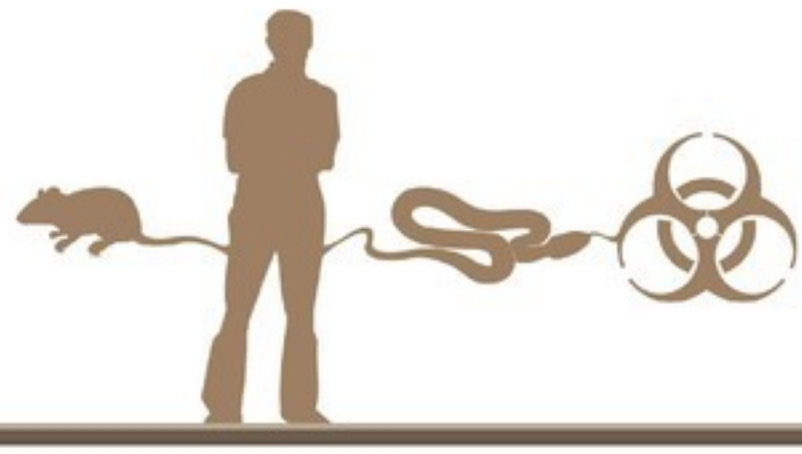
10. Others



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10.01 Changes on aminopeptidases in subcellular fractions of adipocytes from monosodium glutamate obesity

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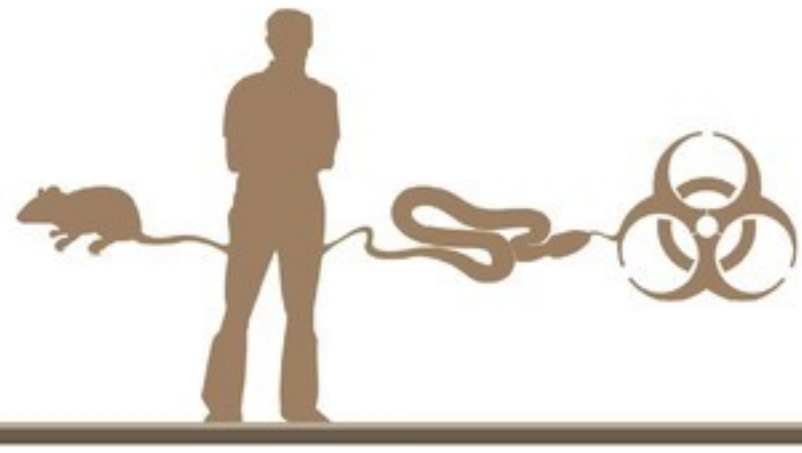
Introduction: Obesity is a consequence of increased fat stores. Multiple neuroendocrine factors can affect the adipose tissue. It is not known if aminopeptidases in adipocytes are engaged with neuroendocrine perturbations induced by the monosodium glutamate (H) neonatal administration in rats leading to obesity. **Objectives:** To identify novel activities of acid (APA), basic (APB), neutral puromycin insensitive (APM) and sensitive (PSA), methionyl (MetAP) and dipeptidyl-IV (DPPIV) in addition to cystyl (CAP)/leucyl (LAP/IRAP) aminopeptidases in membrane (MF) and in high (HDM) and low (LDM) density microsomal fractions from abdominal adipocytes in a model of obesity provided by the monosodium glutamate neonatal administration in rats. **Methods:** Peptidase activities (pmoles substrate hydrolysed/min/mg protein) quantified by fluorometry using β-naphthylamide derivative substrates. **Results and Discussion:** Vmax: APM> LAP/IRAP> MetAP> APB> PSA> CAP> DPPIV> APA. Affinity: APA> DPPIV> PSA> MetAP> LAP/IRAP> APM> CAP> APB. Efficiency: APM> PSA> MetAP> LAP/IRAP> APA> DPPIV> APB> CAP. Obese (H) presented higher APA-HDM, APA-LDM, APM-LDM and PSA-LDM, and lower APA-MF, APM-MF, CAP-MF and DPPIV-MF than controls (C). Comparison among % activities in each fraction (ANOVA $p<0.0001$; Tukey-Kramer $p<0.05$, different letters) and between C and H for each activity in each fraction (unpaired two-side Student's t-test $p<0.05$, asterisk) showed: APA-MF C=17±2^{ab}, H=18±3^{b}; APA-HDM C=14±1^{a}, H=22±2*; APA-LDM C=8±1^{a}, H=9±1^{ac}; APB-MF C=3±0.9^{a}, H=8±1^{b}*; APB-HDM C=H=0; APB-LDM C=28±7^{b}, H=10±2^{ac}; APM-MF C=23±0.7^{b}, H=22±2^{ab}; APM-HDM C=8±4^{a}, H=18±12; APM-LDM C=26±7^{b}, H=41±7^{b}; CAP-MF C=23±6^{b}, H=5±0.3^{b}*; CAP-HDM C=4±1^{a}, H=6±1; CAP-LDM C=2±0.3^{a}, H=0; DPPIV-MF C=14±1^{ab}, H=3±0.2^{b}*; DPPIV-HDM C=10±2^{a}, H=8±0.8; DPPIV-LDM C=7±2^{a}, H=2±1^{a}*; LAP/IRAP-MF C=H=0; LAP/IRAP-HDM C=H=0; LAP/IRAP-LDM C=8±3^{a}, H=2±1^{a}; MetAP-MF C=19±5^{b}, H=43±10^{a}; MetAP-HDM C=43±10^{b}, H=20±1; MetAP-LDM C=17±6^{ab}, H=12±3^{a}; PSA-MF C=H=0; PSA-HDM C=21±6^{a}, H=26±5; PSA-LDM C=3±1^{a}, H=23±8^{c}*. Data show for the first time a diversified profile of activities of APA, APB, APM, CAP, DPPIV, MetAP and PSA in addition to LAP/IRAP in different subcellular compartments of the adipocyte from abdominal fat with different patterns of distribution between obese and control rats, suggesting a role of these adipocyte aminopeptidases in MSG-obesity.

Supported by: Fapesp, CAPES, CNPq



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10.02 Good Laboratory Practice (GLP) Applied to Basic Research - Implementation of GLP in the Special Laboratory on Pain And Signaling, Butantan Institute

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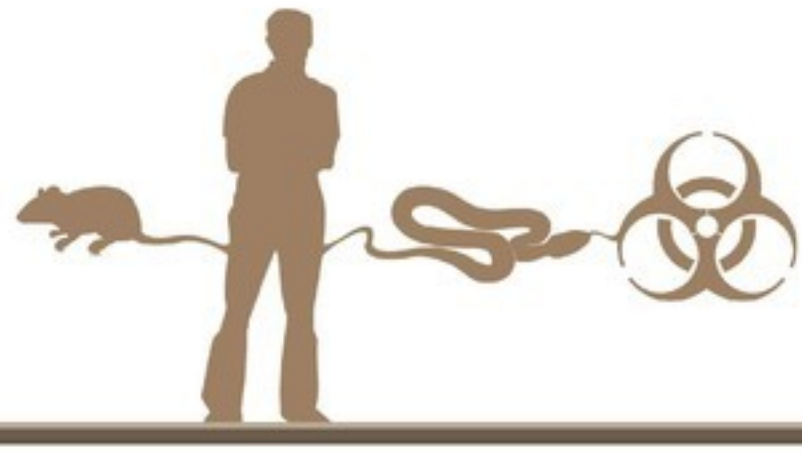
Special Laboratory on Pain And Signaling, Butantan Institute, Sao Paulo, SP, Brazil

Introduction: The Special Laboratory on Pain and Signaling (LEDS), Butantan Institute was initiated in March 04th, 2010. This laboratory has been conducting basic research and innovation activities aiming to characterize: a) the pathophysiology of the local effects caused by animal venoms and toxins, b) the physiology and pathophysiology of pain and inflammation, and c) novel compounds endowed with analgesic and/or anti-inflammatory activities, with possible therapeutic application. These activities resulted in partnerships with Brazilian pharmaceutical companies. **Objectives:** Based on these partnerships, we have been requested to conduct our experimental procedures in compliance with Good Laboratory Practice principles. For this purpose, several approaches have been carried out, including the rebuilding and adequacy of our research building and test facilities, according mainly to the international guidelines for animal care and housing as well as for the development of in vivo and in vitro experimental procedures. This work describes our experience in implementing GLP in our laboratory. **Methods:** GLP experts from Butantan Institute were assigned to help our laboratory in the GLP implementation and dissemination. LEDS staff, employees and fellowships assume responsibilities in order to meet the GLP goals. The approaches include the development of Standard operating procedures (SOPs), standard controls, instrument calibration and personnel training. Chemicals, reagents and solutions are labeled to indicate identity, preparation date and person in charge. Standard study protocol format is followed, according to SOPs and GLP regulations. Records of all these activities are maintained. We also needed a system for independent evaluation of all activities. This was achieved by setting up a Quality Assurance Unit that conducted inspections of facilities and studies, and report their results to the laboratory. **Results and Discussion:** The GLP has been gradually but successful implemented to meet the appropriate goals. Experimental protocol controls have been established, and laboratory instruments and test facilities are maintained to a high working standard. Setting up a GLP system implies long-term commitment by every staff member in the laboratory and this has been well accepted by technical and scientific staff. In addition, the small size of our group and well-defined management structure allowed us to overcome some of the GLP implementation challenges. It is important to stress that keeping the GLP is a high cost proposition.

Supported by: FINEP



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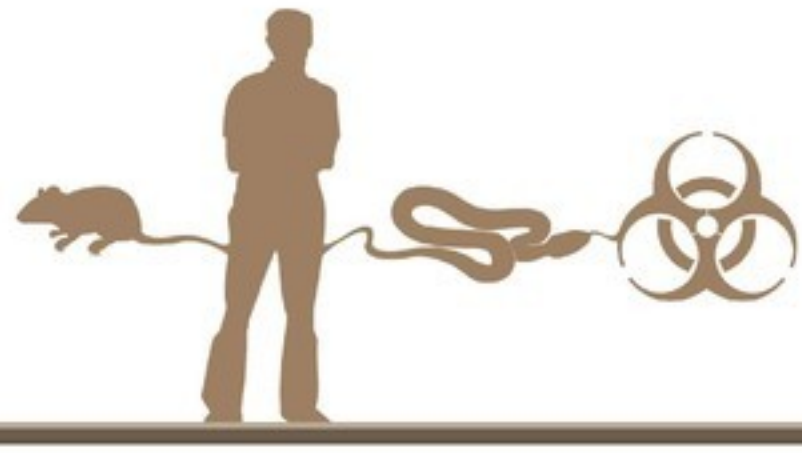
10.03 Inventory of specimes and type-specimens of Zoological Collections of the Butantan Institute

Costa AV[1], Barros TA[1], Leite CG[1], Badari, JC[2], Barros-Battesti DM[1]
[1]Special Laboratory of Zoological Collections, Butantan Institute; [2]Laboratory of Parasitology/Entomology, Butantan Institute, São Paulo, SP, Brazil

Introduction: The Zoological Collections of Butantan Institute are distributed into five significant collections: Acari, Entomological, Arachnological, Myriapods and Herpetological, organized within each laboratory. Duo to the fire in 2010 at the premises of Arthropods and Herpetology buildings, the two respective collections had significant losses. The Acari and Entomological collections deposited in the Laboratory of Entomology/Parasitology are preserved. A new building that will store the collections is in final stage of construction. In 2011, the Institution has received support from Fundação Butantan and FAPESP to develop the INFRA Project (09/54921-4). **Objectives:** Present the estimated number of types and material of the IBU Zoological Collections after fire, such as the destination of these collections. **Methods:** The collections are being computerized, and will soon be available online. The information of type-specimens and material of the collections were rescued from index cards and collection-books. **Results and Discussion:** The Acari Collection contains 11,000 lots cataloged, and about 150,000 specimens. Currently 10,175 lots are computerized, and from this material, 175 are type-specimens. The Entomological Collection includes 3,000 data from scanned specimens. From this material, 8 are type-specimens of Hemiptera (Reduviidae, Triatominae), 136 from Diptera (117 Brachiceras and 19 Nematoceras). Some material from the collection of arachnids and myriaphods has been compromised and 80% of the Herpetological Collection. In addition to the specimens there were not burned, remains the collection-books, allowing computerization that one day were among the largest in number of species. The Collection of Araneae has 87 collection-books, of which 82 books are already computerized, a total of 164,000 records. From this material, 296 are type-specimens, 6 collection-books of Opiliones, totaling 10,549 scanned data, of which 81 are type-specimens. In the initial survey to be applied in the computerization, there are 3 collection-books on Escorpiones (30 are type-specimens); and 2 collection-books of small orders (4 are type-specimens), as well as 5 collection-books of Myriapods, totaling 3,500 records, where 40 are records of type-specimens. The Herpetological Collection has 81,600 records and already 71,000 computerized. Within this material, 1,220 specimens were type-specimens. The survey of all specimens that were affected by the fire will take place only after the transfer of the collections to the new facility.

Supported by: FAPESP



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10.04 Implantation of computerized systems of the zoological collections and reception of Butantan Institute

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Laboratório Especial de Coleções Zoológicas, Instituto Butantan, São Paulo, SP, Brazil

Introduction: The zoological collections of the Butantan Institute are distributed into five significant collections: Acari, Entomological, Arachnological, Herpetological and Myriapods. Due to historical and scientific importance, the institution, in 2011, received financial support from FAPESP and Fundação Butantan - INFRA Project (09/54921-4). The implementation of the program allowed the acquisition of Sophia program, to attend the digitization of data from records and collection-books of the collections. Parallel to this work, followed by the computerization of the reception of animals collected in projects, brought by Vital Brazil Hospital or donated by the public. At the same time, the Collections web page is being made, and will be available at the IBU web site. **Objectives:** Present the programs used to computerize and feed the databases of each collection, and also the receipt of animals collected. Make available the database record on the internet to be accessed by the interested ones. **Methods:** The software "Sophia Collection" elaborated by the company "Crescer Informática", was developed in Delphi programming language, using the SQL Server database. The software "Animal Reception" was developed by the IBU programming team, using the Scriptcase program. **Results and Discussion:** The software "Sophia Collection" allows the dissemination of classified information, in several formats, for example: .doc; .xml; .pdf; html, and also the inclusion of new collections, with unlimited number of data. The organization of the collections through the "Sophia Collection" software, involves 5 basic processes: 1. Organization; 2. Recovery; 3. Dissemination; 4. Circulation, and 5. Zoological Cataloguing Module. The "Animal Reception" software, implemented on April 9th this year, is available at www.sistemas.butantan.gov.br/recepcao_de_animais. It is organized into 6 windows: a) Registration; b) Edit classification; c) Inclusion-Classification; d) 2nd copy; e) Summary, and f) Receipt and Tracking, the latter is in development process. The Collections web page began to be developed by the Technical Productions Center of the "Centro de Divisão Cultural" on May 17. Once it is completed, will allow the availability of information from Collections in Portuguese and English. The page will be linked to the "Sophia Collection" software, and will be available through a link on IBU web page.

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10.05 The Núcleo de Documentação of the Instituto Butantan and the preservation of scientific and institutional memory of public health in São Paulo – proposal for a guide creation

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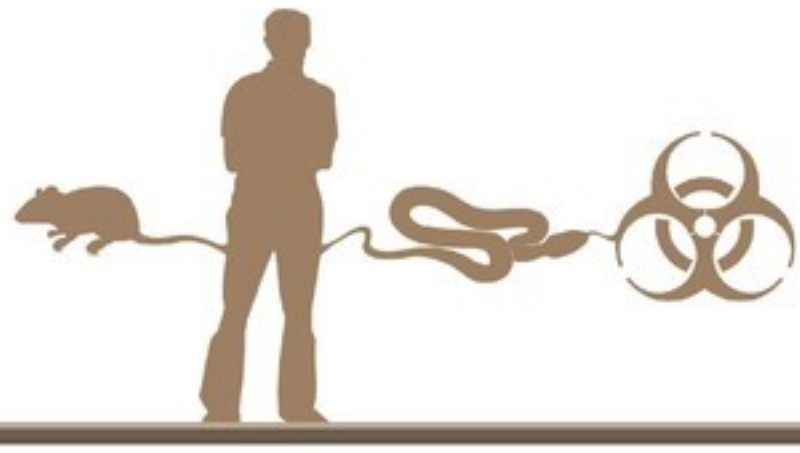
Introduction: the Núcleo de Documentação of Instituto Butantan, created in January 2010 by the Decree n. 55.315, aims preservation and divulgation of scientific memory, besides supporting actions in museology and sciences history field. It is also its mission the development of methodological research in holdings administration area, allowing a policy of document management development within the institution. It develops actions in order to identify and diagnose the archives of the Institution and the creation of working methods for information processing and documents organization. **Objectives:** development of holdings diagnosis, with identification of main archive groups and its quantification to elaboration of proposal for a guide creation. **Methods:** Archive diagnosis is the first step in the process of information maintenance, anticipates holdings organization and offers guidance and priorities in its treatment. The identification accomplished so far consisted in surveying the office of origin, documental type and inclusive dates. This work was part of actions undertaken with the Departamento de Gestão of the Sistema de Arquivos do Estado de São Paulo in 2011, in order to accomplish a diagnosis from historical documentation at Instituto Butantan and at the Museu de Saúde Pública Emílio Ribas (Musper). The first step of this work aimed the identification of documents dated until the year 1940, so it would be in accordance with the State Decree n. 48.897, of August 27, 2004, which defines that documents produced, received or accumulated by the state public administration state dated until 1940 are subjects of archival value, so cannot be eliminated. **Results and Discussion:** At Butantan, we have, as first result, a summary characterization of each section, the main document types and inclusive dates. At Musper, it was accomplished an identification of the main units of Secretaria da Saúde by sampling. This survey is presented in a *guide* format: one of the main research instruments of an archive, containing basic information about the institution and its holdings. The development in the field of science history and technology is related inherently to institutional scientific memory preservation programs, such as proposed by the Núcleo de Documentação, which primary challenge consists in dealing with the lack of awareness and information from scientists and employees in regards of preservation and divulgation of daily produced documents, which in last resort represents its memory. The actions of the Núcleo demonstrate a preoccupation from the Instituto Butantan in the rescue, preservation and divulgation of its institutional scientific memory.



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10.06 In vitro activity of piplartine analogues against *Schistosoma mansoni*

Freitas RP[1], Fokoue H[2], Miyasato P[1], Yamaguchi L[2], Kato MJ[2], Nakano E[1]
[1]Laboratório de Parasitologia, Instituto Butantan; [2]Laboratório de Produtos Naturais, Instituto de Química, Universidade de São Paulo, São Paulo, SP, Brazil

Introduction: Schistosomiasis is a helminth infection caused by worms of the genus Schistosoma. The disease affects around 200 million people in 74 countries remaining a serious public health problem. Currently, praziquantel is the only drug recommended by WHO for the treatment of schistosomiasis; however, occurrence of resistance to praziquantel has been reported. In this context, searching for new active and safe schistosomicides is needed. In our screening on extracts and pure compounds from plants of Piperaceae family, the amide piplartine showed a potent in vitro schistosomal activity, causing their immobility at the concentration 9.5 μM. **Objectives:** With the purpose of determining the regions of piplartine responsible for the biological activity, several analogs of piplartine were obtained in order to determine structural requirements for schistosomal activity. **Methods:** For the *in vitro* assays, analogs were pre-dissolved in 3% DMSO before dilution in RPMI medium. Five worm pairs were exposed to 50 and 100 μg/mL of each analog in 24-well culture plates and incubated for 120 hours. Positive control group was exposed to 3 μg/mL praziquantel and negative control to 0.003% DMSO. **Results and Discussion:** We assessed the motility of the schitosomas 120 h period, the worms motionless were removed and considered dead. Among 25 tested analogs, 3 exhibited activity in both concentrations. There was no difference in sensitivity between male and female worms. The substitution of 5,6-dihydropyridin-2(1H)-one ring of piplartine completely eliminated biological activity, and compared to piplartine, a decrease in the activity was observed with the addition of 1,3-dicyclohexylurea group. The lowest concentration of piplartine for killing 100% of the parasites was in the range of 9.5 μM while for the analogues the lowest concentration were 79.8μM; 103.63 μM; 223.29 μM respectively. Major changes in functional groups, mainly in the 5,6-dihydropyridin-2(1*H*)-one ring of piplartine reduces or inhibits the schistosomal activity. Additionally, the reduction in biological activity was also observed with the modification of 1,2,3-trimethoxyphenyl ring as well as after the removal of double bond.

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10.07 Effects of low power laser on pain control and functional recovery after crush injury in rats

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[1]Special Laboratory of Pain and Signaling, Butantan Institute; [2]University of São Paulo, Pharmacologic Department, São Paulo, SP, Brazil

Introduction: Peripheral nerve injury occurs commonly in our daily life. May result in motor impairment, sensory, autonomic and often in persistent neuropathic pain, or chronic characterized by spontaneous pain, burning, accompanied by allodynia and hyperalgesia. To try to accelerate peripheral nerve regeneration, the development of physical resources has been very promising, such as low power laser. **Objectives:** To compare three different parameters of low power laser on pain and functional recovery in rats subjected to nerve crush injury. **Methods:** Male Wistar rats were subjected to sciatic nerve injury, performed by clamping. The animals were divided into four groups with five animals each, namely: Group 1: dosage of 3 J, Group 2: dosage of 6 J, Group 3: dosage of 20.4 J; Group 4: no treatment, Group 5: Sham, Group 6: Naive. Groups 1, 2 and 3 were treated on the first postoperative day lasting 21 consecutive days. To evaluate the functional activity, we used the sciatic functional index (SFI). For assessment of mechanical hyperalgesia, we used the paw pressure test in rats. The assessment of thermal hyperalgesia was performed by test planting. The allodynia was assessed by quantitative assay, in response to tactile stimulation applied to the paws of the rats. Statistical analysis was performed two-way ANOVA and Bonferroni post-test with significance level $p < 0.05$. **Results and Discussion:** The clamping technique for peripheral nerve injury was effective, i.e., the control group had pain and motor dysfunction compared with the groups naive and false-operated. We observed statistical differences between the SFI in Group 4 and Group 2 ($p < 0.05$) and Group 3 ($p < 0.01$) and between groups 2 and 1 (21, $p < 0.01$). There was observed the phenomena of hyperalgesia and allodynia in groups treated with laser. The clamping technique for peripheral nerve injury was effective, i.e., the control group had pain and motor dysfunction compared with the groups naive and false-operated. The low power laser at a dosage of 6 J showed benefits for the control of nociception and on functional recovery in this experimental model.

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10.08 Role of quality assurance in research laboratories at Butantan Institute

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Introduction: The Quality Assurance in the research laboratories that compose the Division of Scientific Development (DDC) at Butantan Institute, has started working in 2009, when the Board of Directors of the institution approved the proposal for implementation of Good Laboratory Practice (GLP) in the research areas that needed these standards or had interest in joining the Quality Management program. **Objectives:** We work in partnership with the Board of the Butantan Institute, Quality Assurance (Production Division), Animal Central Facility, Engineering Division, Administration Division, Division Cultural and suppliers. The Quality Assurance System covers from quality management, through planning, risk analysis, analysis of plans and designs, fitness areas, documentation, training and education, inspections, equipment, waste management, animal houses to innovation and pursuit of excellence as a research institution. **Methods:** There are currently eight laboratories already working on deployment of GLP and the adoption of standards, legislation and relevant documentation. These labs are tailoring to the needs and compliance requirements as well as adopt a quality culture among its employees. They are: Laboratório Especial de Dor e Sinalização (LEDS), Laboratórios de Parasitologia, Coleções (LECZ), Laboratório de Bacteriologia, Laboratório de Artrópodes, Laboratório de Herpetologia, Laboratório de Bioquímica e Biofísica e o Museu Biológico. New laboratories are being recruited to gradually broaden our spectrum of activity. **Results and Discussion:** Each laboratory, on its own way and time, have been adapting to the new reality and conditions of GLP, seeking to improve the infrastructure of the laboratories areas, as well as training and qualification of personnel, documentation, updating the compliance of rules, legislation and optimization of results. All this effort reflects the recognition of the work and it is visible reliable results in productivity and recognition, experiments traceability and excellence of research conducted. Constant improvement is the path to certification in these areas.

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10.09 Action of *Bothrops jararaca* kininogen (BjKgn) on cytokines processing proteases and macrophages functions.

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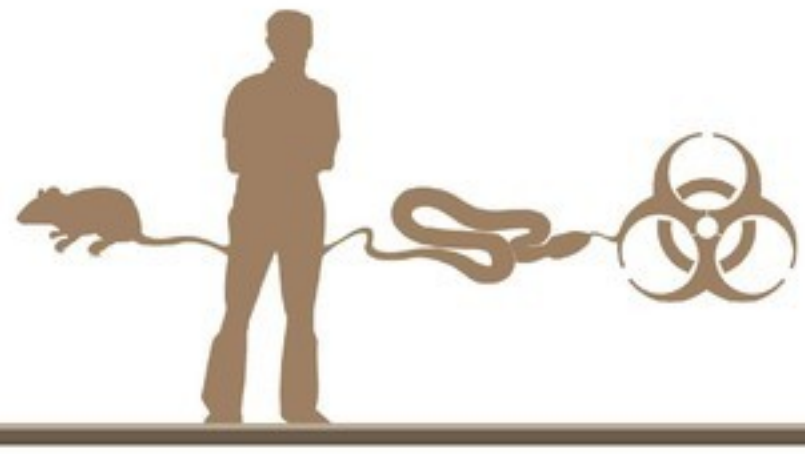
[1]Laboratório de Fisiopatologia, [2]Laboratório de Imunoquímica, Instituto Butantan, São Paulo, SP, Brazil

Introduction: A potent inhibitor of cysteine proteases isolated from the plasma of *Bothrops jararaca*, BjKgn, possesses similar characteristics to mammalian kininogen. This protein showed inhibitory activity against Jararagin (JAR), a hemorrhagic metalloproteinase isolated from the venom of the aforementioned snake. Studies showed that JAR is able to induce the release of tumor necrosis factor (TNF), a cytokine-related to the venom dermonecrotic activity. The TNF-alpha is expressed on the cell surface and cleaved by the action of a metalloproteinase named TACE (TNF-converting enzyme), which is also expressed on the cell surface and releases the soluble and active TNF-α. Another important cytokine is the interleukin-1β (IL-1β), which in turn is processed intracellularly by ICE (Interleukin converting enzyme), a cysteine protease that belongs to Caspases family. The synthesis and release of cytokines are mainly carried out by macrophages. **Objectives:** To test the possible inhibitory effect of BjKgn on TACE and ICE activities and on macrophages functions, such as spreading, phagocytosis, production of hydrogen peroxide (H_2O_2) and nitric oxide (NO). **Methods:** For the studies with ICE and TACE we used specific fluorogenic substrates for each enzyme and different concentrations of BjKgn. On the tests for spreading and phagocytosis we used resident or elicited macrophages, and for the production of H_2O_2 and NO only cells elicited with thioglycollate 4% were used. The cells were adhered to glass round coverslips and incubated in the presence or absence of different concentrations of BjKgn. **Results and Discussion:** The results showed that BjKgn were capable of inhibiting ICE under a competitive inhibition mechanism and constant inhibition (Ki) of 4.2 μg. Relating to TACE, preliminary data showed an inhibitory action of BjKgn, but the mechanism and Ki hasn´t been determined yet. The biological and metabolic activities of macrophages apparently were not affected by the protein, since the groups treated with BjKgn presented the capacity of spreading, phagocytosis and production of H_2O_2 and NO, similar the control groups. The results corroborated with other studies confirming that BjKgn is a potent inhibitor of cysteine proteases and capable of inhibiting the metalloproteases TACE and JAR. As long as macrophages were not influenced on biological and metabolic activities by BjKgn, we intend to check if this protein affects the production of TNF-α or IL-1β by these cells.

Supported by: CAPES, FAPESP and CNPq



10.10 Description Nymphal Stage of *Ornithodoros mimon* (Acari: Argasidae)

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[1]Curso de Pós-Graduação em Ciências Veterinárias, Departamento de Parasitologia Animal, Universidade Federal Rural do Rio de Janeiro, Seropédica, RJ, [2]Laboratório Especial de Coleções Zoológicas, Instituto Butantan, São Paulo, SP, Brazil.

Introdution: *Ornithodoros mimon* Kohls, Clifford & Jones, 1969 is an argasid ticks common on Chiroptera, very aggressive for humans, originally described from larvae collected on bats *Mimon crenulatum* (E. Geoffroy) from Bolivia and *Eptesicus brasiliensis* (Desmarest) from Uruguay. Later, this tick was also registered in others bats species from Argentina and Brazil. *Ornithodoros mimon* was for long time known only by descriptions of the larva stage, this become difficult its morphological separation from other closer species belonging to the genus. The Laboratory of Parasitology of Instiute Butantan has a colony of *O. mimon*, which originally by adults and nymphs collected in the municipally of Araraquara, SP. The description of adult stage was made recently, while the nymphal instars have not been described. **Objectives:** This paper aims to describe for first time all nymphal instars (N1, N2 and N3) of *O. mimon* based on optical microscopy and scanning electron microscopy. **Methods:** Fifteen specimens of each instar were selected for morphological studies. The specimens were sacrificed in fever water and fixed in 70% alcohol. The morphometric were taken from 10 specimens of each instar by means of a stereoscopic microscope Nikon SMZ 745T and software Nis-F. **Results and Discussion:** The nymphs of *O. mimon* have characteristics similar to other species that parasitize bats, but there is morphological distinction between instars. Also differ from other ticks of the genus *Ornithodoros*, especially those who are buried in the soil and parasitize other host species.

Supported by: CNPq, CAPES and FAPESP

10.11 Presence of respiratory viruses in horses of Brazil

Mancini DAP[1], Pereira ASP[1], Mendonça RMZ[1], Kawamoto AHN[1], Alves RCB[1], Pinto JR[1], Mori E[2], Richtzenhain LJ[2], Jorge Mancini-Filho[3].
[1]Virology Laboratory, Butantan Institute; [2]Department of Preventive Veterinary Medicine and Animal Health, Faculty of Veterinary Medicine and Zootechnics, University of São Paulo; [3]Department of Food and Experimental Nutrition, Faculty of Pharmaceutical Sciences, University of São Paulo, SP, Brazil.

Introduction: Equines are very susceptible to respiratory viruses, such as influenza viruses and also it has been cited the parainfluenza virus. Respiratory diseases have adversely impacted economies all over the world. **Objectives:** In this regard, this study was intended to evaluate the presence of influenza and parainfluenza viruses in unvaccinated horses from some regions of the state of São Paulo, Brazil. **Methods**: Blood serum collected from 72 horses of different towns in the state of São Paulo, Pirassununga (39), Águas de Lindóia (25) and Mairiporã (08), was heat-deactivated (56°C/30min) and treated with Kaolin for the removal of non-especific inhibitors. It was then tested by the hemagglutination inhibition technique for specific antibodies to equine influenza and parainfluenza type 3 viruses using the corresponding antigens from these viruses. **Results and Discussion**: About 98.6% (71) and 97.2% (70) of the horses responded with antibody titers of ≥80 HIU/25µL to influenza type A subtypes H7N7 and H3N8, respectively. All horses (72) responded with titers of ≥80 HIU/25µL against the parainfluenza virus. The difference between antibody average titers for influenza A subtypes H7N7 and H3N8 was not statistically significant ($P>0.05$). The average titers for influenza and parainfluenza viruses, on the other hand, showed a statistically significant difference ($P<0.001$). These results indicate a better antibody response by horses to Parainfluenza-3 than to the equine influenza virus. This study suggests evidence of the concomitant presence of two subtypes of equine influenza A (H7N7 and H3N8) and the parainfluenza type 3 viruses in horses in Brazil. Thus, it is advisable to vaccinate horses against these respiratory viruses.

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10.12 Effect of punicic acid from pomegranate seeds (*Punica granatum*, L) on antioxidant enzymes in animal model

de Melo ILP[1], Mancini DAP[2], Pinto JR[2], Mancini-Filho J[1]
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Introduction: Punicic acid (CLNA-cis-9, trans-11, cis-13) is a conjugated isomer of alpha linolenic acid (LNA-cis-9, cis-12, cis-15) showing trans configuration in its structure. It is present in about 70-80% in pomegranate seed oil and has been studied aiming their participation in physiological processes. **Objectives:** This study proposed to determine the effect of punicic acid of pomegranate seed oil on antioxidant enzymes in rat liver. **Methods:** Twenty four male Wistar rats were divided into three experimental groups: control (H_2O); CLNA 2% and CLNA 4%, which were administrated daily by orogastric intubation for forty days. After this period the animals were sacrificed and the livers were evaluated for fatty acid profile by CG, the peroxidation level by TBARS, and the activity of the hepatic enzymes superoxide dismutase (SOD) and catalase (CAT), as well its gene expression. Statistical analysis was performed by Tukey test. **Results and Discussion:** In the liver of the rat groups receiving 2% and 4% of pomegranete oil was not observed the presence of punicic acid, however were detected the both conjugated linoleic acids cis-9, trans-11and trans-10, cis-12 in the concentrations of 3% and 5% respectively. No were observed differences in peroxidation level in the three experimental groups. The activities and gene expression of antioxidant enzymes SOD and CAT in both groups that received pomegranate oil were similar to the control. The results show that the presence of punicic acid of the pomegranate seed oil is metabolized in the liver to conjugated linoleic acid (CLAs) c9, t11 and t10, c12 and that did not alter peroxidation level. The activities and gene expression of antioxidant enzymes (SOD and CAT) were preserved in the rat livers. These observations may contribute to the expansion of the knowledge of physiological mechanisms of action of the punicic acid from pomegranate seeds.

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10.13 Test regarding the preferred material for the nesting of isogenic mice from lineage *BALB/c*

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Introduction: The mouse is an altricial species. Due to its fragility, newly born mice require intense maternal care for their survival and development. The act of creating a nest is a behavior that characterizes this species and is an important stage of the maternal behavior. The nest provides a better heating of the pups, intensifies the contact between the mother and her litter and facilitates the endeavors of the mother in reducing pre-weaning mortality. In nature, mice use fur and plants to build nests. In labs, the supply of materials for nesting is one of the better methods of environmental enrichment, since the material used will influence the quality of the nest and consequently will have potential to alter the zootechnical levels of the colony. **Objectives:** To evaluate the mice's preference concerning four different kinds of nesting materials. **Methods:** We used 20 couples of *BALC/c* mice, of a controlled sanitary standard, raised and kept in a standardized environment. We developed an apparatus made by 4 cages connected to each other by pvc pipes that allowed the animal locomotion between all cages. Each cage of the apparatus has water and specific rations for the species (ad libitum). In each cage was put, weekly, a different kind of nesting material: card paper tubes, disposable polypropylene caps, cotton and gauze, all autoclaved. Each system housed a couple with 28 days of age. The test was performed until the third birth, for each female. The material initially and in greater quantity used for nesting indicated a preference for a specific kind of enrichment. **Results and Discussion:** The enrichment for means of nesting, as expected, demonstrated to be beneficial to the mice. However, it was evident that there was a preference to the use of the disposable polypropylene cap.

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10.14 Characterization of genes coding for the Complement System C3 component from *Loxosceles* spider venom gland

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Introduction: The human complement system is composed by more than 30 proteins and many of them have conserved domains that allow tracing the phylogenetic evolution based on amino acid linear sequences and their tertiary structures. The studies of vertebrate and invertebrate genomes revealed that many domains of mammals complement components are found in both deuterostomes and protostomes. The origin of the complement system has probably occurred with the appearance of C3 and factor B, the only components found in some protostomes and in cnidarians, suggesting that the alternative pathway represents the most ancient complement pathway. Recently, a gene with similarity to C3 genes from invertebrates of Limulidae Family was identified in the transcriptome of *Loxosceles laeta* spider venom gland. This finding suggests that the central component of the complement system, C3, may be expressed on venom gland from *Loxosceles* spiders, playing a role in the defense mechanisms. **Objectives:** This study aims to clone and characterize the C3-like component from *Loxosceles* venom gland and phylogenetically analyze its deduced amino acid sequence. **Methods:** Specific cDNA fragments for the component C3 were obtained from the total RNA from *Loxosceles* spider venom gland, which were amplified by using RT-PCR technique and degenerate primers. The resulting cDNA sequences and their respective deduced protein sequences were analyzed using bioinformatics tools. **Results and Discussion:** It was possible to identify representative domains of the alpha-chain from the vertebrates C3 component in the partial deduced amino acid sequences of the *Loxosceles* C3 (Lox-C3), including two domains of alpha-2-macroglobulin (A2M), an anaphylatoxin domain (ANATO), a thioester-containing domain (TED) and C345R domain. Similarity analyses indicated that Lox-C3 shares a major identity (~54%) with horseshoe crab C3-like sequences. Alignments made with these and other sequences present on database showed strong conservation of such domains in others organisms. These results, although preliminary, allow us to infer that the component C3 from *Loxosceles* spiders have the same composition of domains and chains found in organisms of the Limulidae Family.

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10.15 Knowing to organize: the Instituto Butantan and its documental production

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Introduction: classification and organization of documents of an institution is the knowledge that the researcher needs to have to understand the institution itself. The study of institutional competences covers both the knowledge of administrative organization, as the recognition of subjects, people and key dates to institutional historical research. **Objectives:** identifying the institutional units who generate documents in a regular basis is an important step in the process of classifying activities middle and end of the institution and it is essential for the documents distribution in series (documents of the same kind produced by a particular organ): a consequence and an expression of the activities of the institution in exercise of their functions. **Methods:** the process for the identification of the institutional units follows a few steps: 1) creation of organogram of the institution that provides its organic structure; 2) studies of the units that composes the institution and its referred responsibilities and 3) survey of the documentary typologies. For that, in the case of Instituto Butantan, we analyze de Annual Reports, which are the official documents that portray the activities and actions undertaken in each institutional unit, as well as the nomenclature used to identify them. To support and guide our assessment about the transformations observed over time, we also surveyed the legislation, seen as the legal regulations governing the institution's position as a public agency. Another consulted source were the Butantan human resources file, which contains employees information and resources for hiring, offering conditions to assess the volume of staff available for the development of their functions. **Results and Discussion:** from May 2011 to August 2012, we raised the organizational structure from 1901 to 1946. We identified the number of contracted employees, in addition to their functions exercised and their occupied positions. Through institutional documentation and the information provided by the organograms, we can describe the functions of the institutional internal units, its dates and the kind of documents generated by each unit. The Instituto Butantan is considered an organ of public administration, which also has the competence to issue documents by following the guidance of a certain administrative procedure, as well as having to be responsible for the management of the documentation which it originates.

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10.16 Absence of *Borrelia burgdorferi* transovarian transmission by artificial feeding ticks

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Introduction: In Brazil, human cases of Lyme-simile disease, known as BYS (Baggio-Yoshinari Syndrome) are diagnosed since the early 90's, with cases being recorded in the states of São Paulo, Rio de Janeiro, Mato Grosso do Sul and Amazonas. The diagnoses are based on clinical symptoms, *Borrelia burgdorferi* antigen positive serology (American strain G39/40) and infestation history, because the etiological agent is unknown. **Objectives:** Artificially feed partially engorged female ticks to assess transovarian transmission of *B. burgdorferi*. **Methods:** With the use of selected patient's blood with suspected disease as material for artificial feeding of *Amblyomma cajennense* and *Rhipicephalus sanguineus* ticks species, through capillary tubes, aiming to promote the agent multiplication in the vector for further isolation. Likewise, rabbits blood samples inoculated with *B. burgdorferi* (American strain) were used in artificial feeding by means of capillary tubes of both species of ticks, to evaluate the possibility of infection from both, vector and postures. These evaluations were performed by PCR (Polymerase Chain Reaction) molecular analysis. The blood obtained from 6 suspicious patients was transferred by artificial feeding to 10 females of *A. cajennense* and 10 females of *R. sanguineus*. The *B. burgdorferi* inoculums in rabbit's blood were prepared for the feeding of 10 *A. cajennense* and 36 *R. sanguineus* female ticks. **Results and Discussion:** All specimens fed with blood from suspicious patients were negative for *Borrelia* by PCR, like cell cultures derived from four egg masses of each species. Moreover, the females fed with rabbit blood inoculated with *B. burgdorferi*, which were positive by PCR, positions held, but the egg masses were all negative for the pathogen, indicating the absence of transovarian transmission, supporting the literature.

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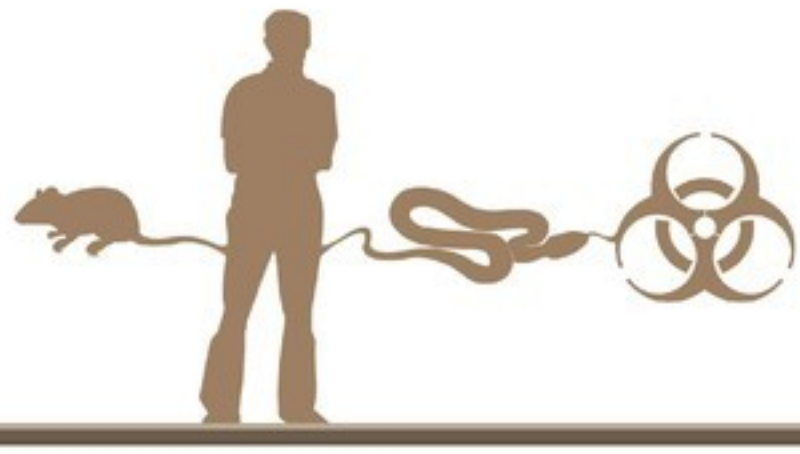

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10.17 Investigations on Hemostatic Mechanism in *Crotalus durissus terrificus* snake

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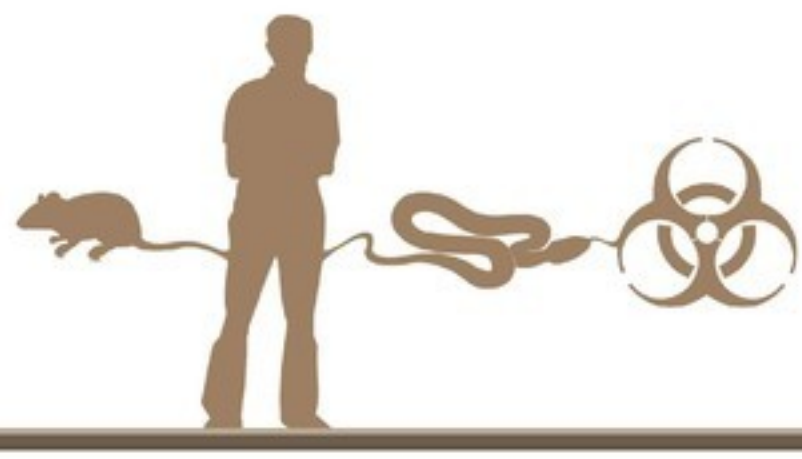
Introduction: Most of reptiles present high levels of natural anticoagulant, showing prolonged clotting time. However, the mechanisms of coagulation and thrombocytes function are not well known. **Objectives:** The aim of this study was to investigate the hemostatic mechanism of the *Crotalus durissus terrificus* (*C.d.t*) snakes. **Methods:** Blood was collected by abdominal aorta of five adult *C.d.t.* snakes anesthetized with sodium thiopental. The clotting time in whole blood (without anticoagulant) was performed in glass tubes. The prothrombin time (PT), activated partial thromboplastin time (APTT) and thrombin time (TT) were performed in Start® coagulometer (Diagnostica Stago). For activation of the extrinsic pathway, macerated aorta was used. The level of fibrinogen was determined by colorimetric method. The thromboelastographic assays were performed using Rotem System® (Pentapharm). **Results and Discussion:** The results of this study show considerably longer times for whole blood clotting time (35 ± 5.5minutes), PT (52.7 ±5.9s), APTT (227.8 ±104.3s) and TT (24.4 ±2.4s) comparing with human plasma. The amount of thrombocytes (13.5 ±2.7 x10^9/L) was similar to the other species of snakes and well aggregated with collagen (2.5µg/mL). The NATEM test assesses clot formation in whole blood with saline solution, without anticoagulant. In this test, the clotting time (CT, 2972 ±777.8 seconds, s), the clot formation time (CFT, 855.5 ±86.5s) and the angle α (determines the speed of clotting formation, 52 ±28°) were more prolonged than that of normal human blood. Furthermore, the maximum firmness of the clot (MCF, 21.7 ±8.2mm) was shorter than in human. The INTEM test evaluates the intrinsic pathway by activation with ellagic acid. In this test, the TC was prolonged (2154.4 ±319.1s) and the graphic showed low amplitude, which probably is related to the low amount of coagulation factors or the presence of coagulation inhibitors. The EXTEM test evaluates the extrinsic pathway through the activation by thromboplastin. In this test, we observed a slight prolongation of CT (196.3 ±42.1s) and CFT (221.5 ±80.1s). The angle α (65.3 ±6.9°) and MCF (57 ±17.6mm) were similar to human values. The FIBTEM test specifically analyzes the role of fibrinogen, since thrombocytes are inhibited with cytochalasin D in this process of clot formation. Although the normal level of fibrinogen (2.5 g/dL), FIBTEM presented low-amplitude graphic. Thus, the hemostasis in *C.d.t* is effective, characterized by predominant activation of extrinsic pathway, and the intrinsic system seems controlled with the presence of plasmatic inhibitor in higher concentration than in mammals.

Supported by: CAPES and CNPq



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10.18 Functional characterization of the Elongation Factor Tuf from *Leptospira*: an example of a moonlighting protein

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Introduction: Leptospirosis is a spirochetal disease caused by pathogenic members of the genus *Leptospira*. After penetrating the host, pathogenic *Leptospira* have the ability to disseminate and to trigger a specific immune response. In the last years great efforts towards the development of an effective vaccine against human leptospirosis are being undertaken. Therefore, the identification of virulence factors is of great relevance. Of special interest are the proteins present on the surface of the bacterium, supposed to interact with host molecules. The Elongation Factor Tuf (EF-Tu), a cytoplasmic protein responsible for critical steps in protein synthesis, has been shown to play a newly found role in host immune system evasion and tissue invasion in some prokaryotes. Multiple functions performed by a single protein is a phenomenon known as "moonlighting". This special class of proteins is usually found in different compartments of the cell. **Objectives:** In this work we aimed to functionally characterize the leptospiral EF-Tu protein with regard to its interaction with human complement regulators, extracellular matrix components (ECM), and coagulation cascade molecules. **Methods:** The gene coding for leptospiral EF-Tu was cloned, the protein was expressed in *E. coli* and the recombinant His-tagged protein was purified by nickel-affinity chromatography. Antibodies against the protein were produced in mice. Cellular localization of the protein was assessed by Triton X-114 detergent solubilization and phase partitioning, and also by immunoelectron microscopy. Protein conservation was evaluated by immunoblotting using different *Leptospira* species. The interaction of EF-Tu with laminin, collagen I and IV, plasma and cellular fibronectin, elastin, fibrinogen, plasminogen, Factor H and C4b Binding Protein was performed by ELISA. **Results and Discussion:** *Leptospira* EF-Tu is higly conserved among different *Leptospira* species, and is both a cytoplasmic and membrane associated protein. Moreover, EF-Tu binds all ECM molecules tested and also strongly interacts with plasminogen in a dose-dependent manner. Binding to this component of the hosts´ fibrinolytic system may be of great relevance to the bacterium, facilitating its dissemination through the organism. This is the first characterization of a "moonlighting" protein from *Leptospira*. Given that these proteins serve as virulence factors in a number of important pathogens, understanding their function may contribute to the development of therapeutic strategies to counteract the disease.

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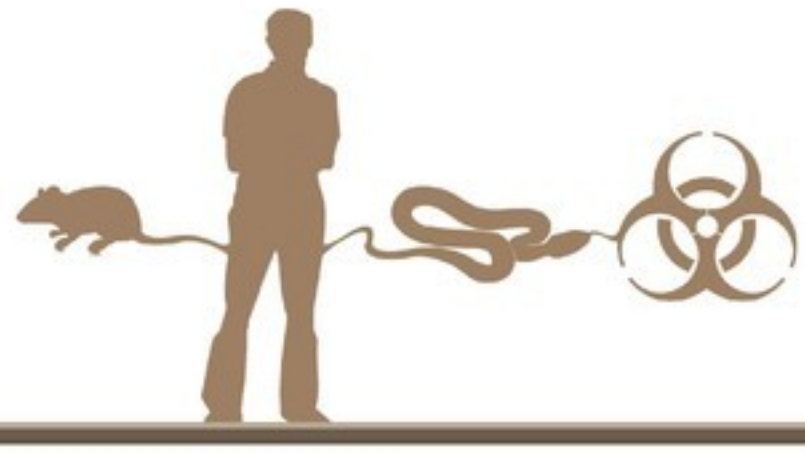

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10.19 PMA promotes apoptosis of malignant transformed H-RasV12 human keratinocytes while stimulate proliferation of its normal counterpart

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Introduction: *Croton* genera (family *Euphorbiaceae*) include ornamental plants often involved in accidental home poisonings due to toxins produced by its secondary metabolism. *Croton tiglium* oil seed is rich in PMA (Phorbol-12-myristate-13-acetate), a diterpene commonly used as tumor promoter in mouse skin carcinogenesis model, mimicking DAG (diacylglycerol) as an activator of PKC (Protein Kinase C) isoforms. Paradoxically, it was also seen inhibiting a wide range of tumoral cells. Despite PMA is already in phase I of clinical trial for haematological malignancy, its molecular mechanism of action is still poorly understood. **Objectives**: To understand the effects of PMA in normal and malignantly transformed human keratinocytes. **Methods:** We used HaCaT cell line expressing the oncogene H-RasV12 constitutively and transfected with empty vector. While PMA stimulated DNA synthesis and mitosis entry of normal keratinocytes, its malignant counterpart was inhibited by this compound, being this effect reverted with the PKC inhibitors Gö6976 and bisindolylmaleimide I. PMA also suppressed colonies growth in agarosis suspension and decreased the thickness of HaCaT H-RasV12 stratification in organotypic culture. Sustained treatment with PMA induced malignant keratinocytes DNA fragmentation and stimulated caspase 3/7 activity. PMA also stimulated transient increase in normal HaCaT Reactive Oxygen Species (ROS) levels while induced high and sustained ROS generation in H-RasV12 transformed cells. **Results and Discussion**: Our findings suggest that PMA promotes growth of "normal" human keratinocytes while suppresses growth of H-RasV12-driven malignant keratinocytes by inducing apoptosis and ROS generation in PKC-dependent fashion. These antagonic effects can be explained by differences in cellular ROS generation: in normal cells PMA induces low and transient increases in ROS levels, which are known to be proliferative, while induces high and sustained lethal ROS levels in malignant keratinocytes. This redox approach can be promising in cancer therapy and deserves further investments.

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