24 h of incubation with the supernatant from bacterial cultures grown in DMEM, were observed after 5 h when 1% tryptone was added to the DMEM medium. These findings are important not only for the detection of Sat in aEPEC, but also because they open the possibility of discovering new toxins with the use of tryptone. This will help improve the understanding of aEPEC pathogenesis, which is known to show highly heterogeneous virulence factors and which is, up to this time, practically unknown.

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