10. Others

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10.01 Process validation methodology applied in immunobiological production at Butantan Institute

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Introduction: Validation of the production process is a requirement of Good Manufacturing Practice (GMP) and consists in documented evidence that the immunobiological production process is effective and reproducible. The validation process starting with equipment qualification (Installation Qualification - IQ, Operation Qualification - OQ and Performance Qualification - PQ) such as the bioreactor, followed by Media Hold Test that simulate the production process with the bacteriological culture medium. The last step in process validation is performed with the product. **Objectives:** The aim of this study was to describe a methodology applied in order to validate the immunobiological production process according to the national requirements (ANVISA) and World Health Organization (WHO). **Methods:** The measuring instruments and control process of the bioreactor were calibrated. All specifications of the equipment parts were checked in IQ. OQ was performed by functional tests, such alarms and critical point test while simulating the production procedure. PQ consisted of three cycles of the sterilization process with the empty equipment, and the temperature was monitored by sensor and biological indicator geometrically distributed inside the bioreactor. Three batches of the Media Hold Test were performed using tryptic soy broth (TSB) and the samples collected during the test were evaluated by sterility and bioburden tests. The environment was monitored by viable and non-viable particles. Process validation was carried out with three consecutive batches of immunobiological product, and the quality control tests were performed during the production and in the final product. **Results and Discussion:** IQ demonstrated that the equipment was in accordance with the project and user requirements. The results of functional tests demonstrated that the equipment was certified by OQ. In PQ, all sterilization cycles showed a temperature at 121 ± 1°C and pressure at 1.2 ± 0.1 bar, and the biological indicator proved the efficiency of the sterilization process. The result of sterility test in the Media Hold Test showed no microbial growth after 14 days at 37 °C and the bioburden test showed that the amount of microbial particles acquired during the process was in accordance with the requirements for the immunobiological production. The quality control tests performed during process validation demonstrated that the product met the criteria established by ANVISA and WHO requirements.

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10.02 Comparative studies between monogamous and polygamous mating in the Swiss mouse strain

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Introduction: The Central Animal House of Butantan Institute sets high demands for laboratory animals, requiring quality standards and providing animal well-being. These factors create a challenging scenario to administer, since the Swiss mice reproductive lifetime decreases over time, the interval between birthing increases and proliferation decreases, leading to low productivity. **Objectives:** The objective of the present study was to perform a comparative study of two mating types: monogamy (1:1) and polygamy (2:1) in order to verify and define the mating types that better match with demand and to maintain quality standards, providing animal well-being. **Methods:** Swiss mice were housed in polypropylene boxes and kept in rooms with flow set, protected by sanitary barriers (autoclave, air filtration system, differential pressure), with ambient temperature of 22±2°C, light cycle of 12 L:12 D and free access to water and feed. The animals were divided into two types of mating systems: monogamous (n = 25 females : 25 males) and polygamous (50 females : 25 males). Animals were mated to 60 days old and kept together until the 7th birth. After this period, the groups were analyzed as to birthing intervals, number of births per female, and number of weaned as well as pre-weaning mortality. **Results and Discussion:** In both mating types analyzed, intervals between births was reduced in 2nd to 4th parturition, but after this period, the intervals gradually increased. The interval average between births from 1:1 mating was 27.41 days. However, in the polygamous system, it was 25 days. Moreover, it was observed that the average weaned between two mating systems was similar, around 7.19 and 7.3 animals weaned per female respectively for monogamous and polygamous groups. The mortality rate of pre-weaning in both groups was low (0.20 and 0.27% of monogamous and polygamous groups, respectively). The highest yield from monogamous mating occurred on 3rd parturition with 8 born per female. In the 2:1 system, the highest production occurred at the 4th birthing, reaching 8.5 animals per female. There was no significance for the traits analyzed between mating systems, suggesting a need for more studies with larger numbers of animals with the aim of total elucidation of these results. However, we can affirm that the polygamous system (2:1) appears to be more suitable for intensive animal production with high demands, as it requires less space and material, which reduces labor hours and the skilled workforce needed, allowing the optimization of production.

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10.03 Snake welfare: "Curious" food items offered to feed snakes coming to Butantan Institute

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Introduction: The Butantan Institute in its history has stood out in the knowledge of snakes. The institution has received snakes from all over Brazil since 1903, when Vital Brazil set established his effective work on environmental education. Thanks exclusively to the cooperation of suppliers of venomous animals, we could develop this work. The snakes sent to Butantan Institute came in different types of boxes, some suitable for this transportation and others with less safety, such as pet bottles, cardboard boxes and a number of other kinds of containers. To accommodate the snake in the container used for transportation, some supplies were added, in addition to moistened cotton (as recommended), a little soil as substrate, newspaper, rags and even small branches and leaves, presumably to better accommodate the animal besides some food items. All snakes received were destined for different research areas of the Institute and used for several purposes: for antivenom production and also for research, systematic studies, such as general biology and physiology, public exhibition and environmental education. Besides, some specimens were included and registered in the Herpetological Collection and others used for feeding ophiophagous snakes. **Objectives:** As the diet of snakes is poorly known we recorded here different food items offered to the snakes by the suppliers. **Methods:** Only items clearly placed to feed the snake which could not have come together with the substrate or vegetation placed in the container were considered during a one-year investigation period. **Results and Discussion:** We recorded food items such as slugs, ball-armadillos, ants, spiders, beetles and other arthropods. In most cases, prey offered could be ingested by the snake, concerning size relationship, but items did not belong to the usual known diet list. We also recorded food for human feeding purposes (rice, beans, hotdog, lettuce, cauliflower, bread, cookies and a coconut sweet piece). Such information emphasizes the supplier's concern regarding the snake's welfare despite the fear of being bitten. Moreover, it corroborates previous environmental educational studies carried out in the Laboratory of Herpetology, Butantan Institute.

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10.04 Searching for the control of nuclear DNA replication in trypanosomes

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Introduction: Chromosomal replication begins with the assembly of the prereplication complex (pre-RC) at replication origins. In eukaryotes, the pre-RC is composed of the ORC complex containing six proteins, Orc1-Orc6, two proteins called Cdc6 and Cdt1, and the minichromosome mainenance (MCM) complex, which is composed of Mcm2 to Mcm7 proteins and shows helicase activity, essential for DNA replication. Once pre-RC is organized in the chromatin, origins become licensed to replicate. Since ORC, Cdc6, and Cdt1 are required for loading MCM onto DNA, but are not required for the continued MCM-DNA interaction, the downregulation of their expression and/or activity at the end of G1 represents, in eukaryotes, an effective way to block DNA replication. Trypanosomes do not contain an ORC complex, Cdc6 or Cdt1. Instead, they contain a protein homologous to Orc1 and Cdc6, called Orc1/Cdc6 which is a component of pre-RC. Orc1/Cdc6, however, does not seem to be involved in DNA replication control, since it is bound to DNA during the entire cell cycle. **Objectives:** Therefore, we wondered if Mcm proteins could be involved in this control in trypanosomes. **Methods:** In this work, we searched for sequences in trypanosome databases, and we found 10 genes for *T. cruzi* and 8 genes for *T. brucei* annotated as Mcms. By alignment analysis, we identified the likely Mcm7 gene. *T. cruzi* Mcm7 was then cloned and expressed by a prokaryote system. The recombinant protein rTcMcm7 was used to immunize mice. **Results and Discussion:** The antibody obtained was able to recognize the *T. cruzi* recombinant Mcm7 protein as well as the putative *T. brucei* recombinant Mcm7, expressed by Sf9 cells using the baculovirus system. The anti-rTcMcm7 serum will be now used in Western blotting and immunofluorescence assays in order to analyze the expression and localization of Mcm7 during the cell cycle of trypanosomes.

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10.05 Comparative evaluation of antioxidant activity of raw and processed pequi fruit (*Caryocar brasiliense* Camb.)

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Introduction: The pequi is a characteristic tropical fruit that is cultivated in the Central-West and Northest regions of Brazil. This fruit is very popular in Brazil and normally is consumed with a rice and chicken meal. The pequi fruit is normally sold in the raw or processed forms. The phenolic compounds present in many foods, including fruits, vegetables and spices, exhibit antioxidant properties. **Objectives:** The objective of this study was to evaluate the thermal processing effect on pequi's phenolic composition, its antioxidant activity "in vitro" and in cellular metabolism in MDCK cultivated cells. **Methods:** The phenolic compounds were evaluated by the Folin-Ciocateau method. The antioxidant activity was measured by β-carotene/linoleic acid and DPPH• methods. Cell viability was measured by MDCK cell cultivation at 37 °C in L15 medium in six 24- well plates. The phenolic compounds in aqueous extract of raw and processed pequi fruit were 57.36 mg/100 g and 45.18 mg/100 g, respectively. **Results and Discussion:** The antioxidant activity measured by β-carotene/linoleic acid was 90.70% and 85.01% in raw and processed pequi fruit and in the DPPH• the half maximal effective concentration EC50 in μg/mL was 289.9 and 240.6, respectively. The viability of MDCK cells was 68.6% and 64.9% for raw and processed pequi extracts. Therefore, both induced higher cell viability levels than observed in the control. Thermal processing induces a minor decrease in the content of phenolic compound and antioxidant activity, as well as in cell viability in response to aqueous pequi extract, which was not a significant loss with regard to the importance of these phytochemical compounds in pequi.

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10.06 New technique for undoing slides and recovery of type specimens and material of mites (Acari) in deteriorated Hoyer's medium

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Introduction: The Acari Collection of the Instituto Butantan (IBSP) includes a large slide collection. This collection has many types of mites that are deteriorated. Since 1996, we have tried to recover the types, dismounting slides and remounting them based on a traditional technique using Hoyer's medium. However, most of the types were impregnated with old gum arabic, and the remounted slides were not satisfactory so far. **Objectives:** The aim of this study was to recover important material of types and other specimens in mite collections that are in deteriorated Hoyer´s medium. **Methods:** A) Undoing slides: After copying the data label, the slides were placed in a Petri dish with distilled water to 55ºC in an oven to remove the sealant. Usually, the slide could be separated from coverslip after around 24 h. If the coverslip still stayed attached to slide, the process was repeated. When the coverslip was free, it was carefully removed with a micropin to avoid damaging the specimen. The specimen was removed with a small paint brush and placed into a cavity slide. Sometimes the specimen still stayed fixed in old and dirty glue from Hoyer's medium, and in this case, it was placed in a Petri dish with distilled water to 80ºC until the specimen appeared clean. For each specimen, a clean cavity slide and paint brush were used, to avoid contaminating the next mite. B) Remounting slides: A drop of Hoyer's medium was placed on a new clean slide. The mite specimen was placed in the ventral position over it, and the slide was heated at 55ºC for 10 min for fixation. Hoyer's medium was replenished, and specimen covered with a coverslip; the slide was then placed at 55ºC for a week or more. The excess around the coverslip was removed with a blade and the slide returned to the oven at 55ºC until completely dry. The slide was labeled. Finally, the coverslip was sealed with Glyptal sealant. Material recovered was photographed and prepared in CorelDraw. **Results and Discussion:** The techniques used to date were not efficient in the undoing of mite slides, because they failed to take into account the differences in their external structures. In most cases, the specimens were damaged, not allowing the identification of basic characters for their taxonomy. The same did not occur with this new technique that uses only heated distilled water. It was excellent in separating the coverslip from the slide and in diluting the Hoyer's medium completely without damaging the specimen. Another reason to remove the sealant with water is that other chemical products are toxic, and can penetrate and deteriorate the specimen. With this new technique of undoing slides, the types and other material of mites of the IBSP Acari Collection have been successfully recovered.

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10.07 Cellular absorption and antioxidant activity of free phenolic acids from pomegranate (*Punica granatum* L.)

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Introduction: Oxidation reactions are of major significance in both human physiology and food preservation. Oxidative stress in humans has been associated with several diseases, and food rancidity with oxidative spoilage. The phenolic acids present in many fruits and vegetables possess antioxidant activities. **Objectives:** The present study aimed to evaluate the antioxidant activity of free phenolic acids obtained from pomegranate pulp and its transport across MDCK cultured cells, focusing on protection from oxidation. **Methods:** The antioxidant activity was measured by β-carotene/linoleic acids and DPPH• methods. The phenolic acids were determined by high performance liquid chromatography (HPLC), based on their spectral characteristic and retention time, comparing with standards. The transwell plate technique with MDCK cells cultivated at 37°C in L15 medium was used to measure the transport of 70 μg/0.15μL of pomegranate free phenolic acids (FPA) using gallic acid as control. **Results and Discussion:** The antioxidant activity of FPA measured by β-carotene/linoleic acids was 68.18% and by DPPH• was half maximal effective concentration, EC50 =1.04 μg/mL. The principal phenolic acids transported by MDCK cells after 40 min were caffeic, catechinic, feluric, protocatechinic, sinapic and fumaric at 25% of the aforementioned initial level and against 35% of gallic acid as control. Thus, phenolic acids from pomegranate were absorbed by MDCK cells, which can confer oxidative protection in them. The free phenolic acids from pequi fruit have antioxidant activity and are absorbed by these cells. These properties could be useful in regular diets and cosmetics industry.

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10.08 An investigation of the host range of human influenza viruses

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Introduction: Studies on the host range of influenza viruses have been of great importance to determine the role of animals, once unlikely links, in the virus transmission chain. **Objectives:** This study aimed to investigate the circulation of the influenza virus in cats in Brazil. **Methods:** Domestic cats, seen at the clinic of the Faculty of Veterinary Medicine at the University of São Paulo, were grouped according to gender and age (young and adult). Serum samples were collected and, prior to titration, were examined for antibodies to influenza A and B viruses by the hemagglutination inhibition (HI) test using the corresponding antigens from the circulating viruses in Brazil. **Results and Discussion:** In cats between 6 and 20 years old, 20 % responded with high antibody titers (≥640 HIU/μL) against human influenza type A (H1N1). Lower percentages of the animals in the same age group, 11% and 8%, showed the same high titers in response to human influenza types A (H3N2) and B, respectively. In the gender group, 17 % of males and 8% of females showed a poor antibody response against the influenza A (H1N1) virus (titers of ≤20 HIU/μL). Protective titers of ≥40 HIU/μL against human influenza viruses suggest viral infection transmitted to the domestic cats by humans. In conclusion, our results show that domestic cats, like other mammals, may play a role in interspecies transmission and spread of the influenza virus.

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10.09 Chronic treatment with BPP-5a and BPP-10c attenuates the hypertension and cardiac hypertrophy in spontaneously hypertensive rat (SHR)

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Introduction: Some BPPs isolated from *B. jararaca* venom have an antihypertensive effect independent of ACE inhibition. A recent study proposed that the activation of AsS enzyme is a mechanism for the antihypertensive effect caused by BPP-10c, which raises an NO-dependent effect. **Objectives:** The aim of this study was to evaluate the effect of chronic treatment with BPPs 5a and 10c on cardiovascular parameters and cardiac hypertrophy in SHR. **Methods:** Experiments were conducted in male SHRs (±300 g). Blood pressure measurements were performed by a non-invasive tail cuff method. Rats were anesthetized (tribromoethanol, 250 mg/kg, i.p.) for subcutaneous insertion of mini-osmotic pumps (Model 2002, ALZET) containing BPP 5a, 10c (n=5-8) or vehicle (0.9% NaCl) (n=7). The doses administered of each BPP were 71 and 710 nmol/kg/day. Cardiovascular parameters were determined at 0, 1, 4, 7, 10 and 14 days. At the end, blood samples (6 mL) were collected and the heart was removed. The left ventricle (LV) was weighed and mass index (LVMI) was calculated by the ratio LV/body weight (mg/g). L-Arginine (L-Arg) and NO plasma levels were measured by HPLC and NO analyzer, respectively. Statistics: Student's *t* test or one-way ANOVA; $p<0.05$. **Results and Discussion:** Compared to vehicle ($p<0.05$), both BPPs reduced blood pressure throughout treatment. At a dose of 71 nmol/kg/day, BPP-5a and BPP-10c reduced mean arterial pressure (MAP) (-24±5 and -26±2 mmHg, respectively). At 710 nmol/kg/day, reductions in MAP by BPP-5a and BPP-10c were -17±3 and -26±3 mmHg, respectively. There were no differences in the range of antihypertensive effect between peptides and doses. In addition, slight bradycardic effect (-20±7 bpm) was found only in animals treated with BPP-10c (71 nmol/kg/day). LVMI was smallest after BPP-5a and 10c treatments (71 nmo/kg/day) compared to control (2.53±0.05 and 2.57±0.03 *vs.* 2.76±0.11 mg/g; $p<0.05$). Based on LVMI results, LV histological investigation is required. Preliminary results with 71 nmol/kg/day showed that BPP-5a increases L-Arg levels (2.92±0.63 nM, $p<0.05$), while BPP-10c decreases L-Arg levels (0.38±0.06 nM, $p<0.05$) compared to control (0.8±0.03 nM). Despite L-arg differences, no changes in NO levels were observed after either treatment, compared to control. Chronic treatment with BPPs at both doses caused a long-lasting antihypertensive effect, such as reduced cardiac hypertrophy in SHR. Results concerning plasma levels indicate mechanistic differences between BPPs 5a and 10c. However, further studies are needed to elucidate the mechanism(s) involved. Nevertheless, these peptides may be considered potential pharmacological tools to treat cardiovascular diseases.

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10.10 Evaluation of the in vitro activity of piplartine against schistosomula and adult flukes of *Schistosoma mansoni*

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Introduction: Schistosomiasis is a neglected tropical disease, considered a severe public health problem worldwide. Praziquantel is the only effective drug currently used against all schistosome species, and as a consequence, parasite resistance remains a major challenge. Thus, the search for antiparasitic compounds from natural sources, mainly from plants, has been encouraged. **Objectives:** The aim of the present study was to determine the effect of piplartine, an amide isolated from *Piper tuberculatum* (Piperaceae), against schistosomula and adult flukes of *Schistosoma mansoni*. **Methods:** Schistosomula and adult (male and female coupled) were each incubated in vitro using piplartine over a wide concentration range (1–200 μg/ml). The efficacy of piplartine was examined regarding: a) schistosome survival; b) reproductive fitness of adult worms; c) motor activity; and d) alterations in the tegument of *S. mansoni* as determined by means of laser scanning microscopy. **Results and Discussion:** Piplartine significantly reduced worm motor activity and caused death in schistosomes of all larval- and adult-stages within 24 h at 25 and 5 μg/ml, respectively. At the highest sub-lethal concentration for adult worms (2 μg/ml), an inhibition of 75% in egg laying was observed despite the parasites remaining coupled. In addition, piplartine at 5 to 200 μg/ml induced morphological changes in the tegument and suckers in adult worms. These findings revealed that piplartine is an effective compound against larval and adult stages of *S. mansoni in vitro*, and that this natural amide is promising for further development as an antischistosomal drug.

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10.11 In vitro activity of dermaseptin, cationic antimicrobial peptide, against *Schistosoma mansoni*

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Introduction: Schistosomiasis is a neglected tropical disease that remains of considerable public health significance worldwide. Since the mainstay of schistosomiasis control is chemotherapy with a single drug, praziquantel, drug resistance is a concern. Dermaseptins (DSs) are cationic antimicrobial peptides found in the skin secretion from frogs of the genus *Phyllomedusa*. Besides the fact that DSs are active against a large spectrum of microorganisms, they have been considered promising candidates for new anti-infective drugs. As an advantage, it has been demonstrated that this compound does not induce significant cytolysis against mammalian blood cells. **Objectives:** The aim of the present study was to determine the effect of dermaseptin 01 (DS 01), on *Schistosoma mansoni* adult worms. **Methods:** In this study, the viability of 49-day-old adult worm pairs of *S. mansoni* was assessed *in vitro* with incubation in RPMI 1640 medium with different concentrations of DS 01. Worm motor activity, egg output (oviposition), tegumental alterations, and survival of parasites were monitored on a daily basis for 5 days using a confocal microscope and a stereomicroscope. **Results and Discussion:** Dermaseptin 01 at 100 μg/ml reduced worm motor activity and caused death of all worms within 48 h in RPMI 1640 medium. At the highest sub-lethal concentration of antimicrobial peptide (75 μg/ml), a 100% reduction in egg output of paired female worms was observed. Additionally, dermaseptin 01 induced morphological alterations in the tegument of *S. mansoni*, and a quantitative analysis carried out by confocal microscopy revealed extensive destruction of the tubercles in a dose-dependent manner in the range of 50-200 μg/ml. As stated previously, the dermaseptin family of peptides may have potential use as therapeutic drugs, as they are not toxic to animals or plants. Consistent with the data reported above where the schistosomicidal activity of dermaseptin 01 was demonstrated, combined with its effect on reproductive fitness and tegumental alteration in adult worms, it is clear that these peptides should be considered important candidates as antihelminthic agents.

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10.12 Cardiovascular effects of synthetic analogues of BPP-10c: in vivo and in vitro assays

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Introduction: BPPs from *B. jararaca* venom were used for the development of captopril, a powerful ACE inhibitor. Recently, *in vivo s*tudies showed that BPP-10c reduced blood pressure of SHRs, without affecting ACE activity. Also, BPP-10c boosts argininosuccinate synthetase (AsS) activity, increasing plasma L-arginine levels. **Objectives:** The aim of this study was to develop stable and active synthetic molecules with similar cardiovascular effects found for the natural molecule, BPP-10c. **Methods:** In vivo assays: *1) Cardiovascular parameters:* 24 h after femoral catheterization, SHRs and Wistar rats (WT) (±300 g) received i.v. injections of: BPP-10c, analogues A and B (71 nmol/kg); or vehicle (0.9% NaCl) (n=5-6 each). Cardiovascular parameters were monitored for 6 h. At the end, blood samples were collected for plasma levels. *2) Bradykinin potentiation (BK):* After measuring standard hypotensive responses evoked by BK (0.5 and 1.0 μg) in anesthetized WT, i.v. bolus injections of BPP-10c, analogues A and B (60 nmol/lg) or vehicle (0.9% NaCl) were given (n=4-5 each). Afterward, injections of BK (0.5μg) were repeated every 5 min. In vitro assays: *1) AsS activity:* kinetic assays using colorimetry were performed with BPP-10c and analogues. It was based on inorganic phosphate produced from pyrophosphate, by cleaving with pyrophosphatase. *2) Quantification of NO:* HEK-293 cells were treated with specific inhibitor of AsS and/or stimulated with 1, 20 and 30 μM BPP-10c and analogues. NO values in cell medium were determined by NO analyzer and compared to a nitrate standard. *3) Plasma levels*: L-arginine levels were measured by HPLC. Statistics: Student's *t* test or one-way ANOVA; $p<0.05$. **Results and Discussion:** Compared to vehicle (-9±4 mmHg), analogues A and B reduced mean arterial pressure (MAP) of SHR (Δ: -26±3 and -26±2 mmHg, respectively; $p<0.05$) and did not change MAP of WT, which means an antihypertensive but not hypotensive effect. However, MAP changes caused by both analogues were smaller than those for BPP-10c (-53±6 mmHg). Analogue B was more effective in potentiating BK (306 %) than either analogue A (212%) or BPP-10c (232%). Unlike what was found for BPP-10c, AsS activity was not changed by any analogue. L-arginine levels found after treatment with analogues were lower in WT than in SHR (analogue A: 0.42±0.04 and 0.61±0.05 nM; analogue B: 0.46±0.07 and 0.86±0.14 nM, respectively). Only analogue B increased NO production (A: 8.44±1.9; B: 26.2±1.6 vs. vehicle: 8.9±0.7 nmol/10^6 cells). Analogue A seems to act independently of NO-related mechanisms. Further studies using different approaches are needed to develop active and stable molecules useful in treating hypertension.

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10.13 Snake diversity in upper Tietê municipalities

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Introduction: São Paulo state, Brazil, has ca. 80% of the Atlantic Rainforest remains, but this number is decreasing due to the anthropic influence on this biome. It is believed that the Atlantic Rainforest has ca. 130 reptile species, but many may have been already lost. Originally, this biome was largely distributed on the coast of Brazil, having about 1.2 million square km. Currently, only 7% of it still exists (2% in conservation units). The study area: Salesópolis, São Luis do Paraitinga, Biritiba Mirim and Mogi das Cruzes are within the Atlantic rainforest domain, where there are many river springs, including the Tietê river spring (Salesópolis). The climate is humid (rainfall is between 2,100 - 2,400 mm a year and the relative humidity, around 70%). **Objectives:** The aim of this study was to compile a list of snake species of the region and to characterize their species richness and abundance. **Methods:** The data presented here are records of the collection "Alphonse Richard Hoge" – Instituto Butantan, collected from 1989 to 2009. From this data, we estimated the family and regional richness and relative abundance. **Results and Discussion:** A total of 1,146 specimens were collected in the study area during the period considered. The most abundant family was Dipsadidae (67%), followed by Colubridae (14%), Viperidae (11%), Elapidae (0.5%) and Boidae (0.3%). The commonest species found were *Bothropoides jararaca* (27% of the records), *Caudisona durissa* (13%), *Oxyrhopus guibei* and *Sibynomorphus neuwiedi* (9% and 7%, respectively). These last ones are non-poisonous and easily mistaken for poisonous ones by the public, which is the reason why they may have been brought to Instituto Butantan. Mogi das Cruzes showed the largest abundance (31 species) and followed the pattern described above. Biritiba Mirim had 21 species, with *B. jararaca* as the most abundant, but, unlike the other municipalities, only one *C. durissa* was found. The whole region has undergone an intense vegetation suppression (mainly for coffee plantations and ranching). *C. durissa* is known to be an alien species in the Atlantic rainforest. It is, probably, present there due to the impact caused in the last decades. Salesópolis and São Luis do Paraitinga recorded 20 species each. The most abundant for Salesópolis were *B. jararaca, Oxyrhopus clathratus* and *O. guibei*. São Luis do Paraitinga had a larger record for *C. durrisa* (90 specimens) than for *B. jararaca* (75 specimens). The third and fourth scores were for *Xenodon neuwiedi* and *X. merremii*. The results here may have some bias, as most of the snakes were collected by the local population, many times, because they are similar to poisonous snakes. Species not fit in this case were rare on the records. Mogi das Cruzes is the most urban of them; this increases the chance of human encounters.

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10.14 Kinetic parameters calculated from application in MATLAB

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Introduction: The treatment experimental data is very important in a biotechnological process, and for that there is a need for a suitable fast tool that supplies reliable results. **Objectives:** This work is an application developed in MATLAB, including all the results and analysis involved in biotechnological processes. **Methods:** Starting from the fundamental kinetic variables of such bioprocess as cell concentration, and metabolite and product concentrations, the program developed in MATLAB calculates several parameters, such as growth rate, and several conversion factors, and it allows for smoothing of experimental data, using a spline algorithm. The program imports data from Microsoft Excel. Conversion factors are certain starting from the kinetic correlation of variables during the exponential phase. **Results and Discussion:** The use of that application results in less time spent for this treatment and also in a standardization of results. This application shows better results in its analysis, besides presenting the information in a clearer way to be understood by anybody. Besides, the results are standardized and obtained in a shorter time, using a single program.

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10.15 Genetic and morphological variability of *Aedes aegypti* populations from metropolitan area of São Paulo based on microsatellite loci and wing morphometrics

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Introduction: *Aedes aegypti* (Diptera: Culicidae) is a mosquito species of medical interest, where it is vector of etiological agents of diseases such as dengue and yellow fever. Its geographical distribution is tropical and subtropical, and its habits are synanthropic and anthropophilic. In the absence of a vaccine to combat dengue virus, the alternative is to control the vector. **Objectives:** Population studies are important to develop control strategies, and with the lack of population studies of this mosquito in the State of Sao Paulo, our purpose was to characterize *Ae. aegypti* populations combining microsatellite molecular markers and phenotypic wing study. **Methods:** Samples of *Ae. aegypti* were collected at four different locations in the state of São Paulo (SP): Butantã (BUT), Guarulhos (GUA), Osasco (OSA) and Suzano (SUZ), all located in the metropolitan area of Sao Paulo city. Locations were separated by a minimal distance of 13.5 km and a maximal distance of 50 km. For the genetic analysis five microsatellite *loci* were used, and for geometric morphometric analysis, wings were analyzed regarding 18 landmarks. **Results and Discussion:** The number of alleles observed for each *locus* differed between populations: six for 38/38 *locus*, five for T3A7 and 34/72 *loci*, and four for AED19 and C2A8 *loci*. A high frequency of heterozygotes was observed at *loci* 34/72 and C2A8, surpassing the rate of homozygotes, whereas the opposite was observed at *loci* AED19 and 38/38. For the *locus* T3A7, homozygotes and heterozygotes occurred at equivalent frequencies. The population of SUZ showed a 90-bp allele at *locus* 38/38 which was not present in other populations. Multivariate analysis of wing shape showed small interpopulation differences between BUT, GUA and OSA. Mahalanobis Distance Analysis revealed that the population of the BUT is morphologically closer to GUA than to OSA, for both sexes. Such morphological distances were not correlated to geographical distances between the same locations. The five *loci* examined were polymorphic. A deficit of heterozygotes was observed at 38/38 and AED19 *loci*, a pattern that may have been caused by the presence of null alleles. The microsatellite *loci* revealed possible diagnostic alleles for GUA, OSA and SUZ. Morphometric analysis showed morphological variability in wings, which, however, does not indicate population structure. The two population markers used here apparently have different degrees of resolution for microevolutionary events, and microsatellite DNA was slightly more sensitive in revealing population structure.

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10.16 Prolyl oligopeptidase and aminopeptidases in adipocytes

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Introduction: Adipose tissue has important endocrine functions whose disruption is related to the high incidence of pathological states, such as obesity. Among the molecular signals and regulators of lipogenesis and lipolysis in adipose tissue are several peptides susceptible to hydrolysis by exo- and endopeptidases. **Objectives:** The aim of this study was to detect prolyl oligopeptidase (POP) and the diversity of representative aminopeptidase activities, i.e., acid (APA), basic (APB), puromycin-insensitive (APM) and puromycin-sensitive neutral (PSA) and dipeptidyl peptidase IV (DPPIV) in adipocytes. **Methods:** Adipocytes were isolated from eight healthy male Wistar rats by the method of Rodbell. Briefly, the retroperitoneal fat was collected and incubated (30 rpm, 37°C for 1 h) in appropriate buffer with type 2 collagenase (1.25 mg/mL) and then centrifuged (200 rpm, 25°C for 1 min). The supernatant, which contains the suspension of adipocytes, was aspirated, suspended in 20 mM Tris-HCl buffer (pH 7.4, 20°C) (1.67 mL/g of initial fat), sonicated (20 s at 20% of amplitude) and centrifuged (1,500 rpm, 4°C for 10 min). The lower layer formed was aspirated, homogenized in the same buffer with 0.1% Triton X-100 (800 rpm for 3 min) and centrifuged again (1,500 rpm, 4°C for 10 min). After this last centrifugation, the lower layer formed was collected and subsequently submitted to fluorometric measurements of peptidase activities using synthetic naphthylamide substrates. **Results and Discussion:** Peptidase activities were expressed as picomoles of hydrolyzed substrate/min/mg protein and presented as mean±s.e.m. (n=8) as the following results: APA (14±6), APB (4331±480), APM (137±22), DPPIV (83±18), PSA (156±34) and POP (25±6). This is the first report describing the existence of peptidase activities belonging to the M1 (APA, APB, APM and PSA) family in addition to insulin-regulated aminopeptidase (EC 3.4.11.3., LAP/IRAP, VP165, gp160), and to the S9 (DPPIV and POP) family in mammalian adipocytes. The interaction of these novel adipocyte enzyme activities with insulin, angiotensins, bradykinin, oxytocin and vasopressin is now under investigation in our laboratory in order to elucidate new mechanisms of adipocytes acting in energy balance.

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10.17 APM/CD13 and FOS are altered in the hypothalamus of obese and fasting rats

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Introduction: Peptides such as substance P, somatostatin, angiotensin III, vasopressin, kallidin, dynorphin, leu- and met-enkephalin and endorphin exert significant effects on the nutritional status and regulation of energy balance by the central nervous system and are susceptible to hydrolysis by exopeptidases. **Objectives:** Protein (Western blotting) and gene (PCR) expressions, catalytic activity of puromycin-insensitive membrane-bound neutral aminopeptidase (APM/CD13) and *in situ* regional distribution of CD13 immunoreactivity (ir) and FOS(ir) were evaluated in the hypothalamus of rats in order to explore the association of APM/CD13 and cellular activity with monosodium glutamate obesity (MSG) and/or fasting (FD). **Methods:** Induction of monosodium glutamate obesity (MSG) and/or food deprivation was carried out in rats. Western blotting and RT-PCR were used for measurements of protein and gene expression (RT-PCR) of hypothalamic CD13. The distribution of CD13 was immunohistochemically studied by the ABC technique and catalytic activity of APM/CD13 by fluorometry. **Results and Discussion:** Variations in protein and gene expressions of CD13 in relation to controls coincided in the hypothalamus of MSG and MSG-FD (decreased 2- to 17-fold). Compared to controls, the reduction of hypothalamic CD13 content may reflect a negative balance with its regional distribution in the supraoptic, paraventricular, periventricular and arcuate nuclei. CD13(ir) increased in the supraoptic nucleus in MSG (2.5-fold) and decreased in the paraventricular nucleus (2-fold) together with FOS(ir) (1.5-fold) in FD. In MSG-FD, FOS(ir) decreased (7-fold) in the paraventricular nucleus, while CD13(ir) decreased in the periventricular (5.6-fold) and the arcuate (3.7-fold) nuclei. All these changes of CD13 were not related to catalytic activity of APM. The data suggest that CD13 in these hypothalamic areas may function in the regulation of energy metabolism but not by means of APM enzyme activity.

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10.18 Role of HSP70 in the survival of *Biomphalaria glabrata*: evidence based on heat shock

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Introduction: HSP70 (heat shock protein) is a member of an important family of cell proteins involved in the regulation of protein homeostasis. Due to evidence that exposure to a wide variety of stressors, including elevated temperatures, ultraviolet (UV) radiation, heavy metals, and xenobiotics, can induce the heat-shock response, many studies are trying to establish the family of heat shock proteins as biomarkers of environmental stress. **Objectives:** The aim of this work was to investigate the role of HSP70 in protecting organisms from external injury after induction by a sublethal stimulus and a subsequent challenge with a lethal harm. **Methods:** We followed the HSP70 expression in the digestive gland of freshwater snails by Western blotting. Heat shock was chosen as the injurious stimulus for a group of 70 snails *Biomphalaria glabrata*; they were pre-exposed to sublethal temperatures of 33 and 36 °C to induce HSP70, and then exposed to a lethal temperature of 42 °C. Control group was not exposed to sublethal temperatures. The proteins were extracted with RIPA buffer from digestive gland tissues, fractionated in dodecylsufate polyacrylamide gel electrophoresis (SDS-PAGE), transferred to nitrocellulose membrane, and detected with a HSP70-specific antibody. **Results and Discussion:** The animals pre-exposed to sublethal temperatures survived longer to the lethal temperature than the control ones. Western blotting showed that the induction of HSP70 in the digestive gland of pre-induced snails was clearly higher than in the controls, which strongly suggests that HSP70 played a protective role against the lethal effects of heat. *Biomphalaria glabrata* has been used as experimental model by our group. The results found here reinforce the proposal of HSPs as biomarkers of environmental damage, providing further evidence to establish *Biomphalaria glabrata* as a bioindicator in ecotoxicological studies.

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10.19 Serologic incidence of Chagas disease in patients treated at Sanatorinhos Hospital in 2006 and evaluation of two different methods for diagnosis

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Introduction: Chagas disease was named in honor of its discoverer, the Brazilian physician Dr. Carlos Chagas. The disease has a ubiquitous geographical distribution on the American continents, and it is characterized by a generalized infection, with endemic nature and chronic development, by the flagellate protozoan *Trypanosoma cruzi*. Humans are intermediate hosts, and infection occurs via mucosa exposed to contaminated feces of triatomine insects. **Objectives:** The aim of this study was to evaluate the incidence of Chagas disease in serum samples of suspected patients sent to the diagnosis laboratory of Sanatorinhos Carapicuiba Hospital (2006). **Methods:** Serum samples were taken from 993 patients from five different municipalities of metropolitan São Paulo. The samples were analyzed for the presence of anti-*T. cruzi* IgG by commercial Pathozume Chagas ELISA kit (OMEGA) and by indirect immunofluorescence (IFI) with commercial Imuno Com kit (WAMA). **Results and Discussion:** The incidence of positive or indeterminate results in samples was 14.1% (140 patients of 993). Except for samples that came from Itapevi that showed just 5.26% positive results, samples of other municipalities showed equivalent rates of incidence of chagas disease: Vargem Grande (17.24%), Carapicuiba (15.35%), Osasco (13.46%) and Cotia (12.19%). Among the 140 patients with positive results to Chagas disease, we found a higher prevalence in adults from 32 to 87 years (70.71%). Regarding sex, most positive samples were from women (57.14%). When these results were analyzed together with data from medical handbooks of positive patients, we found that physicians adopted similar procedures for Chagas diagnosis in the period. Most suspected patients (65%) received medical attention due to heart disease at the moment of blood collection. Just 25% of patients were hospitalized, but around 50% of patients were not yet diagnosed for Chagas disease. In general, ELISA and IFI are requested for the exact diagnosis of the disease. In our analysis, the two methods showed similar results in 95% of samples (133 patients). Results were divergent or inconclusive in only seven patients (5%).