GOVERNO DO ESTADO DE SÃO PAULO
SECRETARIA DE ESTADO DA SAÚDE
COORDENADORIA DE CIÊNCIA, TECNOLOGIA
E INSUMOS ESTRATÉGICOS DE SAÚDE
INSTITUTO BUTANTAN
SÃO PAULO, SP - BRASIL

GOVERNO DO ESTADO
DE SÃO PAULO

Memórias do Instituto Butantan

VIII Reunião Científica Anual

VIII Annual Scientific Meeting

Volume 63 - December 2006

São Paulo, SP – Brasil

2006

COMISSÃO EDITORIAL

EDITORIAL COMMITTEE

Editor-chefe (Editor-in-Chief) Fan, Hui Wen
Editores Associados (Associate editors) Portaro, Fernanda Calheta Vieira; Nascimento, Ana Lúcia Tabet Oller
Bibliotecária Chefe (Chief Librarian) Silva, Lindalva Santana da
Secretária (Secretary): Teresa EiKo Kawabata.
Assessoria de revisão (Assistant Reviewer): Jim Hesson

APOIO INSTITUCIONAL: INSTITUTO BUTANTAN e FUNDAÇÃO BUTANTAN, Financiamento desta publicação

A partir do volume 56, "Memórias do Instituto Butantan" passa a ser um relatório bienal das atividades científicas e técnicas do Instituto Butantan e a partir do volume 60, passa a conter os resumos da Reunião Científica Anual do Instituto Butantan.

From volume 56, "Memórias do Instituto Butantan" will serve as a biennial report of the scientific and technical activities of Instituto Butantan, and from volume 60, will include the Abstracts of the Annual Scientific Meeting of Instituto Butantan.

Endereço (Address): Biblioteca, Instituto Butantan, Av. Vital Brasil 1500, 05503-900,
São Paulo-SP, Brasil
Telefone (Phone) (011) 3726-7222, Ramal (Extension) 2129/2117
Fax: (011) 3726-1505
e-mail: bibliobut@butantan.gov.br - www.butantan.gov.br

MEMÓRIAS do INSTITUTO BUTANTAN (Secretaria de Estado da Saúde).
São Paulo, SP, Brasil, 1918 -

1990, 52 (1-3, supl.)
Em apenso, a partir de 1990, 52(3): BOLETIM DE BIOTECNOLOGIA
1991, 53 (1, supl. 1, 2)
1992, 54 (1, 2)
1993, 55 (1, 2, supl. 1)
1994-1995, interrompido
1996, 56 bienal, nova forma
1998, 57 bienal
2001, 58 bienal
2002, 59 bienal
2003, 60 annual
2005, 61 annual
2005, 62 annual
2006, 63 annual

ISSN0073-9901
MIBUAH CDD 614.07205

Table of Contents

EDITORIAL

In its 63rd volume, **"Memórias do Instituto Butantan"** continues to present an overview of the diverse activities carried out at Instituto Butantan, based on the scientific program and abstracts of the Annual Scientific Meeting, which is in its 8th year. An initiative of the Division for Scientific Development, this meeting involves the entire Institute that discuss scientific production of Basic and Applied Research, Technological Development, Production and Cultural Development, in the form of round tables and poster presentations.

The 294 posters to be presented in the VIIII Annual Scientific Meeting are covered by different research areas: Venoms, Toxins and Envenomation; Microorganisms and Vaccines; Genetics and Immunoregulation; Biology of Snakes, Arachnids and Aamphibians; Animal Care and Veterinary Diseases; Education and Science Diffusion; and Cellular Biology. The best posters presented by under-graduate, Ms.C. and Ph.D. students will be awarded with Instituto Butantan Prize.

The theme of this meeting "Science and Health: Generating Interdisciplinarity" has been chosen to guide discussions. In recent times there has been an important academic debate at Butantan on how to transfer research-based knowledge to the health policy-making process. There is a consensus concerning the relevance of scientific and technological knowledge and their usefulness to contribute for minimizing public health problems and satisfying the needs of a rapidly changing society. Therefore this meeting intends to discuss how scientific outcomes influence policymaking in health, as well as the difficulties imposed by concrete reality and the political process for implementing innovative proposals.

On a whole we hope this issue provides a general view of the diverse activities being carried out at Instituto Butantan in 2006.

Fan Hui Wen
Editor-in-Chief

Ana M. Moura da Silva
Head of the Division for Scientific Development

INSTITUTO BUTANTAN

Board of Counselors and Divisions

Director

Mercadante, Otavio Azevedo

Directing Council:

Mercadante, Otavio Azevedo (Presidente)
Moura da Silva, Ana Maria
Higashi, Hisako Gondo
Canter, Henrique Moisés
Camargo, Erney Plessmann de
Buss, Paulo Marchiori
Jatene, Adib Domingos
Guralnik, Hernan Chaimovich
Travassos, Luiz Rodolpho R. G.
Kalil, Jorge
Teixeira, Paulo Roberto

Scientific Division

Director

Moura da Silva, Ana Maria

Research Council

Furtado, Maria de Fátima Domingues
Yamanoue, Norma
Lebrun, Ivo
Tambourgi, Denise Vilarinho
Ho, Paulo Lee

Technological Development and Production Division

Director: Higashi, Hisako Gondo

Production Council

Akimura, Olga Kikue
Guidolin, Rosalvo
Moro, Ana Maria
Tenório, Elisabeth Christina Nunes
Prado, Sally Muller Affonso

Cultural Division

Director: Canter, Henrique Moisés

Cultural Council

Puorto, Giuseppe
Ribeiro, Orlando Garcia
Prezoto, Benedito Carlos
Wen, Fan Hui
Martins, Rosana
Piagge, Vânia Dalle

Organizing and Scientific Committee for the VIII Annual Scientific Meeting of Instituto Butantan

Fan Hui Wen - President

Ana Lúcia Tabet Oller do Nascimento - Vice-President

Fernanda Calheta Vieira Portaro

Francisco Oscar de Siqueira França

Giuseppe Puorto

Ida Sigueko Sano Martins

Marcelo Larami Santoro

Maria Aparecida Sakauchi

Marilene Demasi

Mônica Valdyrce dos Anjos Lopes Ferreira

Myriam Elizabeth Velloso Calleffo

Orlando Garcia Ribeiro Filho

Suzana Cesar Gouveia Fernandes

Assistance:

Sergio Marcello Humantschuk

Teresa Eiko Kawabata

Tiago de Paula Oliveira

VIII Annual Scientific Meeting of Instituto Butantan

December 06 – 08, 2006

Scientific Program

Wednesday 06/12/2006

09:00 - 09:15	**Opening Session**
09:15 - 12:00	**Round Table – Insertion of Instituto Butantan in public health, science and technology policies** Coordinator: Nelson Ibañez (Instituto Butantan)
09:15 - 09:45	Balance and perspectives Otavio Azevedo Mercadante - Director of Instituto Butantan
09:45 - 10:15	The politics of São Paulo State for science and technology in health programs Paulo Roberto Teixeira (Coordenadoria de Insumos Estratégicos – SES/SP) The PP-SUS in the São Paulo State Dra. Luiza Heiman (Instituto de Saúde – SES/SP)
10:15 - 10:30	**Coffee break**
10:30 - 11:00	Financial support for research in public health Carlos Américo Pacheco (Universidade Estadual de Campinas)
11:00 - 11:30	The institutes of research in the scenario of Brazilian science Ennio Candotti (President of Sociedade Brasileira para Progresso da Ciência)
11:30 - 12:00	General discussion
12:00 - 13:30	**Lunch**
13:30 - 15:30	**Poster session I**
15:30 - 16:00	**Coffee break**
16:00 - 16:15	**Flashes of the Butantan's memory** The reception of snakes Myriam Elizabeth Velloso Calleffo (Instituto Butantan)
16:15 – 18:00	**Scientific Initiation Award** Coordinator: Ida S. Sano Martins (Instituto Butantan)

Thursday 07/12/2006

09:00 - 12:00	**Round Table – Venoms, toxins and envenomings** Coordinator: Francisco Oscar de Siqueira França (Instituto Butantan)
09:00 - 09:40	Dilemmas and challenges in the therapeutic approach of human envenoming caused by poisonous animals Ceila Maria Sant'Anna Málaque (Instituto Butantan)
09:40 - 10:30	Contributions of experimental research to understand human envenoming caused by poisonous animals Denise Villarinho Tambourgi (Instituto Butantan)
10:30 - 10:50	**Coffee break**
10: 50 - 11:30	The scientific research between the clinics and experimentation Paulo Andrade Lotufo (Hospital Universitário – USP)
11:30 - 12:00	General discussion
12:00 - 13:30	**Lunch**
13:30 - 15:30	**Poster session II**
15:30 - 16:00	**Coffee break**
16:00 - 16:15	**Flashes of the Butantan's memory** Antivenom production Maria Aparecida Sakauchi (Instituto Butantan)
16:15 – 18:00	**Master Award** Coordinator: Orlando Garcia Ribeiro (Instituto Butantan)

Friday 08/12/2006

09:00 - 12:00 **Round Table – New vaccines for emerging diseases**
Coordinator: Ana Lúcia T. Oller Nascimento (Instituto Butantan)

09:00 - 09:40 Viral respiratory emerging diseases: perspectives of immunization
Claudio Pannutti (Instituto de Medicina Tropical - USP)

09:40 - 10:30 Brazil's contingency plan to confront an influenza pandemic
Expedito Luna (Secretaria de Vigilância em Saúde - Ministério da Saúde)

10:30 - 10:50 **Coffee break**

10:50 - 11:30 Implementation of industrial area for influenza vaccine production at Instituto Butantan
Hisako Gondo Higashi (Instituto Butantan)

11: 30 - 12:00 General discussion

12:00 - 13:30 **Lunch**

13:30 - 15:30 **Poster session III**

15:30 - 16:00 **Coffee break**

16:00 - 16:15 **Flashes of the Butantan's memory**
Families at Instituto Butantan
Suzana Cesar Gouveia Fernandes

16:15 – 18:00 **Doctoral Award**
Coordinator: Fernanda C. V. Portaro (Instituto Butantan)

18:00 Closing session

Poster Sessions	Dec 6 Session I	Dec 7 Session II	Dec 8 Session III
1.Venoms, toxins and envenomations	-	1-37	38-74
2.Microorganisms and vaccines	1-31	-	32-61
3.Genetics and immuno-regulation	-	1-15	-
4.Biology of snakes, arachnids and amphibians	1-15	16-29	-
5.Animal care and veterinary diseases	1-6	-	-
6.Education and science diffusion	1-9	-	-
7.Cellular biology	-	1-12	13-25
8.Others	-	1-20	21-39
9.PIBIC program	1-37	-	-

Poster Abstracts

1. Venoms, toxins and envenomations

1.01 Characteristics of *Bothrops* snakes and clinical aspects of human envenomation.

Amâncio FF[4], Saad N[3], Pessoa Jr VP N N[3], Toledo JP N N[3], Fan HW[1], Malaque CMS[1], Almeida-Santos SM[2].
[1]Hospital Vital Brazil, [2]Lab Herpetology, Instituto Butantan; [3]Instituto Infectologia Emílio Ribas, São Paulo; [4]Hosp. Eduardo de Menezes, Belo Horizonte, Minas Gerais.

Introduction: differences between *Bothrops jararaca* snake venom have been described experimentally with some clinical evidence. **Objective**: correlate clinical aspects of *Bothrops* human envenomation with animal biology. **Patients, material and methods**: patients bitten by proven snakes were monitored at Hospital Vital Brazil; animals were analysed in terms of species, sex and total length (TL) at Lab. Herpetologia, Instituto Butantan. Analysis of clinical aspects and length of causative snakes were performed using comparison between proportions, means or medians, whenever appropriate. **Results**: from a total of 276 patients attended in a two-year period (2004-5), 103 were included in this study. All snakes except one were identified as *B. jararaca*, most were female (81 or 83.5%) and mean of TL was 593.4 ± 303.3 mm (median= 461, min= 245, max= 1635 mm). Local effects, expressed by extension of edema and blister formation and occurrence of secondary infection, were more prominent in those bitten by longer snakes (p= 0.0005, p=0.0092 and p=0.00011, respectively). Both female and male snakes were captured in the same season; differences in clinical aspects of envenomation were not observed, probably due to the small sample of male animals and low frequency of complications. **Discussion:** predominance of female snakes captured may be related to their higher mobility thus, increasing the exposure to human contact. Sample should be increased to evaluate severity of envenomation caused by female or male *B. jararaca* snakes.

1.02 Risk factors associated with acute renal failure in patients bitten by *Bothrops* snakes.

Novaes CTG, Malaque CMS, Fan HW, Crespo FG, Medeiros CR, França FOS, Cardoso JLC, Prado JCL, Risk JY.
Hospital Vital Brazil, Instituto Butantan.

Introduction: Considered the main cause of death from snake bites, acute renal failure (ARF) is not fully understood. **Objective**: To evaluate frequency of ARF and the associated risk factors in patients bitten by *Bothrops* snakes. **Patients and methods**: Blood samples were collected from patients with clinical diagnosis of *Bothrops* envenomation treated at Hospital Vital Brazil, Instituto Butantan. Comparison between proportions, means or medians, whenever appropriate, were performed; statistical significance was established when $p < 0.05$. **Results and discussion**: ARF, defined as serum creatinine levels > 1.3 mg/dl, was detected in 12 (11%) patients within the first 24 hours of envenomation. Of these, only 1 was submitted to dialysis. No differences were detected in time between bite and treatment, size of snake and severity since most of patients were considered mildly envenomed. Age was significantly higher in those with ARF (mean= 54 ± 10 years, median= 53) when compared with those without ARF (mean= 33 ± 17 years, median= 31) [p=0.0001]. Coagulation disturbance was equally detected in both groups (p=0.8085) but higher levels of indirect billirrubin (p= 0.013182) and DHL (p=0.0950) were related to ARF. In contrast, platelet count was significantly lower in the ARF group (p= 0.0364). **Conclusion**: Vigorous intravenous liquid infusion should be considered in all *Bothrops* envenomed patients on admission in order to prevent risk of ARF.

1.03 Intravascular hemolysis in human *Bothrops* envenomation: more than we supposed.

Malaque CMS, Crespo FG, Novaes CTG, Fan HW, Medeiros CR, França FOS, Cardoso JLC, Prado JCL, Risk JY.
Hospital Vital Brazil, Instituto Butantan.

Introduction: hemolysis is a common effect of *Bothros* venom observed *in vitro* but fairly studied *in vivo*, especially in human beings. **Objective**: to describe laboratorial profile of suggestive intravascular hemolysis in patients bitten by *Bothrops* snakes. **Patients and methods**: 84 patients attended at Hospital Vital Brazil, Instituto Butantan were followed up; laboratorial tests, including total and indirect bilirrubin, urea and creatinine, DHL, platelets, hematological and coagulation profile were performed before and/or after antivenom therapy. **Results and discussion**: 30 (36%) patients present evidences of intravascular hemolysis, expressed by elevated indirect bilirrubin (mean= 1.26 ± 0.4; median= 1.1, max= 2.5; normal range= <0.7 mg/dl) and DHL (mean= 279.3 ± 87.4; median= 271.5; max= 583; normal range= 100-190 U/L), but no clinical signs. No differences were observed in age or gender of patients, time between bite and treatment or severity of envenomation. Although not statistically significant, a tendency of young snakes to be causative of hemolysis was detected (385.5 x 665.0 mm, p=0.102428). Coagulopathy was more frequent in those with hemolysis (p=0.0005) and platelets count decreased after antivenom therapy (mean= 127.5 ± 60.5, median= 130, min= 48, normal range=150-400x10^3). Urea and creatinine levels were slightly higher, indicating a risk of acute renal failure (p= 0.0005) in this group. **Conclusion**: although mostly subclinical, occurrence of hemolysis should not be neglected in *Bothrops* envenomation.

1.04 Epidemiological aspects of snakesbites caused by *Crotalus durissus terrificus* in São Paulo State, Brazil

Sueiro LR[1], Gonçalves MR[1], Rojas CA[1], Almeida-Santos SM[1].
[1]Lab. Herpetology, Instituto Butantan, São Paulo, Brazil.

Introduction: Snake bites are a major public health problem in Brazil, being the majority of the accidents attributed to the *Bothrops* genus (90%), followed by *Crotalus* (7.7%), *Lachesis* (1.4%) and *Micrurus* (0.5%). **Objective:** To analyze the epidemiological aspects of the crotalic accidents recorded by Hospital Vital Brazil (HVB) and to dissect specimens preserved in the Coleção Herpetologica of Instituto Butantan (IB). **Methodology:** Data were collected from the HVB records from 1959 to 2005. Data regarding to identification, sex and ontogeny of the snakes were analyzed, as well as sex and age of the patients, and date, hour, place and characteristics of the accidents. **Results**: A total of 214 records of accidents with *Crotalus durissus terrificus* were collected. Of these, 65 animals were deposited in the HVB collection, being 54 adults and 11 juveniles; 29 females and 36 males. Victims were predominantly males (78%), between 15 and 40 years old (46%), of which 49% were involved in agricultural activities. Bites were more common in the summer (31%) and autumn (26%), and occurred between 6 am and 12 am (38%). **Discussion:** For rattlesnakes, the peak of accidents is directly related to the reproductive period (when males actively search for females), combat and courtship, which occurs in latter summer and mid autumn, different to the pattern found for *B. jararaca*.

1.05 Bites by *Philodryas patagoniensis* (colubridae snake): an epidemiological study of 301 cases registred at Hospital Vital Brazil.

Hess PL[1,2], Almeida-Santos SM[1], Rocha MMT[1] and Furtado MFD[1].
[1]Lab. Herpetology, Instituto Butantan, [2] Instituto Biociências, USP, São Paulo, Brazil.

Introduction: The low incidence of accidents caused by *Philodryas* is due to the anatomy of the inoculator teeth (located in the posterior region of the maxilla) of these serpents leading to the difficulty to inject their venom, and the non-aggressive behavior of the colubrid snakes. Therefore less than 20 cases of bites by *Philodryas patagoniensis* (Colubridae) have been reported in the literature. **Objectives:** To gather epidemiologic data of cases registered as accidents by *Philodryas patagoniensis*. **Methodology:** Were consulted all of the books of registration of snakes that caused accidents, attended in the Hospital, since 1959 up to present moment of 2006. **Results:** The results showed that happen approximately 6.5 cases a year. The 211 male (70.1%) and 90 female (29.9%) patients, most between 0 and 30 years of age (63.8%), presented mainly from November to March (53.2%), of 06:00 am up to 06:00 pm (67.1%). Most of the registrations were caused by animals in adult life stage (66.1%). **Discussion:** Most of the accidents reaches men and occurred during the hottest months (summer and spring) and during daylight hours. Snakes of the genus *Philodryas* are considered as not poisonous; therefore, people should be aware about the risks the so-called harmless snakes may cause. Precautions should be increase in the hottest season (summer), not only because it's the time people most look for leisure, but also due to the fact that this is the reproductive season for these snakes, when they are more active.

1.06 Bites and stings by venomous animals in the northwestern region of the State of São Paulo, Brazil

Rojas, CA, Gonçalves, MR, Sueiro, LR, Almeida-Santos, S.M.
Laboratório de Herpetologia, Instituto Butantan, São Paulo, Brazil.

Introduction: Records of bites and stings by venomous invertebrates, especially spiders and scorpions, increased considerably after 1988 once it became compulsory to notify authorities. **Objective**: To show the current data on bites and stings by invertebrate species in the northwestern region of São Paulo State and to relate it to biological factors. **Methodology**: In this study we analysed the occurrence of venomous bites and stings by invertebrates registered from 1999 to 2004 in the archives of the Departamento de Vigilancia Epidemiologica of São José do Rio Preto, State of São Paulo. **Results**: We listed a total of 975 cases of bites and stings. Of these, 72 % were by scorpions (*Tityus serrulatus* 74%), 20% by spiders (*Phoneutria* 50%) and the other 8% by bees, caterpillars, etc. The victims were predominantly male between 15 and 40 years old. Hands and arms (53%) were the body parts most frequently injured. Bites occurred equally in urban (52%) and rural (43%) areas. Scorpion stings were more common in the spring, whereas spider bites were more frequent in summer and autumn. The great majority of the victims received aid during the first hour after having been bitten or stung. Ninety-five percent of the victims were cured, 0.4% were left with permanent injuries and 0.2% died. **Discussion**: The scorpion *Tityus serrulatus* caused accidents throughout the year, which can be explained by the fact that this species is parthenogenetic. The peak of spider bite injuries coincides with the reproductive period of the genus *Phoneutria*.

1.07 Experimental *Bothrops jararaca* envenomation in pregnant mice: efficiency of antibothropic serum.

Ferreira KV[1,2], de Tomy SC[1], Spadacci-Morena DD[1].
[1]Laboratory of Pathophysiology, Butantan Institute; [2]Presbiterian Mackenzie University, São Paulo, Brasil.

Introduction: *Bothrops* snakes are responsible for about 90% of snakebites in Brazil and cause high fetal wastage among pregnant women. In this case, the treatment with antibothropic serum is advisable because even if the envenomation is insufficient to provoke clinical signs in the mother, the venom can cross the placenta.However, antivenoms can provoke adverse reactions and, in pregnant women, abortion has been reported. **Objective**: To investigate the injury caused by *Bothrops jararaca* venom and the effect of treatment with antibothropic serum in pregnant mice, studying the morphology of the uterus, in the maternal-fetal interface, and some hematologic parameters. **Methods**: On day 8 of pregnancy, groups of animals were injected with saline or *B. jararaca* venom (2.44mg/kg body weight). Treatment with antibothropic serum was performed after 3 hours, in both groups. On day 9, the uterine horns of mice was removed and processed for light microscopy. Leukocyte count and fibrinogen dosage were also analysed. **Results and Discussion**: Histological observations revealed an organized decidua, morphologically similar to that of saline group animals, but exhibiting few polymorphonuclears, some of them within decidual capillaries and several at the maternal-fetal interface. Plasma fibrinogen levels were similar in both groups (BjV 1.218 ± 0.081 and Sal 1.329 ± 0.088). Total and differential white blood cell counts were not statistically different between groups. Initial results suggest that antibothropic serum probably attenuate the harmful effects of envenomation in pregnant mice.

1.08 Inhibition of platelet aggregation by an ATP/ADPase isolated from *Bothrops jararaca* snake venom.

Santoro ML, Nakasato A, Oliveira A
Laboratory of Pathophysiology, Institute Butantan, São Paulo-SP, Brazil. e-mail: santoro@ butantan. gov.br

Introduction: Nucleotides and nucleosides containing an adenine base interfere intensely with platelet function, either inducing or inhibiting platelet activation under steady state and pathological conditions. Snake venoms contain enzymes that hydrolyze nucleic acids and nucleotides, whose products can potentially modify platelet aggregation. In fact, platelet dysfunction is observed during *Bothrops jararaca* snake envenomation. **Objective:** Herein, an ATP/ADPase present in *B. jararaca* venom was purified and its action on platelet aggregation was investigated. **Methodology and Results**: This nucleotidase was purified by oligo(dT)-cellulose and Blue-Sepharose column chromatography (yield, 0.85%), and showed a relative molecular mass of 126 kDa under non-reducing conditions. *B. jararaca* ATP/ADPase had a catalytic activity 4-fold higher on ADP than on ATP, and it also hydrolyzed bis(p-nitrophenyl) phosphate, a phosphodiesterase substrate. Its activity was inhibited by EDTA and dithiothreitol. **Discussion:** *B. jararaca* ATP/ADPase shows intense inhibitory activity on collagen-induced platelet aggregation, with an IC_{50} of 14 nM, evidencing that it might be also responsible for the platelet aggregation disorder observed in patients bitten by *B. jararaca* snake.
Supported by: FAPESP (04/02223-8) and Butantan Foundation

1.09 Effect of botropic antivenom (BAV) or dexamethasone (Dx) on leukocyte-endotelial interaction induced by *Bothrops jararaca* venom (BjV) in the microcirculation of the cremaster of mice.

Zychar BC, Gonçalves LRC.
Laboratorio de Fisiopatologia, Instituto Butantan, São Paulo, Brazil.

Introduction: The BAV is not efficient to prevent local reactions caused by bothropic venoms. Metabolites of arachidonic acid are main mediators of edema and cellular migration induced by BjV. **Objective:** In this study the effect of BAV or Dx on leukocyte-endothelial interactions induced by BjV injection was evaluated by intravital microscopy. **Methodology**: The BjV (1µg) or saline (100µL) was injected into the scrotal bag of mice. Treatment with Dx (1.0 mg/kg, i.p.) or BAV (0.2 mL, i.v.) was given 1h before or after the BjV injection. The cremaster was exposed 2 (T2) or 24hrs (T24) after the venom injection. Leukocytes in *rolling* and adherent were evaluated during one minute in post-capillary venules (20-40µm) and emigrated cells were counted. **Results:** The BjV induced an increase of adherent and emigrated cells at T2. In T24, the adhesion decreased when compared with T2, but the emigrated cells increased. The pre-treatment with BAV decreased the emigration at T24 and the pre-treatment with Dx inhibited the adhesion and emigration in both times. The same was observed by post-treatment with BAV and Dx. The BAS, *per se*, induced a significantly increase of adhesion and emigration, which was not observed with Dx. **Conclusion:** The BAV does not inhibit cellular inflammatory events induced by BjV. Besides, these events are significantly inhibited by Dx. Studies using Dx associated with BAS on treatment of the inflammatory local reactions induced by BjV are in progress.
Supported by: FAPESP and CAPES.

1.10 Differences in the inflammatory activity of two myotoxic venoms: *Bothrops jararacussu* (Bjssu) and *Crotalus durissus terrificus* (Cdt).

Castro Jr NC, Zychar BC, Gonçalves LRC.
Laboratório de Fisiopatologia, Instituto Butantan, São Paulo, Brazil.

Introduction: The venoms of Bjssu and Cdt are myotoxic, however, with some differences: VCdt induces a systemic myotoxicity and barely no local inflammatory reaction, while VBjssu induces local myotoxicity and an exuberant inflammatory response. In this work, we compared by histological analysis the development of the inflammatory infiltrate (polymorphonuclear neutrophils - PMN) induced by the i.m. injection of VBjssu or VCdt. **Methodology:** Mice (n=3/group) received 50 μg of VBjssu or 3 μg of VCdt into the gastrocnemius. One, 4 or 24 h after injection, animals were sacrificed and the injected tissue was processed for inclusion in paraffin. Sections of 5 μm stained with H&E were analyzed, and PMN infiltrate was estimated in five-fields with an integrative ocular and expressed as Mean ± SD of cells/field. **Results:** In 1st hour, both venoms induced a marked lesion of the muscle cells, with some hemorrhage focuses in the group injected with VBjssu. Four hours after the injection, the group injected with VBjssu presented a conspicuous PMN infiltrate (11.2 ± 1.7), while PMN infiltrate was discreet in mice injected with VCdt (3.8 ± 1.3). The higher infiltrate induced by in VCdt was observed at 24h (7.6 ± 1.2); however, it was less intense than that observed in the VBjssu injected group (12.2 ± 1.5). **Conclusion:** In addition to producing muscular lesions, the VBjssu is a potent flogistic agent. VCdt is a weak flogistic agent and the late PMN migration observed can be secondary to the muscular lesion, being part of the process of tissue regeneration.

1.11 Effect of *Crotalus durissus terrificus* (*Cdt*V) venom on leukocyte-endothelial interaction induced by carrageenan or by *Bothrops jararaca* venom at microcirculation of mice cremaster muscle.

Nunes, FPB[1]; Zychar, BC[1]; Gonçalves, LRC[1]; Sousa-e-Silva, MCC[1].
[1]Laboratory of Pathophysiology, Butantan Institute, São Paulo, Brazil.

Introduction: The *Crotalus durissus terrificus* venom (*Cdt*V) does not induce an inflammatory action at the site of bite and inhibits biological activities of macrophages. **Objective:** The aim of this study was investigate the effect of the *Cdt*V on endothelium-leukocyte interaction induced by *Bothrops jararaca* (*Bj*V) and carrageenan (Cg), at cremaster muscle evaluated by intravital microscopy. **Methodology:** The *Cdt*V (1,5µg) or saline (100µL) were injected subcutaneously, 1h before or after the s.c. injection of the inflammatory agents, *Bj*V (1µg/100µL), or Cg (300µg/100µL) into the scrotal bag of the animals. After 2h, the cremaster was exposed, and 10 minutes after of the microcirculation exposition, the post-capillary venules (20-40µm) were evaluated in a minute and the leukocytes in *rolling*, adherents and emigrated were counted. **Results:** The pré-treatment with *Cdt*V decreased the adhesion (*Bj*V: 68%; Cg: 96%) and increased *rolling* (*Bj*V: 246%; Cg: 371%) when compared with control groups treat with saline. The same decrease of adhesion (*Bj*V 72%; Cg: 54%) and increase of *rolling* (*Bj*V: 185%; Cg: 90%) were observed at post-treatment. **Discussion:** The *Cdt*V inhibits the endothelium-leukocyte interaction induced by the inflammatory agents used (*Bj*V and Cg), decreasing the adhesion and consequently the cell migration to the tissue. This inhiitory effect contributes for the anti-inflammatory action of the *Cdt*V.
Supported by: CNPq, CAPES and FAPESP.

1.12 Comparison of the leukocyte-endothelial interaction induced by snake venoms: *Bothrops jararaca* (VBj) and *Crotalus durissus terrificus* (VCdt).

Zychar BC, Nunes FPB, Sousa-e-Silva MCC, Gonçalves LRC.
Laboratório de Fisiopatologia, Instituto Butantan, São Paulo, Brazil.

Introduction: VCdt, unlike VBj, does not induce marked local reactions. In this study, the leukocyte-endothelial interaction induced by VBj and VCdt was evaluated at different time intervals by intravital microscopy. **Methodology**: VBj (1µg), VCdt (1.5µg) or sterile saline (100µL) were injected in s.c. of the scrotal bag of mice. After 1, 2, 4 and 24 hr of the injection, the cremaster was exposed. Ten min after the exposition of the microcirculation, the number of rolling and adherent cells was evaluated for 1 min and the migrated leukocytes were counted in a portion of 100 µm of post-capillary venules (diameters of 20-40µm). **Results**: Significant differences were observed between VBj and VCdt in relationship to the rolling in the 2nd hr (VBj=7.7±1.2; VCdt=18.3±1.2) and 24th hr (VBj=22.2±1.9; VCdt=11.0±0.5); to the adhesion in the 1st hr (VBj=7.6±0.5; VCdt=4.5±0.4) and 4th hr (VBj=15.3±0,7; VCdt=5.1±0.4); and to the migration in the 4th (VBj=20±1.2; VCdt=7.8±1.4) and in the 24th hr (VBj=16±0.5; VCdt=10±0.3). Except for the rolling induced by VCdt, both venoms showed significant differences compared to the control groups. **Conclusion**: VCdt acts as a weak flogistic agent, inducing a discreet adhesion and migration when compared to VBj, which induces an intense inflammatory reaction. These results corroborate with the clinical and experimental observations. The increase of migration observed in the 24th h in the group VCdt may be due to the myotoxic action of that venom.
Supported by: FAPESP, CAPES and CNPq.

1.13 *Bothrops jararaca* (BjV) and *Crotalus durissus terrificus* (CdtV) venoms elicit distinct responses regarding to production of prostaglandins D_2 and E_2, and expression of cyclooxygenases.

Moreira V[1], Zamuner SR[1], Souto P[1], Wallace JL[2], Teixeira CFP[1].
[1]Lab. de Farmacologia, Instituto Butantan, Brasil, University of Calgary, Canada.

Introduction: Prostaglandins, synthesized by cycloxygenases, play relevant roles in many pathophysiological processes including inflammation and hyperalgesia. **Objective**: In this study the profiles of PGE_2 and PGD_2 production secondary to injection of BjV, with inflammatory activity or CdtV, with antiinflammatory and antinociceptive properties, into mice were evaluated, and the ability of these venoms to induce expression of cyclooxygenases -1 (COX-1) and -2 (COX-2) was investigated. **Methodology**: Male Swiss mice were injected i.p. with CdtV (250 μg/kg) or BjV (25 μg/kg). At selected time, in peritoneal washes were determined PGE_2 and PGD_2 concentrations by EIA. Expression of COXs was determined by western blot. **Results**: Intraperitoneal injection of BjV but not of CdtV induced the release of PGD_2 at 30 min and of PGE_2 from 3 up to 12 h after injection. Moreover, BjV up-regulated expression of COX-2 but not of the COX-1, suggesting that expressed COX-2 is the critical enzyme for prostaglandins production in the late periods of BjV effect. In contrast, CdtV does not have any effect on expression of both COX-1 and -2 proteins. **Discussion:** Differences between BjV and CdtV in the ability to regulate prostaglandins synthesis can account for opposite effects with regard to inflammation. Moreover, inhibition of COX-2 by selective drugs may be of value to counteract the severe local inflammation induced by BjV in the victims.
Supported by: FAPESP and CNPq.

1.14 Local oedema induced by *Bothrops moojeni* snake venom (BmV) in mice: role of histamine.

Nascimento NG[1], Sampaio MC[1], Olivo RA[1], Lima SA[1], Teixeira CFP[1].
[1]Laboratory of Pharmacology, Butantan Institute, São Paulo, Brazil.

Introduction: *Bothrops moojeni* snake causes pronounced local tissue damage both in humans and experimental animals, characterized by haemorrhage, myonecrosis and a prominent oedema. The mechanisms responsible for oedema formation are unknown. **Objectives:** In this study participation of histamine in the paw oedema induced by *Bothrops moojeni* venom (BmV) was investigated. **Methodology:** Male Swiss mice (20g) were used. Paw oedema was measured by plethysmography from 15 min up to 24h after injection of BmV (1μg/paw) into subplantar surface of one hind paw and saline into contralateral paw (control). Groups of mice were treated with compound 48/80, 0,6; 1,0; 1,2 and 2,4mg/kg, i.p., consecutively, or promethazine (H1 receptor antagonist; 5,0 mg/kg, i.p.) or cimetidine (H2 receptor antagonist; 15,0 mg/kg, i.p.) or vehicles (controls), 30 min and 2 h before injection of BmV, respectively. **Results:** BmV intraplantar injection caused a marked paw oedema, peacking at 30 min after its injection decreasing to basal levels after 12 h. Inhibiton of mast cell degranulation by compound 48/80 significantly reduced BmV-induced oedema from 1 up to 6h. Treatment with promethazine significantly decreased BmV-induced oedema from 15 min up to 3h, with a pronounced effect at 30 min. Cimetidine also significantly reduced paw oedema (30 min up to 6h) with maximum at 30-60 min. **Conclusions:** These data suggest that histamine, acting via H1 and H2 receptors, is an important mediator for local oedema induced by BmV. Moreover, activation of mast cells may be an important mechanism for BmV-induced local inflamamation.
Supported by: CNPq and FUNDAP

1.15 Effects of two phospholipases A_2 (PLA_2s) isolated from *Bothrops asper* (Ba) snake venom on leukocytes cyclooxygenases (COXs).

Moreira V[1], Zamuner SR[1], Zuliani JP[1], Wallace JL[2], Gutiérrez JM[3], Teixeira CFP[1].
[1]Lab. Farmacologia, Instituto Butantan, Brasil; [2]University of Calgary, Canada, [3]Instituto Clodomiro Picado, Costa Rica.

Introduction: Two myotoxins (MTs) with PLA_2 structure were isolated from Ba snake venom: MT-III is a PLA_2-Asp49 with high enzymatic activity and MT-II, a PLA_2-Lys49, devoid of catalytic activity. However both evoke inflammatory events. **Objective**: In this study we evaluated the effects *in vivo* and *in vitro* of MT-II and III on the expression and activity of the COX-1 and -2, and the release of prostanoids. **Methodology**: Male Swiss mice were injected i.p. with MT-II or III (1 µg/g). At selected time, in peritoneal washes were determined PGE_2 and PGD_2 concentrations by EIA. Expression of the COXs was determined by western blot and peroxidase activity by colorimetric assay in leukocytes. **Results**: MT-II and MT-III induced expression of COX-2 but not COX-1 in peritoneal leukocytes. The COX-2 showed peroxidase activity whereas the constitutive COX-1 activity was not modified. Moreover, these MTs increased the levels of PGE_2 and PGD_2 in the peritoneal exudates. *In vitro* macrophages with MT-II or III (6.5 µg/mL) resulted in increased release of PGE_2 and PGD_2 and expression of COX-2 but not of COX-1. **Discussion**: These data demonstrate the ability of snake venom PLA_2s to induce the expression of active COX-2 protein. This effect may be at least in part due to a direct action of both MTs on macrophages. Induction of expression of COX-2 may be the major mechanism for production of prostanoids induced by both PLA_2s. The catalytic activity of some snake PLA_2s may not be relevant for their stimulating effects on COXs.
Supported by: FAPESP and CNPq.

1.16 Discovery of two new pharmacological activities for proline rich peptides in *Bothrops jararaca* venom

Rioli V[1], Fernandez JH[1], Prezoto BC[2], Konno K[1] Melo RL[1] Klitzke CF[1], Ferreira-Lopes M[3] Ferro ES[4], Camargo ACM[1], Portaro FC[1].
[1]Lab. Esp. de Toxinologia. Aplic. LETA-CEPID/FAPESP [2]Lab. de Farmacologia, [3]Lab. de Imunopatologia, Instituto Butantan; [4]Depto de Biologia Celular e Desenvolvimento, ICB, USP, SP, Brasil.

Introduction: Proline rich peptides (PRP), also known as BPPs (Bradykinin potentiating peptides), have been isolated and characterized from *Bothrops jararaca* snake venom. They are the most potent natural inhibitors of the angiotensin-converting enzyme (ACE), presenting Kis in the order of nM. The BPPs XIe and XIe-AP, had also shown to be inhibitors of oligopeptidases ep24.15 and ep24.16 (with Kis in the order of magnitude of uM). **Objective**: In the present study, two new pharmacological actions of these two peptides had been evaluated in the microcirculation of cremaster and leukocytes rolling in post-capillary venules through intravital microscopy. **Methodology**: The local administration of the peptides (10ug) was monitored by 30 min. **Results**: The BPP XIe demonstrated a powerful pro-inflammatory action through the increase of the number and the flow of leukocytes in venules after-capillaries (five times bigger of the one than the control), not presenting vasodilatation of the arterioles. In contrast, XIe-AP presented little increase in the number of leukocytes, but it demonstrated vasodilatation action of the arterioles (about 100% when compared with the control). **Discussion**: These results suggest that a small structural difference of these peptides, extension of two amino acids in the C-terminal, can cause great differences in its pharmacological action.
Supported by: FAPESP.

1.17 Further characterization of monoclonal antibodies (MoAb) against BaP1, a metalloproteinase from *Bothrops asper*.

Aievoli E[1], Checchi P[1], Tanjoni I[1], Baldo C[1], Serrano SMT[2], Faquim-Mauro EL[1], Gutiérrez JM[3], Moura-da-Silva AM[1], Takehara HA[1] and Fernandes I[1].
[1]Lab. Imunopatologia, [2]Lab Especial de Toxinologia Aplicada, Instituto Butantan, São Paulo, Brazil; [3]Instituto Clodomiro Picado, San José, Costa Rica.

Introduction: We previously reported that six MoAb against BaP1 (MABaP1) were produced recognizing only conformational epitopes of BaP1 (P-I class Snake Venom MetalloProteinase-SVMP) and *B. asper* venom and not recognizing 27 venoms from different families. **Objective:** We analyzed the ability of IgG1 MABaP1s to cross react with other SVMPs: BJ-PI, Moojeni Protease A (MPA), neuwiedase and jararhagin to study the role of these enzymes in envenomation. **Methodology:** Venoms or enzymes were used to coat plates or dotted on nitrocellulose membrane and after blocking, MABaP1 were added and detected using anti-IgG peroxidase. The dissociation constant (K_d) of MABaP1-7 was determined by indirect ELISA. **Results:** By ELISA, MABaP1-7 recognized neuwiedase and *B. neuwiedi*, with titres similar to BaP1 and *B. asper* (512 to 2048). By dotblot, the MABaP1-7 presented the same pattern of recognition plus MPA, but only for conformational epitopes. The K_d of this MoAb was 5.9 nM. The other MABaP1s did not cross-react with any sample assayed. **Discussion:** These results suggest that MABaP1-7 may be an important tool in understanding the role of P-I class SVMP in envenomation.
Supported by: FAPESP and FUNDAP.

1.18 Effects of *Bothrops jararaca, Bothrops jararacussu* and *Crotalus durissus terrificus* snake crude venoms on the contractile activity of the vas deferens

Sciani JM[1], Garcez-do-Carmo L[2], Conceição IM[1].
[1]Lab. Pharmacology, Instituto Butantan, SP, [2]Department of Pharmacology, UNIFESP, São Paulo, Brazil

Introduction: In previews studies it was verified that the crude venoms of *Bothrops jararaca* (Bj) and *Bothrops jararacussu* (Bjs) snakes potentiate the electrical field stimulation-induced contractions on rat isolated *vas deferens (VD*). The aim of this study was to further characterize this effect and verify if this observation could be also extended to *Crotalus durissus terrificus* (Cdt) venom. **Methods:** After an equilibration period of 30 min VD was submitted to continuously stimulatory trains of 10 s duration at frequency of 0.01 trains per second (with stimulus of 5 Hz, 0.1 ms and supramaximum voltage). After the maximum contraction has become stable (about 15 minutes), 0.1 mg/ml venoms were added and the effects were recorded by 60 min. Data were expressed as percent of control curve (obtained immediately before venom the venom addition). **Results:** The Bj venom enhanced the maximum phasic and tonic contractions by 59.88% and 122.37%, respectively. This potentiation is followed at the end period of experiment by a decreased in both phasic and tonic contraction to 50.78% and 20.98% of control values, respectively. Bj and Cdt potentiated tonic contraction by 49.18% and by 46.73%, respectively. **Conclusions:** These results indicated that all studied venom could enhance sympathetic neurotransmission in VD. However, Bj venom also produced an inhibitory effect that is indicative of tissue damage.
Supported by: FAPESP and Butantan Foundation

1.19 Comparison of the proteolytic activity of P-I and P-III snake venom metalloproteinases on plasma and extracellular matrix proteins.

Oliveira AK, Assakura MT, Camargo ACM, Serrano SMT.
Laboratório Especial de Toxinologia Aplicada-CAT-CEPID, Instituto Butantan, SP, Brazil.

Introduction: Snake venom metalloproteinases (SVMPs) have been shown to be active on plasma and extracellular matrix proteins and therefore they are involved in the hemostasis imbalance observed upon envenomation. **Objectives**: The objectives of this study were to isolate a P-I metalloproteinase from *Bothrops jararaca* venom and to compare its proteolytic activity with that of a hemorrhagic P-III SVMP, HF3, on plasma and extracellular matrix proteins. **Methodology**: Hemorrhagic activity was determined on mouse skin. Proteolytic activity was analysed by incubation of the enzymes at 37ºC with plasma and extracellular matrix proteins followed by SDS-PAGE. **Results**: BJ-PI was isolated from *B. jararaca* venom by gel filtration chromatography (FPLC system). Based on the molecular mass and identification by mass spectrometry it was possible to classify BJ-PI as a P-I class enzyme. This enzyme is highly active on casein however it is not able to cause hemorrhage. Both HF3 and BJ-PI degraded fibrinogen, fibrin, fibronectin, vitronectin, collagen type IV, collagen type VI and laminin showing different hydrolysis profiles. Deglycosylation of HF3 with N-glycosidase F caused destabilization of protein structure and loss of hemorrhagic activity.**Discussion:** The preliminary results of this study suggest that the presence of disintegrin-like and cysteine-rich domains in HF3 confer to this enzyme substrate specificity different than that of BJ-PI, which is important for the in vivo hemorrhagic activity displayed by HF3.
Supported by: FAPESP.

1.20 Hydrolysis of extra-cellular matrix proteins by snake venom metalloproteinases

[1]Baldo C, [1]Tanjoni I, [1]Della-Casa MS, [1]Clissa PB, [2]Valente RH, [1]Lebrun I, [1]Lopes-Ferreira M. [3]Rodrigues VM, [1]Moura-da-Silva AM.
[1]Lab. Imunopatologia, Instituto Butantan, SP [2]Depto. Fisiologia e Farmacodinâmica, IOC, FIOCRUZ, RJ
[3]Lab. Química de Proteínas e Produtos Naturais, Universidade Federal de Uberlândia, MG, Brasil.

Introduction: Hemorrhagic SVMPs have been extensively studied and their effects are associated with proteolysis of extra-cellular matrix (ECM). However, P-I and P-III SVMPs similarly hydrolyze ECM proteins while only P-III SVMPs induce significant hemorrhage suggesting that other molecular structures are involved in this effect. **Objective**: To compare the effects of jararhagin (Jar), as a model of P-III SVMP, and two non-hemorrhagic P-I SVMPs isolated from *B. neuwiedi* venom on ECM and plasma proteins. **Methodology**: Neu1 and Neu2 were isolated by Superdex 75 HR 10/30 and Mono-Q HR 5/5 column. Toxins were characterized by amino acid composition and mass spectrometry. The proteolysis of laminin I, collagen I and fibrinogen by SVMPs was evaluated by SDS-PAGE. The fibrinolytic activity was assayed in fibrin-agarose plates. The binding of SVMPs to collagen I and fibrinogen was also evaluated by a solid-phase assay. **Results**: The isolated toxins showed identical apparent molecular mass of 25 kDa, but different isoeletric properties. After 6 hs, Jar, Neu1 and Neu2 were equally able to hydrolyze collagen I and fibrinogen. However, the effect of Jar occurred after 30 min incubation period while Neu1 and Neu2 effects were detectable only after 1 h. Jar was also more effective to degrade fibrin. The hydrolysis of laminin was not observed. Jar binded to collagen and fibrinogen in concentrations as low as 0.19 e 192 nM respectively. The binding of Neu1 and Neu2 to these proteins was not detected. **Discussion:** Our data showed that P-III SVMPs binds with high affinity to ECM proteins. This binding may enhance catalysis and locate P-III SVMPs closer to basal lamina *in vivo* experiments. These results should be considered as an important step for induction of hemorrhage.
Supported by: FAPESP

1.21 A new mechanism of action for the Bradykinin-Potentiating Peptides (BPPs).

Guerreiro JR[1], Gomes CL[1], Oliveira EF[1], Linares E[2], Silva CA[1], Augusto O[2], Serrano SMT[1], Camargo ACM[1].
[1]Centro de Toxinologia Aplicada CAT/CEPID, [2]Departamento de Bioquímica do IQ/USP;

Introduction: The effect of the Bradykinin Potentiating Peptides (BPPs) from *Bj* venom has always been associated with the inhibition of the ACE. However, recent biodistribution experiments in mice have shown that the peptide BPP10c (QNWPHPGIPP) is accumulated in the kidneys, same in the presence of a 1000-fold excess of Enalaprilat (inhibitor of ACE) indicating that the BPP10c has a different molecular target. **Objectives:** We used affinity chromatography to search for proteins that might interact with BPP10c. **Methods and Results:** The resin was prepared by coupling the peptide to the HiTrap column and used to capture proteins from the cytosolic and membrane fractions of kidney. Mass spectrometry analysis showed argininosuccinate synthase (ASS) as the main protein with affinity for this peptide. This enzyme participates in the urea cycle in the liver. However, its tissue distribution is not restricted to the liver, being found in various organs, and also in the vascular endothelium where it plays a role in the L-arginine–NO pathway. This pathway involves the activity of three enzymes: ASS, argininosuccinate lyase and eNOS, being the ASS the rate-limiting enzyme in the L-arginine–NO pathway. In agreement, we show that the BPP10c is able to increase the enzymatic activity of ASS *in vitro*. Moreover, the peptide caused an increase in the production of the NO by endothelial cells in culture. **Conclusions:** These results led us to propose a new mechanism for the anti-hypertensive effect of BPP10c in which the interaction of the peptide with ASS causes an increased catalysis rate with consequent increase in the generation of the NO.
Supported by: FAPESP.

1.22 Morphological alterations on spermatogenesis of mice caused by low molecular weight fraction (LMWF) from the *Bothrops jararaca* snake venom.

Gilio JM[1], Borella MI[2], Parreira FS[2], Portaro FCV[1], Camargo ACM[1], Silva CA[1].
[1]Laboratório Especial de Toxinologia Aplicada-CAT/CEPID, Instituto Butantan/ São Paulo-Brasil; [2]Laboratório de Endocrinologia de Peixes, Instituto de Ciências Biomédicas, USP/ São Paulo-Brasil.

Introduction: The low molecular weight fraction obtained from *Bothrops jararaca* snake venom (LMWF) comprises a series of bioactive peptides. The main components of the LMWF are the BPPs (Bradykinin Potentiating Peptides), also know as Proline Rich Peptides (PRPs). One of its mechanisms of action is related to the Angiotensin Converting Enzyme (ACE) inhibition. At moment, two ACE isozymes are known: Testicular ACE (tACE) is expressed in germ cells exclusively during spermatogenesis and Somatic ACE (sACE) that acts in the blood pressure regulation. **Objectives**: Evaluate the possible morphologic alterations in the seminiferous epithelium of mices dealt caused by LMWF. **Methodology**: Male Swiss mice (30-35g) were treated or not with the LMWF (60μg/animal/day for 15 days). Testis were removed, dehydrated, stained with Mallory trichromic and about 50 sections were examined at different sites for each testis using an Zeiss photomicroscope. **Results**: The morphologic analysis of the treated animal's testis presented disruption in the germinal epithelium (adluminal compartment), degeneration of germinal cells, presence of atypical cells in the lumen of it and an increase of the intertubular compartment. **Discussion**: These data indicate such effects can occur through the PRPs interaction with the gACE, causing an alteration in all the process of spermatogenesis. Therefore, possible inhibitors of this enzyme open new perspectives for the medical development of contraceptive with property in animals, including humans.
Supported by: CEPID-FAPESP and UNIEMP.

1.23 Recombinant expression of the cysteine-rich domain of HF3, a hemorrhagic P-III metalloproteinase from *Bothrops jararaca* venom.

Menezes MC[1], Assakura MT[1], Olivo RA[2], Della-Casa MS[3], Camargo ACM[1], Teixeira, CF[2], Serrano SMT[1]
[1]Lab. Especial de Toxinologia Aplicada-CAT-CEPID, [2]Lab. de Famacologia, [3]Lab. de Imunopatologia, Instituto Butantan, SP, Brasil

Introduction: HF3, a P-III class metalloproteinase isolated from the *B. jararaca* venom, is a potent hemorrhagic toxin, which degrades extracellular matrix proteins and inhibits platelet aggregation. We have shown that native HF3 and its recombinant disintegrin-like/cysteine-rich domains (DC/HF3) were able to increase αMβ2-mediated phagocytosis of opsonized-zymosan particles by macrophages. Recently, our studies on the role of the cysteine-rich domain of P-III class metalloproteinases pointed to its function as a cell-surface-receptor-binding site and/or a substrate recognition exosite. **Objective:** Our goal was to obtain the recombinant cysteine-rich domain of HF3 in order to test its ability to inhibit platelet-aggregation and to activate phagocytosis by macrophages. **Methodology:** The cDNA sequence coding for the cysteine-rich domain was subcloned into the expression vector pGEX-4T2, which allows the expression of soluble recombinant proteins in fusion with Glutathione S-transferase (GST) and transformed in *E. coli* DH5α cells. **Results and Discussion:** The recombinant fusion protein GST-C/HF3 purified by affinity chromatography on Glutathione Sepharose 4B showed to be essentially homogeneous by SDS–PAGE and by Western blot using an anti-HF3 antibody. The cys-rich domain (C/HF3) was obtained after the cleavage of the fusion-protein with thrombin, and analysis by N-terminal sequencing confirmed the expression of C/HF3. Authenticity of the recombinant-protein was also analyzed by mass spectrometry.
Supported by: FAPESP.

1.24 Increased survival of mice bearing melanoma cells treated with snake venom toxin jararhagin.

Durvanei A. Maria[1], Ruth C. Vassão[2], and Itamar R.G. Ruiz[2].
[1]Biochem. Biophys. Laboratory, [2]Genetics Laboratory, Butantan Institute, São Paulo, Brazil.

Introduction: Snake venom toxins have been envisaged as suitable tools regarding metastases inhibition. Tumor cells morphology and biological properties are altered following toxins treatment, as ligands of integrin receptors. Mice injected with MM cells pre-treated with jararhagin, from *B. jararaca*, had a significant decrease on the number of metastasis. **Objective**: To evaluate the efficacy of jararhagin treatment on hematopoiesis and survival of mice bearing B16F10 MM cells. **Methodology**: Mice were injected with of 12 ng jararhagin once a week for 3 to 9 weeks. Total erythrocytes and differential leukocytes, from blood, peritoneal, bronchoalveolar, and bone marrow washes were counted. **Results**: Erythrocytes counts doubled ($p<0.001$), and total leukocytes reduced to half ($p<0.001$), compared to controls. The survival rate varied from 14 % to 25 % among treated, compared with control mice. **Discussion**: Tumor cells had the same effect on hematopoiesis as did jararhagin alone, but the effects were not cumulative. The increased survival of jararhagin-treated mice was significant, but experiments with enlarged samples are needed to confirm the data.
Supported by: CNPq.

1.25 Viperistatin/Alkaline phosphatase fusion protein: an enzymatic marker for alpha 1 beta integrin

Butera D[1,2,3], Chammas R[1,3], Moura da Silva AM[2].
[1]Depto. Radiologia, FM/USP, SP; [2]L. Imunopatologia, I. Butantan, SP; [3]CTC/CEPID, SP.

Introduction: Viperistatin (Vp) is a selective inhibitor of $\alpha 1\beta 1$ integrin, which is up regulated during angiogenesis and shows a different pattern of expression in neoplastic cells. In this way, the detection of this integrin will be an interesting approach to study processes such as angiogenesis and cancer. **Objective:** To construct and validate a molecular marker based on alkaline phosphatase (APv)-tagged Vp. **Methodology:** Vp gene was synthesized, cloned into the pLIP6-GN vector and expressed in fusion with APv. VpAPv was detected by SDS-PAGE and pNPP AP substrate. The selectivity of the molecule was tested against purified integrins in a one-step dot blot using the NBT/BCIP substrate for developing. **Results:** The Vp gene was correctly cloned and expressed as detected by DNA sequencing and SDS-PAGE, respectively. In addition it preserved is enzymatic AP activity and selectivity. It clearly discriminated $\alpha 1\beta 1$ from other 3 $\beta 1$ integrins. **Discussion:** Our data present a novel tool, VpAPv, with potential use in diagnosis of disorders where the $\alpha 1\beta 1$ integrin is involved.
Supported by: FAPESP

1.26 Crotoxin inhibits neuropathic pain and the development of neuromas

Nogueira-Neto F[1,2], Amorim RL[2], Brigatte P[1], Picolo G[1], Ferreira Jr WA[1], Nicoletti JLM[2], Conceição IM[3], Cury Y.[1]
[1]Laboratory of Pathophysiology and [3]Pharmacology, Butantan Institute, SP, Brazil; [2]FMVZ-UNESP, Botucatu, SP, Brazil.

Introduction: *Crotalus durissus terrificus* snake venom (CdtV) induces analgesia mediated by opioid receptors. The factor responsible for this effect, named crotalphine, was recently isolated from crude venom. In addition to this factor, recent data have indicated that crotoxin (CTX), the main neurotoxic component of CdtV, exerts antinociceptive effect in an experimental model of cancer pain. The aim of the present study is to evaluate the effect CTX on neuropathic pain and to determine the mechanisms involved in this effect. **Methods:** Neuropathic pain was induced by neurectomy of the sciatic nerve of male Wistar rats. Hyperalgesia and neuromas development were evaluated over a 64-day period after surgery. Hyperalgesia was assessed using the rat paw pressure test. The presence of neuromas was determined by histological analysis. **Results**: Hyperalgesia was detected 2h after surgery and persisted for 64 days. Neuromas were fully developed on day 64. CTX (0.01mM) applied to the proximal and distal nerve stumps, immediately after nerve transection, blocked hyperalgesia. The analgesic effect was observed 2h after CTX treatment and persisted for 64 days. CTX-induced analgesia was blocked by atropine and yohimbine. Methylatropine, methysergide, atenolol and naloxone did not alter this effect. Histological analysis showed that CTX delays the development of neuromas. **Conclusions:** CTX inhibits the development of neuropathy and induces a long-lasting analgesic effect on neuropathic pain mediated by central muscarinic receptors and α-adrenoceptors.

1.27 Effect of *Crotalus durissus terrificus* snake venom on the integrity of endothelial cells monolayers *in vitro*.

Matsubara MH, Fernandes CM and Teixeira CFP.
Laboratory of Pharmacology, Butantan Institute, São Paulo, Brazil.

Introduction: *Crotalus durissus terrificus* venom (CdtV) induces systemic effects which interfere with both central nervous and coagulation systems. Despite of the importance of the endothelium in homeostasis, the effect of this venom on endothelial cells (EC) are still unknown. **Objectives:** In this study the effect of CdtV on the integrity of endothelial cell monolayers was evaluated. **Methodology:** Murine endothelioma cell line (tEnd) was cultured in RPMI medium with 10% SFB and seeded in 96 well microplates for formation of monolayers. After reaching confluence (48 hours) EC monolayers were incubed with CdtV (10, 50 and 100 μg/mL) or RPMI (control). After 1, 4, and 24h, the monolayers were stained with crystal violet, and integrity of endothelium was determined by spectrophotometry, at 620 nm. **Results:** Incubation of EC monolayers with CdtV caused a time-dependent detachment of cells. At 10 and 50μg/mL CdtV induced a significant detachment of monolayers from 4 up to 24 hours. The highest concentration caused cell detachment since the first hour of incubation with maximum at 24 hours (89% of detached cells). **Conclusions:** These data show for the first time the ability of CdtV to affect the integrity of endothelium. This effect may have implications for the vasculature under CdtV action.

Supported by: CNPq and FUNDAP.

1.28 Sex differences in the pain threshold and in the antinoceptive effect of Crotalphine, an opioid analgesic obtained from *Crotalus durissus terrificus* snake venom (CdtV).

Britto L[1], Picolo G[1], Cury Y[1]
[1]Laboratory of Pathophysiology, Butantan Institute, SP, Brazil

Introduction: Crotalphine (CRP), an analgesic peptide synthesized based on the natural analgesic obtained from CdtV, induces potent and long-lasting antinociception mediated by κ and δ opioid receptors, evaluated in male rats and mice. Sex differences in the pain threshold and in the opioid-induced antinociception have been reported in human and animals. **Objective:** The purpose of the present study was to investigate differences in pain sensation and in the antinociceptive effect of CRP between male and female Wistar rats. The onset, peak, intensity and duration of the prostaglandin E_2 (PGE_2)-induced nociception and CRP-induced antinociception were also evaluated. **Methods:** Hyperalgesia was determined using the rat paw pressure test applied before and at different times after PGE_2 (50,100,150 ng/paw) injection. For antinociception evaluation, CRP (5 or 7,5 μg/rat, p.o) or morphine (MOR, 5 mg/kg, s.c,) were administered immediately before or 1h after PGE_2 injection (100 ng/paw), respectively, and the test applied 3h after hyperalgesic agent. **Results:** Female rats showed lower basal pain threshold and responded to lower doses of PGE_2 than male rats. MOR produced significantly greater antinociception in male while CRP produced greater antinociception in female rats. **Conclusions:** Sex differences could be observed between male and female rats in relation to pain threshold and opioid-induced antinociception. Besides that, CRP is able to induce antinociception in both male and female rats.

Supported by: CNPq and COINFAR Pesquisa e Desenvolvimento.

1.29 Effect of crotoxin (CTX) on circulating lymphocytes and lymphoid organs

Zambelli VO[1], Sampaio SC[1], Spadacci-Morena DD[1], Britto LRG[2], Cury Y[1].
[1]Instituto Butantan – Lab. Fisiopatologia, [2]ICB - USP - Fisiologia e Biofísica

Introduction: Data have shown the CTX, the main neurotoxin of *Crotalus durissus terrificus* venom and its subunit phosfolipase A_2 (PLA_2) inhibit macrophage activity and that LO-derived lipid mediators mediate this effect. Our previous work showed that the toxins promotes leukocyte endothelium adhesion and reduce the number of lymphocytes in blood and lymph, but the mechanisms involved in these effects were not known. **Objectives:** This study aimed to investigate the effect of CTX and PLA_2 on lymphoid tissue, on lymphocyte proliferation, as well as to investigate the involvement of LO-derived lipid mediators on the effect of CTX or PLA_2 on circulating lymphocytes. **Methods and Results:** The lymphoid organs of Wistar rats were removed 2h after s.c. injection of CTX (18µg/rat), PLA_2 (10.4µg/rat). Paraffin-embedded tissue sections were stained with HE for histopathological analysis. The expression of T and B lymphocyte was determined by immunohistochemistry. CTX or PLA2 reduced (42% and 51%, respectively) the number of circulating lymphocytes of Wistar rats, 2h after the treatments. Zileuton blocked this inhibitory effect. CTX and PLA_2 promoted, in mesenteric lymph nodes, follicular stimulation. Hyperplasia of spleen white pulp was also detected. Toxins treatments increase T and B lymphocyte immunostain and decrease lymphocyte proliferation. **Conclusions:** These data indicate that toxins promote morphological alterations and increase the number of lymphocytes in lymphatic tissue. LO-derived lipid mediators mediate the decrease in the number of circulating lymphocytes caused by the toxins.
Supported by: CAPES and CNPq

1.30 Effect of *Crotalus durissus terrificus* snake venom and crotoxin on cytokine secretion by macrophage.

Sousa-e-Silva, MCC[1] Sampaio, SC[1], Brigatte, P[1], Curi, R[2], Cury Y[1].
[1]Laboratory of Pathophysiology, Butantan Institute Brazil, [2]Department of Physiology and Biophysics, Institute of Biomedical Sciences, University of São Paulo, São Paulo, Brazil.

Introduction: Previous studies showed that *Crotalus durissus terrificus* snake venom (CdtV) and crotoxin (CTX), the main toxin of CdtV, modulate macrophage function, inhibiting the spreading and phagocytic activity of theses cells. Macrophages, through production of various cytokines, modulate inflammation and innate and adaptative immune responses. **Objective:** To investigate the effect of CdtV and CTX on cytokine secretion by macrophages. **Methods:** Peritoneal macrophages, obtained from Wistar rats, were incubated with CdtV or CTX for 2h and incubated with LPS (5µg/ml) for 24 h or with RPMI medium. In order to induce a phagocytic process, particles of opsonized zymosan were subsequently added to the RPMI incubation medium for 2h. The levels of IL1-α, IL-1β, IL-6 and TNF-α in the culture supernatants were determined by ELISA. **Results**: CdtV inhibited secretion of IL-1α (0.5µg/ml: 50%; 1.0µg/ml: 70%) and IL-1β (0.25µg/ml: 129%; 0.5µg/ml: 91%; 1.0µg/ml: 28%) induced by LPS. The phagocytic process increased cytokine levels (63%). This increase was inhibited by CdtV (IL-1α, 0,5µg/ml: 63% and IL-1β, (0.5µg/ml; 1.0µg/ml: 100%). CdtV did not alter TNF-α and IL-6 secretion. Incubation with crotoxin (0.08; 0.15; 0.3 µg/ml) did not modify cytokine secretion induced by LPS or opsonized zymosan. **Discussion:** The CdtV inhibits the secretion of IL-1α and IL-1β. This effect is not mediated by crotoxin.
Supported by: FAPESP

1.31 Anti-inflammatory action of *Crotalus durissus terrificus* snake venom in mice

Nunes FPB[1], Sampaio SC[1], Sousa-e-Silva MCC[1].
[1]Laboratory of Pathophyisiology, Butantan Institute, São Paulo, Brazil.

Introduction: The *Crotalus durissus terrificus* (*Cdt*V) induces a mild inflammatory response and inhibits the macrophages function. Recently we demonstrated that *Cdt*V, administrated by s.c. route, at pre-treatment (1h,7,14 or 21 days) and at post-treatment (1h), inhibits vascular and cellular events of inflammatory process induced by carrageenan (Cg). **Objective:** In this study, we investigated the effect of pre and post-treatment of *Cdt*V by o.v. route on cell migration induced by Cg and the anti-inflammatory effect of this venom when compared with other drugs: Dexametasone (Dx) and Indometacine (Ind). **Methodology:** The *Cdt*V (1,5µg s.c.) (8,8µg o.v.) or saline (50µL) (control) or Dx (1mg/kg) or Ind (4mg/kg) were injected in mice, before or after the intraperitoneal injection of Cg (300µg) or saline (200µL). Total count was realized at Neubauer´s chamber and differential count was performed in smears stained with a panchromatic dye. **Results:** The *Cdt*V (o.v.) induced an inhibition of cell migration, 41% at pre-treatment (1h) (*Cdt*V 0,60 ± 0,06; control 1,09 ± 0,06) and 38% at post-treatment (1h) (*Cdt*V 0,65 ± 0,05; control 1,05 ± 0,06). The treatment of *Cdt*V was more efficient to inhibit the cell migration (*Cdt*V 0,95 ± 0,11; control 1,47 ± 0,10) when compared with Dx (Dx 1,22 ± 0,10; control 1,47 ± 0,10) and Ind (Ind 1,37 ± 0,14; control 1,47 ± 0,10). **Discussion:** The same inhibitory action of the *Cdt*V by s.c. route, was observed by o.v. Comparing with anti-inflammatory drugs (Dx or Ind), the *Cdt*V (s.c.) was more efficient to inhibit cellular events of inflammation, confirming the anti-inflammatory action of this venom.
Supported by: CNPq.

1.32 The gene family of crotamine and variations of its content in venoms of the South American rattlesnake *Crotallus durissus*

Collares M de A[1]; Corrêa PG, Suzuki H[2], Furtado MFD[1], Oguiura N[1]
[1]Lab. Herpetologia and [2]Lab. Biologia Celular, Instituto Butantan, SP, Brazil

Introduction: Crotamine was first isolated from the venom of *C. d. terrificus* and belongs to a group of closely related small basic polypeptide myotoxins commonly found in *Crotalus* venoms. Its content, averaging 17% (w/w) of the crude venom, varies according to the snake's geographical location. The crotamine-positive rattlesnake has the crotamine gene with 1.8 kbp organized into three exons separated by two introns. **Objective:** It was determined the amount of crotamine in venoms of *C. durissus* from some Brazilian states. **Methods and Results:** The crotamine was quantified by ELISA and the protein content determined by the Lowry method. The crotamine gene region between 5crot and 3UTRas primers was amplified by PCR and sequenced. The quantity varied from 0.006 to 0.5 µg of crotamine per µg of protein in the venom. The venoms were classified into three categories which were plotted on a map. We found a large number of crotamine-plus venoms in the state of Paraná, including venom with a crotamine concentration exceeding 10%. We analyzed crotamine gene sequences of specimens from PR, SP, MS, GO which showed an identical sequence to that described by Rádis-Baptista et al. (2003). The changes found in sequences were point mutations and complete deletion of intron 2. **Conclusions:** These results indicated that crotamine is a gene family, since the same rattlesnake may have up to four different sequences. The rattlesnakes with the highest amount of crotamine showed no alteration in their crotamine genes.
Supported by: FAPESP and FUNDAP.

1.33 Neurotoxicity induced by Phospholipases A_2 (PLA_2) Isolated From Brazilian Coral Snake (*Micrurus lemniscatus*) in cultured hippocampal neurons

Carvalho ND[1], Casais e Silva LL[3],Lebrun I[2], Afeche SC[1], Sandoval MRL[1].
[1]Pharmacology, Butantan Institute; [2]Biochemistry and Biophysics, Butantan Institute; [3]Physiology, UFBA.

Introduction and objective: The neurotoxicity from *Micrurus* venoms from Americas has been poorly studied. The aim of this study was to investigate the effects of two PLA2s, MI-8 and MI-9, isolated from the venom of *M.lemniscatus* in cultured hippocampal neurons. **Method**: Neurons were dissociated from hippocampi of E18 Wistar rat embryos. After treatment with trypsin and deoxyribonuclease neurons were cultured in B27-supplemented Neurobasal Medium. Cultures were kept at 37^0C in 5% CO_2 and plated at a density of 200 neurons/mm^2. After incubation of cultures neurons (at day7) with MI-8 or MI-9 (10 – 1000 ng/ml), for 24h assessment of neuronal injury was made by using Ethidium Bromide. It was counted 200 neurons in 10 fields in each coverslip. PLA2 activity was previously determined. **Results:** Cultured neurons survival was significantly decreased when compared with the control group (ANOVA and Dunnett test): control (25,59±6,66); 10 (26,7±14,0), 100 (16,37±10,75) or 1000 (15,0±10,44) ng/ml ; MI-9 10 (16,8±16,32); 100 (13,66±4,09); 1000 (1,68±3,97) ng/ml. **Conclusion:** MI-8 and MI-9 caused neuronal death mainly by apoptosis in neuronal culture. The toxicity might be associated with the binding of these toxins with specific brain site and/or a β-BuTx –like effect at the pre-synaptic terminal. β-Bungarotoxin the most investigated pre-synaptic PLA2 neurotoxin from elapid venom applied to cultured rat hippocampal neurons induced apoptotic cell death.These PLA2 can be a useful tool for exploring the death- signaling pathways of neurotoxicity.
Supported by: FAPESP and Butantan Foundation

1.34 Immunochemical characterization of snake venoms from *Micrurus* genus

Tanaka GD[1], Furtado MFD[2], Portaro FCV[3], Sant' Anna OA[1], Tambourgi DV[1].
[1]Laboratory of Immunochemistry, [2]Laboratory of Herpetology, [3]Center of Applied Toxinology-CAT/CEPID, Institute Butantan – São Paulo – SP.

Introduction: Envenomation caused by snakes of the genus *Micrurus* results in respiratory arrest and death by muscle paralysis few hours after envenoming. **Objectives**: The characterization of some biological activities – phospholipasic, hyaluronidasic and proteolytic – from venoms of *M. ibiboboca, M. frontalis, M. corallinus, M. hemprichii, M. spixii, M. fulvius, M. altirostris, M. surinamensis and M. lemniscatus*, and the evaluation of their antigenic cross-reactivity against the anti-elapidic serum produced by Instituto Butantan, through the immunization of horses with a mixture of *M. corallinus* and *M. frontalis* venoms. **Results:** Analysis of the biological properties as well as of the protein composition showed a variation of components and toxicity of *Micrurus* venoms. ELISA and Western blot assays showed low antibody titers and a varied capability of the anti-elapidic serum to recognize the different *Micrurus* venoms. **Conclusion:** These data clearly indicate that *Micrurus* venoms exhibit a diversity of composition and toxicity, and that the elapidic serum is not able to fully recognize the components of these distinct venom species.
Supported by: FAPESP, FUNDAP and CNPq

1.35 Identification of Putative Antigenic Candidates to an Antielapidic Serum Based on the Analysis of *Micrurus corallinus* Transcriptome

Leão LI[1,2], Ho PL[1,2], Junqueira de Azevedo ILM[1,2].
[1]Centro de Biotecnologia, [2] Departamento de Genética e Biologia Evolutiva, USP, SP

Introduction: The transcriptomic characterization of venom glands has proved to be a fast and efficient way to describe the general composition of toxins from these animals, at least regarding the gene expression related to them. **Objectives**: We have generated 1438 "Expressed Sequence Tags" (ESTs) from *Micrurus corallinus* venom gland, a snake from Elapidae Family, commonly found in tropical forest areas. **Methods and Results**: The 1438 sequences were grouped in 611 clusters that were built in a pipeline of softwares in LINUX system, specially adjusted to the characteristics of a project of medium scale ESTs generation for venom gland projects. Among these clusters, we have obtained 7 putative types of toxins that had their sequences partial or totally described for the first time. Likewise the transcripts related to toxins, the transcripts related to celular proteins represent each around 46% in this databank. The general proportion of toxins include: three-finger proteins (24%), phospholipases A_2 (PLA_2s) (16%), lectin type C (5%), and others. The databank allowed not only the identification of putative toxins, but also celular transcripts, being the majority probably involved in physiologicals functions. The major part of these molecules shows an involvement in gene and protein expression reflecting the high especialization of the tissue to toxin synthesis. **Conclusion**: The transcriptomic databank helped an analysis of gene expression and it allowed the identification of probable vaccines candidates to a future recombinant antielapidic serum.
Supported by: FAPESP.

1.36 Preliminary Study of Venoms of Five Colubrid Species from Brazil.

Zelanis APP[1,2], Rocha MMT[2] and Furtado MFD[2]
[1]Department of Physiology, Biosciences Institute, USP, [2]Herpetology Laboratory, Butantan Institute, São Paulo – Brazil.

Introduction: The polyphyletic family Colubridae comprises several species of rear-fanged snakes with toxin-secreting Duvernoy glands, some of which are capable of causing human envenomation with systemic and/or local damage. **Objectives:** To characterize, in a preliminary study, certain properties of five colubrid venoms from Brazil: *Philodryas olfersii*, *P. patagoniensis*, *P.nattereri*, *Thamnodynastes strigatus* and *Tomodon dorsatus*. **Methodology:** The proteins and carbohidrates contents were analyzed by colorimetric methods, eletrophoretic patterns by SDS-PAGE, proteolytic activity upon different substrates (casein, gelatin and fibrinogen) and phospholipasic activity using cromogenic substrate. **Results:** Electrophoretic profiles showed bands ranging from 90 to 14 kDa in molecular mass. Protein content was lower than verified for Viperid venoms (*B.jararaca*). In contrast, the carbohydrate content was higher (up to 8%) compared to *B.jararaca* venom. All venoms showed proteolytic activity upon casein, gelatin and fibrinogen, in addition to low phospholipase A_2 activity. **Discussion:** Results suggest variability in some aspects of colubrid venom composition and activity, providing an increase in knowledge of new toxins.
Supported by: CNPq

1.37 Analysis of the venom of *Thamnodynastes strigatus* (Serpentes: Colubridae)

Feraiorni[1] GG, Rocha[1] MMT, Furtado MFD[1]
[1]Herpetology Laboratory, Butantan Institute, São Paulo, Brazil

Introduction: In Brazil, most snake bites are caused by poisonous snakes, but some data have shown that approximately 20-40% of snake bites are caused bynon-poisonous snakes. Most of the non-poisonous snakes belong to the Colubridae family including the species *T. strigatus,* an opistoglyphous snake. The injuries caused by them are characterized byintense local action, with pain, edema and hemorrhage. **Objective**: To analyze the biological and biochemistry activities of the *T. strigatus venom*. **Methodology:** The electrophoretic pattern of *T. strigatus* venom was analyzed by SDS-PAGE, as well as the following biological actions: hemorrhage, miotoxicity, edema, median lethal dose and three enzymatic actions: proteolytic activities of casein and gelatin, phospholipasic A_2 and hialuronidasic activities. All results were analyzed using as pattern *B. jararaca* venom. **Results:** Electrophoretic profiles present three major bands in 21, 14 and below 14 kDa, showing proteolytic activities in casein and gelatin, phospholipasic, hialuronidasic as well important edematogenic, hemorrhagic and miotoxic activities. **Discussion:** The results showed that the venom *T. strigatus* was more active than *B. jararaca* venom in hialuronidasic, hemorrhagic and miotoxic activities.

1.38 Characterization of certain local activities induced by the toxic secretion of *Philodryas patagoniensis* (Serpentes: Colubridae)

Hess PL[1,2], Rocha MMT[1] and Furtado MFD[1]
[1]Lab. Herpetology, Instituto Butantan, SP, Brazil; [2]Instituto Biociências, USP, SP, Brazil.

Introduction: *Philodryas patagoniensis* presents venom causing local actions similar to those found in botropic accidents; therefore, the knowledge of this venom is important to improve the differential diagnosis of the accidents. **Objective**: To characterize certain local actions induced by this venom. **Methodology**: Formation of edema in the mouse paw was measured plethysmographically; local hemorrhage was evaluated by colorimetric method of hemoglobin determination. The miotoxic activity was evaluated by histology. **Results**: Edematogenic activity peaked in 30 min (53.33 ± 4.22 %). The edematogenic minimum dose was of 0.82µg, reduced by EDTA ($p < 0.05$). The local hemorrhage was 6 hours max after the injection (3.1± 0.01mg Hb/g tissue), and the haemorrhagic minimum dose was of 1.98µg, being also reduced by EDTA and PMSF ($p < 0.05$). Histological analysis of mice gastrocnemius muscles, shows myonecrosis beginning 30 min after the injection, regressing after 24 hours, presenting light regeneration of the muscular fibers. **Discussion:** These data indicate molecules similar to those present in the botropic venoms, responsible for the local damages, such as metalloproteinases and serine proteinases, in addition to the occurrence of miotoxicity, which suggests a narrow similarity in the composition, but maybe differences in mechanism of action of the venoms of the families Colubridae and Viperidae.

1.39 Purification of patagonstatin, an inhibitor of collagen-induced platelet aggregation, from *Philodryas patagoniensis* snake venom

Peichoto ME[1], Leiva L[1], Acosta O[2], Tanaka-Azevedo AM[3], Santoro ML[3].
[1]School of Exact Sciences and [2]School of Veterinary Sciences, University of Northeastern Argentina, Argentina; [3]Laboratory of Pathophysiology, Institute Butantan, Brazil.

Introduction: Venoms of Colubridae snakes are a rich source of novel compounds, which may have applications in medicine and biochemistry. **Objective**: In the present study we isolated a potent inhibitor of collagen-induced platelet aggregation from *Philodryas patagoniensis* venom **Methodology and Results**: Patagonstatin is a single-chain protein, with a molecular mass of 25.3 kDa under non-reducing conditions and 27.4 under reducing conditions. Purification of this protein was accomplished by chromatography on Mono-Q and Phenyl-Sepharose columns. Patagonstatin was homogenous by SDS-PAGE and hydrolyzed neither azocasein nor fibrinogen. Mild hemorrhage developed in mouse skin after i.d. injection of patagonstatin (20 µg of protein produced a hemorrhagic spot of 2.40 mm in diameter). Subcutaneous injection (20 µg) of patagonstatin induced edema, which peaked at 2 h. Patagonstatin showed no platelet pro-aggregating activity *per se*, but it inhibited collagen-induced platelet aggregation, with an IC_{50} of 9.1 nM. Ristocetin- and ADP-induced platelet aggregations were not inhibited by patagonstatin. **Discussion**: This is the first report on the isolation and characterization of a platelet aggregation inhibitor from the venom of a colubrid snake. Due to its potent action on collagen-induced platelet aggregation, patagonstatin may be used on studies of platelet physiology.
Supported by: FAPESP (04/02223-8,04/02224-4), Fundação Butantan and CONICET(PhD scholarship).

1.40 Some aspects of the venom proteome of the Colubridae snake *Philodryas olfersii* revealed from a Duvernoy´s (venom) gland transcriptome

Ching ATC[1], Rocha MMT[2], Paes-Leme AF[3], Pimenta DC[3], Furtado MFD[2], Serrano SMT[3]; Ho PL[1], Junqueira-de-Azevedo ILM[1] .
[1]Centro de Biotecnologia, Instituto Butantan, [2]Laboratório de Herpetologia [3]Laboratório Especial de Toxinologia Aplicada, CAT-CEPID, Instituto Butantan, São Paulo, Brazil

Introduction: The interest about venoms of Colubridae is increasing due to its importance in human envenoming accidents. *Philodryas olfersii* (Colubridae) has a Duvernoy's gland that produces a secretion exhibiting high hemorrhagic activity. **Methods and Results:** A cDNA library from *P. olfersii* venom gland was constructed for the generation of an *Expressed Sequence Tags* database (dbEST). The 2194 ESTs were grouped in 1285 clusters. About 30% of all transcripts corresponded to toxin sequences, revealing the presence of major toxin classes from the Viperidae family. The P-III class of metalloproteases is the predominant but also serine proteases, C-type lectins, CRISPs, and C-type natriuretic peptides (CNPs) were found. Their corresponding proteins were detected in a 2-D gel electrophoresis and identified by mass spectrometry. Phylogenetic analysis of the CNP precursor showed it as a linker between the multifunctional precursors found in *Viperidae* and *Elapidae* snakes. We suggest that these precursors constitute a monophyletic group derived from the vertebrate CNPs. Other sequences were investigated and suggested as possible toxin candidates. **Conclusions:** The *P. olfesii* dbEST is the first effort to massively identify cDNA sequences from a Colubridae species. It matches the venom composition, provides an overview of Colubridae venom and supports the important role of metalloproteases in the hemorrhagic effect elicited during *P. olfersii* envenoming (FEBS Lett. 580(18):4417-22, 2006).
Supported by: FAPESP and Butantan Foundation

1.41 Proteomic analysis of the venom of the opisthoglyphous colubrid snake *Phalotris mertensi*.

Paes Leme AF[1], Zelanis A[2], Menezes MC[1], Rocha MMT[2], Pimenta DC[1], Camargo ACM,[1] Furtado MF[2], Serrano SMT[1]
[1]Lab. Especial de Toxinologia Aplic CAT-CEPID, [2]Lab. Herpetologia, Inst. But., SP, Brazil.

Introduction: Compared with the venoms of many front-fanged species of snakes (e.g. Viperidae, Elapidae and Atractaspidae) in which the pharmacology, biochemistry, and mechanism of action of many of the toxins have been extensively studied, the toxic nature and mode of action of the venom toxins of colubrid snakes are less understood. Although the genus *Phalotris* occurs from the central region of Brazil down to Paraná State, nothing is known about the composition of its Duvernoy's gland secretion. **Objective and Methodology:** In this work, the complexity of the venom from *P. mertensi* was examined by various protocols for measuring its enzymatic activities, and by gel electrophoresis. **Results:** The venom has proteolytic activity on casein, gelatin and fibrinogen. Determination of its coagulant activity on plasma showed a specific activity 20 times higher than that of *Bothrops jararaca* venom. Moreover, the venom showed hyaluronidase, phospholipase A2 and myotoxic activities. The general profiles of this venom by 1D-PAGE and 2D-PAGE were similar to the profiles reported in our previous works on *Bothrops* venoms. 2D-PAGE showed spots dispersed across the pI and molecular mass range of the gel. Finally, by using specific staining methods applied to 2D-PAGE we examined subpopulations of the venom proteins (metalloproteinases, serine proteinases, phospholipases A2 and glycoproteins). **Discussion:** These results may accelerate research into this colubrid venom and may provide insights into novel treatments for colubrid envenoming.
Supported by**: FAPESP and CAT/CEPID.**

1.42 Biological and immunochemical characterization of spider venoms from the Ctenidae family

Okamoto CK[1], Gonçalves-de-Andrade RM[1], Bertani R[1], Portaro FCV[2], Manzoni-de-Almeida D[1], Tambourgi DV[1].
[1]Lab. of Imunochemistry and [2] Center of Applied Toxinology, Butantan Institute, São Paulo, SP.

Introduction: *Phoneutria* nigriventer spider envenomation is considered of medical importance in Brazil, causing intense local pain, pulmonary neurogenic shock, and edema, among other reactions. **Objectives:** to compare the biological and immunochemical properties of venoms from *Phoneutria nigriventer* and *Oligoctenus medius, a* species belonging to the Ctenidae family, whose venom has not been characterized. **Results:** SDS-PAGE analysis showed a different pattern of bands in these venoms. *Phoneutria nigriventer* and *Oligoctenus medius* venoms exhibit proteolytic, gelatinolytic, phospholipasic and hyaluronidasic activities. The proteolytic activity detected in *P. nigriventer* venom is mainly associated with the presence of serinoproteases and in the *Oligoctenus* venom with serino- and metalo-proteases. Anti-aracnidic serum showed high cross-reactivity by immunoblotting with *O. medius* venom. *Oligoctenus medius* venom was not lethal to BALB/c mice when tested in the concentrations from 5 to 100 □g/animal; however, it can cause some neurological alteration. **Conclusion:** Our data show that although *O. medius* venom contains most of the biological properties exhibited by *P. nigriventer* venom, its toxicity as tested in a murine model is reduced.
Supported by: FAPESP and CNPq

1.43 Action of Gd^{+3} and Zn^{+2} ions on the dermonecrotic activity of the SMases D from *Loxosceles* spider venoms

Fernandes-Pedrosa MF[1], Andrade SA[1] and Tambourgi DV[1].
[1]Laboratório de Imunoquímica, Instituto Butantan, SP, Brazil.

Introduction: Bites by *Loxosceles* spiders can produce severe clinical symptoms, including dermonecrosis, thrombosis, vascular leakage, hemolysis, and persistent inflammation. The causative factor is a sphingomyelinase D (SMaseD) that cleaves sphingomyelin into choline and ceramide 1-phosphate and has intrinsic lysophospholipase D activity toward LPC. We have cloned and expressed the fully active recombinant sphingomyelinases from *L. laeta* (SMase I) and *Loxosceles intermedia* (SMases P1 and P2). The recombinant toxins were endowed with all biological properties ascribed for the whole venoms, including dermonecrotic and complement-dependent haemolytic activities and the ability of hydrolysing sphingomyelin. **Objectives:** The aim of this study was to investigate the effect of Gd^{+3} and Zn^{+2} ions on the dermonecrotic inducing activity of the *Loxosceles* SMases D. **Methods:** Samples of the SMases I, P1 and P2 were incubated with increased concentrations of Gd^{+3} and Zn^{+2} ions (10mM, 20mM, 40mM) for 30 min at 37°C and then tested for dermonecrotic activity on rabbit model. **Results:** Data obtained showed that both ions were able to neutralise the necrotic reaction induced by SMase I from *L. laeta* and SMases P1 and P2 from *L. intermedia*. **Conclusion**: These data suggest that Gd^{+3} and Zn^{+2} ions can compete with Mg^{+2} present in the active site of the SMases D blocking their toxic activity.

Supported by: FAPESP and CNPq.

1.44 Tetracycline protects against dermonecrosis induced by *Loxosceles* spider venom

Paixão-Cavalcante, D[1], van den Berg, C[2], Gonçalves-de-Andrade, RM[1], Fernandes-Pedrosa, MF[1], Okamoto, CK[1] and Tambourgi, DV[1]
[1]Laboratório de Imunoquímica, Instituto Butantan, Brazil; [2]University of Wales, College of Medicine, UK.

Introduction: Envenomation by spiders belonging to the *Loxosceles* genus (brown spider) often results in local dermonecrotic lesions. We have previously shown that *Loxosceles* sphingomyelinase D (SMase D), the venom component responsible for all the pathological effects, induced the expression of matrix metalloproteinases (MMPs) in rabbits and in human keratinocytic cells. We also showed that the SMase D induced apoptosis and MMP expression of keratinocytes was inhibited by tetracyclines. **Objectives:** the aim of this study was to further investigate the ability of tetracyclines to inhibit or prevent the dermonecrotic lesion induced by *Loxosceles* venom *in vivo* and *in vitro* models. **Results:** Primary cultures of rabbit fibroblasts incubated with increasing concentrations of venom or SMase D showed a decrease in cell viability, which was prevented by tetracyclines. *In vivo* experiments showed that topical treatments with tetracycline of rabbits, inoculated with crude *Loxosceles intermedia* venom or recombinant SMase D, significantly reduced the progression of the dermonecrotic lesion. Furthermore, tetracyclines also reduced the expression of MMP-2 and prevented the induction of MMP-9. **Conclusion:** Our results suggest that tetracycline may be an effective therapeutic agent for the treatment of cutaneous loxoscelism.

Supported by: FAPESP, CNPq and The Wellcome Trust

1.45 Extraction of venom, maintenance in captivity and dispersion of scorpion species of medical importance in State of São Paulo.

Candido DM[1] and Bárbaro KC[2]
[1]Laboratory of Arthropods, [2] Laboratory of Immunopathology, Butantan Institute, São Paulo, SP.

Introduction: *Tityus serrulatus* (*Ts*) and *Tityus bahiensis* (*Tb*) are responsible for most of the reported accidents in Brazil. In the last few years the distribution of these animals expanded including the urban areas of the State of São Paulo. **Objectives**: To determine the best conditions of captivity and the frequency and time interval between venom extractions in laboratory; morever, to estimate the dispersion of *Ts* and its domain over *Tb* habitats in S. Paulo. **Methodology**: Two hundred *Ts* specimens were divided in 4 groups (A, B, C and D) of 50 individuals each. A: animals not to be extracted; B, C and D were extracted every 30, 60 and 90 days, respectively. Dispersion data were compiled from the reception of animals in the Lab. Arthropods (2000-2005). **Results**: After 13 months of extraction, all groups had similar mortality indices. Average mean venom quantities decreased approximately 55% in groups B and C. No reduction in venom quantity amount was observed in group D. The number of cities in S. Paulo, in wich *Ts* and *Tb* were reported, increased 405% and 148%, respectively, from 2000 to 2005. **Discussion**: The best interval between extraction was 90 days, since it did not affect venom production. Areas presenting sanitation problems, garbage accumulation, as well parthenogenetical reproduction of scorpions contribute to animal expansion and settlement. *Ts* showed the best capacity of habitat occupation, even though it is an introduced species. On account of *Tb*, it is a native species of S. Paulo, but the destruction of its natural environment deforestation, associated to the conditions described above, favour its dispersion.

1.46 Differential effects of *Tityus bahiensis* scorpion venom in rat isolated vas deferens, jejunum and aorta.

Casasco BS[1, 2], Garcez do Carmo L[1], Conceição IM[2].
[1]Lab. Pharmacology, UNIFESP, [2]Lab. Pharmacology, Butantan Institute, SP, Brazil.

Introduction: The effects of *Tityus serrulatus* scorpion venom have been well studied in the autonomic nervous system. However, no detailed studies of the pharmacological effects of *Tityus bahiensis* scorpion venom (VTb) have been carried out. **Objective:** The aim of this study was to analyze the actions of the VTb in rat vas deferens (VD), jejunum (JE) and aorta (AO). **Methods and Results:** Venom induced contraction that was studied by performing concentration and time-response curves. The effects of tetrodotoxin (0.3 μM – TTX) on contraction induced for the VTb were also analysed. Data were expressed as percent values of initial KCl contraction. The VTb promoted contraction that was concentration-dependent in VD, JE and AO, with maximum values (mean±SEM, n=6) of 324±22%, 118±8% and 50±9%, respectively. In VD and AO the TTX abolished total contractile response of VTb. In JE TTX suppress the initial contractile response to VTb. However, this initial block of contraction is followed by a large increase in final phase of contraction curve. **Conclusions:** We concluded that VTb induce contraction in VD, JE and AO but these effects are distinct depending on the preparation studied.
Supported by: CAPES, FAPESP and Butantan Foundation

1.47 Prenatal exposition of dames treated with *Tityus bahiensis* venom during pregnancy: effects on the offspring and in the adult life

Dorce ALC[1], Dorce VAC[1], Nencioni ALA[1].
[1]Lab. Pharmacology, Institute Butantan

Introduction: In Brazil *T. serrulatus* and *T. bahiensis* are considered the most dangerous scorpions. The venom toxicity is well known, however, it is well understood if it causes damage to the offspring of dames that received it. **Objective:** To check possible toxic effects of the venom of *T. bahiensis* on offspring and in adult life when administrated to pregnant rats. **Methodology:** Pregnant rats were separated in experimental groups and injected with 2,5mg/kg of the venom and saline s.c on the 10^{th} (PN10) and 16^{th} (PN16) gestational days. The pups and the adult were evaluated according to physical and behavioral development. In PN10 it was observed in pups: advance on the ear unfolding, incisor eruption and vaginal opening; decrease of time of palmar grasp and surface righting and increase of general activity ($p<0.05$). In adult life it was observed in males decrease of general activity and locomotion, in activity box and in the enriched environment; in females decrease on the time to stop swimming on forced swimming ($p<0.05$). In PN16 it was observed in pups: advance on the ear unfolding and incisor eruption; delay on the hearing stream opening and testicle declivity; decrease in the time of palmar grasp and on the surface righting; increase of time of negative geotaxis and of general activity ($p<0.05$). In adult life it was observed on females decrease on the time of social interaction ($p<0.05$). **Conclusion:** Moderate envenomation causes alteration in offspring and in adult life whose dames were treated during pregnancy.
Supported by: Butantan Foundation

1.48 Hippocampal effects of a toxin isolated from *Tityus serrulatus* scorpion venom: a behavioural, eletroencephalografic and histophatologic study.

Freitas MM[1], Dorce VAC[1], Lebrun I[2], Nencioni ALA[1].
[1]Pharmacology Laboratory, [2]Biochemitry and Biophysic Lab, Butantan Institute, São Paulo – Brasil.

Introduction: Scorpion venoms are composed among other substances by neurotoxins that act on ionic channels, mainly sodium and potassium. Previous studies showed that some toxins of *Tityus serrulatus* venom (vTs) have epileptic and neurotoxic effects.**Objective:** The aim of this study was to investigate the effects of IV–IV toxin isolated from this venom in the hippocampus of rats. **Methodology:** Male Wistar rats (220 – 250g) were anesthetized and positioned in a stereotaxic frame. Stainless steel guide cannulas and bipolar twisted electrodes were chronically implanted in the hippocampus. One day after sugery the animals were injected with 1µg/µl of toxin (n= 6) or Ringer solution (control group, n= 6) After the injections, continous eletroencephalografic recording (EEG) and observations of animals behavior were performed for periods of 4h. Seven days after the injections the animals were sacrificed and perfused. The brains were removed and prepared for histological analysis. **Results:** The EEG showed intense epileptic-like discharge often accompanied by behavioral alterations as wet dog shake and mioclonia. The histopathological analysis showed neuronal death in CA1, CA3 and CA4 ipsilateral to injection and in CA4 contralateral. **Discussion:** The toxin IV–IV cause neuronal death and have convulsive effect.

1.49 Comparative studies among centipede venoms

Lira MS[1], Malta MB[1], Soares SL[1], Rocha GC[1], Knysak I[2], Martins R[2], Guizze SPG[2], Santoro ML[3] and Barbaro KC[1].
[1]Lab. of Immunopathology; [2]Lab. of Arthropods, [3]Lab. of Pathophysiology, Butantan Institute, SP, Brazil.

Introduction: Envenomation by centipedes are characterized by burning pain, paresthesia and edema, sometimes evolving to superficial necrosis. **Objetive:** The aim of this work was to compare some properties of *Otostigmus pradoi* (Op), *Cryptops iheringi* (Ci), and *Scolopendra viridicornis* (Sv) venoms. **Methodology:** Zymograph, SDS-PAGE, ELISA and Western blotting (WB) were employed to characterize all venoms. Hemorrhagic, myotoxicity, edema and nociceptive activities were carried out in mice. Hemolytic and coagulant activities were evaluated in human O+ red blood cells. **Results:** By SDS-PAGE (4-20%), differences were noticed among venoms, with similar components distributed above 131 kDa. Caseinolytic and gelatinolytic activities were observed in all three venoms. Fibrinogenolytic and hyaluronidase activities were only observed in Sv and Op venoms. Most of these enzymatic components are metalloproteinases. Cross-reactivity was detected among all venoms by ELISA and WB using species-specific sera raised in rabbits. All venoms could induce nociception, edema and myotoxicity. Only Sv had direct hemolytic activity and could induce a discrete hemorrhagic activity in mice. No coagulant activity was detected in centipede venoms. **Discussion:** All three venoms showed differences and Sv venom presented more toxic activities, which can explain the severity of the clinical picture observed in human accidents by this species.
Supported by: FAPESP (03/04527-1) and FUNDAP.

1.50 Can a lipocalin be proteolytically active?

Ramos OHP[1]; Chudzinski-Tavassi AM[1]
[1]Laboratório de Bioquímica e Biofísica, Instituto Butantan, São Paulo, SP.

Introduction: LOPAP (*Lonomia obliqua* prothrombin activator protease) is a lipocalin related to the coagulant syndrome triggered during envenoming by this species. **Objective**: To find molecular features related to LOPAP's proteolytic activity. **Methods**: Molecular models were constructed for LOPAP's momomeric and tetrameric forms using Modeller 8v2. Then, models were checked for the presence of serinoprotease-like catalytic residues using Catalytic Site Atlas (CSA). The residues indicated by CSA were refined by rotamer searching, and the entire model was submitted to energy minimization using GROMOS96 implemented in a Swiss PDB Viewer. Since "*in vitro*" observations indicated that the catalytic activity of LOPAP is positively affected by the presence of calcium, the GG software was employed for calcium binding site prediction. **Results**: The Lopap monomer model shows the characteristic lipocalin basket-like □-barrel formed by eight □-strands and a conserved □-helix. The search for serinoprotease active residues using CSA indicated residues that could be related to the serinoprotease-like activity of LOPAP. The GG software found two putative calcium binding sites, one of which is probably related to the stabilization of the putative active site. The tetrameric LOPAP model suggests that the active site and the hydrophobic pocket entrance remains accessible for the solvent in each momoner. **Discussion**: These results indicate structural features that could be related to LOPAP's serinoprotease-like activity.
Supported by: FAPESP and CNPq

1.51 Identification of the hemolymph amino acids from *Lonomia obliqua* to optimization in cultures of insect and mammalian cells

Sousa APB[1], Swiech K[2], Moraes RHP[3], Suazo CAT[2], Pereira CA[1] and Mendonça RZ[1].
[1]Laboratório de Imunologia Viral, [3]Laboratório de Parasitologia, Instituto Butantan, São Paulo, Brasil; [2] Departamento de Engenharia Química, Universidade Federal de São Carlos, SP Brasil.

Introduction: A protein of 51 Kda was isolated which has an antiapototic effect in Sf-9 insect cell culture (Sousa et al, 2005). Hemolymph has been used for optimization of the S_2 and S_2AcGPV_2 insect cells and as culture medium supplement in the CHO-K1 animal cell cultivation (Raffoul et al, 2005). **Objective:** To identify amino acids presents in hemolymph to optimization cultures cells. **Methodology**: Hemolymph was collected, centrifuged at 1000 rpm for 10 min, filtered at 0.22 µm membrane and stored at 4ºC. SF-9 cells were grown in flasks containing Grace´s medium supplemented with 10% fetal bovine serum (FBS) with hemolymph 1% v/v. S_2 and S_2 $AcGPV_2$ cells cultures were investigated using TC-100 medium with 10% of FBS, and also with hemolymph 1%. The cell CHO-K1 was cultured in DMEM media supplemented with 10% fetal calf serum at 37ºC in a 10% v/v CO_2 incubator with microcarrier Cytodex 1 at a concentration of 3g/L. The hemolymph was loaded on a Reverse-Phase column; amino acid concentrations were analyzed by the Pico-Tag System using HPLC. **Results and discussion**: Seventeen amino acids were identified in hemolymph. In cell CHO-K1, a positive influence of the hemolymph was observed with 52% higher cell concentration. In S_2, Sf-9 and S_2AcGPV_2 cells, high viability and optimization during the cells culture was observed.
Supported by: FAPESP and CAPES

1.52 Optimization of SF-9 cell culture using hemolymph from *Automeris sp*.

Sousa APB[1], Moraes RHP[2] , Pereira CA[1] and Mendonça RZ[1] .
[1]Laboratório de Imunologia Viral, [2]Laboratório de Parasitologia, Instituto Butantan, S.P. Brasil.

Introduction: More recently we have identified proteins from *Lonomia obliqua* hemolymph that presents a potent protecting effect in insect cell culture, which extende the cellular viability increasing at least twice the cellular yields.(Maranga et al, 2003). It was isolated and purified of the hemolymph from *lonomia obliqua* a protein of 51 Kda which has an antiapototic effect in *Spodoptera frugiperda* Sf-9 cell insect (Sousa et al, 2005). **Objetive**: In the present study we investigated the benefits of *Spodoptera frugiperda* Sf-9 insect cell culture medium supplementation with hemolymph from *Automeris sp.* **Methodology**: Hemolymph was collected, centrifugation at 1000 rpm for 10 min, filtered a 0,22µm membrane and stored at 4ºC. Sf-9 cells were grown in flaskes containing Grace´s medium supplemented with 10% fetal bovine serum (FBS), with hemolympf from *Automeris sp* 1% v/v. All experiments were carried out in 100 mL shake flaskes with working volume of 13 mL. The cultures were performed in a orbital Shaker at 100 rpm and 28ºC, starting with a inoculum of $3{,}5x10^5$ viable cells/mL. **Results and discussion**: Addition of hemolymph from *Automeris sp*. 1% (v/v) to Grace´s medium supplemented with 10% of fetal bovine serum(FBS) increased the Sf-9 cell growth when compared to values achieved with Grace´s medium with 10% of FBS. High cell viability was maintained during the cell culture resulting in a final cell density of $1{,}45x10^6$ cells/mL.
Supported by: FAPESP and CAPES.

1.53 Optimization of SF-9 cell culture using hemolymph from *Podalia sp*.

Sousa APB[1], Moraes RHP[2] , Pereira CA[1] and Mendonça RZ[1] .
[1]Lab. de Imunologia Viral , [2]Laboratório de Parasitologia, Instituto Butantan, São Paulo Brasil.

Introduction: Recently we have identified proteins from *Lonomia obliqua* hemolymph which present a potent protecting effect in insect cell culture, and extend the cellular viability thus increasing, at least, twice the cellular yields (Maranga *et al*, 2003). A protein of 51 Kda was isolated and purified from the hemolymph of *Lonomia obliqua*, which has an antiapototic effect in *Spodoptera frugiperda* Sf-9 cell insect (Sousa et al, 2005). **Objetive**: In the present study we investigated the benefits of *Spodoptera frugiperda* Sf-9 insect cell culture medium supplementation with hemolymph from *Podalia sp.* **Methodology**: Hemolymph was collected, centrifugated at 1000 rpm for 10 min, filtered in a 0,22μm membrane and stored at 4ºC. Sf-9 cells were cultivated in flaskes containing Grace´s medium supplemented with 10% fetal bovine serum (FBS), with hemolymph from *Podalia sp* 1% v/v. All experiments were carried out in 100 mL shake flaskes with working volume of 13 mL. The cultures were performed in an orbital Shaker at 100 rpm and 28ºC, starting with a inoculum of $3{,}5x10^5$ viable cells/mL. **Results and discussion**: Addition of hemolymph from *Podalia sp*. 1%(v/v) to Grace´s medium supplemented with 10% of fetal bovine serum (FBS) increased the Sf-9 cell growth when compared to values achieved with Grace´s medium with 10% of FBS. High cell viability was maintained during the cell culture resulting in a final cell density of $1.43x10^6$ cells/mL.
Supported by: FAPESP and CAPES

1.54 Bee venom encapsulation within liposomes: a challenge for liposomology.

Silva TC[1,2], Lopes AC[2] and Costa MHB[1,2]
[1]Lab. Microesferas e Lipossomas – C. de Biotecnologia I. Butantan, SP; [2] Dep. de Clínica Médica UNIFESP - SP, Brasil.

Introduction: For individuals with a specific allergy to Hymenoptera stings, the venom immunotherapy **(VIT)** may be a relatively effective treatment option. Treatment failures do however occur and VIT may cause frequent systemic allergic side effects, mainly in honeybee venom allergy persons. The Immunotherapy is expensive and time consuming. New strategies to improve safety and efficacy of this treatment are therefore of general interest. **Objectives:** We propose here a systematic approach to study the basic and biotechnological problems related with the development of safe formulations of bee venoms within liposomes to be used in VIT. **Methods and Results:** Liposomes could increase the immunogenic capacity, decrease the toxicity and decrease the quantity and frequency of the venom doses during the VIT. However, bee venom mellitin and phospholipase destruct the phospholipid membranes. Our central idea® in modifying the VIT is the combination of three different tools: encapsulation of *chemically modified venoms* within stabilized *liposomes*. Here we present the results of the interaction of modified bee venom (BVm) with artificial membranes. The liposome turbidity of formulation containing pbb (inhibitor) within the lipid membrane was constant during their incubation with BVm. This fact was interpreted as 100 % of protection against aggregation. In contrast it was observed 90% of protection in incubations of BVm with pbb membrane free. It was observed 62% of rodamine leak out from those liposomes pbb free and incubated with BVn (native bee venom). We strongly believe that this formulation will be non toxic and that its immunogenicity will be retained. **Conclusions**: We expected that less quantities of the antigen will be required to obtain the same benefits in venom immunotherapy.
Supported by: FAPESP (05/04514-2 and 00/14228-3) CNPq (491171/2005-6) and CAPES.

1.55 Proteomic analysis of low molecular weight peptides from the skin secretions of *Phyllomedusa hypochondrialis*.

Conceição K[1], Konno K[1], Melo RL[1], Antoniazzi MM[2], Jared C[2], Lopes-Ferreira M[3], Pimenta DC[1].
[1]LETA – CAT/CEPID; [2]Dept. Cellular Biology, [3]Immunopathology Lab., Butantan Institute, São Paulo, SP, Brazil.

Introduction: Amphibian skin secretions may provide new molecules with biotechnological applications, which should play different roles, either in the regulation of physiological functions of the skin or defense against predators or microorganisms. **Objective:** We targeted the identification of novel peptides with bradykinin-like structure present in the skin of this frog. **Methodology:** Glandular secretions of *P. hypochondrialis* specimens were purified by rp-HPLC after solid phase extraction. MS fingerprinting was performed by MALDI-MS. Peptides were de novo sequenced on an ESI-Q-TOF-MS/MS. Peptides were synthesized by a Fmoc-strategy. BK activity was assessed by guinea-pig ileum contraction and intravital microscopy. **Results and Discussion:** First, the SPE-processed sample was fractionated into 29 pools. The fractions were monitored by MALDI-ToF mass spectrometry for evaluation of molecular masses and purification grade. These pools were screened for low molecular weight peptides (700 to 1100 Da) through ESI-Q-TOF mass spectrometry. Several peptides were sequenced, and three presented sequences that slightly resemble Bradykinin. These peptides were sequenced and identified as KPLWRL-NH2 (Phypo 3), RPLSWLPK (Phypo 5) and VPPKGVSM (Phypo 7a). Bradykinin-like peptides were chosen since the direct administration of the crude skin secretion has several effects on the circulatory system, including inflammation.
Supported by: CNPq, CAPES and FAPESP.

1.56 Identification of a novel bradykinin potentiating peptide (BPP) from the skin secretion of *Phyllomedusa hypochondrialis*.

Conceição K[1], Konno K[1], Melo RL[1], Antoniazzi MM[2], Jared C[2], Gilio JM[1], Silva CA[1], Lopes-Ferreira M[3], Borella MI[4], Camargo ACM[1], Pimenta DC[1].
[1]LETA - CAT/CEPID; [2]Dept. Celullar Biology, [3]Immunopathology Lab., Butantan Institute, SP, [4]Dept. of Cellular Biology and of Development, Instituto de Ciências Biomédicas/USP, SP.

Introduction: Bradykinin is often associated to inflammatory processes. Classical studies showed that there existed a factor in the venom of *B. jararaca* which potentiated bradykinin. This factor (BPPs) could inhibit the angiotensin-converting enzyme. **Objective**: We describe the isolation and biochemical characterization of a novel BPP, from the skin of *P. hypochondrialis*. **Methodology**: Glandular secretions of *P. hypochondrialis* specimens were purified by rp-HPLC after solid phase extraction. MALDI-MS fingerprinting was performed and peptides were sequenced on a ESI-Q-TOF-MS/MS. BK potentiation was assessed by guinea-pig ileum contraction and by MAP measurements on anesthetized rats. **Results and Discussion**: We were able to purify and characterize a novel BPP from the skin secretion of *P hypochondrialis* that was able to inhibit ACE, lower the blood pressure and potentiate smooth muscle contraction. The skin secretions of the members of the Phyllomedusinae frog family are widely known to be one the richest sources of bioactive peptides. This novel peptide has a typical BPP motif. Moreover, in vivo and in vitro assays were able to clearly demonstrate that this peptide meets all the functional requirements for a bioactive BPP. And mostly important, physiologically, at the level of the frog skin and its mechanisms of defense, the presence of BPPs can make all the well documented BKs and BRPs extremely powerfull weapons.
Supported by: FAPESP, CAPES and CNPq.

1.57 Activity of *Phyllomedusa hypochondrialis* Secretion Against Visceral and Cutaneous Leishmaniasis and its Citotoxicity Against Mammalian Cells

Tempone AG[1]; Reimão[1] JQ, Teixeira D[1], Antoniazzi MM[2] & Jared C[2].
[1]Lab. Toxinologia Aplicada Dept. Parasitologia Inst. Adolfo Lutz, São Paulo, [2]Lab. Biologia Celular, Inst. Butantan - São Paulo.

Introduction: Leishmaniasis is a parasitic disease afflicting 12 million people worldwide. Amphibian skin secretions have been used to develop chemotherapies for tropical diseases. **Objectives**: To study the *in vitro* activity of *Phyllomedusa hypochondrialis* crude secretion against promastigotes of *Leishmania* (L.) *chagasi* and *L.* (L.) *braziliensis* as well as its mammalian citotoxicity. **Methodology**: 50% Effective Concentration (EC_{50}) data were obtained after 48h incubation with crude secretion in 96-well microplates. Macrophages were obtained from BALB/c mice and incubated with secretion for 48 h. Viability of parasites and mammalian cells was determined using MTT assay. **Results**: Our work demonstrated a considerable activity of crude secretion with an EC_{50} of 87.73 µg/mL against *L. braziliensis* and an EC_{50} of 153.5 µg/mL against *L. chagasi*. The citotoxicity against peritoneal macrophages resulted in an EC_{50} of 139.0 µg/mL. Pentamidine presented an EC_{50} of 0.096 µg/mL against *L. chagasi* promastigotes and demonstrated strong citotoxicity against mammalian cells (EC_{50} 4.22 µg/mL). **Discussion**: *P. hypochondrialis* secretion was effective *in vitro* against Leishmaniasis, with *L. braziliensis* more susceptible than *L. chagasi* promastigotes. Despite citotoxicity, secretion was at least 33-fold less toxic than pentamidine. Further isolations of active compounds could be of interest in developing new drugs against neglected diseases such as Leishmaniasis.
Supported by: FAPESP (2005/00974-9) and Butantan Foundation

1.58 Comparative study of biological and biochemical activities of venoms of Brazilian fishes with medical importance

Boletini-Santos D[1], Erlichman C[1], Kadura A[1], Ramos AD[1], Conceição K[2], Pimenta DC[2], Charvet-Almeida P[3], Figueiredo SG[4], Haddad JrV[5]; Lima C[1], Lopes-Ferreira M[1].
[1]Lab. Imunopatologia, Instituto Butantan; [2]Lab. Especial de Toxinologia Aplicada (CAT/CEPID), Instituto Butantan, São Paulo; [3]Univ. Federal da Paraíba; [4]Dep. Ciências Fisiológicas, Universidade Federal do Espírito Santo, Vitória; [5]Dep. Dermatologia, Universidade Estadual Paulista, Botucatu.

Introduction: In Brazilian waters many species of venomous fishes are found, but 5 species are frequently involved with human accidents: *Potamotrygon orbignyi* (*Po*), *Cathrops spixii* (*Cs*), *Pseudoplatystoma fasciatum* (*Pf*), *Scorpaena plumieri* (*Sp*), and *Thalassophryne nattereri* (*Tn*). Usually, the clinical features induced by these fish venoms are pain, edema, and necrosis. **Objective:** In this study we compared the biological and biochemical activities induced by these different fish venoms. **Methodology:** Toxic activities (nociception-10 µg, edema -10 µg, necrosis -30 µg) and alterations in the microcirculatory net were induced in Swiss mice by application of 10 µg of venoms, which were analysed by 12% SDS-PAGE and chromatography. Gelatinase, phospholipase A_2 (PLA_2) and proteolytic activity were also determined. **Results:** *Tn* induced the highest level of nociception and edema, and the venoms of *Sp* and *Po* induced the lowest levels of nociception or edema, respectively. Only *Tn* venom induced necrosis and hemolitic activity. Alterations in microcirculation were not observed after application of *Sp* venom. All venoms presented proteolytic activity, and *Tn* venom was devoid of PLA_2. The SDS-PAGE and zymography showed a high similarity between *Pf* and *Cs* venoms. The chromatography shows that *Po*, *Cs*, *Pf*, and *Tn* presented a similar band around 18 kDa and *Sp* presented proteins with high molecular weight (> 100 kDa). **Discussion:** This is the first study that compares the toxic activities and biochemical characteristics of the venoms from the major Brazilian venomous fishes: the venom of *Tn* showed the highest toxic activities and *Sp* venom presented the lowest, but with systemic effects.
Supported by: FAPESP and FUNDAP.

1.59 Comparative studies between secretory cells of sting apparatus and venoms obtained from freshwater and marine stingrays

Barbaro KC[1], Lira MS[1], Malta MB[1], Soares SL[1], Santoro ML[2], Pedroso CM[3], Antoniazzi MM[3], Jared C[3], Cardoso JLC[4], Garrone Neto D[5] and Haddad Jr V[4.6].
[1]Lab. Immunopathology, [2]Lab. Pathophysiology, [3]Lab. Cellular Biology, [4]Hospital Vital Brazil, Butantan Institute, [6]Dermatology Department, UNESP, Botucatu

Introduction: Injuries caused by stingrays mainly cause pain and edema. Cutaneous necrosis is commonly observed in accidents by freshwater stingray. The aim of this work was to characterize some aspects of venoms and the sting apparatus of freshwater *Potamotrygon falkneri* (Pf) and marine *Dasyatis guttata* (Dg) stingrays. **Methods:** Zymograph, SDS-PAGE, ELISA and Western blotting (WB) were employed to characterize Pf and Dg venoms. Evaluation of lethality, dermonecrosis, myotoxicity, edema and nociceptive activities were carried out in mice. Hemolytic and coagulant activities were evaluated in human O positive red blood cells. **Results:** By SDS-PAGE, both venoms had similar patterns above 80 kDa. Major bands were located around 10 and 15 kDa. Lethal, dermonecrotic and myotoxic activities were detected only in Pf venom. Edematogenic activity was similar and dose-dependent in both venoms. Nociceptive activity was demonstrated in both venoms. No direct hemolysis and coagulant activities were observed in both venoms. By ELISA and WB, many components of both venoms, mainly above 50 kDa, were recognized using species-specific sera produced in rabbits. Both venoms presented gelatinolytic, caseinolytic and fibrinogenolytic activities. Hyaluronidase activity was detected only in Pf venom. Morphological differences between Pf and Dg venom apparatus were found. The position and the number of the cells which comprise sting tegument were different. **Discussion:** Our experimental results might explain the different clinical pictures presented by patients wounded by freshwater and marine stingrays.

Supported by: FAPESP (03/06874-1).

1.60 Biochemical variation between the mucus and the sting venom from the catfish *Cathorops spixii*

Ramos AD[1], Conceição K[2], Pimenta DC[2], Lopes-Ferreira M[1]
[1]Lab. Imunopatologia, [2]Lab. Esp. de Toxinologia Aplicada (CAT/CEPID), Instituto Butantan, SP.

Introduction: Common in Brazil, *Cathorops spixii* is one of the most abundant venomous fishes of the southeastern coast of the State of Sao Paulo, and consequently it causes several accidents. Its venomous apparatus includes a single milled sting in the distal portion of each breastplate and dorsal fins and contains three different venoms. The sting venom is found in the glandular epithelium covering it and the mucus venom is dispersed on the skin. **Objectives:** The aim of this study was to characterize the biochemical variations between these venoms (mucus and sting). **Methodology:** Twenty micrograms of the venoms were analyzed by 12% SDS-PAGE and 1.5 mg were submitted to RP-HPLC on a C18 analytical column. The obtained fractions were analyzed by mass spectrometry. Moreover, both venoms were analyzed by LC-ESI-Q-TOF/MS. **Results:** The electrophoretic profile of the venoms showed differences in the protein content of each venom being the mucus richer, containing proteins with molecular masses between 45 and 35 kDa, around 25 kDa and between 18 and 14 kDa. The sting venom contained heavier proteins that are not detected in the mucus (>116 kDa), bands around 66 kDa, around 25 kDa and 18 kDa. After RP-HPLC profiling of the venoms, one could observed that the mucus presented only four significant peaks while the sting presented six peaks, being two of them common to the mucus. **Discussion:** By analyzing these results, the mucus seems to contain more toxins than the sting; moreover, the peaks present in both venoms may be due to a contamination by the mucus in the removal of the sting.

Supported by: FAPESP.

1.61 Evaluation of immune response induced in mice by mucus and sting fish venoms from *Cathorops spixii*

Grund LZ[1], Junqueira M[2], Lopes-Ferreira M[1], Lima C[1]
[1]Lab. Imunopatologia, Instituto Butantan, [2]Dep. Dermatologia, Univ Estadual Paulista. Botucatu

Introduction*:* Catfishes, especially *Cathorops spixii*, are the venomous fishes that cause the greatest number of accidents in Brazil. These accidents affect mainly fishermen, swimmers and tourist and are characterized by painful injury, intense edema and some systemic symptoms. **Objective:** Evaluate the profile of the immune response developed after injection of mice with mucus and sting venoms from *C. spixii.* **Methodology:** Balb/c mice were immunized at days 0 and 7 with 100 μg of mucus or sting venoms adsorbed in 1,6 mg of $Al(OH)_3$. The animals were bled at days –1, 7, 14 and 21 for venom-specific IgM, IgG, IgG1 or IgG2a antibody levels determination by ELISA. Fourteen days after the first immunization, animals were sacrificed to obtain the spleen, and then cellular suspension was restimulated with venom (10 μg/mL) or Concanavalina A (5 μg/mL) for 24, 48, 72 and 96 hours. **Results:** Immunization with mucus and sting venoms induced similar levels of venoms-specific IgM in the primary or secondary response and the mucus venom induced higher levels of IgG in the secondary response. However, the sting venom induced levels of IgG1 and IgG2a while mucus venom induced mainly IgG1 subclass. Furthermore, we observed that spleen cells restimulated with sting venom exhibited higher levels of IFN-γ, IL-10 and IL-5 while mucus venom induce mainly of IL-4 and IL-5. **Discussion:** These results suggest that high levels of IgG1 and IgG2a induced by sting venom can be related with the profile of cytokine induced (IFN-γ and IL-5) suggesting that this venom can induce the development of both types of lymphocytes Th1 and Th2. Already, mucus venom induce the activation of Th2 lymphocytes producing IL-4 and IL-5 cytokines that can promote the synthesis of IgG1 antibodies.
Supported by: FAPESP and FUNDAP

1.62 Effect of *Thalassophryne nattereri* venom in adhesion and viability of HeLa cells.

Komegae EN[1], Lopes-Ferreira M[1], Lima C[1].
[1]Laboratory of Imunopatologia, Instituto Butantan, São Paulo, Brazil.

Introduction: Several studies have been carrying with venom of aquatic animals in the attempt of identifying toxins with activity antiproliferation for be used in tumors (Newman & Cragg, 2004). In this sense, the poison of the fish Brazilian *Thalassophryne nattereri* stands out for being capable to block the selective recruitment of leucocytes. **Objective:** This work had as objective evaluates the effect of this venom in the adhesion and survival of a cellular type. **Methods:** The extracted poison of the fish after the compression of the base of the spine was quantified as for the tenor of proteins. After acquire maxim confluence, cells of carcinoma of human cérvix (HeLa, CCL-2, ATCC, 4 x 105/mL) were cultivated in presence of 0,2, 2 or 20 g/mL of the venom of *T. nattereri* for 24 hours. After the cultivation, the cells were stained with violet crystal to 0,05% for the determination of the cellular adherence and, for the evaluation of the viability was used quantitative method of reduction of MTT (0,5 mg/mL/100 L). **Results:** The crescent increase in the concentration of the venom (from 0,2 to 20 g/mL) it induced a significant loss so much in the adhesion of the cells HeLa to the surface of the plate (9,3%, 9,4% and 8,4%, respectively) as in the viability (65,64%, 65,87% and 2,51%, respectively). **Conclusions:** These results show that damage of the adherence of the cells to the substratum is not related with the cellular death induced by the venom, once in smallest concentrations (0,2 and 2 g/mL) approximately 66% of the cells are viable, suggesting that the venom of *T. nattereri* can act blocking the connection of the cells to adhesive receivers in extracelular matrix.
Supported by: FAPESP

1.63 The role of Natterinas, majoritary toxins of the *Thalassophryne nattereri* fish venom in local inflammatory response in mice.

Pareja-Santos A, Lopes-Ferreira M, Lima C.
Lab. Imunopatologia, Instituto Butantan, São Paulo.

Introduction: Natterins, isolated toxins from *Thalassophryne nattereri* (niquim) fish venom, is a family of 5 toxins which present homology amongst themselves and molecular mass around 30-45 kDa. **Objetive:** The group of majority toxins Natterin 1, 2 and 3 was used for the evaluation of the inflammatory response in mice. The kinetic of the leukocyte recruitment, cytokine and chemokine levels and matrix metalloproteinases 2 and 9 activity after administration of Natterins in the footpad of Swiss mice was determined. **Methodology:** After 6, 24 and 48 hours or 7, 14 and 21 days the animals injected with 10 μg of Natterins were was sacrificed and the footpad was processed for cellular suspension collection and inflammatory mediators were determined in supernatant of footpad homogenates. The leukocyte cell counts were performed using a hemocytometer using Hema3-stained cytospin preparations. **Results:** Natterins were not able to induce leukocyte influx into footpad of mice at 6 h after injection, that was characterized by a significant reduction of macrophages and lymphocytes compared with PBS-group, but after 7 days Natterins provoked an intense recruitment of neutrophils, macrophages, and lymphocytes. The footpad levels of PGD2, IL1-β, IL-6, MCP-1 were augmented after 6 hours and IL1-β and TNF-α after 7 days. High MMP-9 activity was detected in footpad supernatant at 6 h. **Discussion:** We can suggest that the impared cellular influx provoked by Natterins was independently of cytokines or chemokines production, and it could be related with the high MMP-9 activity in the footpad.
Supported by: FAPESP and FUNDAP.

1.64 Evaluation of the role of Nattectina, the lectin from the *Thalassophryne nattereri* venom, in the innate and specific immune response

Saraiva TC[1], Orii NM[2], Ramos AD[1], Conceição K[3], Pimenta DC[3], Lima C[1], Lopes-Ferreira M[1].
[1]Lab. Imunopatologia, [2] Lab. Medicina Tropical, USP, [3]LETA-CAT/CEPID, Instituto Butantan/SP.

Introduction: Recently, it was identified a C-type lectin in the venomous fish *T. nattereri* denominated Nattectin. **Objective**: Evaluate the innate immune response induced by Nattectin and consequently its role in the DCs and adquired immune response activation. **Methodology**: After isolation by RT-HPLC, the toxin (20 μg/mL) was ip injected in BALB/c mice for evaluation of cellular recruitment and inflammatory mediators, cytokine production by splenic cells. The animals were bled at days 0, 7 and 14 for detection of antibody levels. DCs obtained after bone marrow cell culture (7 d, GM-CSF) were stimulated with venom (0,02 μg/mL) or Nattectin (10 μg/mL) for 24 h. The expression of surface molecules and MMPs was analyzed by FACS and immunohistochemistry, respectively. **Results**: Nattectin induced a significant cellular recruitment, mainly neutrophils at 1 and 6 h followed by macrophages at 24 h. One hour after the injection the levels of IL-6, IL-1β, MCP-1 and KC were detected, however in 6 or 24 h only IL-1β and MCP-1, respectively. Nattectin induced the PGE_2 and LTB_4 at 6 h. After immunization, mice produced elevated levels of IgG, represented by IgG1 and mainly IgG2a, and splenic cells produced high levels of IL-10 in 24 and 48 h and IFN-γ only in 72 h. The major cells obtained after culture (70%) were $CD11b^+$ and $CD11c^+$ cells. $CD11c^+$ cells were $B220^-$ and MHC II^+. DCs presented low levels of CD80, CD86, and CD40. Venom induced up-regulation of costimulatory molecules expression, and Nattectin induced the production and nuclei and cytoplasm localization of MMP-2 and MMP-9. **Discussion**: These results suggest that Nattectin induce the acute phase of inflammatory response as well as the development of Th1 response. These results might be related with activity of Nattectin in myeloid dendritic cell maturation.
Supported by: FAPESP

1.65 Neuro and cytotoxic effects of polar extracts of dinoflagellates from Brazilian coast

Naves JL[1], Rangel M[2,3], Prado MP[2], Santelli GMM[2], Freitas JC[1,3]
[1]Depto. de Fisiologia, Instituto de Biociências da USP; [2]Depto. de Biologia Celular e do Desenvolvimento, ICB-USP; [3]CAT/CEPID, Instituto Butantan, São Paulo, Brazil.

Introduction: We investigated the effects of polar extracts (PE) obtained from species of dinoflagellates, *Prorocentrum mexicanum* and *P. gracile* from the southeastern Brazilian coast. **Objective:** Evaluate the effects of the extracts in terms of neurotoxicity to isolated crustacean nerves and cytotoxicity against T47D breast cancer cells. **Methodology:** Leg nerves from blue crabs were used in neurotoxicity assays. The action potentials were recorded before, during and after addition of the extracts to the crustacean nerve. In cytotoxicity tests, T47D cells were treated with PE for 48h, fixed and submitted to immunofluorescence reactions, and then analyzed under a confocal laser scanning microscope. **Results:** Neurotoxicity: the PE of *P. mexicanum* (20µg/mL) induced depolarization and a rapid blockade of crustacean nerve action potential conduction. *P. gracile* PE was inactive. Cytotoxicity: T47D cells exposed to 430 and 118 µg/mL of PE of *P. mexicanum* and *P. gracile,* respectively*,* detached from the substrate, losing their control morphological characteristics. **Discussion:** The neurotoxic effect of *P. mexicanum* PE is not clearly due to an unspecific increasing in ion permeability, or to the blockade of potassium channels. Results of the citotoxicity assay provided evidence of apoptotic events induced by both PE. This activity has been previously observed for okadaic acid and yessotoxin, which could be present in the PE.
Supported by: Capes, FAPESP.

1.66 Effects of sponge peptides on gap juntion channels in liver cells

Pfister SC[1], Rangel M[1,2], Prado MP[1], Ferreira RAS[1], Konno K[2], Santelli GMM[1].
[1]Depto. de Biologia Celular e do Desenvolvimento, Instituto de Ciências Biomédicas da USP, São Paulo, Brazil; [2]Lab. Especial de Toxinologia Aplicada-CAT/CEPID, Instituto Butantan, São Paulo, Brazil.

Introduction: Connexins are membrane proteins that form gap junction channels (GJC) between adjacent cells. Connexin 43 (Cx43), the most widely expressed member of the connexin family, has a rapid turnover rate and its degradation involves both the lysosomal and ubiquitin-proteasome pathway. **Objetives:** The goal of this work is to study if the cytoskeleton alterations induced by sponge depsipeptides are associated with changes of connexin assembly or degradation in plasma membrane. **Methods:** Normal liver (BRL3A) and hepatocarcinoma (HTC) cell lines were submitted to treatment with peptide solutions at different conditions. Immunofluorescence reactions and Lucifer Yellow assays by Scrape Loading and Dye Transfer (SL/DT) of the cells were analyzed under a confocal laser scanning microscope. **Results:** The treatment with sponge peptides, like geodiamolide B (Geo B), showed a probable role in enhancing or preserving GJC both in BRL3A and in HTC-transfected cells. Geo B presented a clear effect on actin filaments but its effect is more apparent in tumor cells than in normal ones. The treatment with proteasomal inhibitors enhanced gap junction plaques (GJP). **Discussion:** The geodiamolides could interfere with the delivery of connexins to the degradation structures, keeping connexins assembled and the accumulation of GJP.
Supported by: FAPESP and CNPq.

1.67 Characterization of the analgesic effect of the compound LAS390 purified from sea anemone *Bunodosoma cangicum* venom

Ferreira Jr WA[1,2], Zaharenko AJ[1], Gutierrez VP[2], Konno K[3], Freitas JC[1], Picolo G[2] and Cury Y[2].
[1]Department of Physiology, Institute of Biosciences, USP; [2] Laboratory of Pathophys and [3]Center for Applied Toxinology-CAT/CEPID, Butantan Institute, São Paulo- Brazil.

Introduction: LAS390 is a low molecular weight and non-peptidic compound purified from *B.cangicum* venom that induces analgesia in rats. The aim of the present study is to characterize this analgesic effect and to determine the participation of opioid receptors and K^+ channels in this effect. **Methods:** Pain threshold was assessed using the rat paw pressure test. The LAS390 was administered in different doses (1.5, 15 and 150nM) intraplantarly. Pain threshold was evaluated before and at different times after treatments. Glybenclamide (80µg/paw), apamine (10µg/paw), charybdotoxin (2µg/paw), TEA (640µg/paw) and 4-aminopiridine (100µg/paw), K^+ channels blockers, were used to evaluate the involvement of K^+ channels in antinociceptive effect of LAS390. Naloxone (5µg/paw), an inespecific opioid receptor antagonist, was used to evaluate the involvement of opioid receptors in this analgesic action. **Results:** LAS390 induced antinociception in all doses that was detected until 120 min after LAS390 administration. The antinociception was not altered by glybenclamide, apamine, charybdotoxin, 4-aminopiridine and naloxona. On the other hand, TEA, a voltage-gated K^+ channel blocker, reversed the antinociception induced by LAS390. **Conclusions:** LAS390 induces analgesic effect that is mediated by voltage-gated K^+ channels.
Supported by: CNPq and FAPESP

1.68 Fragmosis in casque-headed tree-frogs: a comparison between *Corythomantis greeningi* and *Aparasphenodon brunoi* skin secretion.

Ono RK[1], Antoniazzi MM[1], Lopes-Ferreira M[2], Lima C[2], Rodrigues MT[3], Haddad C[4], Jared C[1]
[1]Lab. Biologia Celular e [2]Lab. Imunopatologia, Instituto Butantan, [3]Depto. Zoologia ICB-USP; [4]Depto. Zoologia, UNESP, Rio Claro.

Introduction: *Corythomantis greeningi* (Cg) and *Aparasphenodon brunoi* (Ab) are casque-headed hylids presenting fragmotic behaviour, which consists in protecting of the body by entering holes in trees (Cg) or in bromeliad axils (Ab). It was already demonstrated that Cg possess a spiny head with many cutaneous granular glands producing quite potent toxins. There is no morphological or toxinological information about the skin of Ab. **Methodology:** The skin secretion of both head and body of the two species was collected in water by stimulation with a teeth brush. After liophilization the content of protein was determined and samples were analised by electrophoresis and the pharmacological action was screened using intravital microscopy. **Results:** Both species presented different head and body electrophoresis profiles: in Cg the difference was more evident, with bands more intense in the head and others in the body. Preliminary conclusions show that in Cg, the body secretion is more different from that of the head when compared to Ab. However, there is a thick common band in both species. The intravital analysis reveals myofibrilar hypercontration and thrombus formation. **Discussion:** Electrophoresis and pharmacological data support our previous conclusion that Cg uses its head as a poisonous weapon by scraping the spiny and secretory head when manipulated. Ab, despite presenting co-ossification, do not have spines in the head and, when manipulated, do not releases so much secretion when compared to Cg.
Supported by: CNPq, FUNDAP and Butantan Foundation

1.69 Comparative study of native and recombinant forms of FXa inhibitor, from *Amblyomma cajennense* (Acari: Ixodida) (Fabricius, 1787)

Simons MS[1], Batista IFC[1], Lopes A[1], Konno K[2] and Chudzinski-Tavassi, AM[1]
[1]Biochemistry and Biophysics Laboratory, [2]CAT- CEPID, Butantan Institute, São Paulo, SP, Brasil.

Introduction: Ticks are obligate ectoparasities and importante vector of many diseases. *Amblyomma cajennense* tick is a principal vector for different pathologies as babesiosis, Maculosa fever and Lyme-like disease. Bloodsucker saliva contains a complex mixture of bioactives proteins and small molecules, which interfere in the physiological mechanisms in their host. **Objectives**: To compare the kinetic parameters of two Factor Xa inhibitors: one of them is a native protein purified from the *A. cajennense* saliva and the other one is a recombinant protein obtained from the cDNA library of the tick. **Methods**: The native protein was obtained from tick saliva which was collected according the Tachell's method, the inhibitor was isolated in a Resource-Q column (FPLC system) and the recombinant protein was obtained from the cDNA library from the glands and it was expressed by using the *E. coli* system and purified by Ni-sepharose column. To analyze the kinetic parameters of both proteins the chromogenic substrate (S-2765) hydrolysis was evaluated and the data compared. **Results and conclusion**: The native inhibitor showed MW 7.5 KDa, a Ki = 0.04 nM, and the best pre-incubation time was 15 min; when the native inhibitor was incubated to the FX no interaction was observed. On the other hand, to the recombinant inhibitor the Ki = 0.78 nM, the best pre-incubation time was 20 min. The obtained data suggest that the inhibitor shows a competitive pattern of inhibition, and when recombinant inhibitor is incubated with the FX an interaction is observed resulting in a decrease in the inhibition.

Supported by: FAPESP and FINEP

1.70 Characterization of the protein profile of botulinum type A toxin production

Colombini M[1], Oliveira JM[2], Fratelli F[3], Prado SMA[3], Higashi HG[4] and Trezena AG[2]
[1]Serviço de Controle de Qualidade, [2]Vacinas Anaeróbicas, [3]Serviço de Bacteriologia, [4]Divisão de Desenvolvimento Tecnológico e Produção, Instituto Butantan, São Paulo, Brasil.

Introduction: *Clostridium botulinum* is an anaerobic bacteria consisting of 7 different serotypes (A-G). The toxins produced by *C. botulinum* are of large molecular size in their culture supernatants. These complexes are formed by association of the neurotoxin (150 KDa) with non-toxic components. **Objective:** This study characterized electrophoretically the forms of toxin found during the production process. **Methods:** Samples of culture supernatants of *C. botulinum* type A, strain ATCC25763, cultivated in Proteose-peptona medium, were analyzed by SDS-PAGE using 6% polyacrylamide gel. **Results:** The electrophoretic profile showed markedly increasing bands of approximately 220, 182, 107, 72, 54 e 35 kDa. The bands of 220 and 182 kDa may correspond to botulinum toxin associated with non-toxic components. The band of 107 and 54 kDa may correspond to a high and light chain of botulinum toxin, respectively. The bands of 72 and 35 kDa may correspond to non-toxic proteins. A markedly visible band of 53 kDa, observed throughout cultivation, including the time immediately following inoculation of bacteria in the fermentor, probably corresponding to the medium of culture. It was not possible to observe low molecular mass bands in this type of gel. **Discussion:** The results show the increasing pattern of botulinum toxin type A produced *C. botulinum* culture. The electrophoretic profile of toxin was similar to that described in the literature. Future analysis by Western Blotting may distinguish the toxin compound of the culture medium.

Supported by: Butantan Foundation.

1.71 Biochemical profile from the mucus of the slug *Phyllocaulis boraceiense*s (Thomé, 1972)

Toledo-Piza AR[1], Franzolin, MR[2], Sant'Anna OA[2], Lebrun I[3], Nakano E[1], Kawano T[1]
[1]Laboratório de Parasitologia, [2]Lab. Especial de Microbiologia, [3]Lab. de Bioquímica e Biofísica
Instituto Butantan – Av. Vital Brazil, 1500 – CEP 05503-900 – São Paulo – SP

Introduction: Many invertebrates, like mollusks, have been used as source of compounds with antibiotic activity. **Objectives:** Biochemical profile of the mucus from the slug *Phyllocaulis boraceiense*s was studied with the objective to verify which fractions contain this compound. **Methods and Results:** Assays to quantify proteins, lipids, amino acids, free and bound glucose associated with other substances, mass spectrometry, electrophoretic profile and a high performance liquid chromatography were carried through. Crude samples were collected using a spatula and a saline solution (NaCl-0.06%). This material was stored in freezer at –70ºC, after that an extraction with acetonitrile was performed, and then the material was liofilizated. The results in terms of concentration were: protein-1.15 x 10^{-4}mg/ml; lipids-6.9 x 10^{-5}mg/ml; amino acid analysis –only few amino acids were detected, probably the material degraded; free glucose–not detected; glucose in association with another compounds-600μg/ml. The mass spectrometry shows a compound with 17.5kDa molecular weight and probably masses corresponding to monomers, dimmers and trimmers. In the HPLC assay some bands of protein were detected, these results were in accordance to that obtained in the electrophoretic profile. **Conclusion:** These data suggested that mucus is a source of protein and probably will be able to have an antimicrobial action.
Supported by: FAPESP.

1.72 Distinct immune response in B10.A and A/J mice immunized with antigen extract of *Lagochilascaris minor*

Spadafora-Ferreira M[1], Pichiteli CR[1], Tambourgi DV[1], Carvalhaes MS[2]
[1]Laboratory of Immunochemistry, Instituto Butantan, São Paulo, SP; [2]Sector of Immunology, DMIPP, UFG, Goiânia, GO

Introduction: Lagochilascariasis is an infection caused by *L. minor*. Experimental life cycle was described in mice as intermediate host and cats as definitive host. A/J mice are resistant and B10.A susceptible to infection. **Objectives**: Study the *in vitro* immune response of splenocytes from immunized mice to the parasite crude extract antigen (CEA) and to ConA by proliferation and cytokine analysis. **Methods**: Mice were immunized ip with 5 μg of CEA, challenged one week later, and sacrificed at day ten. Control mice received saline. Splenocytes proliferation to ConA and CEA was measured after 4 days of culture. IFNγ and IL-10 was detected in culture supernatants after 48h stimulation by ELISA. **Results**: Splenocytes proliferation (mean cpm±sd) in control and immunized B10.A was: 1733±385 and 1551±496, without antigen; 19705±5936 and 24762±6608 with ConA; 2561±641 and 5544±2022 with CEA, while in control and immunized A/J was: 3818±3735 and 6661±1723, without antigen; 16926±9637 and 25632±7542 with ConA; 1728±1688 and 15420±4308 with CEA. IFNγ (pg/ml) induced by ConA was 2272±1214 and 839±440 in control and immunized B10.A, and 2924±3469 and 17909±10933 in control and immunized A/J mice. IL-10 (pg/ml) induced by ConA was 171±22 and 113±61 in control and immunized B10.A, and 182±108 and 279±94 in control and immunized A/J. **Discussion**: CEA induced proliferation to ConA in both strains but a higher proliferation to CEA in A/J mice. CEA induced IFNγ and IL-10 in A/J in contrast to inhibition of both cytokines in B10.A, suggesting a distinct immune response in these strains.
Supported by: CNPq

1.73 The effect of the wild type and K409A mutant rhsp65 of *Mycobacterium leprae* on systemic Lupus Erythematosus

Marengo EB[1], Portaro FCV[2], Moraes LV[3], Fernandes B[2], Carvalho LV[1], Tambourgi DV[1], Camargo ACM[2], Sant´Anna, OA[1].
[1]Lab. Imunoquímica, Instituto Butantan, [2]LETA – CAT/CEPID, [3]Lab. de Imunologia Clínica, ICB–USP.

Introduction: The chaperones hsp60s guide several steps during protein synthesis being abundant in prokaryotic and eukaryotic cells and highly conserved during evolution. Both the bacteria and mammalian hsp60 are the major targets for the immune response against infectious agents and they had been implicated in autoimmune diseases and chronic inflammation. Mutagenesis studies showed that the putative catalytic site at the C-domain of *M. leprae* hsp65 ($Thr^{375}Lys^{409}Ser^{502}$) carries out the main proteolytic activity of this molecule. **Objectives:** In the present study the putative pathophysiological role of wild type (WT) and K409A mutant recombinant hsp65 of *M. leprae* in autoimmune diseases was under evaluation. **Methods and Results:** Spontaneous Systemic Lupus Erythematosus [SLE] developed by the [NZB/NZW] F_1 hybrids was the model choose. The individual anti-DNA and anti-hsp65 antibody titers were determined until 1 year. The results showed that the treatment with the hspWT abbreviate the mean survival time of the animals in 46% when compared to control ($p<0.001$) and there is no ascite development; these mice presented more precocious anti-DNA antibodies than the control ones ($p< 0.001$), suggesting the possible role of hsp increasing the exposition/expression of nuclear antigens. Moreover, the involvement of WT rhsp65 correlates to the age of administration and is dose-dependent. Groups treated with the K409A mutant behaved as the control mice. **Conclusions:** These data clearly indicate the role for hsp65 in the physiopathogenesis of this autoimmune disease.

Supported by: FAPESP

1.74 Induction of chronic and persistent airway inflammation in a murine model of asthma

Kabbara MF[1], Lopes-Ferreira M[1], Vargaftig BB[2], Lima C[1].
[1]Lab. Immunopatologia, Instituto Butantan, [2]Dep. Farmacologia, Universidade de São Paulo.

Introduction: Most of animal models of airway allergen inflammation are restricted to appointing acute changes, occurring after short periods of allergic exposure. But few authors use animal models which reproduce functional or structural changes typical of lung remodeling. **Objectives:** In this study we reproduced a persistent lung inflammation after chronic allergen exposure. **Methods:** A fragment of heat-coagulated hen's egg white alone (EWI) was implanted s.c. in BALB/c mice and 14d later they were exposed to 1% aerosolized ovalbumin for 3 days during 3 consecutive weeks. At 24h and 45d after the last challenge they were bled and sacrificed for analysis. **Results:** Repeated allergen-exposure caused a significant increase of leukocyte infiltration for up to 45d. Lung eosinophilia was only observed at 24h, and at 45d it was observed the predominance of neutrophils and macrophages in BAL. IL-4 remained high in the lung for up to 45d, IL-5 was not determined, and IFN-γ and eotaxin were only observed at 45d or at 24h, respectively. Plasma levels of IgG1 anti-OVA determined via ELISA remained significantly high for up to 45d, but with anaphylatic activity only at 24h. Total and anaphylactic IgE in lung were determined until 45d. Only MMP-2 was detected in BAL at 24h and 45d. **Discussion:** Our results suggest that repeated allergen exposure may develop a lung eosinophilic inflammation that in the absence of the allergen is substituted by the neutrophilic and macrophagic influx, accompanied by high levels of IL-4, IFN-γ, eotaxin, and IgE. Then, we can suggest that in the absence of the allergen exposure the inflammation changes its cellular profile and these alterations can determine the lung remodeling.

Supported by: FAPESP

2. Microorganisms and vaccines

2.01 Evaluation of the immune response of mice immunized with a vaccine based on *Lactococcus lactis* expressing the variable fragment of β intimin.

Alves PCP[1], Carbonare CB[2], Tino De Franco M[1], Vulcano AB[1], Carbonare SB[1].
[1]Lab. Imunogenética, Instituto Butantan, São Paulo; [2]Lab Imunologia de Mucosas, ICB-USP, São Paulo.

Introduction and Objective: Intimin is an important virulence factor of enteropathogenic *E. coli* (EPEC), the main etiological agent of infantile diarrhea. Antibodies anti-intimina inhibit the adhesion of the EPEC to the host cells, suggesting its protective capacity. Our vaccine is based on *L. lactis* transformed with the expression vector pMD112 cloned with the variable fragment of β intimin. Our aim was to evaluate the immune response evoked by this vaccine in different routes and immunization schedules**. Methods and Results**: Groups of Balb/c mice were immunized with vaccine, control *L. lactis* or only the vaccine vehicle, in different immunization schedules and by different routes (exclusively oral, subcutaneous or intranasal; or subcutaneous followed by oral or intranasal). We also used 25mg/dose of the adjuvant monophosphoril lipid A (MPL) in some immunization schedules. The immune response to intimin was evaluated by ELISA in sera and feces. Our results showed that there was a great variation in the responses of the various individual animals. The exclusively oral or the combined immunization schedule with subcutaneous followed by oral vaccine with 10^{10}cfu gave us a good seric response to intimin, but the response of secretory antibodies was very low in all immunization schedules. The intranasal schedules are still being evaluated and it will be compared with the other immunization schedules. **Discussion**: These results show the viability of the use of the vaccine of intimina based on transformed *L. lactis* and indicate the way to achieve the best immunization schedule.
Supported by: FAPESP and CNPq.

2.02 Seric and secretory antibodies reactive with intimins α, β e γ in healthy Brazilian adults

Zapata-Quintanilla LB[1], Carbonare CB[2], Oliveira RR[2], Carbonare SB[1].
[1]Lab. Immunogenetica, Instituto Butantan; [2]Lab. Imunologia Mucosas, Dep. Imunologia, ICB/USP.

Introduction; Intimin is an outer membrane protein present in enteropatogenic (EPEC) and enterohemorragic (EHEC) *Escherichia coli*, able to induce attaching and effacement lesion on enterocyte. The main subtypes of intimin presents in EPEC and EHEC prevalent in Brazil are α, β e γ. **Objectives:** To determine the concentration of antibodies reactive with intimins α, β e γ in serum and colostrum samples from healthy Brazilian adults. **Methods and Results:** Seric IgG and secretory IgA antibodies anti-intimin in 100 serum and 54 colostrum samples were determined by ELISA with purified proteins obtained from recombinant bacteria (conserved and variable regions of intimins α, β e γ). In general, the antibody concentrations of colostrum are higher than serum samples. The concentrations of anti-intimin γ in colostrum were lower, and the concentrations of anti-conserved intimin in serum were higher compared to the others groups. There were a high correlation between anti-intimins α and β, characteristics of the prevalent EPEC in our population, both in the sera (r = 0.85) as in the colostrums (r = 0.98). The presence of anti-intimin antibodies in healthy Brazilian people was expected in face of the low incidence of EHEC and EPEC infection in adults. The high concentration of anti-intimin antibodies in colostrum samples may be related with the high protective effect of breastfeeding against EPEC infection in infants. **Conclusions:** The establishment of a reliable cutoff value for these antibodies could be an important tool for diagnosis purposes.
Supported by: CNPq and FAPESP

2.03 Oral vaccination of rabbits with an intimin vaccine based in transformed *Lactococcus lactis:* immune response and challenge with rabbit enteropathogenic *Escherichia coli* (REPEC)

Carbonare CB[1], Alves PCP[2], Vulcano AB[2], Carneiro-Sampaio MMS[1], Carbonare SB[2]
[1]Lab Imunologia Mucosas, ICB-USP, SP; [2] Lab Imunogenética, Inst. Butantan, SP.

Introduction: *Lactococcus lactis* are non-pathogenic and non-invasive lactic acid bacteria, and have been applied as antigen carriers, regarding vaccination of children, elderly and immunodepressed patients. Intimin is an adhesin that mediates the intimate adhesion of enteropathogenic and enterohemorrhagic *E. coli* to the host cell. **Objectives:** Our aim was to evaluate the immune response of rabbits after oral vaccination with *L. lactis* transformed with the expression vector pMD112 cloned with fragments of intimin (Lc-pKR110) in rabbits and their behavior after challenge with REPEC. **Methodology:** The vaccine (10^{10}cfu or 10^{11}cfu/0.5ml) was orally given to 15 New-Zeland rabbits. The control groups received the vehicle (n=9) or *L. lactis* (n=10), in different immunization schedules. After the immunization, the rabbits were challenged with $3x10^8$ufc of REPEC and followed during 15 days for the observation of clinical manifestations. **Results and Discussion:** The majority of the rabbits presented diarrhea episodes and the mean weight loss was of 21%. The vaccinated animals survived longer (mean 9 days) despite of the greater loss of weight, counting of colonies and severe clinical manifestations. The colony counting in feces reached $3.3x10^7$ to $6.0x10^8$ufc when the animals died. The non-vaccinated animals had survived in mean 3 days. Some vaccinated rabbits (7/15) have developed significant levels of anti-intimin seric antibodies. After the challenge with REPEC there was a significant increase in the levels of seric and secretory antibodies in the majority of the surviving rabbits (20/21).
Supported by FAPESP

2.04 Antibodies reactive with α, β and γ intimins in serum and colostrum samples of healthy Brazilian mothers and cord sera of their newborns

Nuñez SP[1], Carbonare CB[2], Zapata-Quintanilla LB[1], Carbonare SB[1].
[1]Lab Imunogenética, Inst. Butantan, SP; [2]Lab Imunologia Mucosas, ICB-USP, SP.

Introduction: Intimin is an outer membrane protein present in the diarrheagenic *Escherichia coli* able to induce attaching and effacement lesion onto the host enterocyte. The main subtypes of intimin present in the *E. coli* prevalent in Brazil are α, β e γ. **Objectives:** Our aim is to evaluate the naturally acquired immunity and the protection of the newborn children against *E. coli* infections, by means of the determination of antibodies reactive with intimins α, β e γ in serum and colostrum samples from healthy Brazilian mothers and in the cord sera of their newborns. **Methodology and Results:** Serum and colostrum samples are being obtained from 50 healthy women as well as cord sera from their newborns. Purified proteins consisting of conserved and variable regions of intimins α, β e γ were obtained from recombinant bacteria. Seric IgG and secretory IgA antibodies anti-intimin in serum and colostrums are being determined by ELISA and immunoblotting with the purified intimins. This research is presently in course, but preliminary results showed that concentrations of secretory antibodies in colostrum are higher than serum samples. The presence of anti-intimin antibodies in healthy Brazilian people has confirmed our previous results with whole cell ELISA and immunoblotting with crude bacterial extracts. **Conclusions:** The presence of anti-intimin antibodies in colostrum samples may be related with the high protective effect of breastfeeding against EPEC infection in infants.
Supported by: CNPq and FAPESP

2.05 Expression of different EPEC β-intimin fragments in lactic acid bacteria

Ferreira, PCD[1,2]; Campos, IB [1,2]; Ho PL[1,2]; Oliveira MLS[1,2].
[1]Centro de Biotecnologia, Instituto Butantan, São Paulo, SP, Brazil; [2]Pós Graduação Interunidades em Biotecnologia, Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, Brazil.

Introduction: Enteropathogenic *Escherichia coli* (EPEC) is a common etiological agent of severe diarrhea in infants, responsible for a large number of morbidity and mortality in developing countries. One of the virulence factors that promote the intimate adhesion of EPEC with enterocytes in the small intestine is intimin, a 94kDA outer membrane protein, essential for full virulence. As a result, activation of several signaling proteins in the host cells and rearrangement of the cytoskeletal lead to the formation of attaching and effacing lesions (A/E lesions), disrupting the membrane. Based on antigenic variations, intimins are classified in several subtypes that can be grouped into nine families; among them β intimin is one of the most frequently subtype found in clinical isolates. Lactic acid bacteria are commensal microrganisms present in the gastrointestinal mucosa of health individuals that have been used as carriers for antigen presentation in different models. **Methodology and Results:** In this work different β-intimin fragments corresponding to the conserved region, to the variable region or to both regions were cloned into a vector based on the *Lactococcus lactis* P1 promoter. The recombinant vector was used for transformation of *L. lactis* and *Lactobacillus casei*. Intracellular expression of the different fragments in *L. lactis* and *L. casei* extracts was analyzed by Western-blot. **Conclusions:** The recombinant bacteria are now been tested for the induction of the immune system upon oral immunization of mice.

Supported by: FAPESP, CNPq and Butantan Foundation

2.06 Neutralizing activity of secretory antibodies in colostrums and milk samples of Brazilian women

Santos SMR, Ferreira TL, Carbonare SB, Tino de Franco, M.
Laboratório de Imunogenética, Instituto Butantan, São Paulo, SP.

Introduction: Rotavirus is the most common cause of severe, dehydrating diarrhea in children worldwide. There is no consensus about protective effect of breast-feeding against rotavirus infection, but some authors reported that breastfeeding is associated with significant decrease in the immunogenicity of rotavirus vaccines **Objective:** Our aim is to study the ability of colostrum and milk to inhibit cytopathic effect of 2 rotavirus serotypes in neutralization assays. **Methodology**: SA-11 (G3) and G9 rotavirus serotypes are cultivated and titrated in MA-104 cells. Colostrum and milk samples (n=30) with known levels of anti-rotavirus antibodies, are incubated with a known concentration of virus and the mixtures are added to a monolayer of MA-104 cells. The cytopathic effect is evaluated after 48 hours by neutral red colorimetric assay and correlated with the anti-rotavirus specific antibodies. **Results:** The results indicate the levels of inhibition of cytopathic effect and the correlation with levels of specific antibodies. Taken together these results may indicate the colostrums and milk ability to neutralize rotavirus serotypes. **Discussion:** The ability of colostrum and milk anti-rotavírus specific antibodies to neutralize rotavirus may be important in studies concerning protective effects of breast-feeding against rotavirus infection and in anti-rotavirus vaccination strategies.

Supported by: FAPESP and FUNDAP

2.07 Experimental infection of AIRmin mice by oral route with enteropathogenic *Escherichia coli*.

Vulcano AB[1], Amaral JA[1], Cabrera WH[1], Tino De Franco M[1], Ribeiro Filho OG[1], Alves PCP[1], Carbonare C B[2], Carbonare SB[1].
[1]Laboratório de Imunogenética, Instituto Butantan, SP; [2]Laboratório de Imunologia de Mucosas, ICB-USP.

Introduction: Enteropathogenic *E. coli* (EPEC) are important agents of infantile diarrhea, able to produce attaching and effacing lesions on the enterocyte membrane. **Objectives:** As the *in vitro* models are limited, we intend to search an *in vivo* model for this infection. AIR min strain mice were selected for minimal inflammatory response and showed low resistance in experimental *Salmonella* infection. Our present aim was to obtain an oral infection model of EPEC in AIR mice and to compare various bacterial doses and two animal ages. **Methods and Results:** Groups of five AIRmin mice, aged 4 or 8 weeks were infected with various doses of live EPEC by oral route and observed for 45 days. Serum and feces samples were collected before and after infection were analyzed by ELISA. The intestines of two animal of each group were submitted to histology. The orally given EPEC caused no death in the mice group that received the higher dose of bacteria, in contrast with the same bacteria given intraperitonally (LD_{50}=$3x10^7$ cfu). The intestines of the infected animals showed a modified histology compared to control animals, with flattened apical portion of the villi and alteration in the glicocalix. The oral infection of AIRmin mice with EPEC was not able to cause death even in high doses as $2X10^{10}$ cfu, but caused histological alterations in intestinal villi. **Conclusion:** The establishment of an experimental model for EPEC can be used in future studies of infection and protection by passive immunotherapy or vaccines.
Supported by: FAPESP

2.08 Experimental infection of airmin mice by intraperitonial route with enteropatogenic *Escherichia coli*

Vulcano AB[1], Amaral JA[1], Cabrera WH[1], Tino De Franco M[1], Ribeiro Filho OG[1], Alves PCP[1], Carbonare C B[2], Carbonare SB[1].
[1]Lab Imunogenética do Instituto Butantan – São Paulo/SP; [2]Lab Imunologia de Mucosas, ICB-USP

Introduction: Enteropathogenic *E. coli* (EPEC) are important agents of infantile diarrhea, able to produce attaching and effacing lesions on the enterocyte membrane. As the *in vitro* models are limited, we intend to search an *in vivo* model for this infection. AIR min strain mice were selected for minimal inflammatory response and showed low resistance in experimental *Salmonella* infection. **Objectives:** Our present aim is to obtain an intraperitonial infection model of EPEC in AIRmin mice and determinate the LD_{50} value for two animal ages. **Methods and Results:** Groups of five AIRmin mice, aged 4 or 8 weeks were infected with various doses (10^7, 10^8 and 10^9 cfu) of live EPEC by intraperitonial (i.p) route and were observed for 45 days. Serum and feces samples were collected before and after infection of the surviving animals, and were analyzed by ELISA. The values of LD_{50} calculated by Reed & Müench method were 4.21×10^7 cfu for younger animals and 3.1×10^7 cfu for older animals. The levels of seric and secretory antibodies were higher in animals that received the higher bacterial doses. Our study established i.p infection models of AIRmin mice with EPEC and determined the two LD_{50} values for animals with two ages. In the next steps, we will compare these results with the ones obtained by oral infection experiments. **Conclusion:** The establishment of an experimental model for EPEC can be used in future studies of infection and protection by passive immunotherapy or vaccines.
Supported by FAPESP

2.09 α- and entero- hemolysin activities detected in atypical enteropathogenic *Escherichia coli* (EPEC).

Magalhães CA, Rocha LB, Piazza, RMF.
Lab Bacteriology, Instituto Butantan, São Paulo, Brazil.

Introduction: Different from typical, atypical EPEC are isolated from diarrhea outbreaks in infants, adults and animals even in developing and industrialized countries. No specific virulence factors have been associated with this pathotype. **Objectives:** We investigated in this work the presence of toxins associated with atypical EPEC that shows hemolytic effects on blood agar. **Methods and Results:** Twenty-five isolates of atypical EPEC representing the main serotypes were pre cultivated at 37°C under aeration, for 16-18 h, in different culture media: Tryptic Soy Broth (TSB), Evans Medium, Minimal Medium (MM) and Tryptic Soy Broth containing 0.25% of lactose (TSB-L). For hemolytic activity detection, 2 μl of bacterial grown were platted onto blood agar supplemented with 10mM calcium chloride and 5% defibrinated sheep erythrocytes (freshly prepared by washing three times in PBS). After 3 and 24 h of incubation at 37°C, blood agar plates were examined by the presence of hemolysis zones around the bacterial dot. Our finding shows that 1 strain (0,04%) was Hlyα positive and 4 strains (0,16%) Ehly positive. **Conclusions:** Besides, differences in activity of these hemolysins were observed; presence of lactose or glucose as carbon sources during the growth affects the expression of this protein, suggesting that different enzymatic pathways have activated expression of the toxin.
Supported by: FAPESP

2.10 O111 Polysaccharide as a candidate for the development of a conjugated vaccine against pathogenic *E. coli*

Ozaki CY, Santos MF, Sircili MP, Culler HF, Franzolin MR, Ruiz RC, Domingos MO.
Lab. Bacteriologia, Instituto Butantan, São Paulo, Brazil.

Introduction: The serogroup O111 of *E. coli* can be found in three different categories of pathogenic *E. coli*, namely Enteropathogenic *E. coli* (EPEC), Enterohaemorrhagic *E. coli* (EHEC) and Enteroaggregative *E. coli* (EAEC). The development of a vaccine effective against all pathogenic categories of O111 *E. coli* will be very important for public health, since this serogroup is the one mainly responsible for child diarrhoea in endemic areas of Brazil. **Objective**: To determine whether the O111 polysaccharide is a good candidate antigen for the development of a vaccine against all pathogenic categories of O111 *E. coli*. **Methodology**: Serum used was obtained from a rabbit immunized intravenously with O111 bacteria. Flocculation was employed to assay for recognition of live *E. coli* bacteria. Levels of bacteria in the cell adhesion, biofilm and phagocytosis tests were determined either by reading the optic density or by visualizing or by counting of colony-forming units. **Results**: Antibodies from immunized rabbits recognize and aggregate samples of all three categories of live O111 bacteria derived from human, cat and dogs, but not any other serogroup. These antibodies completely inhibit *in vitro* the adhesion of O111 *E. coli* to an epithelial cell line derived from cervix, but do not interfere with adhesion of any other serogroup. Furthermore, biofilm formation is 82.15% inhibited on glass and 90.9% on plastic surfaces. These antibodies also increase the phagocytosis of O111 *E. coli* by macrophages. **Discussion**: O111 polysaccharide is a good candidate antigen for the development of a conjugated vaccine effective for all three categories of O111 diarrhoegenic *E. coli*.

2.11 Antibiotic susceptibility rates of atypical Enteropathogenic *Escherichia coli* (EPEC).

Franzolin MR, Culler HF, Bianchi PA.
Lab. Bacteriology, Instituto Butantan, São Paulo, Brazil.

Introduction: Atypical EPEC has been associated with infantile diarrhea in developing and in industrialized countries. Antimicrobial resistance in *E. coli* has been reported worldwide, being highly variable from geographic zone to another as well as they present significant differences in various populations and environments. **Objective:** This study was carried out to establish the antimicrobial resistance patterns of atypical EPEC strains isolated from children younger than 10 years old with and without diarrhea, living in Salvador, Bahia. **Methodology:** Atypical EPEC strains were tested for susceptibility to 12 antimicrobials with the Kirby-Bauer disc diffusion method, utilizing Muller-Hinton agar. **Results and Discussion:** All bacteria tested were sensitive to ceftriaxone, ciprofloxacin and gentamicin. 59.7% of strains showed resistance to at least one agent. The strains presented resistance to ampicillin (45.4%), trimethoprim/sulfamethoxazole (44.5%), tetracycline (34.5%) and streptomycin (26.0%), while they presented low resistance to chloramphenicol (4.2%), cephalotin (3.4%), amikacin (2.5%), ceftazidim (0.8%) and nalidixic acid (0.8%). We found an elevated percentage of strains (14.3%) that showed multiple drug resistance, indicating the necessity of controlling the spread of antibiotic-resistant *E. coli*. Resistance to at least two classes of antimicrobial agents in *E. coli* has an increasing impact on available therapeutic options.
Supported by: FAPESP

2.12 Enteroinvasive *Escherichia coli* (EIEC) isolated from infants with diarrhea in Salvador - Brazil.

Castro ICP and Franzolin MR.
Lab. Bacteriology, Instituto Butantan, São Paulo, Brazil.

Introduction: Enteroinvasive *Escherichia coli* (EIEC) is an important agent of pediatric diarrhea and dysentery in developing countries, being biochemically, genetically and pathogenically related closely to *Shigella* spp. The characteristic features of EIEC strains are the presence of a virulence plasmid (pInv) capable of entering epithelial cells and disseminating from cell to cell. **Objective:** To evaluate the occurrence of EIEC strains in children with acute diarrhea, living at Salvador - BA. A total of 80 *E. coli* strains negative to the patotypes of EPEC, EAEC, ETEC and STEC, verified by polymerase chain reaction (PCR) were studied through biochemical tests. **Methodology:** Eighty *E. coli* strains were analyzed in respect to lactose fermentation, motility and lysine descarboxilation using standard methods. **Results and Discussion:** 39 *E. coli* isolates were non-lactose fermentative and non-motile, of which 28 strains were lysine descarboxylase negative, while 11 were positive for this test. The 28 samples presented biochemical characteristics compatible with EIEC, and were serogrouped with respect to O type of EIEC. The Congo red binding was assayed, and the invasion plasmid (pInv) was investigated by PCR. Our results suggest low frequency of EIEC strains in these patients.

2.13 Analysis of the capacity of biofilm formation in strains of atypical Enterophatogenic *Escherichia coli* (EPEC)

Culler HF, Sircilli MP and Franzolin MR.
Lab. Bacteriology, Instituto Butantan, São Paulo, Brazil.

Introduction: Atypical EPEC are emergent pathogens that cause infantile diarrhea in developing countries. A biofilm is a structured community of bacterial cells enclosed in a self-produced polymeric matrix adhering to an inert or living surface. This organization type is extremely advantageous in protecting against dehydration and resistance to antimicrobial agents. **Objective**: To verify the growth capacity of biofilm in atypical EPEC strains in abiotic surfaces (polystyrene and glass). **Methodology**: Thirty-two strains of atypical EPEC isolated from patients with diarrhea of Salvador (BA) were studied. Biofilm formation was quantified spectrophotometrically at 595 nm in an ELISA reader, after crystal violet staining and solubilization of the bacteria fixed on plates of polystyrene 24-well culture dishes, with and without glass coverslips. **Results and Discussion**: All strains of atypical EPEC analyzed in both surfaces produced biofilm, and 87.5% presented greater growth on the glass. The atypical EPEC strains presented less biofilm (OD < 0.100 in plastic and OD < 0.200 in glass) than the prototype strain of EAEC (042) (OD < 1,180 in plastic and OD < OD 1,326). The next stages of this project include the analysis of others 90 strains. The strains with larger index of biofilm formation will be tested in epithelial cells. The presence of *bfpA* and *espA* genes will be identified and characterized in the strains using PCR. Biofilms may be responsible for persistent diarrhea.
Supported by: FAPESP

2.14 Comparative proteomic analysis of the outer membrane and periplasmic fraction between typical and atypical enteropathogenic *Escherichia coli*.

Taddei CR[1], Oliveira FF[1], Piazza RM[1], Paes Leme AF[2], Serrano SMT[2], Sant´Anna OA[3].
[1]Lab. Bacteriology, [2]LETA-CAT/CEPID, [3]Lab. Immunochemistry - Instituto Butantan, São Paulo, Brazil.

Introduction: The term atypical enteropathogenic E. coli (EPEC) is used to define the EPEC strains that do not have the EAF plasmid. Until now, there are no reported exclusive atypical EPEC virulence factors that could be used to characterize these strains as a new diarrheagenic E. coli patotype. **Objective**: In the present study we analyzed the proteomic profile of outer membrane and periplasmic proteins of typical and atypical EPEC, and compared them looking for the presence of virulence factors that could be related with the pathogenicity of each EPEC group. **Methodology**: The typical EPEC strain serotype O55:H6 and the atypical serotype O55:H7 were used in this study. For the proteins extractions, both strains were grown in Luria Broth (LB) and D-MEM. The OMP fractions were obtained using sarcosine extraction, and the periplasmic proteins were obtained using the methodology of osmotic shock. The fractions were analyzed in 2-dimension electrophoresis, and the spots were identified using MALDI-TOF-MS methodology. **Results**: In the SDS-PAGE analysis of the extractions we identified a better expression of OMP in typical EPEC strain grown in LB. In the periplasmic proteins analysis, we identified a better protein expression in atypical EPEC strain grown in D-MEM. **Discussion**: We are now analyzing the proteins spots in 2DE, and identifying the protein of each EPEC fraction, to better elucidate the mechanism of virulence of the EPEC patotype.
Supported by: Fapesp and CNPq

2.15 The MR/P fimbriae is involved in the aggregative adherence pattern of uropathogenic *Proteus mirabilis*

Rocha SPD[1,2], Elias WP[2], Cianciarullo AM[3], Nara JM[2], Piazza RMF[2], Silva MRL[1], Moreira CG[2], Pelayo JS[1].
[1]Universidade Estadual de Londrina, Londrina; [2]Lab. Bacteriology; [3]Lab. Genetics, Instituto Butantan, SP.

Introduction: *P. mirabilis*, an important cause of urinary tract infection (UTI), presents several virulence factors including the MR/P fimbriae. **Objective**: Characterise the adherence pattern to HEp-2 cells, the biofilm formation capacity and the role of MR/P fimbriae in these phenotypes. **Methodology:** 35 strains of *P. mirabilis* isolated from UTI were evaluated in the HEp-2 cells adherence test and biofilm formation on polystyrene. Also, a non-polar mutagenesis in the MR/P strucutural gene were performed and the effect of this mutagenesis was evaluated by haemaegglutination, SDS-immunoblotting, transmission electron microscopy, adherence to HEp-2 cells and biofilm formation. **Results and Discussion:** All isolates displayed the aggregative adherence (AA) to HEp-2 cells and were able to produce biofilm. Type I fimbriae and AAF/I, II and III fimbriae of EAEC, and the EAEC probe fragment sequence were not detected in all strains. The *mrpA* and *mrpH* genes of MR/P fimbriae were detected in all isolates, as well as MR/P expression, detected by haemagglutination of chicken erythrocytes. The non-polar mutation of *mrpA* in UEL-13 demonstrated that MR/P is involved in the aggregative adherence to HEp-2 cells and in biofilm formation. However, these phenotypes are multifactorial, since the *mrpA* mutation did not abolish both phenotypes. Our results reinforce the importance of MR/P in the virulence of *P. mirabilis* due to its association with AA and biofilm formation, an important step for the establishment of UTI in catheterised patients.

2.16 Antimicrobial susceptibility of bacteria responsible for secondary infection in *Bothrops* snake bites and of bacteria species isolated from the oral cavity of *B. jararaca*.

Maeda CT[1], Franzolin MR[1], Fan HW[2], Malaque CMS[2], Grego KF[3], Calleffo MEV[3], Trabulsi LR[1] and Sant´Anna OA[4].
[1]Lab. Bacteriology, [2]Hospital Vital Brazil, [3]Lab. Herpetology, [4]Lab. Imunochemistry, Instituto Butantan, São Paulo, Brazil.

Introduction: Once abscess formation occurs in snakebite victims, adequate treatment requires prompt surgical drainage combined with administration of appropriate antibiotics. Bacteria isolated from abscesses in *Bothrops* envenoming were predominantly aerobic Gram-negative bacilli (mainly *Morganella morganii*), being similar to those found in the oral cavity of the snakes, representing the source of pathogens. **Objective:** The aim of this study was to investigate the extent of antimicrobial resistance of bacteria isolated from 16 patients and from oral cavity of 22 snakes. **Methodology:** Bacteria isolated and identified from patients and oral cavity of snakes were tested by the Kirby-Bauer method for antimicrobial susceptibility, utilizing commercially available sensitivity discs. **Results:** All bacteria tested were aerobic Gram-negative bacilli and were sensitive to amikacin, ceftazidime, chloramphenicol, trimethoprim /sulfamethoxazole and gentamicin. Resistance was observed to cephalotin (87.5%), ampicillin (37.5%), amoxicilin/clavulanic acid (25%) and tetracycline (18.8%), while the snakes isolates presented resistance to cephalotin (50.0%), tetracycline (36.4%) and ampicillin (31.8%). Only 2 *M. morganii* isolated from patients were resistant to ciprofloxacin and one sample to ceftriaxone. **Discussion:** These results show a variable antimicrobial susceptibility of aerobic Gram-negative bacilli. In order to choose the best antimicrobial therapy, it is important to know the predominant bacteria involved with infection and its antimicrobial susceptibility.
Supported by: FAPESP

2.17 Comparative study of different promoter systems for *in vivo* protein expression loaded in vaccinal *Salmonella enterica* sorovar typhimurium.

Guembes APS, Nakajima E, Martins EAL.
Centro de Biotecnologia, Instituto Butantan, São Paulo, Brazil.

Introduction: Live vaccines using recombinant attenuated pathogens expressing antigens of interest present the advantage of direct immunization avoiding expensive and laborious purification processes. In this view it is interesting to study control systems for expression heterologous protein *in vivo* in attenuated salmonellas. **Objectives:** To compare the *in vivo* expression systems soxRpsoxS, PnirB and PCMV based on pAE vector, carried by the vaccine strain *Salmonella* typhimurium SL3261, using the fragment C of tetanus toxin as report antigen (FC). **Methods:** The vectors pAEsox and pAEnir were constructed to clone genes of interest under control of the expression systems. The FC gene was inserted in these vectors, as well as in pAE and pCDNA, under control of T7 and CMV promoters respectively. Groups of mice were immunized ip with purified FC and with salmonella strains carrying the different vectors for *in vivo* expression. Titles of antibodies were measured and animals were challenged with DMM of the tetanus toxin. **Results:** Immunization with purified FC and SalpAEsoxFC induced higher titles of antibodies and 100% survival in challenge assay. Results with SalpAEnirFC and SalpCDNA-FC were not conclusive and some more data should be collected. **Discussion:** FC was expressed *in vivo* in the salmonella carrying the pAEsox-FC, in enough amounts to sensitize the immune system, conferring the same level of protection as the purified FC. Adjuvant effect of salmonella is being measured by IgG1 and IgG2a contents in the sera.
Supported by: FAPESP

2.18 Expression of Shiga toxin using different enrichment protocols

Rocha LB, Piazza RMF.
Lab Bacteriology, Instituto Butantan, São Paulo, Brasil.

Introduction: The Shiga toxins (Stx1 and Stx2) are a family of bacterial cytotoxins. While Stx1 is secreted into the milieu, Stx2 and its variants are known to be periplasmatic and not secreted into the medium, for this reason several enrichment protocols have been described for Stxs expression. Although, the data on which is the most suitable medium is divergent. **Objective and Methods:** In this study, we evaluated the toxin expression using as growth media: Luria Bertani (LB), Tryptic Soy Broth (TSB), Penassay broth, Brain Heart Infusion (BHI), *E. coli* broth (EC), Evans media, Peptone water and Syncase media. Stx1 and Stx2 expressing *E. coli* isolates were cultivated in the above mentioned media at 37^{o}C (250 rpm) for 24 h. Aliquots of 1 ml were taken every 30 min until 5 h, the absorbance was measured at 588 nm and aliquots were centrifuged at 13,000 x g for 15 min and supernatants obtained. Between 5 and 8 h, aliquots were taken in 1-h intervals and processed as described above. All supernatants were kept at –20^{o}C until use. Besides, for the Stx2 expressing isolate the pellet of each time was incubated with 0.1 mg/ml of polymixin at 37^{o}C (250 rpm) for 30 min and processed as described above. All supernatants were assayed by ELISA and immuno-dot using the polyclonal anti-Stx1 and Stx2. **Results and Discussion:** Stxs expression was variable amongst the tested media, but EC media was the most suitable for both toxin expression, besides this occurred in the mid-log growth phase. For Stx1 at least 1.5 h is necessary for its best expression, on the other hand for Stx2 the expression in the supernatant occurs in 3h, but toxin associated to the bacteria was detected in 1.5h. These results are important contributions in Shiga toxin expressing *E. coli* isolates detection.
Supported by: FAPESP and FINEP

2.19 Hofmeister ions serie protecting the Diphtheria protein damage during water/methylene chloride interface formed on PLGA microspheres encapsulation process.

Namur JAM[1], Takata CS[2] and Bueno da Costa MH[1].
[1]Lab. de Microesferas e Lipossomas – C. de Biotecnologia; [2]Divisão de Desenvolvimento Tecnológico e Produção-I. Butantan, Av. Vital Brasil, 1500, 05503-900 – Butantan, São Paulo, SP, Brasil.

Introduction: Water-in-oil-in-water emulsion technique is the mostly used to encapsulate water soluble proteins into biodegradable microspheres like poly (lactic-co-glycolic acid) (PLGA). It is known that the first emulsification step is the most deleterious to protein stability. This step (W1/O) is created by emulsifying protein aqueous solution within CH2Cl2 phase containing PLGA. In this ambient there is formed hydrophobic interfaces resulting in interfacial adsorption followed by protein unfolding and aggregation. **Methods and Results**: Intending maintain the protein structure in a more soluble conformation we investigate the influence of KSCN, NaH2PO4, NaCl and Cl_2Mg (salts of Hofmeister's ion serie) on solubilization and conformation of the diphtheria toxoid (Dtxd) after first emulsification step. The salts were added in different concentrations to protein solution during the primary emulsion process. The solubility was quantified by uv, KSCN was the best salt to Dtxd solubilization and Cl_2Mg the worst. The structure was analised by fluorescence spectroscopy, Cl_2Mg improve F350/F330 ratio demonstrating Dtxd structure loss, in the presence of KSCN the F350/F330 ratio was not altered. By HPLC Cl_2Mg demonstrated acting as a desorganizing agent on the protein structure and by ELISA KSCN or NaH_2PO_4 made an organizing effect on Dtxd structure reflected by a bigger antibody recognizing to Dtxd. **Conclusion:** The KSCN addition on Dtxd solution should be a good strategy to improve the protein stability on double-emulsification method to PLGA microspheres production.

2.20 Acellular pertussis vaccine using Pulmonary surfactant as adjuvant

Prestes AFRO[1], Horton DSPQ[1], Gebara C[1], Bugelli VC, Kubrusly FS[1], Iourtov D[1], Esteves MIQ[1], Leme W[1], Furuyama E[3], Sakauchi MA[3], Raw I[1] and Dias WO[1].
[1]Centro de Biotecnologia; [2]Seção de Controle de Qualidade; [3]Seção de Vacinas Aeróbicas - Instituto Butantan.

Introduction and Objectives: The adjuvant effect of a pig lung surfactant (PLS) was evaluated in the immune response of mice to an acellular pertussis vaccine (APV), comparatively with alum (AL). **Methods and Results:** The vaccine, mixed with the adjuvant, was given by intraperitoneal (i.p) route and elicited a very high protective effect, showing a dose response curve of survival according to decreasing concentrations of PLS (100, 90 and 50% of survival, respectively). The intranasal (i.n) immunization elicited 60% of protection using APV mixed with PLS, but only 28.6% with alum. It was not detected serum IgG antibodies anti-pertussis toxin (PT) in the animals injected i.n. After i.p immunization, similar serum antibodies titers anti-PT were obtained, using AL or PLS as adjuvants, either when PLS was injected mixed with the vaccine or simultaneously, by i.n route. The animals immunized i.p or i.n with APV plus PLS showed levels of serum IFNγ significantly higher than the induced by the adjuvant alone, suggesting a Th1 tendency of response, that was confirmed by lower ratio of IgG1/IgG2a in the i.p group, compared to AL used as adjuvant. **Conclusions:** Thus it would be interesting to consider other adjuvants, preferentially those able to induce Th2 cells, due to the side effects related to AL. The immunemodulation to Th1 response would simulate the protection elicited by native infection with *B.pertussis*.

Supported by: FAPESP

2.21 Antibody response to whole-cell pertussis vaccine in immunized Brazilian children

Pereira A[1], Pereira ASP[2], Silva CL[3], Rocha GM[3], Sant'Anna OA[2] and Tambourgi DV[2].
[1]Lab. Genética and [2]Imunoquímica, Instituto Butantan, São Paulo, Brazil; [3]Faculdade de Medicina, Universidade de São Paulo, Ribeirão Preto, Brazil.

Introduction: Various vaccine combinations have been developed, aiming to improve immunization programs and increase vaccine coverage. Antibody response in children may vary depending on the vaccination program and the product used. **Objective:** In this study, the antibody response of vaccinated children to different *Bordetella pertussis* strains and their virulence factors, *i.e*., pertussis toxin (PT), pertactin (PRN), and filamentous haemagglutinin (FHA) was analysed, after they had been vaccinated according to the Brazilian vaccination program. **Methodology:** Serum samples from male and female children, from three months to three years and three months old were collected in different periods of time after the completion of the immunization process and tested for presence of specific antibodies by ELISA. **Results:** The maximum peak of antibody response for all bacteria extracts and for PRN antigen occurred after four months and for PT and FHA antigens two months after the completion of the immunization schedule. **Conclusion:** This data indicate that Brazilian whole-cell pertussis vaccine is able to induce antibodies against the main virulence factors of *Bordetella pertussis*.

2.22 Production of *Bordetella pertussis* proteins by different culture conditions

Wolf PS[1], Lopes APY[1], Ferreira VRF[1], Sakauchi MA[2] and Cainelli Gebara VCB[1].
[1]Centro de Biotecnologia, [2]Seção de Vacinas Aeróbicas, Instituto Butantan, SP, Brazil.

Introduction: *B. pertussis* produces several immunomodulatory factors acting on the immune system of the host. The production of these factors can be influenced by the bacteria culture conditions. **Methods and Results:** Two proteins (B1 e B2) were purified by ion exchange chromatography and preparative SDS-PAGE, from a *B. pertussis* soluble fraction extracted from the bacteria growing in CW (Cohen-Wheeler) medium for 30h, heat-inactivated at 56^{o}C for 1 h, and concentrated by 4N citric acid precipitation. These two proteins were identified by mass spectrometry, and tested in mice for adjuvant and immunogenic activities. However, the conditions for bacteria culture were modified to improve the pertussis vaccine production. The present conditions were: bacteria growing for 20 h in SS (Stainer&Scholte) medium, inactivation by formaldehyde solution and concentration by tangential flow filtration. We aimed to compare the recovery of B1 and B2 proteins obtained from both culture conditions. The results (SDS-PAGE analysis and purification) have shown that after Q-sepharose chromatography B1 and B2 correspond to more than 70% of total protein in the former culture conditions and decrease to less than 5% in the later. **Conclusions:** Therefore, since the current conditions of bacteria growing are not satisfactory for the production of the proteins of interest, studies are now in progress to isolate their genes from genomic DNA, cloning and express the recombinant proteins in *E. coli*.
Supported by: FAPESP and Butantan Foundation

2.23 Tetanus Toxin Production in a Media Containing Peptone from Soya

Prado SMA[1], Guarnieri LS[1], Marchi AS[1], Fratelli F[2], Abreu ME[2] and Higashi HG[3].
[1]Serviço de Bacteriologia, [2]Seção de Vacinas Anaeróbicas, [3]Divisão de Desenvolvimento Tecnológico e Produção, Instituto Butantan, São Paulo, Brasil.

Introduction: Instituto Butantan produces large quantities of tetanus toxoid for associated vaccines production and anti-tetanus serum for human treatment. **Objective:** To analyse the growth of *C. tetani* in a media with absence of meat or dairy because it is possible that those components contain undesirable contaminants such as the prion causing BSE or antigenic peptides. In this way tetanus toxin produced in media Müeller e Miller modified by Latham containing peptone from soya in different concentrations was analysed versus the same media with NZ Case TT®. **Methodology:** Lyophilized cultures of *C. tetani* were grown in thioglycollate media. After that this culture was transferred and cultivated in the same conditions. The media containing different concentrations of peptones was dispensed in Erlenmeyers inoculated with of the culture and incubated. Every day samples from the cultures were collected and the toxin titers were determined in Lf/mL. **Results:** The best results had been obtained with the peptone from soya at 8.46%; 11.28% and 14.1%. The result with NZ Case TT® was 90 Lf/mL in the fourth day of culture. The results indicate to be possible the use of peptone from soya for production in large-scale. **Discussion:** The studies will continue to analyse peptones of soya with different specifications and manufactures. Evaluating the tetanus toxin destoxification and the purification of the final product will be necessary in order to attend WHO guidelines for vaccine production.
Supported by: Butantan Foundation

2.24 Evaluation of immune response and protection against pneumococcal challenge in wild type and IL-4 knockout mice immunized with recombinant proteins or DNA vaccines.

Ferreira DM, Darrieux M, Leite LCC, Miyaji EN.
Centro de Biotecnologia, Instituto Butantan - São Paulo – SP

Introduction and Objectives: Several proteins have been investigated as vaccine candidates against infection with *S. pneumoniae*, including pneumococcal surface protein A (PspA) and pneumolysin (Ply). These proteins have been shown to be immunogenic and protective in several animal models. Our previous results suggested that anti-PspA IgG2a antibodies have an important role in complement deposition and protection against intraperitoneal challenge with *S. pneumoniae* in mice. This evidence was reinforced by the fact that animals immunized with recombinant protein and DNA vaccine had similar levels of protection, despite the lower induction of total anti- PspA antibodies by the last group. **Methods and Results:** We have compared the immune response elicited by the recombinant proteins PspA and Ply or DNA vaccine (pSec-*pspA*) expressing the first protein in wild-type (WT) and IL-4 knockout mice (KO). IL-4 KO mice showed increased total antibody levels against both antigens: four to six fold for anti-PspA and fivefold for anti-Ply. We have also evaluated the IgG1/IgG2a ratio of these antibodies and observed that IL-4 KO mice induced significantly higher IgG2a production in all immunized groups. Preliminary results of protection showed high survival rates in immunized groups up to 100% in IL-4 KO mice. **Conclusion:** IL-4 KO mice immunized with pneumococcal antigens as recombinant protein or DNA vaccine show preferential induction of IgG2a antibodies and survival levels similar to WT mice after intraperitoneal challenge with *S. pneumoniae*.
Supported by: FAPESP

2.25 Anti-PspA antibodies: functional aspects and their role in protection against pneumococcal infections in mice

Darrieux M[1], Miyaji EN[1], Mirza S[2], Ren B[2], Hollingshead S[2], Briles D[2], and Leite L[1].
[1]Centro de Biotecnologia-Instituto Butantan,São Paulo . [2]University of Alabama – EUA

Introduction: *S. pneumoniae* is a major cause of pneumonia, meningitis and sepsis. Among the vaccine candidates against this pathogen is Pneumococcal Surface Protein A (PspA), an exposed protein present in all pneumococcal strains, and protective against sepsis in animal models. Due to its structural diversity, an effective PspA based vaccine should include one fragment from each of the two major families (1 and 2). PspA is able to prevent pneumococcal killing by the bactericidal protein lactofferin. Also, PspA interacts with complement C3b, avoiding phagocytosis of the bacteria. **Objective:** The aim of this study was to establish a relationship between the ability of anti-PspA antibodies to abrogate PspA's functions and their protective role against *S. pneumoniae* infections in mice. **Methodology:** We have produced PspA chimeras - containing fused fragments from families 1 and 2 - which were used to immunize BALB/c mice. Sera from the animals were tested for their ability to interact with PspA on the bacterial surface. Anti-PspA antibodies led to an increase in complement deposition on virulent pneumococcal strains bearing PspAs from both families, in FACS analysis, as well as an increased death of different pneumococcal strains in more than one log in presence of apolactoferrin. After intravenous challenge with pneumococcal strains bearing diverse PspAs, the immunized mice showed up to 100% protection.
Supported by: FAPESP and CAPES

2.26 Whole cell pneumococcal vaccine using porcine lung surfactant as adjuvant protects mice against pneumococcal nasopharingeral colonization.

Silva APGB[1], Sbrogio-Almeida ME[1]; Liberman C[1], Takagi M[1], Maimoni Gonçalves V[1], Dmitri Iourtov[1], Kubrusly FS[1], Malley R[2], Anderson P[3], Leite LCC[1], Dias WO[1].
[1]Instituto Butantan – Centro de Biotecnologia; [2]Childrens Hospital Boston and Harvard Medical School; [3]University of Rochester, NY.

Introduction and Objectives: We studied the adjuvant effect of an exogenous natural surfactant from minced porcine lungs, in the humoral immune response and protection of mice, elicited by a killed whole cell pneumococcal vaccine derived from a nonencapsulated mutant of *Streptococcus pneumoniae* (originally a serotype 2 strain, autolisine negative, and carrying a pneumolisin defective gene). **Methods and results:** The vaccine was administered intranasally mixed with pig lung surfactant and elicited significantly higher serum IgG antibodies, evaluated by ELISA against vaccine antigens, when compared with the vaccine alone (IgG antibodies titers 293 and 136, respectively; Mann Whitney p= 0.04). When injected intraperitoneally, the vaccine with surfactant induced significantly higher serum IgG antibodies when compared with the surfactant alone (IgG antibodies titers 720 and 186, respectively; Mann Whitney p= 0.0007). This preparation administered intranasally showed a protective effect in mice, against an intranasal challenge with a capsulated pneumococci (strain 603/serotype 6B), significantly reducing the nasopharyngeal colonization, in relation to the surfactant alone (group immunized with Vaccine+surfactant = 2125 CFU/ml and with surfactant = 10,150 CFU/ml; Mann Whitney p = 0.0033). **Conclusions:** Taken together, our results are promising, and show a new perspective for adjuvants and pneumococcal vaccines.

2.27 Development of conjugate vaccine for capsular polysaccharide serotype 23F and PspA

Csordas FCL[1], Perciani CT[1], Sbrogio-Almeida ME[2], Cabrera-Crespo J[2], Maimoni Gonçalves V[2], Takagi M[2], Raw I[1],Tanizaki MM[1].
[1]Laboratório de desenvolvimento de processos- Centro de Biotecnologia- Instituto Butantan; [2]Laboratório de Fermentação – Centro de Biotecnologia- Instituto Butantan, São Paulo Brasil.

Introduction: In the elderly and children under five years old, *Streptococcus pneumoniae* has been one of the major causes of death by bacterial infection. **Objectives:** In this study, the pneumococcal capsular polysaccharide serotype 23F (PS23F) has been conjugated to pneumococcal surface protein A (PspA) to overcome PS23F thymus-independent nature. **Methods and Results:** Conjugates quantity reduction and vaccine coverage increase is the aim of using PspA. Two different conjugation processes have been used. In both, PS23F has been partially hydrolyzed and its hydroxyl groups oxidized to form aldehyde groups. On reductive amination method, the PS23F hydroxyl groups have reacted with the PspA lysine residues through Schiff base formation and reduced with sodium borohydride. On Hydrazone intermediate method, PS23F hydroxyl groups have reacted with adipic acid dihydrazyde (ADH) which reacted with carboxyl residues of PspA in the presence of carbodiimide. BALB/c mice groups have been immunized with the conjugates and the immunologic response to PspA and PS23F evaluated by ELISA. After the third dose the animals were challenged with serotype 6 pneumococcus virulent strain expressing homologous PspA. Groups immunized with conjugates have not shown significantly higher antibody titers against PS23F than free PS23F groups. Groups immunized with hydrazone method conjugates have shown significantly higher protection than those immunized with reductive amination method conjugates, PS23F and PspA. **Conclusions:** Researches to develop serotype 6B conjugates are going to be initiated.
Supported by: CNPq, and FAPESP

2.28 Cloning, expression and purification of truncated and hybrid fragments of pneumococcal surface protein A (PspA) of *Streptococcus pneumoniae* and immune response evaluation.

Lopes LM[1,2], Bertoncini MDS[1,2], Miyaj E[1], Leite LCC[1] and Lopes APY[1]
[1]Centro de Biotecnologia, Inst. Butantan, SP; [2]Pós graduação Interunidades em Biotecnologia – USP.

Introduction: *S. pneumoniae* is the bacterium that causes pneumonia. Current vaccine consists of capsular polysaccharide of 23 different strains. PspA is a virulence factor of the bacteria and is a potential vaccine candidate. PspA is divided on 3 families and 6 clades, which results in serological differences and consequently suggest the inclusion of PspAs of families 1 and 2. **Objective:** The present study describes the production of PspA 1-3 (clade 1 and 3), and the protection induced by PspA 1, 3 and 1-3 in immunized BALB/c mice against the pathogenic clade 3 strain of *S. pneumoniae* 679/99. **Methodology:** PspA genes were isolated from genomic DNA. The hybrid contains the main immunogenic epitopes linked by the prolin rich region. Fragments of clades 1 and 3 were ligated using the *Xba* I site introduced by PCR in the pGEMT easy vector and than linked in the expression vector pAE-6xHis between *Xho* I and *Kpn* I sites. Proteins were expressed in *E. coli* BL21-DE3 and purified by chelating chromatography. **Results:** The genes were amplified, cloned and expressed. Recovery of the recombinant proteins was about 27 mg/L of culture. These proteins showed protection of mice against the challenge, with survival rates of 33% for PspA 1, 67% for PspA 1-3 and 83% for PspA 3. ELISA assay has demonstrated that B region of α-helix exposed region is the main immunogenic region. **Discussion:** These are expected results showing a good protection for family 2 and a partial protection for family 1, and corroborate with our previous studies on serum cross reactivity among families.
Supported by: FAPESP

2.29 Complement C3 deposition on the surface of *Streptococcus pneumoniae* mediated by anti-PspA antibodies produced in mice after immunization with recombinant *Lactobacillus* strains expressing PspA antigen

Campos IB, Oliveira MLS, Aires KA, Darrieux M, Ferreira DM, Miyaji EN, Leite LCC, Ho PL.
Centro de Biotecnologia, Instituto Butantan, São Paulo, SP, Brazil.

Introduction: *Streptococcus pneumoniae* is an important respiratory pathogen that causes pneumonia, meningitis, otitis media and bacteremia. A currently used vaccine, composed of capsular polysaccharides (PS) derived from 23 different serotypes, has little efficacy in young children, elderly and in patients with immunodeficiencies. PS-protein conjugate vaccines are more effective, but their production is expensive for widespread use. Moreover, the increase in antibiotic-resistant *S. pneumoniae* clinical isolates supports the development of new and more effective vaccines. The use of live recombinant lactic acid bacteria (LAB) as antigen delivery systems represents a promising strategy for mucosal vaccination, since they are generally regarded as safe bacteria able to elicit both systemic and mucosal immune responses. **Objectives and Methods:** In this work, the Pneumococcal Surface Protein A (PspA), an important virulence factor from *S. pneumoniae* was cloned in LAB constitutive expression vectors. Recombinant LAB were able to express PspA intracellularly, as analyzed by Western-blot using polyclonal antibodies produced against recombinant PspA purified from *E. coli.* Recombinant *Lactobacillus casei* and *Lactobacillus helveticus* were administrated in mice intranasally, and they were able to induce anti-PspA IgG in the sera. **Results and Conclusions:** These antibodies were able to bind to two different pneumococci strains and to increase the deposition of complement on the surface of pneumococci, as analysed by flow cytometry.
Supported by: FAPESP, CNPq and Butantan Foundation

2.30 Expression of pneumococcal surface protein C (PspC) in lactic acid bacteria.

Rodrigues FB[1], Ferreira PCD[1,2], Ho PL[1,2], Oliveira MLS[1].
[1]Centro de Biotecnologia, Instituto Butantan, São Paulo, SP, Brazil; [2]Interunidades em Biotecnologia, Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, Brazil.

Introduction: *Streptococcus pneumoniae* is the major agent of pneumonia around the world, causing up to 1 million deaths per year, mainly in developing countries. The high costs with medical care and the appearance of new clinical isolates that are multidrug resistant led to the search for new efficient vaccines to prevent pneumococcal infection. Since the pathogen enters the host through the respiratory mucosa it is desirable that the vaccine induces the production of protective secretory IgA at this site, as well as systemic antibodies. In fact, antibodies produced against pneumococcal antigens were shown to protect mice against colonization and systemic infections. Lactic acid bacteria are commensal microrganisms present in the gastrointestinal mucosa of health individuals that have been used as carriers for antigen presentation in different models. **Methods and Results:** In this work, the gene encoding the PspC antigen from *Streptococcus pneumoniae* was cloned in constitutive lactic acid bacteria expression vectors. *Lactococcus lactis* and *Lactobacillus casei* were transformed with the recombinant vectors and PspC expression was detected in both bacteria either intracellularly or attached to the cell wall, as evaluated by Western-blot. **Conclusions:** The recombinant bacteria will be tested in mucosal immunization experiments in mice.
Supported by: FAPESP and Butantan Foundation

2.31 Expression of recombinant pneumococcal surface A1 protein (fPspA1) of *Streptococcus pneumoniae* in *Escherichia coli* and its purification.

Silva MR, Vidigal PG, Pinto JV, Gonçalves VM, Takagi M and Cabera-Crespo J.
Centro de Biotecnologia – Instituto Butantan.

Introduction: *Streptococcus pneumoniae* is a bacterium that causes pneumonia, bacteremia and meningitis. PspA is a surface protein produced by *S.pneumoniae* considered as a vaccine candidate. cDNA encoding the fragment pspa1 was cloned in pET37b^{+} vector and expressed in *E. coli* BL21DE3. On the industrial production point of view of recombinant protein, it is important to achieve high cell density in order to maximize the volumetric productivities of bacterial cultures resulting in low cost of the process. **Objective**: The purpose of this study was to achieve high production of protein recombinant (fPspA1) growing *E. coli* in complex media using 3 different carbon sources and set up the purification process. **Methods:** The experiments were carried out on bioreactor of 5-10 L with 2YT/HDF and RSB media and glucose, glycerol and fructose as C sources. For the protein extraction, the final cultures of each fermentation run were harvested, centrifuged or microfiltrated; the cell pellet was ressuspended in buffer, disrupted by high pressure. This lysate was centrifuged and the supernatant was microfiltrated. The clarified sample was applied in Q and Ni^{2+} Sepharose column. **Results**: The ($O.D._{600}$) in different C sources were: 43, 38 and 58, for glucose, glycerol and fructose, respectively. The purified protein from 7L of culture was ~39 mg/L culture after Ni^{2+} column followed by Q-Sepharose. **Discussion:** The higher cell density was obtained when used fructose as source of carbon in complex media RSB which resulted in high expression of PspA1 when visualized by SDS PAGE. In relation to purification of this protein, more attention must be dedicated in order to establish the best purifications steps with high yield and purity.
Supported by: FAPESP

2.32 Purification of the capsular polysaccharide produced by *Streptococcus pneumoniae* serotype 6B.

Pinto JV, Goncalves VM, Carmo TS, Cabrera-Crespo J and Takagi M.
Laboratório de Desenvolvimento de Processos, Centro de Biotecnologia, Instituto Butantan, São Paulo.

Introduction*: Streptococcus pneumoniae* is an encapsulated Gram-positive pathogenic bacterium that causes pneumonia, bacteraemia and meningitis. The capsular polysaccharide (PS) is the main virulence factor produced by this bacterium and it is used as an antigen in both current vaccines: 23-valent PS pneumococcal and hepta-valent PS pneumococcal conjugated to a carrier protein. **Objective:** The purpose of this work is to purify the capsular polysaccharide from *S. pneumoniae serotype 6B* following the protocol used to purify the PS from *S pneumoniae* serotype 23 F..**Methods:** The steps used to purify PS follows: microfiltration, supernatant concentration (cut-off 100 kDa), two ethanol precipitations steps, centrifugation, enzymes treatment and ultrafiltration (cut-off 100 kDa). **Results:** The final PS recovery was around 50% and the overall purification factor achieved in relation to protein and nucleic acid was 500 times. **Discussion:** The results have shown that is possible to purify PS from *S. pneumoniae* serotype 6B following the same protocol used to purify PS from *S pneumoniae* serotype 23F, however the last step (tangencial ultrafiltration) must be improved in order to increase the final PS yield.
Supported by: FAPESP

2.33 *Streptococcus pneumoniae* serotype 6B: cultivation under anaerobic and aerobic atmospheres.

Carmo TS, Antonio AP, Gonçalves VM, Takagi M, Tanizaki MM.
Centro de Biotecnologia – Laboratório de Fermentação – Instituto Butantan, São Paulo, Brazil

Introduction: *Streptococcus pneumoniae* is an aerotolerant microorganism and it is the leading agent of upper and lower respiratory tract infections. The ability to colonize and survive in such different environments requires the regulation and synthesis of some key surface structures, and that is correlated to a spontaneous switching or phase variation between two phenotypes: transparent and opaque. All this study was based on a previous study with *S. pneumoniae* serotype 23F developed in the laboratory. **Objective**: In the present study we investigated how changes in the atmosphere of cultivation could result in an increase of capsular polysaccharide (CPS) in the culture supernatant with cell viability maintained at high levels. **Materials and Methods:** *S. pneumoniae* serotype 6B, strain 433/03. The medium composition and batch cultivation were previously described. BioFlo 3000 and 2000 reactors were used to develop the experiments under different conditions. A cell-recycling membrane system appeared to be an alternative way to produce high amount of CPS. **Results**: Three different atmospheres were employed to cultivate the microorganism: nitrogen-sparged, nitrogen-sparged followed by air in the stationary phase and aerobic condition at low O_2 tension (N_2 + air). Higher growth rates were obtained in the cell-recycling system. The presence of air in the atmosphere caused cellular stress and lyses. **Discussion:** The serotype 6B presented different growth rate (biomass and OD600nm) and CPS production when compared with 23F at the same conditions. As an alternative, a cell-recycling reactor maintained the viability and tended to produce high amount of CPS.
Supported by: Fapesp

2.34 Capsular polysaccharide purification from *Haemophilus influenzae* type b using Gel Filtration Chromatography step.

Albani SMF, Takagi M, Pinto JV, Gonçalves VMM, Sbrogio-Almeida ME and Cabrera-Crespo J.
Centro de Biotecnologia- Laboratório de Fermentação- Instituto Butantan, São Paulo, Brasil.

Introduction: *Haemophilus influenzae* type b pathogenic Gram-negative bacterium is responsible for respiratory tract infections and for causing meningitis and pneumonia, especially in children younger than 5 years old and elderly people. The capsular polysaccharide (Hib) from this microorganism is considered the most important virulence factor and currently is purified, conjugated to a protein and used as vaccines against *Haemophilus*. The classical purification processes of these polysaccharides are very complex, including many ethanol precipitations, organic solvents extraction and centrifugation/ultracentrifugation steps. Recently, DNAse, proteinase and tangential ultrafiltration have been used to replace the organic solvents treatment to obtain the purified polysaccharides. However, the ethanol precipitation is still the bottle-neck in these processes because it increases considerably the work volume including 3 centrifugation steps which make the operation process difficult, resulting in low polysaccharide recovery. **Objective:** To assess if gel filtration chromatography (GF) could replace the ethanol precipitations steps, in order to develop a simple, efficient method that could be scaled-up and, consequently, reduce the operation cost. **Methodology:** This technique with a Sephacryl S 500 allowed the separation of polysaccharide from nucleic acids and KDO with a Kd =0.78; Kd =1.04 and Kd=1.24 respectively. Proteins diffuse in this chromatography and it do not produce separated peaks. **Results:** The media of purification of polysaccharides/ nucleic acids was 2 fold and for polysaccharides/ proteins was 2.5 fold. **Discussion:** The addition of magnesium did not change the resolution of the process. Further studies are ongoing to improve the resolution of separation of these biomolecules.

2.35 Optimization of *in vitro* toxin binding inhibition (TOBI) test for estimation of botulinum antitoxin

Souza AS[1], Colombini M[3], Oliveira JM[1], Fratelli F[2], Prado SMA[2], Higashi HG[4] and Trezena AG[1]
[1]Seção de Vacinas Anaeróbicas, [2]Serviço de Bacteriologia, [3]Serviço de Controle de Qualidade, [4]Divisão de Desenvolvimento Tecnológico e Produção, Instituto Butantan, São Paulo, Brasil

Introduction: A method was developed as alternative to the *in vivo* toxin neutralization assay (TNA) for titration of botulinum antibodies *in vitro*. The method *in vitro* was based on toxin binding inhibition test (TOBI) in which the detection antibody was labeled with peroxidase (data previously shown). **Objective:** In order to optimize the method, the antibody was biotinylated and the assay was developed with streptoavidine-peroxidase. This study evaluated titers of botulinum type A antitoxin in the sera of horses by the optimized TOBI and by TNA and correlated the results. **Methods:** For TOBI, microplates are blocked with PBS/BSA 2% solution. After blocking, different dilutions of standard botulinum antitoxin and of horse sera were incubated overnight with the botulinum anatoxin type A (1.0 mg/mL). The next day, these samples were transferred to a second plate previously coated with 27.7 mg/mL of botulinum antitoxin in order to bind the remaining anatoxin that was detected using a biotinylated antitoxin. The titers of the sera were calculated using a linear regression plot of the standard antitoxin. For TNA assay, L+/10 dose of botulinum toxin combined with different concentrations of horse sera were inoculated in mice. The titers were calculated as the lowest dilution of sera that kills 100% of the animals. **Results:** The linear regression plots of the results showed high correlation coefficient (r^2=0.98) between the *in vitro* optimized TOBI and *in vivo* assay. **Discussion:** The new method allows the evaluation of the botulinum antiserum potency in production process minimizing the use of animals.
Support by: Butantan Foundation

2.36 Variations on fermentation conditions affect C*lostridium botulinum* type A growth and toxin production

Oliveira JM[1], Colombini, M[2], Sonobe MH[3], Fratelli F[3], Prado SMA[3], Raw I[4], Higashi HG[5] and Trezena AG[1].
[1]Seção de Vacinas Anaeróbicas, [2]Serviço de Controle de Qualidade, [3]Serviço de Bacteriologia, [4]Centro de Biotecnologia, [5]Divisão de Desenvolvimento Tecnológico e Produção, Instituto Butantan, SP, Brasil.

Introduction: *Clostridium botulinum* is an anaerobic Gram-positive bacterium whose 7 main types, A to G, are characterized by antigenically distinct neurotoxins. Toxin type A was used as therapeutic agent in this study. **Objective:** To analyze the growth and toxin production of *C. botulinum* type A strain ATCC25763, the bacteria were cultivated in a 50 L fermentor using different culture media. The effects of nitrogen or oxygen overlays on toxin production and growth were analyzed. **Methods:** Samples were taken every 4 hours. Growth was measured by determination of optical density of cultures, and toxin concentration was obtained from mouse bioassays. **Results:** Bacterial cultures with NZ Amine® medium attained maximum bacterial growth in a period of 20 to 24 h of culture and maximum toxin concentration in 40 to 44 h. Nitrogen overlay increased the toxin production compared to oxygen overlay but did not modify the temporal profile of toxin production. Proteose-Peptone cultures showed maximum growth within 24 to 28 h. The maximum toxin concentration was obtained in 36 to 40 h. The nitrogen overlay did not alter the toxin production. **Discussion:** Our results showed that toxin concentration determined for Proteose-Peptone medium were about fives times higher than that of NZ Amine®cultures with nitrogen overlay. We observed very similar data during the experiments performed with Proteose-Peptone in a period of two years indicating consistency of the production process.
Supported by: Butantan Foundation

2.37 Production of lyophilized ONCO BCG

Curto EM[1], Guilhen GB[1], Vancetto MDC[2], Prado SMA[2], Higashi HG[3] and Hiss H[1].
[1]Seção de Vacinas Aeróbicas – BCG, [2]Serviço de Bacteriologia, [3]Divisão de Desenvolvimento Tecnológico e Produção, Instituto Butantan, São Paulo, Brazil.

Introduction and Objectives: The lyophilized Onco BCG product is directly sold to the population by Butantan Institute, by medical prescription for bladder carcinoma treatment, as intravesical therapy. Until 12th August 2005 it was presented in liquid form, in small flasks containing 5 mL (40 mg of bacillar mass and viability between 2 and 10 x 10^6 CFU/mg). Due to the short expiration period of only 40 days, the production cost was considerable, since about 37.7% of the produced doses were discarded, because of product expiration. In addition to these costs there are troubles to patients, since the treatment employs one Onco BCG dose per week, during 6 weeks. So, it was recommended a two times withdrawal during the treatment of the medicament from Butantan Institute (3 flasks each time). **Methodology and Results:** Since 30th of August 2005, the product is disposed in lyophilized form, where each ampoule-flask contains the same concentration of bacillar mass and viability. The expiration period however had been expanded to 4 months which brought an economy in treatment, considering that similar imported have high costs (about U$ 500 per complete treatment). **Conclusion:** We have already surpassed a total of 30 lots, produced in lyophilized form since August 2005, performing the social function of Butantan Institute.
Supported by: Butantan Foundation

2.38 Comparison between the ONCO BCG production by submerged and static cultivations.

Curto EM[1], Guilhen GB[1],Silva PPF[1], Morales RA[1], Vancetto MDC[1], Prado SMA[2], Higashi HG[3], Hiss H[1]
[1]Seção de Vacinas Aeróbicas-BCG, [2]Serviço de Bacteriologia, [3]Divisão de Desenvolvimento Tecnológico e Produção, Instituto Butantan, São Paulo, Brazil.

Introduction: The Onco BCG is a prophylactic product, immunostimulant, used in patients with relapse of bladder cancer after transurethral desiccation and treatment of this carcinoma *in situ*. **Objective:** The Butantan Institute interest, on the production of such immunotherapic, by submerged cultivation is: to enhance its viability, increase the final amount of biomass and reduce the size of bacterial clumps, without further dispersion by steel balls. **Methodology:** The Onco BCG production by static cultivation, offered to the patients approximately 1,960 flasks with 5 mg BCG in a liquid suspension of 8 mg/mL. To obtain such a quantity, 300 erlenmeyers were necessary, each with 80 mL of IVM medium incubated during a period of 11 days under static condition, in a room-stove; in contrast, for the achievement of the same production by submerged cultivation, only 50 erlenmeyers are employed as inoculum to ferment a 20 liter quantity containing 10 liters of inoculated IVM medium. **Results:** The final biomass concentration of 19.6g/L, obtained by submerged cultivation in the fermenter, compared to 3.26g/L, obtained by the classical method, provides a culture medium economy of 19.6/3.26=6.0 times. **Conclusion:** In addition to such economy the biomass obtained in the fermenter does not need further steel ball dispersion. An additional advantage is in fermenter, parameters, such as agitation, aeration and pH, may be controlled, as well as less manipulation of the product by the operators.
Supported by: Butantan Foundation

2.39 Determination of the spectrophotometer calibration factor, based on dry biomass, for the preparation of the BCG vaccine,

Ghion GB[1], Evaristo JA[1], Vancetto MDC[2], Prado SMA[2], Higashi HG[3] and Hiss H[1].
[1]Seção de Vacinas Aeróbicas – BCG, [2]Serviço de Bacteriologia, [3]Divisão de Desenvolvimento Tecnológico e Produção, Instituto Butantan, São Paulo, Brazil.

Introduction: Considering bacterial content results and CFU (Colony Forming Units), it was verified that the amount of dry biomass in each lyophilized BCG ampoule was lower than 1 mg. **Objective**: To obtain a product with 1 mg of bacterial biomass per ampoule, containing 10 vaccine doses in which each dose corresponds to 0.1 mg BCG. **Methodology**: Cultivations of *M. bovis* in Sauton medium were filtered to obtain a damp cake, a procedure used for the routine production of BCG vaccine. After weighing, the cake was dried in a moisture analyser with infra-red heating. The relation between dry and moist biomass was multiplied by the spectrophotometer calibration factorbased on moist biomass, converting it to dry biomass. **Results**: Vaccine lots produced using the calibration factor based on dry biomass, presented higher bacterial contents and CFU than lots produced with the factor based on moist biomass. **Discussion**: Our results verified that those lots that had been produced, and measured with calibration factors based on moist biomass, presented low values of bacterial content and CFU since the water presence interferes with the weighing procedure. Since then we have been using this factor based on dry biomass for the vaccine dilution.
Supported by: Butantan Foundation

2.40 Supression of asthma-like responses by recombinant BCG is not associated with increase influx of regulatory T cells to the lung in a murine model of allergic inflammation

Christ APG[1,2], Rodriguez D[2], Keller AC[2], Leite LCC[1], Russo M[2].
[1]Centro de Biotecnologia, Instituto Butantan, SP, [2]Departamento de Imunologia, USP, São Paulo, SP.

Introduction: We have previously shown that recombinant BCG (rBCG) expressing the subunit 1 of pertussis toxin (S1PT) was able to suppress asthma-like responses in a murine model of allergic inflammation. **Aim:** Here, we determined whether suppression of asthma-like responses by rBCG-S1PT is associated with regulatory T cells (Treg). **Methods:** BALB/c mice were s.c immunized at days 0 and 7 with OVA/Alum and i.n challenged with 50ug of OVA at days 14 and 21 (allergic group). rBCG-S1PT group was inoculated with i.n 10^6 CFU of rBCG-S1PT after OVA sensitization and after 30 days was challenged with i.n OVA. Control group received only PBS. Lung and Bronchoalveolar lavage (BAL) cells were collected and FACS analysis performed for the following cell markers: CD4, CD8, CD25, CD45RB, FoxP3 and IFN-γ. **Results** We found that $CD4^+CD25^+CD45RB^{low}$ T cells in the BAL and lung were higher in allergic mice than in the control or the rBCG-S1PT group. Moreover, $CD4^+CD25^+FoxP3^+$ T cells in the BAL were remarkably reduced in the rBCG-S1PT group (6,4%), when compared to the allergic group (11,1%). We also observed an increased proportion of IFN-γ-producing $CD4^+$ T cells in the lungs of the rBCG-S1PT group (0,74%), when compared to the allergic (0,28%) and control (0,034%) groups. **Conclusion:** rBCG suppression of asthma-like features is not due to the recruitment of regulatory T cells. IFN-γ production by lung $CD4^+$ T cells appears to play an important role in down modulation of allergic responses in this model.
Supported by: FAPESP and Butantan Foundation

2.41 Immune response to BHK-21 cell rabies vaccine produced at Instituto Butantan in bovines

Frazatti-Gallina NM[1], Mourão-Fuches RM[1], Ventini D[1], Reis LS[2], Arenas SE[2], Pardo PE[2].
[1]Seção de Raiva, Instituto Butantan, São Paulo, SP; [2] Universidade do Oeste Paulista - UNOESTE, Presidente Prudente, SP, Brazil.

Introduction: A new rabies vaccine for veterinary use hás been developed at Butantan Institute. **Objective and Methods:** This vaccine was obtained from BHK-21 cell cultures infected with a PV virus strain and purified by chromatography, inactivated with β-propiolactone and presented in freeze-dried vials. In this study the immunogenicity of this vaccine was evaluated in Nelore bovines. Thirty-six animals were vaccinated with one dose of the Institute's vaccine (Batch IB-01/03 with 1.7 IU/mL). Blood samples were drawn on days 74 and 150 to determine the neutralizing antibody titers in BHK-21 cells (RFFIT). In these tests, the 2nd International Standard for Rabies Immunoglobulin was used as reference and CVS-11 virus strain for the neutralization. **Results and Discussion:** The mean neutralizing antibody titers obtained on days 74 and 150 after immunization were 3.0 IU/mL and 1.6 IU/mL, respectively and only two of the animals presented antibody titers <0.5 IU/mL. The results obtained showed that the BHK-21 vaccine developed at the Butantan Institute induced a satisfactory immune response in 94% of the animals immunized with only one dose of vaccine.

2.42 Influence of different serum-free media in the rabies virus production

Fujimura SH, Takinami VH, Botelho CRM, Frazatti-Gallina NM.
Seção de Raiva, Instituto Butantan, São Paulo, SP, Brasil

Introduction: Vero cell culture is an important substrate in rabies vaccine production. The fetal calf serum or human albumin used in culture media for cell growth can contain prion contamination. **Objectives**: The use of serum-free medium in this culture is a new strategy for obtaining high purity product. **Methodology and Results**: In this study, the influence of three different serum-free medium in rabies virus production were evaluated: VP-SFM AGT (GIBCO), Ex-Cell™ (Sigma) and HyQ®SFM4MegaVir™ (HyClone). Vero cells (1.6 $x10^6$ cells/flask) were infected with PV rabies virus (MOI= 0.008) and maintained in serum-free media at 34ºC for 11 days. Samples of the supernatants of the cultures were taken every 24h to determine rabies virus titers in BHK-21 cells. The results obtained in the kinetic studies in rabies virus culture infected, maintained in VP-SFM AGT, Ex-Cell and HyQ SFM4MegaVir were $10^{3.8}$–$10^{4.8}$; $10^{2.0}$–$10^{4.6}$ and $10^{2.6}$–$10^{3.7}$ FFD_{50}/0.05ml, respectively. The yield index (virus production x cells growth) obtained during all the experiment were similar in the cultures with VP-SFM AGT (6.08) and Ex-Cell (7.08) media. The values encountered with the HyQ SFM4MegaVir medium were lower than the others (0.48). **Discussion**: The results showed that the VP-SFM AGT and Ex-Cell media are recommended for rabies virus production.

2.43 Vaccination-acquired resistance to murine hepatitis virus is reversed by iNOS inhibition

Tsuhako MH[1] Augusto O[2]; Linares IE[2] and Pereira CA[1].
[1]Laboratório de Imunologia Viral, Instituto Butantan, [2]Instituto de Química, Depto. de Bioquímica, USP.

Introduction: We have previously shown that early and sustained •NO synthesis is important for the control of MHV-3 infection. **Methods and Results:** Relevantly, we report here that administration of the selective iNOS inhibitor, 1400 W, to immunized-resistant mice returned mortality to levels similar to those of non-immunized mice, that are, 63% (A/Ji, n = 8) and 67% (BALB/ci, n = 6) 4 to 6 days after infection respectively. Inhibition of •NO synthesis by 1400 W treatment was detected early by EPR (Electron paramagnetic resonance) 24 h.p.i. and the concentration of nitrosyl complexes was estimated by double integration of EPR spectra using known concentration of tempol as standard. A decrease about 40% and 50% of hemoglobin-nitrosil complexes production in blood was observed in A/Ji mice strain (1.55×10^8 μM by treated mice versus 2.53×10^8 μM by control mice) and BALB/ci mice strain (1.45×10^8 μM by treated mice versus 2.92×10^8 μM by control mice) respectively. It was also possible to detect viral replication in both treated mice strain. The viral titers found in the livers of A/Ji and BALB/ci by 96 h.p.i., 30 and 158 PFU/mg of liver respectively, were similar to titers detected on both control mice. In conclusion, our data strongly support a role for •NO in the development of resistance against MHV-3 infection, suggesting that nitric oxide donors may be a useful adjuvant therapy for controlling some viral infections. **Conclusion:** Further studies are in progress in our laboratory to clarify the mechanism by which •NO limits virus replication and liver injury.
Support by: FAPESP

2.44 Construction of DNA vaccines expressing the E7 oncoprotein from human papilomavirus 16 (HPV-16) genetically fused to *Salmonella enterica* FliCd flagellin.

Bibancos M[1], Cerqueira OLD[1], Szulczewski V[2], Cianciarullo A[2] & Ferreira LCS[1].
[1]Laboratory of Vaccine Development, Department of Microbiology, Institute of Biomedical Science, University of São Paulo; [2]Laboratory of Genetics, Instituto Butantan, São Paulo, Brazil.

Introduction: The human papilomavirus type 16 (HPV-16) is the main etiological agent of cervical cancer. A major drawback in the development of vaccine formulations targeting transformed cells is the reduced immunogenicity, particularly for T CD8+ responses, of the E7 oncoprotein required for HPV-mediated cellular transformation. **Objective:** The objective of the present study was de development of a DNA vaccine inducing enhanced inflammatory response and adjuvant effects toward the E7 protein. **Methodology:** For this purpose a DNA vaccine encoding the HPV-16 E7 protein genetically fused to the *Salmonella enterica* FliCd flagellin. The sequences encoding full length E7 or a deleted version of the protein, encompassing the first N-terminal 59 amino acids, were cloned in frame to the central hypervariable domain of FliCd flagellin, a strong inductor of the innate immune system. Two plasmid vectors encoding the recombinant protein in the cytoplasm of transfected cells or secreted into the extracellular medium, were employed. Transfected mammalian cells were analyzed by immunofluorescence using antibodies directed against flagellin and E7. **Results and Discussion:** The results indicated that all recombinant proteins were expressed and can be used in immunization trials against tumors induced by HPV-16 oncoproteins in animal models.
Supported by: CNPq and CAPES

2.45 Generation of specific mouse polyclonal antibodies to the human papilomavirus type 16 (HPV-16) E7 oncoprotein based on a recombinant protein expressed in *Escherichia coli*

Bibancos M[1], Porchia BFMM[1], Cerqueira OLD[1], Szulczewski V[2], Cianciarullo A[2], Ferreira LCS[1].
[1]Laboratory of Vaccine Development, Department of Microbiology, Institute of Biomedical Science, USP; [2]Laboratory of Genetics, Instituto Butantan, São Paulo, Brazil.

Introduction: Human papilomavirus type 16 (HPV-6) is the main etiological agent of the cervical cancer. Three oncoproteins (E5, E6 and E7) are involved with the neoplasic lesions leading to tumor development. The E7 oncoprotein interacts and inactivated with retinoblastoma suppressor protein (pRB), a tumor suppressing protein. **Objectives:** In this report we describe the expression and purification of an antigenic form of the E7 subunit (amino acids 1 to 59) produced in *Escherichia coli* BL21 strain host and the generation of E7-specific polyclonal antibodies. **Methodology and Results:** Protein expression was achieved in bacterial cells transformed with a derivative of pET28 and protein purification was carried out by affinity chromatography using columns filled with sefarose-niquel beads. The recombinant protein was recovered at a final yield of 1 mg of purified protein from 5 L of culture medium. The recombinant protein product was shown to induce high anti-E7 IgG titers in immunized mice. The recombinant protein preserved epitopes recognized by commercially available E7-specific monoclonal antibodies, as demonstrated in western-blots, and bounded to the E7protein expressed by neoplasic cells. **Discussion:** These results demonstrate that the recombinant E7-derived peptide can be a useful tool either in the development of diagnostic tests and vaccine approaches against tumors induced by HPV-16.
Supported by: CNPq and CAPES

2.46 Expression of HPV-induced proteins in cervical carcinoma cell lines.

Szulczewski V[1], Porto APAGK[1], Troiano PER[1], Spring H[2], Trendelenburg M[2], Pelajo-Machado M[3], Beçak W[1], Armbruster-Moraes E[1], Cianciarullo AM[1].
[1]Laboratório de Genética, Instituto Butantan; [2]Biomedical Structure Analysis Group, German Cancer Research Center – DKFZ, Heidelberg, Germany; [3]Depto de Patologia, IOC, FIOCRUZ,RJ, Brazil.

Introduction: Infection by the high-risk human papillomaviruses (HPVs) is a critical step in the genesis of cervical cancer, and nearly 99% of all cervical cancers contain and express HPV genomes. The viral oncogenes E6 and E7 are responsible for the infected cells transformation and immortalization. The E5 protein can augment cellular immortalization by E6/E7 via mechanisms that are not clearly defined. E5 expression appears to induce many of cellular changes, including enhanced growth factor signaling, the activation of mitogen-activated protein kinase pathways, and the alkalization of endosomes, which may contribute to alterations in endosomal trafficking. There is until now a lot of information at the molecular level, but not knowledge enough about the viral biology related to the host cells interaction. **Methodology and Results:** We are investigating if HPV can uses the ligation mechanism by the human transferrin receptor (TfR) to enter and infect cells, discharging the need of a specific receptor, not still found. Studies are being conducted in order to detect and quantify some genic products from transformed cells in comparison with non-transformed cells, aiming to clarity aspects triggered off by the pathogen-host cell interactions. **Conclusions:** Cervical carcinoma cell lines and non-transformed cells are being assayed by immunofluorescence methods. In preliminary results it was detected a great amount of TfR in a whole cell surface simultaneously the detection of E6/E7 oncoproteins at the cytoplam. Additional studies are being performed to get more information about the correlation of these findings.
Supported by: Butantan Foundation and FAPESP-Proc. 04/15122-5.

2.47 Screening the *Schistosoma mansoni* transcriptome for vaccine candidates by *in silico* selection and protection by DNA vaccines

Farias LP[1], Miyasato PA[1], Araújo BO[1], Tararam CA[1], Garcia JL[2], Kawano T[1], Verjovski-Almeida S[2], Leite LCC[1].
[1]Centro de Biotecnologia - Instituto Butantan, [2]USP Instituto de Química, Brasil.

Introduction: *Schistosoma mansoni* affects 200 million individuals in tropical regions of the world, being endemic in 74 countries, including Brazil. Vaccination would be the most cost-effective approach, but an effective vaccine against schistosomiasis is still to be established. **Methodology**: The *S. mansoni* EST Genome Project has generated 163,000 ESTs. This dataset might represent 92% of a total of 14,000 predicted genes of the parasite (Verjovski-Almeida *et al*., 2003). Automatic annotation based on the Gene Ontology system permitted the identification of proteins which could indicate some potential as vaccine candidates, such as toxins, surface receptors for cellular adhesion, surface proteins and enzymes, and receptors for host factors. We also selected genes up regulated in the lung stage. Due to 55% of the database having unknown functions, we selected delimitated ORFs containing a signal peptide sequence. **Results**: Twenty five selected cDNAs were cloned, either their full-length sequences or extracellular domains, and DNA vaccines were constructed. Mice were immunized and challenged with live cercaria. Reduction in worm burden indicated the level of protection. Six antigens showed significant protection (12-31%). **Discussion**: Characterization of the immune response induced against the respective antigens is being evaluated for correlation with protection. Thus the *in silico* selection of genes combined with screening by DNA vaccines has provided a first evaluation of the protective potential of a set of antigens as vaccine candidates.
Supported by: FAPESP, CAPES and CNPq.

2.48 Studies of three dyneins light chain from *Schistosoma mansoni*

Diniz PP[1], Rocha MO[1], Miyasato PA[2], Gatto, CGLF[1], Kawano T[2] and Martins EAL[1].
[1]Centro de Biotecnologia, [2]Laboratório de Parasitologia - Instituto Butantan, São Paulo, Brasil

Introduction: Schistossomiasis is an important parasitic disease in humans. There are few drugs available for treatment, but infection is recurrent. A preventive treatment is logical and many efforts are being done to develop an anti shistosomiasis vaccine. A family of 18 different Dyneins Light Chain (DLC) was identified in the transcriptome of the *S. mansoni* and some of them were shown to be on the tegument of the adult worm, therefore with chances of being exposed to the host immune system. **Objective:** To study the function and localization of 3 DLCs, starting by producing the recombinant proteins and generating specific antibodies. Evaluate some protective immune response after immunization of mice with the 3 recombinant DLCs. To obtain more information on the complete genes coding these 3 DLCs. **Methodology:** Mice are being immunized with DLCs to raise antibodies and for further challenge with cercaria. The sera would be used for microscopy immune localization assays. **Results:** Transcripts of DLC were cloned and the recombinant proteins expressed in *E. coli* and purified. We are reporting the studies of antigenicity. DNA from adult worms was purified and PCR amplification of DLC genes was performed using primers based on the transcriptome information. **Discussion:** Previously DLC1 was studied shown to be highly antigenic in mice, inducing IgG1 and IgG2a, driven immune response towards Th1. Some protection against cercaria infection was observed. The immunization using 3 DLCs may improve the protection.
Supported by: FAPESP and Butantan Foundation

2.49 Electrophoretic profile of *Leptospira* spp proteins cultivated in medium with different osmolarities

Silva JB da[1],Carvalho E[1], Morais ZM[2], Vasconcellos S[2], Ho PL[1].
[1]Centro de Biotecnologia - Instituto Butantan; [2] Faculdade de Medicina Vet. e Zootecnia - USP

Introduction: Leptospirosis is a zoonosis caused by the spirochetes of the genus *Leptospira*. The severity of the disease can vary widely, being even fatal. There is little knowledge of host environmental factors that affect the expression of *Leptospira* proteins. *In vitro*, some factors, such as grow phase, temperature and salt concentration can promote, increase or inhibit protein expression. **Objectives:** In this work, we cultivated *Leptospira* in medium with different osmolarities to verify possible differences in protein expression. **Methodology:** The medium used was the EMJH supplied with rabbit serum. The medium was also supplied with different salt quantities, based in a salt combination that approximates to human physiological conditions, that we called *osmolarity* 1X: NaCl 62.5mM, KCl 36.25mM and $MgSO_4$ 1.25mM; other variations of salt amount addictions are indicated as a multiple of this value. Two strains of *L. interrogans* serovar Copenhageni and two strains of *L. interrogans* Pomona were grown in an osmolarity gradient: 0X, 0.5X, 1X and 1,5X. Other serovars and even different species of *Leptospira* were grown only in osmolarity 0X and 1X. The eletrophoretic profile of these *Leptospira* extracts was characterized by SDS-PAGE. **Results and Discussion:** We could not observe differences in protein expression in any case. Nevertheless, we observed a visual decrease in *Leptospira* growth in medium with 1,5X osmolarity. A quantitative and specific analysis may reveal possible differences not observed here. Further experiments have to be done for a more detailed characterization of these observations.

2.50 *Leptospira* sphingomyelinases: differential expression in *Leptospira* strains and cross recognition between serum generated against the recombinant proteins.

Carvalho E[1,2,3] and Ho PL[1,3].
[1]Centro de Biotecnologia, Instituto Butantan, SP; [2]Laboratório de Parasitologia, Instituto Butantan, SP; [3]Departamento de Genética e Biologia Evolutiva, USP, SP.

Introduction: Sphingomyelinases (SMases) are toxins released by *Leptospira* that causes hemolysis and are associated to hemorrhagic effects in leptospirosis. **Objectives:** Five loci potentially coding for SMases were found in the *Leptospira* genome. **Methods and Results:** We cloned and expressed three of these toxins (*Lic*10657, *Lic*12631 and *Lic*11040). The purified recombinant SMases were used to generate antiserum in BALB/c mice. In western blot, we observed a cross reaction between LIC10657 and LIC12631-antiserum and between LIC12631 and LIC10657-antiserum. This suggests that LIC10657 and LIC12631 have common epitopes, which might be related to a common ancestral sequence and/or common motifs. To examine differences in SMase expression among *Leptospira* serovars, a western blot was done. We could only detect expression of SMases in one serovar: *L. interrogans* Pomona. In this serovar, we detected the expression of LIC10657 and LIC12631, but not LIC11040. When we used Pomona culture constantly grown in culture media, the LIC10657-antiserum recognized a band with the expected mass of LIC10657. But when we use Pomona that had recently infected hamsters, the band seem before were not present and another band, about 10 kDa higher than LIC10657, are observed. This band could be a modified LIC10657 or even another related protein that can be recognized by anti-LIC10657. **Conclusions:** Nevertheless, this difference of expression may be related to *Leptospira* infection and maintenance of virulence capacity in *L. interrogans* serovar Pomona.

2.51 IgM anti-*Leptospira* levels in Human Clinical Samples

Silva JB[1], Varela TM[2], Pereira MM[2].
[1]Centro de Biotecnologia-Instituto Butantan; [2]Instituto Oswaldo Cruz-Rio de Janeiro.

Introduction: Leptospirosis is provoked by spirochetes of the genus *Leptospira* which infect men and animals. Among pathogenic species, *L. interrogans* is the most important with several serovars that infect humans. In Brazil the disease is considered endemic. Different methods are employed in order to diagnostic human and animal leptospirosis. Microscopic agglutination test (MAT) and ELISA-IgM are the most used. MAT is recognized by OMS as "gold" standard method, and due to its high complexity, its use is restricted to reference laboratories. ELISA-IgM tests are simple and detect IgM antibodies in initial infection phase. In the present work, we analyzed anti-*Leptospira* Immunoglobulin M (IgM) levels in patient serum. **Objectives:** Thirty-six samples received from CRNL-FIOCRUZ-RJ, were analyzed. **Methods and Results:** From these, 14 positive samples to MAT with titles above 1:800; 11 samples with titles below 1:800 and 11 negative samples to MAT test. IgM antibodies were detected by means of immune-enzymatic assay. All positive samples in MAT presented agglutination with more than a serovar, and these samples were reactive to IgM, except one sample that presented high levels in MAT and it was not reative to IgM as well the another sample that presented low titles (1:100) in MAT, and Optical Density three times higher than positive samples in MAT. **Conclusions:** These results demonstrated the need for further evaluation of the kit in relation to false positives and false negatives, and the importance to follow the efforts to develop new simple and rapid diagnostic methods.

2.52 Cloning and expression of antigens from *Leptospira interrogans* serovar Copenhageni.

Nakajima E[1,2], Lopes LM[1,2], Lopes APY[1,2] and Martins EAL[1,2].
[1]Centro de Biotecnologia, Instituto Butantan,[2]Pós graduação Interunidades em Biotecnologia – USP.

Introduction; Leptospirosis is an important zoonosis in Brazil, with high cost for public heath system. The genome of *Leptospira interrogans* serovar Copenhageni was analyzed looking for antigens as vaccine candidates. **Objective:** Since there is no vaccine against leptospirosis available for human use, the goal of our work is to identify antigens, testing their potential to induce protective immunity. **Methodology:** Two systems of antigen presentation are being tested: purified protein and live recombinant vaccine based on attenuated salmonella. Eight chosen genes are being amplified and cloned in vector pAE to produce the recombinant proteins and in vector pAEsox for *in vivo* expression (the "soxRS controlling system", carried by attenuated salmonellas can be activated *in vivo* by oxidative stress and *in vitro* by paraquat). The purified proteins and the live recombinant salmonellas are being used to immunize mice and hamsters in order to investigate differences in the immune response and protective capacity against leptospira infections. **Results**: The proteins (LIC10191, LIC10793, LIC11227, LIC12302, LIC13101, LIC12659, LIC10868 and LIC12631) were expressed hybrid with his tag and purified from *E. coli.* by metal affinity chromatography. Vaccine strain *S. typhimurium* SL3261 carrying the pAEsox constructions were obtained. **Discussion:** The animals are being immunized to obtain specific antibodies for different purposes. Recognition of the proteins by antisera from leptospirosis patients are being investigated.
Supported by: FAPESP and Butantan Foundation

2.53 Evaluation of Immune Response to Experimental Infection of C3H/HeJ Mice with *Leptospira interrogans* serovar Copenhageni

Silva JB[1], Varela TM[2], Pereira MM[2].
[1]Centro de Biotecnologia-Instituto Butantan; [2]Instituto Oswaldo Cruz-Rio de Janeiro.

Introduction: Leptospirosis is provoked by spirochetes of the genus *Leptospira* which infect men and animals. This disease can be caused by several serovar, and clinical manifestations may vary from subclinics to severe. High inflammatory cytokine levels could be associated to disease severity. **Objective:** To establish an experimental model in order to demonstrate chemokines and cytokines participation in immunity and pathogenesis of leptospirosis in C3H/HeJ mice. **Methods and results:** *Leptospira interrogans* serovar Copenhageni from FIOCRUZ was cultivated in EMJH medium to 28°C/ 7 days. Cells were diluted in PBS ($1x10^6$ cells). Bacteria virulence was tested in 21-days *hamsters*. DL_{50} was done in 3 groups of C3H/HeJ mouse infected with 0.5mL of bacteria suspension containing 1×10^5; 2×10^6; 1×10^7 cells, and control not infected. A *hamster* group was inoculated with 2×10^6 cells. Animals were observed during 28 days. Blood of two mice from each group was collected and the serum was separated. Kidney of each animal was macerated to isolate bacteria. Microscopic agglutination test (MAT) was accomplished to analyse antibodies in mice. We observed 100% of death in inoculated *hamsters* in a maximum period of 10 days. There was no death in the group of infected mice until 28 days of inoculation. Only one mouse presented antibody title detected in MAT. This animal presented positive *Leptospira* culture. **Conclusions:** New experiments will be carried out by using inoculum with 10^7 cells and to further analyse cytokine and chemokine profile in different infection times.

2.54 Study of proteins from *Leptospira interrongans* serovar Copenhageni through reverse vaccinology

Fraga TR[1,2], Nakajima E [1,2], Martins EAL [1,2].
[1]Centro de Biotecnologia, Instituto Butantan, São Paulo, Brasil; [2]Pós-Graduação Interunidades em Biotecnologia, USP (ICB) – IPT – IBU, São Paulo, Brasil.

Introduction: Leptospirosis is a widespread zoonosis whose causative agents are pathogenic bacteria from the genus *Leptospira.* The genome from *Leptospira interrogans* Copenhageni was recently sequenced and analyzed by bioinformatic tolls. Several proteins possibly related to its pathogenesis were identified. **Objective:** The aim of this work wass to identify antigens with vaccine potential and to investigate its function. Seven leptospiral putative proteins were selected: three hemolysins (LIC11352, LIC11040 and LIC10657), two lipoproteins (LIC10508 and LIC10509), an outer membrane protein (LIC10537) and an ankyrin like protein (LIC12033). **Methodology:** The genes were amplified from genomic DNA of *L. interrogans* by PCR, inserted in vectors pDEST17 (Invitrogen) or pGEMT-easy (Promega) and subcloned in two expression vectors, pAE and pAEsox. The constructions were confirmed by restriction analysis and DNA sequencing. Protein expression was tested in *E. coli* Bl21(DE3)Star pLysS. **Results:** The cloning and expression steps were successfully performed. The majority of the proteins were expressed in inclusion bodies. **Discussion:** The immunogenic activity of the leptospiral proteins produced in this study is going to be further analyzed, as well as their potential as component for diagnostic kits. In addition, the pAEsox constructions are going to be transferred to attenuated *Salmonella* strains in order to perform immunization with live microorganism.
Supported by: FAPESP

2.55 Identification of a laminin-binding protein expressed by *Leptospira interrogans*

Barbosa AS[1], Abreu PAE[1], Neves FO[1,2], Atzingen MV[1,2], Watanabe MM[1], Vieira ML[1,2], Morais ZM[3], Vasconcellos SA[3] and Nascimento ALTO[1,2].
[1]Centro de Biotecnologia, Instituto Butantan, [2]Interunidades em Biotecnologia, Instituto de Ciências Biomédicas, USP, São Paulo, Brazil, [3]Faculdade de Medicina Veterinária e Zootecnia, USP, São Paulo, Brazil.

Introduction: Pathogenic leptospires have the ability to survive and disseminate to multiple organs after penetrating the host. Several pathogens, including spirochetes, have been shown to express surface proteins that interact with the extracellular matrix (ECM). This adhesin-mediated binding process seems to be a crucial step in the colonization of host tissues. **Objectives:** This study examined the interaction of leptospiral outer membrane proteins with laminin, collagen Type I, collagen Type IV, cellular fibronectin and plasma fibronectin. **Methodology:** Six predicted coding sequences selected from the *L. interrogans* serovar Copenhageni genome were cloned, proteins expressed, purified by metal affinity chromatography and characterized by circular dichroism spectroscopy. Their capacity to mediate attachment to ECM components was evaluated by binding assays. **Results and Discussion:** We have identified a leptospiral protein encoded by LIC12906 (named Lsa24) that binds strongly to laminin. Attachment of Lsa24 to laminin was specific, dose-dependent and saturable. Laminin oxidation by sodium metaperiodate reduced protein-laminin interaction in a concentration-dependent manner, indicating that laminin sugar moieties are crucial for this interaction. Moreover, Lsa24 partially inhibited leptospiral adherence to immobilized laminin. This newly identified surface protein may play a role in mediating adhesion of *L. interrogans* to the hosts. To our knowledge, this is the first leptospiral adhesin with laminin-binding properties reported to date.

2.56 Differential protein expression analysis of virulent and non-virulent strains of *Leptospira interrogans* by two-dimensional gel electrophoresis.

Vieira ML[1,2], Abreu PAE[1], Pimenta DC[3], Vasconcellos SA[4], Morais ZM[4], Nascimento ALTO[1,2].
[1]Centro de Biotecnologia, Instituto Butantan, SP; [2]Pós-graduação Interunidades em Biotecnologia, USP, SP; [3]LETA-CAT/CEPID, Instituto Butantan, SP; [4]FMVZ, USP, SP, Brasil.

Introduction: The bacterial pathogen *Leptospira interrogans* is the causal agent of leptospirosis, one of the most spread zoonosis worldwide. The leptospiral complete genome sequencing revealed several novel proteins that might be involved in pathogenesis and virulence, and may thus constitute important vaccine candidates against leptospirosis. Some bacterial pathogens can modulate their protein expression profile according to the environmental conditions. It is known that leptospires do not express some proteins involved in pathogenesis when cultured *in vitro*, loosing their virulence. **Objectives and Methodology:** Based on these assertions, we set out to investigate aspects of pathogenesis and virulence of these bacteria by analyzing the differential protein expression between cultured-attenuated non-virulent and hamster-derived virulent strains of *L. interrogans* serovar Pomona by proteomics approach. Virulent bacteria were inoculated in hamsters followed by cultivation of kidney and liver-derived leptospires, while the non-virulent were obtained by culture-passage attenuation. Total bacterial protein extracts were separated by two-dimensional gel electrophoresis; the spots were excised from the gel and tripsin-digested; the resulting peptides were analyzed on a MALDI-TOF mass spectrometer. **Results and Discussion:** In preliminary experiments, several proteins were identified, including differentially expressed ones that may play a role in virulence and are, therefore, potential vaccine candidates. Further analyses are being performed in order to identify as many proteins as possible that are expressed only by the virulent strain.
Supported by: FAPESP, CNPq and Butantan Foundation

2.57 Characterization of two novel putative lipoproteins of *Leptospira interrogans*

Atzingen MV[1,2], Abreu PAE[1], Barbosa AS[1], Morais ZM[3], Vasconcellos SA[3] and Nascimento ALTO[1,2] e-mail: m.atzingen@butantan.gov.br.
[1]Centro de Biotecnologia, Instituto Butantan; [2]Pós Graduação Interunidades em Biotecnologia, ICB, USP, São Paulo, Brasil; [3]Faculdade de Medicina Veterinária e Zootecnia de São Paulo, Brasil.

Introduction*: Leptospira* is the etiologic agent of leptospirosis, a bacterial zoonosis worldwide distributed. Due to the extensive serological diversity of leptospires (~250 serovars) a search for conserved surface-exposed proteins that may stimulate heterologous immunity is being pursued. This became possible by combining bioinformatics tools and the available whole-genome sequences of *L. interrogans* serovar Copenhageni. The chosen genes encode for two probable lipoproteins, LIC10368 and LIC10494, predicted to be exported to the membrane and lipidated. **Methodology and Results:** The genes were amplified by PCR methodology and cloned into pDEST17^{TM}, an *E. coli* vector. The recombinant proteins were expressed in fusion with six histidine residues (6xHis-tag) at N-terminus, allowing protein purification by metal-affinity chromatography. After purification by nickel-charged Sepharose beads, the recombinant proteins were analyzed by circular dichroism (CD) spectroscopy that revealed well folded proteins. The recombinants were also evaluated by western blotting using serum from mice immunized with leptospiral cell extracts and both proteins showed reactivity especially with membrane preparation. **Discussion:** These results corroborate with their predicted cellular localization in the bacterial membrane by PSORT program. These recombinants will be further analyzed using serum from human patients diagnosed with leptospirosis. These tests should offer preliminary data if these proteins might be useful for the diagnosis of the disease and/or vaccine development.
Supported by: FAPESP, CNPq and Butantan Foundation

2.58 Characterization of novel surface leptospiral antigens

Neves FO[1,2], Abreu PAE [1], Romero EC[3] and Nascimento ALTO [1,2].
[1]Centro de Biotecnologia, Instituto Butantan, [2]Doutorado Interunidades em Biotecnologia, Instituto de Ciências Biomédicas, USP, [3]Divisão de Biologia Médica, Instituto Adolfo Lutz, SP, Brasil.

Introduction*: Leptospira*, the causative agent of leptospirosis, is a highly invasive spirochete that efficiently colonizes target organs after penetrating the host. **Methodology and Results:** Four proteins (Lsp30, Lsp49, Lsp29 and Lsa24) were cloned, expressed and purified by metal affinity chromatography. These proteins have lipobox sequence tag at the N-terminus and are probably new surface lipoproteins. The structural integrity of the purified proteins was assessed by CD spectroscopy, which revealed alpha-helices for Lsp30 and Lsp29 and beta-sheet for Lsp49 and Lsa24, as predominant molecule populations. The reactivity of these proteins with antibodies present in serum samples from patients diagnosed with leptospirosis was evaluated by western blot and ELISA. Our results showed that Lsp30, Lsp49 and Lsp29 were recognized by antibodies in human serum from patients in the covalescent phase of the disease; two proteins Lsp49 and Lsp29 were also reactive with antibodies present in the initial phase of the disease while Lsa24 showed no reactivity. At present, diagnostic of leptospirosis is performed by MAT (micro agglutination test) which detects anti-LPS antibodies in patient's serum only ca. two weeks after the infection. **Discussion:** Although preliminary, our results suggest that proteins Lsp49 and Lsp29 are good candidates for development of the much needed diagnostic kit for detection of the disease in its early phase. To our knowledge, this is first report of leptospiral proteins capable to give positive results at the beginning of human infection, when MAT is still negative, and deserves a thoroughly investigation.
Supported by: FAPESP, CNPq and Butantan Foundation

2.59 An improved method to calculate antibody avidity index.

Perciani CT[1], Peixoto PS[2],Dias WO[1], Kubrusly FS[1], Tanizaki MM[1].
[1]Centro de Biotecnologia, Instituto Butantan, S.P; [2]Instituto Oceanográfico, USP.

Introduction: The strengh of an antigen-antibody (Ag-Ab) association is measured by a chemical constant, avidity index (AI). Ai is often used in clinical diagnosis and in analysis of vaccine efficacy. Although the assay to determine antibody avidity is a very well established procedure (ELISA with and without a denaturanting agent), the methods for AI calculation are controversial. AI calculation has been performed by numerous ways, all of then, based on calculation of the ratio or percent of bound antigen with and without denaturanting agent in a fixed point of ELISA titration curve, which could be expressed in optical density or in titer. Depending of this fixed point, different AI values is obtained since both titration curves, with and without denaturanting agent, are no parallel. **Objectives:** We present here a new method for AI calculation which is based on the ratio between the area of Ab titration. **Methods and Results:** This method was tested in the sera of newborn mice vaccinated with anti-meningococcal vaccine with two different adjuvants: aluminum hydroxide and an oil/water emulsion of porcine pulmonary surfactant. This method was compared with two other previously published: i) single point determination (J. Med. Virol. 51:242, 1997), ii) end point titer determination (J. Clin. Lab. Anal. 8:16, 1994). **Conclusions:** The method described here based in the area of the whole titration curve avoid the imprecision of the all other ones that uses for AI calculation one specific point of ELISA curve.
Supported: FAPESP and CNPq

2.60 Validation Strategies of Kinetic Turbidimetric Technology

Agostini Utescher CL, Mendes E, Zollo C, Morais JF and Horton DSPQ
Quality Control Service, Instituto Butantan, São Paulo, Brazil

Introduction: The kinetic turbidimetric technique is a quantitative test used for bacterial endotoxins. It is a method to measure either the onset time needed a predetermined absorbance of the reaction mixture or the rate of turbidity development. **Objective:** This study aimed to show the application of this technology in water for injection (WFI) and biological samples. **Methodology:** WFI samples had been tested in three distinct assays, placed in contact with the Limulus Amebocyte Lysate reagent and with an endotoxin standard curves, range 0.05-5.0 Endotoxin Units (EU)/mL. The test counted of positive controls and duplicate analyzes. **Results:** The endotoxins values had been <0.05 EU/mL, coefficient of variation < 5% and correlation coefficient of 0.99. **Discussion:** The results suggest that this technology can be applied for WFI and tests of validation for biological should be initiated.
Supported by: Butantan Foundation

2.61 Validation Procedures Applied to Isolators Technology

Agostini Utescher CL, Zollo C, Mendes E, Silva NG, Morais JF and Horton DSPQ.
Quallty Control Service, Instituto Butantan, São Paulo, Brazil.

Introduction: Isolators are closed equipments that minimize human intervention, offer high level of protection against contamination and can be introduced after validation of critical factors. **Objective:** The present study aimed to performance validation methodologies for sterility tests in isolators system. **Methodology:** The Bio-decontamination capacity was evaluated by distribution of biological indicators. The sterilization agent penetration, hydrogen peroxide, was evaluated by the fertility tests and limited assay. The sensitivity detection was evaluated through the recovery tests of contaminants. **Results:** Absence of growth was observed in all points, presence of characteristic growth of 10-100 CFU in all medium used and peroxide was less than 300 ppb. **Discussion:** The results show that the Isolators technology can be applied to Sterility Tests safely.
Supported by: Butantan Foundation

3. Genetics and Immuno-regulation

3.01 Effect of the snake venom toxin jararhagin on integrin genes expression on human malignant melanoma cells.

Klein A, Capitanio JS, Ruiz IGR.
Genetics Laboratory, Butantan Institute, São Paulo, Brazil

Introduction**:** Several snake venom toxins inhibit tumor cells adhesion, migration and invasion *in vitro* and/or *in vivo*. In order to assess the biological action of these toxins, standard tests have been performed in human and animal tumor cell lines. These tests have confirmed these toxins to exhibit potential therapeutic effects, which are dose and time dependent. **Objective:** To investigate the expression of integrin genes on human malignant melanoma cells treated with the snake venom toxin jararhagin. **Methodology:** Skmel-28 malignant melanoma cells were cultivated and treated with 30 ng, 60 ng and 90 ng of jararhagin, and incubated at 37ºC for 24 hours. The RT-PCR technique was carried out to assess the gene expression of subunits α_v, α_5, α_6, β_1, β_3, and β_6 integrins. All samples were compared with the expression of the GAPDH human constitutive gene (glyceraldehyde-3-phosphate dehydrogenase) as an internal control of PCR reactions. **Results**: The GAPDH gene and the α_v and α_5 integrins were highly expressed in all tumor cells, treated or not with jararhagin. The toxin did not change the low expression levels of the other subunits. Only the integrin β1 was less expressed in the toxin-treated cells, as compared with the untreated control. **Discussion:** These data showed that the jararhagin doses tested on SKmel cells affected gene expression of only integrin β1. Further studies are going on to confirm these data and the expression of other genes involved in adhesion and apoptosis.
Supported by: FAPESP.

3.02 Polymorphic regions on chromosomes 1 and 11 seem to be involved in LPS sensitivity in mice.

Borrego A, Peters LC, Cabrera WHK, Ribeiro OG, Starobinas N, Ibañez OM, and De Franco M.
Laboratório de Imunogenética, Instituto Butantan, São Paulo-SP, Brasil.

Introduction: Lines of mice selected for maximal (AIRmax) or minimal (AIRmin) acute inflammatory reaction (AIR) presented a frequency disequilibrium of *Slc11a1* (formerly *Nramp1*) R and S alleles. AIRmax^{RR}, AIRmax^{SS}, AIRmin^{RR} and AIRmin^{SS} sublines were produced in order to study the *Slc11a1* gene interaction with acute inflammatory Quantitative Trait Loci (QTL) during LPS endotoxic shock. The inflammatory response and LPS susceptibility were higher in AIRmax^{RR} and AIRmin^{RR} compared to AIRmax^{SS} and AIRmin^{SS} counterparts. **Objective**: To analyze microsatellite polymorphisms on chromosomes 1 (around *Slc11a1*) and 11 (at the clusters of cytokines and chemokines) and correlate them with LPS susceptibility. **Methods**: Genomic DNA was extracted from mice tails, and microsatellite genotyping was performed by PCR. Amplicon products were observed in 4.5% agarose gel and the allele frequency disequilibrium was determined by χ^2 test. **Results**: On chromosome 1, two extremely significant regions were identified by analysis of *RR* versus *SS* mice (intraline analysis) the *Slc11a1* gene region (χ^2 = 49.8; $p < 0.0001$), delimited by D1Mit303 and D1Mit132 markers; and another region marked by D1Mit10 (χ^2 = 34.91; $p < 0.0001$). In the interline analysis (between AIRmax and AIRmin mice), only the D1Mit303 alleles were found to be significantly deviated (χ^2 = 45.12; $p < 0.0001$). On chromosome 11, only two markers were found to be significant in interline analysis: the markers D11Mit242 (χ^2 = 62.06; $p < 0.0001$); and D11Mit124 (χ^2 = 66.00; $p < 0.0001$). **Conclusion**: These results indicate that the interaction of the *Slc11a1* alleles with QTL in chromosome 11 modulates the sensibility to the poisonous effects of LPS and the acute inflammatory response.
Supported by: Fapesp and CNPq.

3.03 Cytokine and growth factor mRNA expressions in 12-o-tetradecanoylphorbol-13-acetate (TPA)-treated skin of mice genetically selected for acute inflammatory reaction (Air).

Carneiro PS, Peters LC, Cabrera WHK, Ribeiro OG, Ibañez OM, De Franco M.
Laboratório de Imunogenética, Instituto Butantan, São Paulo-SP, Brasil.

Introduction: Mice genetically selected for maximal (AIRmax) and minimal (AIRmin) acute inflammatory reaction differed in susceptibility to two stage skin chemical carcinogenesis (DMBA-TPA). AIRmax were resistant, while AIRmin were susceptible. **Objectives**: To investigate the mRNA expression of cytokines after TPA treatment of the skin from AIRmax and AIRmin mice in order to evaluate inflammatory alterations initiated by the promoter. **Methodology**: The first group of mice received an application of 10μg TPA and was killed at 6 and 24 hours. The second group received 1μg TPA twice a week for 30 days and then was killed. The skin mRNA expression levels of several cytokines and Caspase-8 were performed by quantitative real-time PCR. **Results**: An increase for all mRNA expression levels after 6h TPA-treatment could be observed; however, differences between AIRmax and AIRmin were shown for TNFα, IL-12 and IL-1β. Also, TNFα, IFNγ, IL-1β, IL-12, and IL-10 RNA expressions increased in AIRmax mice compared with AIRmin, while the AIRmin showed an increase of IL-4 compared with AIRmax 24 hours after TPA-treatment. For 30 days, IL-1α (more expressed in AIRmax), Caspase-8 (more expressed in AIRmin) and TGF-β1 (more repressed in AIRmax) revealed differences. **Discussion**: These data suggest that the balance among cytokine mRNA expressed levels in the skin of AIRmax and AIRmin mice could influence the early events of the resistance/susceptibility to chemically induced skin carcinogenesis.
Supported by: FAPESP, CAPES and CNPq.

3.04 Expression profiling identifies inflammatory genes associated with lung tumorigenesis.

De-Franco M[1], Spinola M[2], Pignatiello C[2], Cabrera WHK[1], Starobinas N[1], Ribeiro OG[1], Ibanez OM[1], Dragani TA[2].
[1]Instituto Butantan, Sao Paulo, Brazil. [2]Istituto Nazionale Tumori, Milan, Italy.

Introduction: AIRmax and AIRmin mice are phenotypically selected for maximal or minimal subcutaneous acute inflammatory response, respectively, and display high inter-line differences in exudated proteins and neutrophil infiltration, as well as in bone marrow granulopoiesis, inflammatory cytokines, and neutrophil apoptosis. AIRmin mice developed a persistent sub-acute lung inflammatory response and a 40-fold higher lung tumor multiplicity than AIRmax mice, which showed a transient lung inflammatory response. **Objective**: To analyze gene expression profiles of these lines compared to the lung cancer resistant C57Bl/6 and susceptible A/J mice. **Methods**: Gene expression profile analysis of urethane-treated and untreated animals was performed using the Applied Biosystems Mouse Genome Survey Microarray containing ~32,000 mouse transcripts. mRNA expression of candidate differentially expressed genes was validated by quantitative real-time PCR and the over-represented biological themes were analyzed with EASE software. **Results**: Urethane treatment modulated the gene expression profile in all four lines. Among the confirmed genes, vanin3 (*Vnn3*) and major histocompatibility antigen E alpha (H2-Ea) commonly appeared for both mouse models. The most represented gene categories in the AIR model were acute phase response, immunoresponse, electron and lipid transport, complement activation and tissue repair. MHC/antigen process and presentation and immunoresponse were the major themes in the inbred model. An interesting gene cluster in chromosome 3 (45.0 cM) was observed. **Discussion**: The study suggests that the expression of a subset of genes may show a strain- and line-specific modulation pattern during inflammatory response and lung tumorigenesis.
Supported by: AIRC, FIRB, FAPESP, CNPq and UICC.

3.05 *Slc11a1 (Nramp1)* alleles interact with acute inflammation loci to modulate wound healing trait in mice.

De Franco M, Peters LC, Borrego A, Ribeiro OG, Starobinas N, Cabrera WHK, Ibanez OM.
Laboratório de Imunogenética, Instituto Butantan, São Paulo, Brazil.

Introduction: Two lines of mice obtained by phenotype-selective breeding for maximal (AIRmax) or minimum (AIRmin) acute inflammatory reaction to polyacrylamide bead subcutaneous injection differ in both selective parameters: neutrophil infiltration and exudated protein. Moreover, they presented a frequency disequilibrium of the solute carrier family 11 a member 1 - *Slc11a1* (formerly named *Nramp1*) *R* and *S* alleles. **Objectives**: To study the interaction of the *Slc11a1 R* and *S* alleles with the inflammatory Quantitative Trait Loci (QTL), AIRmax^{RR}, AIRmax^{SS}, AIRmin^{RR} and AIRmin^{SS} sub lines were produced by genotype-assisted breeding. **Methods and Results**: The wound-healing trait was investigated in AIRmax and AIRmin mice, as well as in F1 and F2 interline hybrids and in *Slc11a1* sub lines, using ear hole closure as phenotypic parameter. 2-mm ear punches were made in 10-15 animals of each group, and the hole was measured for30 days. AIRmax mice showed a significant tissue repair after 30 days (2.0 to 0.52 mm, $p < 0.001$) while AIRmin mice presented no ear closure. Significant differences between male and female AIRmax mice were also observed. The wound-healing trait showed a co-dominance effect in F1 hybrids and a significant ($p < 0.001$) correlation with neutrophil infiltration and protein concentration in F2 populations (n=321). AIRmax^{SS} mice showed complete ear closure, suggesting that *Slc11a1 S* allele favored tissue repair. AIRmax^{RR} wound healing was similar to heterogeneous AIRmax mice. **Discussion**: These results present AIRmax mice regeneration potentials comparable to MLR inbred strain, suggesting, moreover,the involvement of acute inflammation QTL and *Slc11a1* gene in the genetic control of wound healing phenotype.

3.06 Identification of inflammatory QTL involved in experimental arthritis in mice.

Peters LC[1], Borrego A[1], Carneiro PS[1], Canhamero T[1], Cabrera WHK[1], Ribeiro OG[1], Starobinas N[1], Ibañez OM[1], De Franco M[1].
[1]Laboratório de Imunogenética, Instituto Butantan, São Paulo-SP, Brasil.

Introduction: Allele frequency disequilibrium analysis of microsatellite markers was initially performed in lines of mice selected for maximal or minimal acute inflammatory reaction. Significant polymorphic regions identified in AIR Selection were analyzed in AIR sublines homozygote for *Slc11a1 R* and *S* alleles. These sublines differed in incidence and severity of pristane-induced arthritis, suggesting that *Slc11a1* or some other closely linked gene interacts with inflammatory QTL to modulate PIA. **Objective:** To identify inflammatory QTL involved in PIA development. **Methodology:** Arthritis development and microsatellite genotyping in chromosome 1 and 11 were performed. **Results:** Marker regression analysis showed two regions that seem to be associated with experimental arthritis. The first one, near *Slc11a1* gene on chromosome 1, marked by *D1Mit132* microsatellite was related to incidence and severity phenotypes and *D1Mit236* related only to severity. Another region was identified in chromosome 11, co-localized with cytokine and chemokine gene clusters, marked by *D11Mit242*, *D11Mit4* and *D11Mit124* microsatellites. The analysis of chromosome 11 markers allowed for definition of a confidence interval (CI) associated with a severe arthritis phenotype. **Conclusion:** Collectively these data support the theory that inflammation modifier QTL identified on chromosome 1 and 11 could be involved in PIA development.
Supported by: FAPESP.

3.07 Differential susceptibility to polycyclic aromatic hydrocarbon induced hematotoxicity I n mice genetically selected for high or low acute inflammatory response.

Katz ISS[1], Albuquerque LL[1], Souza VRC[1], Cabrera WHK[1], De Franco M[1], Ibañez OM[1], Ribeiro OG[1].
[1]Laboratory of Immunogenetic, Butantan Institute, São Paulo, Brazil

Introduction: The metabolism of Polycyclic aromatic hydrocarbons (PAHs) depends on aryl hydrocarbon receptor (Ahr) activation. An allelic polymorphism was found in the *AHR* gene between inbred mouse lines correlating with a decrease in binding affinity of the receptor to PAHs and a consequent variation in hematotoxicity susceptibility. Two lines of mice genetically selected for maximal (AIRmax) or Minimal (AIRmin) local Acute Inflammatory Response (AIR) to a non immunogenic substance differ in susceptibility to hematotoxicity induced by PAHs. **Objective:** To examine the effects of DMBA and Benzene metabolites on bone marrow and circulating cell numbers and to determine the allelic polymorphism of the *AHR* gene in AIR selected mice. **Methodology:** AIR mice were treated with one dose of 50mg/kg ip DMBA or 75mg/Kg phenol and hydroquinone for 3 days twice a day. Twenty-four and 48 hours after ip injections, the bone marrow and circulating cells were identified and enumerated. **Results:** After DMBA treatment, the depletion of total bone marrow cells was substantial at 24h, only in AIRmin. This reduction is reflected in the pool of the circulating leukocytes. In contrast, both AIR mice presented similar hemocytotoxicity to Phenol and Hydroquinone. According to these results, $AHR^{b1}$and AHR^{d} alleles were found to be fixed in AIRmin and AIRmax mice, respectively. **Discussion:** This differential allele fixation implicates the *AHR* gene in the control of Acute Inflammatory Response and in susceptibility to PAHs effect on selected mice.
Supported by: FAPESP/CNPq.

3.08 Cytokines and metabolizing enzymes induced by epicutaneous applications of 7, 12-dimethylbenzo (a) ANTHRACENE (DMBA) in mouse lines genetically selected for acute inflammatory reaction (AIR).

Souza VRC, Cabrera WHK, De Franco M, Ribeiro OG, Ibañez OCM.
Instituto Butantan - Laboratório de Imunogenética. São Paulo/ SP.

Introduction: AIRmax mice are resistant and AIRmin susceptible to carcinogenesis induced by DMBA followed by TPA. When skin carcinogenesis was induced by repeated DMBA doses alone, AIRmin mice developed contact hypersensitivity reaction (CHS), followed by skin and lung tumors. The aryl hydrocarbon receptor (Ahr) plays an important role in DMBA metabolism. Upon binding to the agonist this transcription factor increases the expression of CYP 450 enzymes. An allelic polymorphism was found in *Ahr* gene in AIRmax correlating with a decreased binding affinity to DMBA and resistance to carcinogenesis. **Objectives:** To study expression of mRNA for cytokines and CYP enzymes in the skin of DMBA treated AIRmax and AIRmin mice. **Methods:** Mice received 5 epicutaneous doses of 50 µg DMBA in 0.1ml acetone or acetone alone. Total RNA was extracted from skin fractions at 48 h after the last dose, and expression levels of cytokines and P450 enzymes mRNA were scored by real-time PCR **Results:** upregulated levels of IL-1β (23.9x), TNF-α (4.8x), IL-6 (12x) and TGF-β1 (3.5x) and of CYP1B1 (5.2x) mRNA were found in DMBA treated AIRmin mice only. **Conclusion:** The profile of cytokines induced in AIRmin mice is consistent with CHS elicitation by DMBA. The increase in CYP1B1 correlates with the cutaneous bioactivation of DMBA dependent on high affinity binding to Ahr. The results indicate mechanisms underlying the differential susceptibility of AIRmax and AIRmin mice to skin carcinogenesis.
Supported by: FAPESP and CNPq.

3.09 IL-10 and IFN-γ-Dependent mechanisms are responsible for the immunomodulatory effect induced by PAS-1 (Protein from *Ascaris suum*) on lung inflammation

Araújo CAA[1] and Macedo-Soares MF[1]
[1]Laboratory of Immunopathology, Butantan Institute, São Paulo/SP, Brazil

Introduction and Objectives: In this work, we investigated the roles of IL-12, IFN-γ and IL-10 induced by PAS-1, an immunomodulatory protein from *Ascaris suum,* on the modulation of lung inflammation. **Methods and Results:** IL-12$^{-/-}$, IFN-γ $^{-/-}$ and IL-10 $^{-/-}$ C57BL/6 mice were immunized on days 0 and 7 by intraperitoneal route with OVA (50 μg) or PAS-1 (300 μg) or OVA (50 μg) + PAS-1 (300 μg) plus $Al(OH)_3$ (7,5 mg). On days 14 and 21, they were challenged with OVA (50 μg) or PAS-1 (300 μg) or OVA (50 μg) + PAS-1 (300 μg). On day 23, we analyzed number of eosinophil and cytokine production (IL-4, IL-5, IL-13, eotaxin, IL-12, IFN-γ and IL-10) in bronchoalveolar lavage by ELISA. Our results demonstrated that IL-12 $^{-/-}$ mice OVA+PAS-1-immunized presented significant reduction of eosinophils, IL-4, IL-5, IL-13 and eotaxin in relation to IL-12 $^{-/-}$ mice OVA-immunized, respectively, 94.34%, 87.61%, 95.89%, 98.45%, 99.09%. On the other hand, eosinophils, IL-4, IL-5, IL-13 and eotaxin from IFN-γ $^{-/-}$ and IL-10 $^{-/-}$ mice OVA+PAS-1-immunized were not significantly different in comparison to IFN-γ $^{-/-}$ and IL-10 $^{-/-}$ mice OVA-immunized. Moreover, IL-12$^{-/-}$, IFN-γ $^{-/-}$ and IL-10 $^{-/-}$ mice OVA+PAS-1-immunized produced increased levels of IL-12, IFN-γ and IL-10 (except the cytokine for which they were knocked out) in comparison to mice OVA-immunized: IL-12 (respectively, non-detected (ND), 98.25%, 98.16%), IFN-γ (respectively, 96.54%, ND, 98.62%) and IL-10 (respectively, 93.3%, 98.62%, ND). **Conclusion**: Our results demonstrated that the immunomodulatory effect induced by PAS-1 is mediated by IFN-γ and IL-10, but not by IL-12.

Supported by: CAPES and FAPESP.

3.10 *Ascaris suum* earlier larval stages secrete PAS-1, an immunomodulatory protein

Oliveira CF[1], Araújo CAA[1] and Macedo-Soares MF[1]
[1]Laboratory of Immunopathology, Butantan Institute, São Paulo/SP, Brazil

Introduction: We have demonstrated that PAS-1 is an immunomodulatory protein found in *Ascaris suum* adult worm extract. However, it was not highlighted the nature of PAS-1 (secreted and/or constitutive protein). **Objectives:** In the present study, we investigate the release of PAS-1 in culture of *A. suum* eggs. **Methodology:** Embryonated eggs obtained from adult worm uteri (600,000 eggs) were incubated with 5.25% sodium hypochlorite/sodium hydroxide, pH 13-14 and they were incubated under agitation for 5 hours at room temperature. Eggs were washed and CO_2-bubbled for 1.5 hours at room temperature to deshell and permit their eclosion. The egg solution was pelleted, ressuspended in DMEM medium and cultivated in 6 well plates (~100,000 eggs/well) for 30 days at 37°C, 5% CO_2. Supernatants were collected each 3-4 days after the cultivation. The presence of PAS-1 in culture supernatants was evaluated by ELISA (using anti-PAS-1 monoclonal antibody, MAIP-1). **Results and Discussion:** Our results demonstrated that *A. suum* eggs in culture secrete PAS-1 since day 3. But, the secretion of PAS-1was peaked on day 8. Moreover, in cultures that presented only non-ecloded eggs, PAS-1 was not detected. On the other hand, when eggs were ecloded, PAS-1 secretion was significant in comparison to PAS-1 secreted by non-ecloded eggs ($p < 0.001$). In conclusion, our results demonstrated that PAS-1 is a secreted product from *Ascaris suum*, at least by earlier stages of the life cycle.

Supported by: FAPESP

3.11 High molecular weight components from *Ascaris suum* inhibit the OVA specific response by a mechanism dependent on MYD88 adapter molecule

Silva SR[1], Jacysyn JF[1], Macedo MS[1] and Faquim-Mauro EL[2]
[1]Dept of Immunology, ICB-USP, SP; [2] Instituto Butantan, São Paulo, Brazil.

Introduction: High molecular weight (P1) components from *A. suum* inhibit *in vivo* the expression of the molecules involved in antigen presentation in DCs from OVA+PI-primed mice and the ability of these cells to induce T cell proliferation compared to DC from OVA-primed mice. **Objective**: To evaluate the *in vitro* ability of PI to modulate the expression of MHC-II and co-stimulatory molecules by activated $CD11c^+$cells from OVA-primed mice and the potential of these cells to stimulate OVA-specific T cells, plus the role of the Myd88 protein in its *in vivo* suppressive effect. **Methodology**: $CD11c^+$ cells from mice primed with OVA in CFA were pulsed with OVA or OVA+P1 for 18h, labeled with anti-MHC-II, -CD80, -CD86 or -CD40 mAbs, and analyzed by flow cytometry or cultivated with OVA-specific T cells. **Results**: PI suppressed the MHC-II and co-stimulatory molecules expression by $CD11c^+$cells from OVA-immunized mice pulsed with OVA+P1 compared to that obtained on $CD11c^+$ cells pulsed only with OVA. Reduction of 53% in the proliferation of OVA-specific T cells was obtained when they were cultivated with $CD11c^+$cells pulsed with OVA+PI. In addition, MHC-II and co-stimulatory molecule expression was reduced by 43-50% on cells from OVA+PI-primed C57BL/6 WT mice compared to those from OVA-primed mice. In contrast, no difference was obtained in Myd88KO mice primed in either way. **Discussion**: PI has a potent modulatory effect on DCs and this effect is related to Myd88 signaling pathway.
Supported by: CNPq and FAPESP.

3.12 Colocalization of CD5 with CD3, B220 and CD3-B220 cells: a regulatory receptor

Loureiro I[1], Araújo C[1], Vancetto MD[1], Araújo-Jorge T[2] and Henriques-Pons A[2].
[1] Laboratório de Imunopatologia-Butantan Institute, SP, [2]Oswaldo Cruz Institute, RJ, Brasil

Introduction: In mice, cells presenting both T and B markers (CD3 and CD45R-B220, respectively) in which Peyer's patch (PP) levels were determined, have been reported. The CD5 activation and expression regulation are respectively, in concatenation to the TCR engagement and integration to the level of avidity with the ligand. CD5-based PP B220-CD3 double positive cells (DP) were identified. **Objective:** To investigate CD5, CD3 and B220 overlapping receptors, in search of an intimate interactivity. **Methodology:** A lab-Tek chamber slide system was used to stain the nonimmune virgin (NV) and lactating (NL) female Balb/C PP cellular suspensions using the same Ab and technique used in flow cytometry. Cells were left to adhere overnight and mounted for confocal microscope acquisition. Overlap coefficient and Pearson's (R^2) correlation coefficient were generated by LSM software upon fluorescent cellular image of CD3, B220 and CD5. **Results:** Total CD5 cell counts increased in the NL group, although neither of the two CD5-based subsets, i.e. B-1a and CD3-B220 DP, was included in the count. The B220 and CD3 receptors, whose overlay coefficient (O.C.) is 1.0 and colocalization correlation R^2= 0.85 were scattered in a few areas however. Masks of superimposed B220 with CD5, and CD5 with CD3 (O.C.= 1, and R^2= 0.97 and 0.84, respectively) were brightness differentiated. **Discussion:** Intimate interactivity between CD3- with CD5-receptor and between B220- with CD5-receptor was observed. The B220-CD3 markers are also aligned in the confocal image microscopy; however, these two receptors do not interact.
Supported by: FAPESP.

3.13 Microsatellite analysis in actinic keratosis and squamous cell carcinoma of the skin.

Cabral LS[1], Festa Neto C[2], Sanches Jr JA[2], Ruiz IRG[1].
[1]Genetics Laboratory, Butantan Institute; [2]Dept Dermatology, School of Medicine University of Sao Paulo, Brazil.

Introduction: Genomic instability characterizes cancer cells, being detected by polymorphic markers as microsatellites. **Objective:** To search genetic alterations in human skin cancers by microsatellites analysis. **Methodology:** DNAs were obtained from 8 actinic keratosis (AKs), 24 squamous cell carcinomas (SCCs), and 4 basal cell carcinomas (BCCs), matched to controls. Primers for microsatellites D6S251, D6S252, D9S50, D9S196, and D9S287 were used. **Results:** Microsatellite instability and/or loss of heterozygosity were observed in 25 % AKs, and 33 % SCCs. BCC tumors were not altered. **Discussion:** The histological differentiation grades of SCCs were not correlated with the changes observed. Although fewer alterations were found in benign AKs than SCCs, further studies are needed to improve the clinical discrimination among SCCs.
Supported by: FAPESP

3.14 Microsatellites study in paraffin-embedded melanocytic lesions.

Lozano LF[1], Festa Neto C[2], Sanches Jr JA[2], Ruiz IRG[1].
[1]Butantan Institute, Genetics Lab.. [2]Dermatology Dept., School of Medicine, USP, Brazil.

Introduction: Cutaneous malignant melanoma (MM) represents roughly 5 % of skin cancers and 1 % of all malignant tumors. It is a multifactor disease, with both genetic and environmental factors involved. Epidemiological studies have demonstrated the presence of numerous nevi and sun exposure as major MM risk factors. Some genes related to MM propensity are located on chromosome 9, where microsatellites alterations are often found. **Objective:** To analyze microsatellites instability and loss of heterozygosity, in search of alterations related with the malignant progression from nevi to MM. **Methodology:** DNA was extracted from 29 paraffin-embedded melanocytic lesions (several types of nevi and MMs) and PCR amplified with primers for D9S287 and D9S280 microsatellites. **Results**: Alterations in D9S287 were found in 7 % tumors, as observed in polyacrylamide gel. No alterations were detected in D9S280 at the agarose gel level. **Discussion:** Although those microsatellites are located next to the important suppressor gene PTCH1, on 9q22.3, the few alterations observed in this study do not associate them to the development of the lesions. The use of paraffin-embedded materials is quite important since it allows the study of large number of samples from archives and the establishment of better statistical inferences.
Supported by: CAPES, FAPESP.

3.15 The effect of the ordered silica SBA-15 as adjuvant in modulation of immuneresponsiveness

Carvalho LV[1], Ruiz RC[2], Commodaro AG[1], Fantini MCA.[3], Marengo EB[1], Matos JR[4], Mercuri LP[4], Cabrera W[5], Pagani E[6], Scivoleto R[7], Ribeiro OG[5], Tambourgi DV[1, 8], Sant´Anna OA[1,8].
[1]Lab. Imunoquímica - Instituto Butantan [IBU], [2]Lab. de Bacteriologia – IBU, [3]Laboratório de Cristalografia - Instituto de Física - USP, [4]Instituto de Química - USP, [5]Lab. de Imunogenética - IBU, [6]Cristália Produtos Químicos Farmacêuticos, [7]Instituto UNIEMP, [8]Centro de Toxinologia Aplicada – IBU – São Paulo, Brazil.

Introduction and Objectives: The ordered mesoporous silica SBA-15 is a polymer that possesses hexagonal porous uniformity, thermal and hydrothermal stability and exhibits potential applications ranging from adsorption and catalysis. Based in its physical and structural properties the aim of this work is evaluate its effect adjuvant in modulation of immuneresponsiveness. **Methods and Results:** Mice genetically selected for high or low antibody response (H_{IVA} or L_{IVA}) with 2-3 month old were immunized with different concentrations (0.1, 1, 10 or 100µg/animal in final volume of 0,2ml) of Serum Bovine Albumin (BSA) in SBA-15, or $Al(OH)_3$, or in Incomplete of Freund Adjuvant (IFA) by the intramuscular or oral route. Blood was collected at different period after immunizations and ELISA was used for IgG titration. The silica SBA-15 was capable to improve and positively modulated the immune response of the low L_{IVA} mice, which produced antibodies title similar to the H_{IVA}. The IgG antibody titration obtained by at 14 days after immunization were: 10,0 ± 1,4 BSA:SBA-15 and 7,0 ± 0,8 BSA:$Al(OH)_3$ for oral route; by intramuscular route were: 12,1 ± 0,9 BSA:SBA-15 and 11,1± 0,9 BSA:IFA; while the mice H_{IVA} immunized by the oral via the titles were: 9,5 ± 1,3 BSA:SBA-15 and 10,7 ± 0,6 BSA:$Al(OH)_3$ and by intramuscular route were: 11,2 ± 0,3 BSA:SBA-15 and 12,5 ± 0,5 BSA:IFA. Other in vitro experiments show the increase induction of yeasts phagocytosis after macrophage stimulation with these nanoparticules. **Conclusion:** The results demonstrated the ability the SBA-15 to increase the immunogenicity of antigens and positively modulate the immune response of the L_{IVA} mice. Thus, SBA-15 seems to be a nanostructured particle useful for vaccine delivery, being capable to improve responsiveness which is safer and more effective.

Supported by: CNPq, CAT – CEPID/FAPESP, Cristália Produtos Químicos Farmacêuticos.

4. Biology of snakes, arachnids and amphibians

4.01 Anurans from Serra do Mar State Park, Cunha-Indaiá Nucleus, São Paulo State, Brazil

Batistic RF[1], Serafim H[1,2], Franco FL[1], Ienne S[2] & Cicchi PJP[3]
[1]Lab. Herpetologia, Inst. Butantan, São Paulo, Brazil, [2]Depto. Genética, Inst. Biociências-USP, São Paulo, Brazil, [3] Depto. Zoologia, Inst. Biociências-UNESP, Botucatu, São Paulo, Brazil

Introduction: The PESM – Cunha-Indaiá Nucleus is a State Park located in the Serra do Mar, 950 to 1561 meters above sea level, covered by remnants of the Tropical Rainforest. The isolation of the region and the poor knowledge of the local fauna make its inventory essential for future studies on its conservation. **Objective:** To verify the current situation of the anuran species richness in this protected area. **Methodology**: From Feb 2004 to March 2005, five collection expeditions were performed, lasting 5 days each. Two methods were employed: diurnal and nocturnal active searches and pitfall traps with drift fences. **Results**: 23 anuran species were sampled: *Brachyicephalus cf. vertebralis, Chaunus ictericus*, *Dendrophryniscus brevipollicatus*, *Aplastodiscus leucopygius*, *Bokermanohyla circumdata*, *Dendropsophus microps*, *D. minutus*, *Hypsiboas faber*, *H. pardalis*, *H. polytaenius*, *Scinax crospedospilus*, *S. hayii*, *S. perpusillus, Trachycephalus mesophea*, *Leptodactylus marmoratus*, *Eleutherodactylus binotatus*, *E. guentheri*, *E. parvus*, *Hylodes asper*, *H. lateristrigatus*, *Paratelmatobius lutzii*, *Physalaemus* sp., *Proceratophrys boiei* and *P. appendiculata*. **Discussion:** Although not all species from the region were sampled, this inventory may serve for future comparisons of species distribution, monitoring of environmental conditions *vs.* species occurrence and improved knowledge about anuran restricted to a high altitude rainforest.
Supported by: BIOTA/FAPESP, proc. number 99/08291-5.

4.02 Rescue and exploitation of fauna: Hydroelectric Peixe angical- Tocantins.

Calleffo MEV[1], Barbarini CC[2] and Puorto G[2]
Lab. de Herpetologia[1]; Museu Biológico[2], Instituto Butantan, SP, Brasil.

Introduction: Butantan Institute (IB) participated in the fieldwork on the Hydroelectric Exploitation of Angical fish in the city of Peixe and the state of the Tocantins, taking place in the Tocantins River from January to April 2006. This fieldwork relied on logistic support of Enerpeixe S/A in the environment of the dam and adjacent cities. **Methodology:** The herpetological fauna and arthropods were rescued from the hydroelectric dam's lake in boats. The macro environ as well as the surrounding micron environment were explored. Specimens were collected during the day from vegetation and floating trunks and mainly on foliage of trees and partly submerged buritis palms. Due to species diversity, a variety of capture techniques were used, including large snap hooks and lassos. The collected specimens were placed in appropriate containers. In field, the selection of specimens occurred through: individual register, sex, biometric data and photography. **Results:** In total, 68 serpent specimens distributed in 17 genus were collected; 11 genus of lizards with 132 specimens; 39 specimens of amphisbenideos of three species, a species of turtles and one of crocodile, and 496 specimens of amphibians of approximately 15 genus. **Discussion:** This material screened on arrival to the IB had some destinations, exposition, extraction of poison, biotery, laboratories, collection, etc. As little is known of the fauna of this region, inventories such as this can contribute to the study of new species and their biological data.
Supported by: Butantan Foundation and Enerpeixe S/A.

4.03 Daily activity patterns of Neotropical Squamata, in captivity

Costa ACOR da[1], Hauzman E[1], Parpinelli L[1], Scartozzoni RR[1]
[1]Lab. Herpetology, Instituto Butantan, São Paulo, Brasil.

Introduction: Studies about Squamata's daily activity in nature are difficult to obtain and field meetings are scarce. Therefore, video surveillance equipments can be use to record and study Squamata's daily activities. **Objective:** Evaluate the efficacy of video surveillance equipments with the purpose of studing Squamata's behavior and daily activities patterns. **Methodology:** Twenty three snakes from eight species, one Anguidae lizard and one Amphisbaenidae species, arrived in the Instituto Butantan (IB), were located individualy in glass terrariums with variable sizes and soil substrate. Each specimen was monitored during seven days (24 hours) with mini cameras and infra-red light for nocturnal records. **Results:** The snakes *Liotyphlops beui* (n = 11), *Leptotyphlops koppesi* (n = 1), *Anilius scytale* (n = 1) and *Tropidophis paucisquamis* (n = 2) showed nocturnal activity, while *Tomodon dorsatus* (n = 2), *Ptychophis flavovirgatus* (n = 1), *Thamnodynastes strigatus* (n = 2), and *Psomophis joberti* (n = 3) presented diurnal activity. The lizards and amphisbaenid, *Ophiodes* sp. (n = 4) and *Leposternon microcephalum* (n = 4) presents diurnal activity. **Discussion:** The daily activity can be related to ambiental factors and food availability throughout the day, as well as reproduction, physiological restrictions and ecological parameters (predation, competition). The present study demonstrates the importance of captivity studies agregated to field information, which can supply subsidies to understanding Squamata's activities patterns.
Supported by: FAPESP

4.04 Reproductive Biology, annual activity and daily activity of *Leposternon microcephalum* (SQUAMATA, AMPHISBAENIDAE) from Southeastern Brazil

Santos LC[1], Almeida-Santos SM[1]
[1]Lab. Herpetology, Instituto Butantan, São Paulo, Brazil

Introduction: Amphisbaenians are fossorial reptiles, and their habits difficult the study of their reproductive biology. *Leposternon microcephalum* is an Amphisbaenidae species, wide spread at South America. **Objective:** This study aimed to describe the reproductive cycle of *L. microcephalum* and to relate it with annual activity. Besides, we verified the correlation between annual and daily activities and climatic variables. **Methodology:** We analyzed the registers of animals received at Instituto Butantan to infer the peaks of annual activity. That data were correlated to climatic data obtained at Universidade de São Paulo Meteorology Station. Two couples of *L. microcephalum* were maintained in captivity, and their daily activity was registered by cams and a software for images capturing. Specimens preserved on zoological collections were dissected, and their testes and vas deferens, or follicles, measured. **Results:** Daily activities were concentrated between 10h and 18h. We observed correlation between annual activity and precipitation, and also between daily activity and temperature. Females have secondary vitellogenesis concentrated on November, and testes size increases on September. **Discussion:** Apparently, precipitation and temperature are decisive variables for this species activities. Data suggest seasonal reproduction. Mating probably occurs on November, on the early rainy season. Possibly, there is sperm storage by the male, in the vas deferens, between September and November.
Supported by: FAPESP

4.05 Reptile fauna disappearing in Northern Paraná State

Stender-Oliveira F.
Laboratório de Herpetologia, Instituto Butantan, São Paulo-SP, Brazil and Pós Graduação em Ecologia, Instituto de Biociências, Universidade de São Paulo-SP, Brazil.

Introduction: The semi-deciduous broadleaf forest in the past covered most of the northern region of Paraná State. In the last sixty years this area has been suffering immense fragmentation, reducing the forest to less than 2% of the original. **Objective**: To verify how changes in the natural habitats interfere with the reptile fauna. **Methodology**: Data was collected from between the parallel 24º south and the river Paranapanema in the north as well as from the Instituto Butantan and História Natural do Capão da Imbuia museums whose collection is over90 years old. **Results**: Approximately 62 species of reptiles were registered for this region. The species occupy in the old forest formations (40.3%; N=25) followed by natural open areas (32.2%, N=20), riparian vegetation (16.2%; N=10), cosmopolitan (9.7%; N=6) and urban (1.6%; N=1) zones. Currently the majority of the reptile fauna are composed of species living in open formations (agricultural and cattle ranches, with remnants of scrubland). **Discussion**: These results reveal an "inversion of fauna" probably due to deforestation, where ordinary species or from open areas take place of forest or riparian ones. So it was possible to notice that estenoic, arboreal, endemic and rare species, like: *Bothrops cotiara*, *Echinanthera cyanopleura*, *Imantodes cenchoa*, *Oxyrhopus clathratus* and *Tropidodryas serra* were substituted by eurioic, opportunistic and poisonous species like: *Crotalus durissus*, *Liophis miliaris*, *Oxyrhopus guibei*, *Sibynomorphus mikanii* and *Tupinambis merianae*.

4.06 Inventory of snake fauna of Jacupiranga, SP, southeastern Atlantic Forest, Brazil.

Peccinini-Seale DM[1], Sena MA[1], Augusto V[1], Passoni JC[1], Duarte MR[2]
[1]Instituto de Biociências, USP, SP; [2]Lab. de Herpetologia, Instituto Butantan, São Paulo, Brazil

Introduction: Jacupiranga, state of São Paulo (24º 41´S 48º 00´W, altitude 33 m) has several outlying communities. Several field trips in anthropic and disturbed areas showed intense agricultural activity. The most striking aspect of devastation in the searched areas was the enchroachment of the banana plantations throughout the Mata Atlântica mountains. **Methods:** Collections were done by active search during March 2004 and June 2005. A larger number of dead snakes samples were obtained from roads and donations of workers on the plantations of Canha and Pindauba. **Results and Discussion:** The most common snake species were *Bothrops jararaca* and *B. jararacussu*. This study included 23 snake species from Jacupiranga; Colubridae: *Chironius bicarinatus*, *C. exoletus*, *C. fuscus C. laevicollis*, *C. multiventris, Dipsas neivai, Echinantera cephalostriata*, *Erythrolamprus monozona*, *Helicops carinicaudus, Liophis amarali, L. miliaris, Oxyrhopus clathratus, Sibynomorphus neuwiedii, Sordellina punctata, Spilotes pullatus*, *Xenodon neuwiedii, Imantodes cenchoa, Tomodon dorsatus*, *Tropidodryas serra;* Viperidae: *Bothrops jararaca, B. jararacussu*; Elapidae: *Micrurus corallinus.* Records at Instituto Butantan donations from 1990-2005 showed only 8 snake species from this area. This study identified 15 new snake species from this region, including *L*. *amarali,* not recorded in the last 43 years. The conservation of the high diversity observed requires attention, such as extension of protected areas from Jacupiranga State Park. **Supported by**: BIOTA/FAPESP

4.07 Sexual Size Dimorphism (SSD) in Brazilian Pitvipers

Freire NFM[1] and Sant'Anna SS[2]
[1]Centro de Ciências Biológicas e da Saúde da Univ. Presbiteriana Mackenzie, São Paulo, Brasil;
[2]Lab. de Herpetologia, Instituto Butantan, São Paulo, Brasil

Introduction: Sexual size dimorphism (SSD) occurs in many taxon groups of snakes, with females presenting a trend for larger body size than males. The tail length shows significant dimorphism, with males usually presenting larger relative tail length. Dimorphism has already been documented in head size, body shape, corporal coloration, scalation and in ecology of snakes. **Objectives:** This paper verified the SSD, regarding body size and mass in certain Brazilian pitvipers. **Methods:** Data on snakes received by Laboratory of Herpetology of Instituto Butantan between 1985 and 2006 were used, totaling 3,203 animals (2,323 adults and juveniles, and 880 newborns) from 11 different species. In all species, females presented snout-vent length (SVL) and mass average greater than males. **Results and Discussion:** The degree of sexual dimorphism was calculated through Gibbons and Lovich indices (1990) (mean size of the larger sex divided by mean size of smaller sex, arbitrarily expressed as positive if female are larger, and negative if males are larger). Male and female newborns showed no differences in SSD. However, we observed that SSD is evident in *Bothrops jararacussu* (0.46), followed by *B. alternatus* (0.35), and *B. jararaca* (0.31), with *Crotalus durissus* (0.007) showing the least SSD.

4.08 Body bending: a cryptic defensive behavior in arboreal snakes

Rodrigues MG[1, 2], Marques OAV[2] and Sazima I[3]
[1]Lab. Ecologia Animal, Universidade Estadual Paulista, São José do Rio Preto, Brazil; [2]Lab. Herpetologia, Instituto Butantan, São Paulo, Brazil; [3]Depto. Zoologia and Museu de História Natural, Universidade Estadual de Campinas, Campinas, Brazil.

Introduction: Snakes display a great variety of defensive tactics against predation. For example, the arboreal colubrids *Pseustes poecilonotus* and *P. sulphureus* bend their bodies when disturbed while on the ground or among branches, which renders them similar to a bent vine or a liana section on the forest floor or vegetation. **Objectives:** Here we report on similar behaviours for two additional arboreal Neotropical snake species. **Methods and Results:** While performing other field studies, a snake was coincidentally observed adopting this posture. An Amazonian green whiptail (*Philodryas viridissimus)* was observed crossing a dirt road in the Amazon Forest near the Von den Steinen River. Upon approaching closer, the snake apparently increased its bending behaviour. A tiger ratsnake (*Spilotes pullatus*) was lying across a trail in a section of Atlantic Forest at Campinas, southeast Brazil. The multiple and regular bending in the snake's body made it closely resemble a piece of the liana genus *Bauhinia* (Caesalpinaceae). Bending the body is a behaviour shared by at least four Neotropical snake species that occur, in forests habitats where lianas and bent sticks are frequently found on the ground. The genera *Pseustes* and *Spilotes* belong to the subfamily Colubrinae and may be closely related; however, the genus *Philodryas* is assigned to the Xenodontinae. **Discussion:** Their very similar defensive displays possibly evolved independently in the two lineages and may be regarded as behavioural convergence.

Supported by: CNPq and FAPESP

4.09 Defensive behaviour of *Bothrops jararacussu*- simulations in captivity of meeting with human beings

Teixeira RN[1], Barreira GFS[1], Pisciolaro AM[2] e Sant'Anna SS[2]
[1]Centro de Ciências Biológicas e da Saúde da Univ. Presbiteriana Mackenzie, São Paulo, Brasil
[2]Laboratório de Herpetologia, Instituto Butantan, São Paulo, Brasil.

Introduction: To avoid its diverse predators and increase the chances of survival, the snakes use varied defensive mechanisms, like the escape and the strike. The strike happens when meeting the predator or the threat is inevitable, resulting into an ophidian accident. The snakes of the genus *Bothrops* are responsible for around 73.1% of these accidents registered in Brazil. **Objectives:** On this experiment, twenty-five *B. jararacussu,* just arrived at the Butantan Institute, were used (males and females, young and adults). **Materials and Results:** Simulations of meeting human beings (capture with the hand, with the "*laço de Luz*", trample snake, and handling the hook) were carried out in an arena of 1 x 1.5 m, with one snake per time. On most experiments, and usually during all the time, the behaviors of flicking tongue (82.9%) and vibration of the tail (76.3%) were observed, both are not specific defense behaviors, and the first one is used by the snake to analyze the environment (chemoreception), and the second behavior is a form to indicate the snake's potential threat. The manipulation and trampling showed high percentage of strike and poisoning (26.3% and 63.1%, respectively), probably because those stimulus are the ones that most stress and threat the snakes. **Discussion:** The results of the stimulus of the hook and the "*laço*" did not show those sorts of behaviors; therefore they are safe instruments to handle the snakes, causing less stress to them.

4.10 Compared Osteology of *Bothrops insularis* Amaral, 1921 (Serpentes:Viperidae: Crotalinae)

Lucas MSB[1,2], Buononato MA[3], Puorto G[1]
[1]Museu Biológico-Instituto Butantan, São Paulo, Brasil; [2]Centro universitário Nove de Julho (Uninove), São Paulo, Brasil; [3]Naturae consultoria ambiental, Goiânia- GO, Brasil

Introduction: The golden lancehead (*Bothrops insularis*) snake endemic of Queimada grande island was described by Amaral in 1921. The specie evolved from an ancestral *Bothrops jararaca*. The island is located approximately 35 km from the southern coast of São Paulo, with a 43 ha area originally covered by Atlantic forest. **Objectives:** To identify and characterize the osteology of *B. insularis* and to determine whether there is a relationship between Jararaca (*Bothrops jararaca*) and the Brazilian lancehead (*Bothorops moojeni*). Amaral in 1921 proposed that, they are similar to *B insularis*. **Materials**: Of three species, 45 dsarticulated skulls were used, measured and compared. The average correlates and relations between length and width *of B. insularis*' skulls bones were measured. **Results and Discussion:** The results already observed indicate that in *B. insularis* that there is a predominance in larger female body and head size in relation to the male body and head sizes, as observed in the morphology of the other species. Also it was observed that *B. insularis* presents a bigger maxilla than the teeth, in relation to *B. moojeni* and *B. jararaca* probably due to their diet of birds, their principal food. Its tooth also has the greatest angle. Therefore, in addition to possessing different outward behaviors and anatomy for adjusting to life on the island, it also possesses osteology, in which its bones are suitable for capturing game and food.

4.11 Use of the predicting modeling of ecological niche to map the *Bothrops jararaca* potential distribution in the state of São Paulo, Brazil.

Couto FM.
Laboratório de Herpetologia, Instituto Butantan, São Paulo.

Introduction: The predicting ecological niche modeling is an important tool to predict species potential distribution, mainly in studied areas. Among the countless existent models, stands out the most recent Maxent. **Objectives**: This study proposes the use of the predictive model Maxent, to map the *Bothrops jararaca* potential geographic distribution, in São Paulo State, Brazil. **Methods**: The 151 *B. jararaca* records occurrence found in Instituto Butantan (IB) collection, Museum de Historia Natural from Universidade de Campinas (UNICAMP) and the Projeto BIOTA collected data,, will be combined with climatic surfaces (temperature and precipitation) and topography, to predict the species occurrence in São Paulo. **Results**: The predictive model presented 97% of success in comparison to the known *B*. *jararaca* distribution in São Paulo. It showed a larger distribution in south Minas Gerais, north Rio de Janeiro, Parana coast and Santa Catarina north coast.
Discussion: the predictive ecological niche models are important tools for unknown areas. Although the method considerates only environmental variables and not historical factors, competition and extinction as limitant factors in the species dispersion, it shows a great success, being a useful tool to propose ecological, phylogenetic and taxonomic hypotheses.

4.12 Analysis of *Bothrops jararaca* coagulation inhibitor (BjI) similar proteins in *Bothrops alternatus*, *Bothrops jararacussu* and *Crotalus durissus terrificus* snake plasmas

Morais KB[1], Grego KF[2] and Tanaka-Azevedo AM[1].
[1]Laboratório de Fisiopatologia, [2]Laboratório de Herpetologia, Instituto Butantan, São Paulo, Brasil.

Introduction: *Bothrops jararaca* coagulation inhibitor (BjI) is a high molecular weight protein isolated from *B. jararaca* snake blood, which inhibits thrombin coagulant activity (Tanaka-Azevedo et. al. 2004). **Objective:** To compare the anticoagulant effect of *Bothrops alternatus*, *Bothrops jararacussu* and *Crotalus durissus terrificus* snake plasmas and as well as to investigate the presence of BjI similar proteins in these species. **Methodology:** The snake blood was collected by caudal puncture and the plasma was separated by centrifugation. Thrombin time was performed and the snake plasma proteins were analyzed by SDS-PAGE. Antibodies against BjI were obtained in mice by subcutaneous injection of purified BjI (5 μg). The antiserum containing anti-BjI was used to confirm whether the prolonged thrombin times found in these snake plasmas were caused by the presence of immunological protein similar to BjI by western blotting experiments. **Results:** The same as *B. jararaca* plasma, the poisonous snake plasmas also presented a prolonged thrombin time (31.6 s, 44.1 s and 45.4 s for *B. alternatus, B. jararacussu* and *C. durissus terrificus*, respectively) when compared to the control (10 s). The snake plasma proteins analyzed by SDS-PAGE showed similar profiles. **Discussion:** Our results confirmed the presence of BjI similar proteins in *B. alternatus, B. jararacussu* and *C. durissus terrificus* plasmas by western blotting. The aim of this study was to purify these proteins to compare them with BjI.
Supported by: FAPESP (nº 04/02224-4 e 04/13434-0).

4.13 Altered levels of pyroglutamyl aminopeptidase activity in the vagina and vas deferens are related to reproductive cycle of the snake *Crotalus durissus terrificus*

Marinho CE[1,3], Di Siervi CR[1], Yamasaki SC[1], Villarroel JS[1], Almeida-Santos SM[2], Silveira PF[1].
[1]Lab Pharmacology and [2]Lab Herpetology, Instituto Butantan; [3]Dept Physiology, Instituto de Biociências/USP

Introduction: In *C. d. terrificus* occurs the storage of viable sperm in the female genital tract during the interval between autumnal courtship/mating and spring ovulation. **Objective:** To evaluate throughout the year, in the vagina (VG) and vas deferens (VD) of this snake, the activity of the broad-spectrum pyroglutamyl aminopeptidase (PAP I), which cleaves the TRH analog, fertilization promoting peptide, a known regulator of mammalian sperm function. **Method:** VG and VD were ultracentrifuged to obtain soluble (S) or solubilized membrane-bound (M) fractions. PAP I activity were fluorometrically evaluated. **Results:** Levels of M PAP I in both VG and VD did not significantly vary along the year. The peaks of S PAP I in VG (554±69, n=9) and VD (529±91, n=4) occur respectively in winter (postmating period and sperm storage in female) and summer (premating period and peak of spermatogenesis). During the other seasons the levels of S PAP I did not differ significantly and were between 165-300 in VG and 67-271 in VD. **Discussion:** Seasonal variations on catalytic activity in these tissues demonstrate that PAP I plays a role on the reproductive function of *C. d. terrificus.* The pattern of these variations suggest that PAP I may be part of the mechanism shared by male and female to keep the long term viability and sperm capacitation in this snake.
Supported by CAPES, FAPESP and CNPq

4.14 Survival and mortality rates of three species of colubrid snakes kept in captivity for venom extraction

Braz HBP[1], Parpinelli AC[1, 2], Rocha MMT[1] and Furtado MFD[1]
[1]Laboratory of Herpetology, Instituto Butantan, SP; [2]Department of Physiology, Instituto de Biociências, Universidade de São Paulo, São Paulo, Brazil

Introduction: Owing to increasing interest on colubrid snakes venom, some species have been kept in captivity aiming venom acquisition and storage. It was adopted intensive breeding system due to the advantages in maintenance. **Objective:** In this study we evaluated survival and mortality rates and the likely factors that influence them in *Erythrolamprus aesculapii*, *Philodryas olfersii* and *P. patagoniensis* kept in captivity at Laboratory of Herpetology. **Methodology:** It were utilized 20 specimens of *E. aesculapii*, 30 of *P. olfersii* and 53 of *P. patagoniensis* donated by the population to Instituto Butantan between 2004 and 2006. All specimens were subjected to intensive breeding and periodically fed with mice (to *Philodryas* spp.) or with colubrid snakes (to *E. aesculapii*). Venom extraction occurred monthly without any kind of anesthesia. **Results:** For all species, females collected during dry season had higher survival rates than those collected during rainy season. Males had larger survival rates than females. Rainy season also was the period in which occurred the largest mortality rates. **Discussion:** We suggest that captivity constraints may be less felt in dry season due to the low activity levels and likely energy accumulated during rainy season. Moreover, in rainy season females are often collected gravid or after egg-laying, thus likely debilitated. This fact is corroborated by males that had larger survival than females.
Supported by: FUNDAP and CAPES.

4.15 *Spilotes pullatus anomalepis* (Serpentes: Colubridae): Reproduction.

Duarte MR.
Laboratório de Herpetologia, Instituto Butantan, São Paulo, SP, Brazil.

Introduction: Available data on *S. pullatus* reproduction is very scant, despite the wide distribution of this species in the Americas. **Objectives and Methods**: Data on some aspects of *S. pullatus anomalepis* reproduction are described for twenty two dissected females between 1996-2003. Courtship and mating was observed in captivity in June. **Results and Discussion:** The smallest mature female had 139 cm SVL and 43 cm Tail. Oviductal eggs (n = 7 and n = 11) for two different snakes was recorded in September and October representing 11.4 and 20.7% of the relative clutch mass (RCM) respectively, and non-vitellogenic follicles 0.2% of the total body mass in both of them. Intra-clutch variation in egg ellipticity ranging from 0.40-0.83 cm. One additional gravid snake not represented in this sample had eight eggs that was easily detected by palpation in September. On 13 November 2003, a female (160 cm SVL, 56 cm Tail, 815 g mass after laying eggs) laid 12 eggs (ellipticity range 0.52-0.73; length range 4.1-6.3 cm; width range 2.7-3.4 cm; total egg mass 327g range 20-38 g representing 40.1% RCM). A single male emerged (35 cm SVL, 11.5 cm Tail, 15 g mass) after 113 days of incubation. According to the literature, incubation period in captivity was 72 days, but due the wide range in distribution of this species, some differences in this traits is plausible. The reproductive cycle of *S. pullatus anomalepis* seems to be markedly seasonal.

4.16 *Spilotes pullatus anomalepis* (Serpentes: Colubridae): Courtship and Mating.

Duarte MR[1] & Albuquerque CE[1]
[1]Laboratório de Herpetologia, Instituto Butantan, São Paulo, SP, Brazil.

Introduction: In Brazil, *S. pullatus* occurs in the Atlantic Forest (including some coastal islands), Cerrado, Caatinga, Amazonia and Pantanal. Despite this widespread distribution and relative commonness, data on courtship and mating are unavailable for this species. **Objectives:** Here we describe courtship and mating in captivity for *S. pullatus anomalepis*. **Methods and Results:** A male (142 cm SVL, 50 cm Tail, 915 g mass) from Cotia, SP, Brazil (23° 36´S 46° 55´W) was released in an indoor box in July 2, 2003 together with a female (182 cm SVL, 56 cm Tail, 1.545 g mass) from Domingos Martins, ES, Brazil (20° 21´S 40° 39´W) kept in this same box since June 12, 2003 without food and water *ad libitum*. On July 10, 2003 at 11:15 am, 21.7°C, 61% UR%, courtship and mating behavior was observed for four hours. Male and female were positioned side-by-side while the male flicked its tongue quickly with fast lateral movements of the head along the female body. The male with slow intermittent ondulatory tail movements twisted the female tail for a period of one hour to an hour and a half. The intromission was performed by a left hemipenis, conspicuously "inflating" the vagina *ca*. 10 cm above the cloacal area. At 13:52 pm the mate was suddenly interrupted by the male, which remained with everted hemipenis outside for three minutes. After the copulation a transparent gelatinous substance (plug) with *ca*. 4 cc³ leaked from the female cloaca.

4.17 Effects of incubation temperature on developmental rates and morphological traits in hatchlings of *Oxyrhopus guibei* (Serpents, colubridae)

Braz HBP[1], Almeida-Santos SM[1] and Furtado MFD[1]
[1]Laboratory of Herpetology, Instituto Butantan, São Paulo, Brazil

Introduction: In oviparous reptiles, females can manipulate phenotype of their offspring through nest-site selection. Temperature experienced by embryos during development may affect substantially several physiological, behavioral and morphological traits. **Objective:** Our goal is to investigate the influence of temperature in traits such as incubation period, body sizes and body shapes (mass and tail length relative to snout-vent length) in hatchlings of the false-coral snake *Oxyrhopus guibei*. **Methodology:** Eggs of 9 clutches of *O. guibei* were incubated in mean temperature of 24,3±2,1°C (low treatment) and eggs of 6 clutches in mean temperature of 28,2±2,3°C (high treatment). After hatching, all hatchlings had incubation period, snout-vent length, tail length and mass recorded. **Results:** Incubation period in low treatment (mean=123,6; range 91-145 days) was larger than in high treatment (mean=80,5; range 71-91 days). The thermal regime during incubation also affected body sizes and body shapes both in males and females. In high treatment hatchlings were smaller and thinner than hatchlings of low treatment, however had larger tails. **Discussion:** Shorter incubation periods may be advantageous because eggs undergo less risk in nest (*e.g.*, predation), moreover, hatchlings have more time to feed before the onset of dry season. Some morphological traits affected may be relevant for survival of the hatchlings. Thus, female nest-site selection has major consequences for the phenotypes and, hence, for fitness.
Supported by: FUNDAP.

4.18 Seasonal and daily activity in the neotropical snake *Liotyphlops beui* (Anomalepididae).

Parpinelli L[1], Marques OAV[1]
[1]Lab. Herpetology, Instituto Butantan, São Paulo, Brazil.

Introduction: Data on activity patterns remain completely unknown for many snakes, and almost nothing is known about the ecology of the Anomalepididae. **Objective:** In this study we characterize the seasonal and daily activity of *Liotyphlops beui* from São Paulo city. **Methodology:** The seasonal activity pattern was inferred from the number of snakes brought each month to the Herpetology Lab, Instituto Butantan by lay people from 1994 to 2003. A total of 13 captive snakes were monitored for 10 days by Digital Surveillance System for records of their daily activity. **Results:** Around 80% of specimens were collected between October and March (rainy season). The daily surface activity of the snakes was recorded from 18:00 to 01:30 h, with a peak of activity between 19:00 to 21:00h. When food was available, the snakes show more extended activity than in the absence of food. There was no significant difference between daily activity in the dry and rainy season. **Discussion:** The data obtained here indicate that *L beui* has a seasonal activity pattern associated with the reproductive status and climate throughout the year. Apparently nocturnal activity is a phylogenetic constraint in Scolecophidia since similar daily activity pattern was described for Typhlopidae and the Leptotyphlopidae species. In this group of snakes, temperature, food availability, and predation may restrict daily activity to nocturnal periods.
Supported by: FUNDAP

4.19 Predation on *Trachycephalus mesophaeus* (Anura, Hylidae) by *Chironius exoletus* with comments on possible diet convergence

Rodrigues MG[1, 2]
[1]Lab. Ecologia Animal, Universidade Estadual Paulista, São José do Rio Preto, Brazil; [2]Lab. Herpetologia, Instituto Butantan, São Paulo, Brazil

Introduction: The neotropical colubrid snakes of the genus *Chironius* inhabits rainforest in Central and South America. These snakes are diurnal, arboreal to terrestrial and feed upon frogs. Arboreal species, as *Chironius exoletus* feed mainly on Hylidae anurans. **Objectives:** Here we report a new item, *Trachycephalus mesophaeus* (Hylidae), in the gut of an individual of *C. exoletus*. **Materials and Results:** The record was obtained in the Herpetological Collection of the Museu Nacional do Rio de Janeiro. This is the first record of *T. mesophaeus* as prey of *Chironius.* Another species of *Trachycephalus* (*T. venulosus*) was documented as prey of others snake species as *Leptophis ahaetulla*, *L. mexicanus* and *Liophis poecilogyrus*. However unsuccessful attempts to swallow those prey were observed in two snakes (*Drymarchon corais* and *Leptodeira annulata*). Although nothing is recorded about *T. mesophaeus* secretion, *T. venulosus* exude an abundant sticky and milky secretion by skin when manipulate. This secretion may provide protection against predator, similar to salamanders and other anurans. **Discussion:** These data indicate an ability of some species of snake from different lineages to handle and swallow *Trachycephalus* species, a dangerous hard-to-eat prey, indicating a probably diet convergence. **Supported by**: FAPESP

4.20 *Tropidodryas striaticeps*: Reproduction

Travaglia-Cardoso SR, Albuquerque CC
Lab. Herpetology, Instituto Butantan, São Paulo, Brazil.

Introduction: Snakes from genus *Tropidodryas* exclusively inhabit the Atlantic rainforest in southeastern and southern Brazil (Thomas & Dixon, 1977), and also Bahia state (Argôlo, 1999a,b), constituting two recognized species: *T. serra* (Schlegel, 1837) and *T. striaticeps* (Cope, 1869), having semiarboreal habits and diurnal activities, feeding on lizards, birds and rodents (Thomas & Dixon, 1977). **Objectives:** This summary presents information about oviposition, hatching, clutch size, sex ratio and size of newborns of *T. striaticeps,* a snake with range distribution to ES, MG, PR, RJ, SC, SP (Amaral, 1937), RS (Puorto *et al.*, 2001) and BA (Argôlo, 1999b). **Methods and Results**: One female *T. striaticeps* (IB 65086: 840mm SVL, 225mm TL and 150g after oviposition) collected in Arujá – SP brought to Instituto Butantan laid 8 eggs on January 16th, 2002. The eggs averaged 39.6mm in length (37.0 - 43.0), 19.0mm in width (16.9 – 20.8) and 8.5g in mass (7.3 – 10.1). The RCM was 0.40. The eggs were incubated at temperatures varying from 20 to 27°C. Hatchings occurred after 162 days of oviposition, in a period of 13 days between the first and the last hatching. Male newborns (n=5) averaged 250mm SVL (230 – 270), 77mm TL (75 – 80) and 7.7g (6.4 – 8.7). Female newborns (n=3) averaged 238mm SVL (220 – 250), 75mm TL (70 – 80) and 7.3g (6.7 – 8.0). No stillborns were observed. Sexual dimorphism did not occur in the SVL, TL and mass. **Conclusions:** It would be interesting to study and analyze adult specimens to verify the possibility of ontogenetic variation in morphometric data. This is the first report on laying, hatching and birth of *T. striaticeps*.

4.21 Sexual dimorphism on *Tropidodryas serra* and *Tropidodryas striaticeps*

Travaglia-Cardoso SR[1], Albuquerque CC[1], Puorto G[2]
[1]Lab. Herpetology, Instituto Butantan, S.P; [2]Museu Biológico, Instituto Butantan, S.P., Brazil

Introduction: There are two recognized species from genus *Tropidodryas*: *T. serra* (Schlegel, 1837) and *T. striaticeps* (Cope, 1869), which inhabits exclusively the Atlantic rainforest and both have semiarboreal habits and diurnal activities, feeding on lizards, birds and rodents (Thomas & Dixon, 1977). **Objectives and animals:** We present herein data on sexual dimorphism of this species, based on the analysis of 69 specimens of *T. serra* and 195 specimens of *T. striaticeps*, from southeastern and southern Brazil, deposited in the collections of the Instituto Butantan (n= 68 *T.serra*: 23 females and 46 males; 175 *T. striaticeps*: 112 females and 83 males) and Museu de Ciências da PUC-RS (n=1 *T.serra*; 20 *T. striaticeps*). **Results and Discussion:** The regression of the tail length by SVL was significant for both sexes in both species: *T. serra* (r^2 = 0,88 - males; r^2= 0,91 - females), *T. striaticeps* (r^2= 0,92 - males; r^2= 0,91 - females). Sexual body size dimorphism was evident only in *T. striaticeps*, females being significantly larger than males (t = 4,38; gl= 158; P < 0,0001). Males presented longer relative tail lenght than females in both species (*T. serra*: t = 2,69; gl= 63; P = 0,0089; *T. striaticeps*: t = 15,69; gl= 159; P < 0,0001). Sexual dimorphism where females present larger body and smaller tail is commonly found among the snakes.

4.22 Structural analysis of the digestive tract of Arachnida

Valvassori JR[1], Ribeiro AF[3], Bertani R[2], Terra WR[4], Lopes AR[1]
[1] Laboratorio de Bioquimica e Biofísica, Instituto Butantan; [2] Laboratorio de Imunoquimica, Instituto Butantan; [3]Laboratorio de Microscopia eletrônica, Instituto de Biociências,USP and [4]Laboratório de Bioquímica de Insetos, IQ-USP

Introduction: Although there is a lot of studies of spider and scorpions due to their venoms, studies of their digestion are rarely described. **Objectives:** Thus enzymatic and structural studies on digestive tract from the giant spider *Nephilengys cruentata* and the yellow scorpion *Tityus serrulatus* have been done. **Materials:** Adult females from *Nephilengys cruentata* and *Tityus serrulatus* were collected and dissected under steromicroscope and fixed in Bouin to histological studies. **Results and Discussion:** The digestive tract from the spider is ramified compounding the hepatopancreas were it was possible to identify two different celular types. The digestive tract from *Tityus serrulatus* is less ramified and it is possible to identify its intestine and the hepatopancreas. Homogenates of hepatopancreas from these two species were used in dot blotting experiments using antibodies raised against insect digestive enzymes (amylase, aminopeptidase, cysteine proteinase, chitinase). With the exception of chitinase antiserum all antibodys recognized enzymes in both samples. Due to that tissues are being fixed in order to observe then using electronic microscopy .
Supported by: FAPESP and CNPq

4.23 Spiders (Araneae) from the Archipelago of Alcatrazes, State of São Paulo, Brazil

Bertani R[1] and Martins R[2]
[1]Lab. Imunoquímica, [2]Lab. Artrópodes, Instituto Butantan, São Paulo, Brazil

Introduction and Objectives: The Archipelago of Alcatrazes is in the north coast of the State of São Paulo, 35 km from the continent (24º06'S, 45º41'W). It has an area of 135 ha and mountainous relief, the main peak reaches 316 meters in altitude. The main island is covered with Atlantic Rain Forest, mainly palm trees, and extensive bromelials on the higher lands. There is at least one species of reptiles and two of amphibians endemic to the Archipelago, which makes the study of its flora and fauna important. Concerning spiders, there are 7 recorded species, collected in 1920 by an expedition of the Paulista Museum. These spiders were studied by the arachnologist Cândido de Mello-Leitão, who described one genus and three new species. **Results and Discussion:** During the 42ª research expedition to the Alcatrazes Archipelago, from August 17 to 19,2005, 218 spider specimens were collected, using an entomological umbrella and manual collecting by day and night. Of these, 130 (60%) were immature. Adult specimens of 2 mygalomorph families and 18 araneomorph families were found, with 39 genera and 39 species: Mygalomorphae: Nemesiidae (1), Theraphosidae (2), Araneomorphae: Anyphaenidae (2), Araneidae (4), Caponiidae (1), Corinnidae (2), Ctenidae (4), Dictynidae (1), Linyphiidae (2), Mimetidae (1), Miturgidae (1), Philodromidae (1), Pholcidae (2), Salticidae (3), Segestriidae (1), Selenopidae (1), Sparassidae (2), Tetragnathidae (1), Theridiidae (6), Thomisidae (1).

Supported by: FAPESP

4.24 Arachnids of the Parque Estadual Turístico do Alto Ribeira, Iporanga, SP.

Bertani R, Nagahama RH, Shoji D, Ortega DRM, Tambourgi DV, Gonçalves-de-Andrade RM
Laboratório de Imunoquímica, Instituto Butantan

Introduction: The Parque Estadual Turístico do Alto Ribeira it is one of the most ancient parks of the São Paulo state. The environmental importance of this geologic site, located at the left margin of the Ribeira de Iguape River, south of the São Paulo state lies in the association of tropical Atlantic forest and caves system. The Atlantic Forest presents an impressive wealth of biological diversity. Studies about the araneofauna of the forestall area of this karst are practically inexistents. The existing literature generally focuses on ecologic and taxonomic studies about spiders found inside the caves. **Objectives and Methods:** In order to characterize the forestall spider fauna of PETAR, a preliminary study was carried out in June and August 2005. The spiders were captured in the Santana, Lajeado and Casa de Pedra nucleus. **Results and Discussion:** The results include five Mygalomorphae and thirty two Araneomorphae families. The captured Mygalomorphae species spiders belong to the Actinopodidae, Dipluridae, Idiopidae, Nemesiidae and Theraphosidae families. Among the Araneomorphae spiders, there were specimes of Anapidae, Anyphaenidae, Araneidae, Caponiidae, Corinnidae, Ctenidae, Deinopidae, Hahniidae, Linyphiidae, Lycosidae, Mimetidae, Miturgidae, Ochyroceratidae, Oonopidae, Palpimanidae, Philodromidae, Pholcidae, Pisauridae, Salticidae, Segestriidae, Senoculidae, Sicariidae, Sparasiidae, Scytodidae, Tetragnathidae, Theridiidae, Teridiosomatiidae, Thomisidae, Titanoecidae, Trechaleidae, Uloboridae e Zoridae. The Araneidae family was the most abundant, followed by Theridiidae and Salticidae. The richness of the spider species diversity in this portion of the Atlantic Forest of São Paulo state could be proved in this preliminary inventory and reinforce the necessity of the ecological studies about the araneofauna in this important bioma of the Brazil.

4.25 Spider phylogeny (Araneae: Araneomorphae) based on predatory behavior sequences

Huffenbaecher C[1], Alberts CC[2], Japyassú HF[3]
[1]Instituto De Psicologia, USP, [2]Lab Vertebrados, UNESP, Assis, Brasil, [3] Lab. Artrópodes, Instituto Butantan, São Paulo, Brasil

Introduction: The order Araneae is one that shows the highest levels of diversity in the world and can be found in almost all ecosystems. In recent decades, numerous comparative data for this group have been collected, including morphologic, molecular and behavioral ones. **Objectives**: To create a phylogenetic reconstruction of the Order based on predatory behavior sequences. Stereotyped behaviors have been used in phylogenetic reconstruction research. **Methodology:** We analyzed adult females from Lycosidae, Uloboridae, Tetragnathidae and Theridiidae that were offered *Tenebrio molitor* larvae as prey, a very uncommon item in its diet. **Results**: As result, we obtained a non oriented cladogram showing closer proximity between Tetragnathidae and Theridiidae, whereas the position of Uloboridae and Lycosidae were not resolved in respect to the former Families. **Discussion:** Further analysis of at least one specie pertaining to an out-group is necessary in order to arrive at a definitive conclusion in affirming the monophily of orbiculariae.

4.26 Comparative tenacity analyze in orb weaver spiders.

Kawamoto TH[1,2], Japyassú HF[1]
[1]Instituto Butantan., São Paulo, Brasil, [2]Instituto de Psicologia da USP, São Paulo, Brasil.

Introduction: The phylogeny of spider groups typically has cribellate spiders in association with more diverse ecribellate groups. In the case of Orbiculariae the higher diversification of the ecribellates (Araneoidea) is traditionally explained by the superiority of the viscid orbweb. However this explanation is not satisfactory since detailed studies show that the cribellate silk can be equally effective. Objectives: For that reason we consider the hypothesis that the reduction of tenacity in viscid orbweavers could have exposed these spiders to a higher microhabitat diversity, which in turn would lead to the higher diversification of Araneoidea. Materials: To test this hypothesis we evaluated the response of one cribellate (*Zosis geniculata*, Uloboridae) and one ecribellate (*Metazygia rogenhoferi*, Araneidae) orbweb spider to a reduction in the availability of preys. We measured the changes in site tenacity and in web investment on a daily basis. Results and Discussion: The ecribellate spider does not change its site tenacity or web investment. The cribellate spider enhances site tenacity and reduce web investment as a response to prey shortage. These results agree with the hypothesis that a cheaper ecribellate orbweb leads to low tenacity. More frequent microhabitat desertion lead to the exploration of a higher diversity of microhabitats, which, in long run, could be the cost explanation to the higher diversification of Araneoidea.

Supported by: CAPES

4.27 Orb web construction metabolism: cribellate versus ecribellate spiders.

Kawamoto TH[1,2], Kaneto GE[3], Japyassú HF[1]
[1]Instituto Butantan., São Paulo, [2]Instituto de Psicologia, [3]Instituto de Biociências-USP, SP, Brasil.

Introduction and Objectives: Like many other taxonomic groups of spiders, Orbiculariae has at its base cribellate spiders (Deinopoidea) associated with a more diverse ecribellate sister group (Araneoidea). In Orbiculariae this major diversification is classically explained by the superiority of the viscid thread. But the cribellate silk can be more effective in some contexts, so that thread effectiveness does not explain completely the diversification of the group. Thus we investigated another hypothesis: the diversification of Araneoidea as a consequence of low cost viscid web. **Materials and methods**: We measured metabolic rate (using oxygen consumption) at rest and during web building of one cribellate (*Zosis geniculata*, Uloboridae) and one ecribellate (*Metazygia rogenhoferi*, Araneidae) spider, with an intermittent respirometry system. **Results and Discussion:** The ecribellate spider has higher rest metabolism, suggesting that it is more prompt to activity. On the other hand, *Z. geniculata* lower consumption could be an evolutionary response to a low relative energetic intake due to a more expensive web: the capture spiral length built with cribellate silk is three times more costly than the viscid one. Considering also another study of tenacity in these same spiders, it appears that viscid orb web diversification is indeed correlated with the onset of a low cost web. The reduction in viscid orb web cost allows a more active microhabitat occupation, resulting in exposure to a higher variety of selective pressures. This potentially increases diversity of this group through evolution.
Supported by: CAPES

4.28 Kleptoparasitic activity of *Argyrodes elevatus* in webs of achaearanea sp. and *Latrodectus geometricus* (Theridiidae, araneae)

Silveira MC[1] & Japyassú HF[1]
[1]Laboratório de Artrópodes, Instituto Butantan.

Introduction: Interspecific kleptoparasitism in spiders occurs in many families, but few studies explore this behaviour in detail. **Objective and methods:** This work describes the kleptoparasitic activity of *Argyrodes elevatus*. Two adult females of *Achaearanea* sp. and one adult female of *Latrodectus geometricus* were maintained in the laboratory and fed *ad libitum* with *Grillus* sp. Two *A. elevatus* were included in each web. **Results and Discussion**: We observed seven successful theft events, all of them in *Achaearanea* sp. webs. When the host runs to capture a second cricket, after immobilizing the first, the kleptoparasite immediately approaches the first prey, cut the threads around it, carrying it away from the host web. During the theft, *A. elevatus* cut the host web threads substituting them with its own thinner threads, thus reducing the probability of being detected by the host. Nevertheless, the host frequently perceives the kleptoparasite vibrations and runs towards them. The host (*Achaearanea* sp.) was able to catch the kleptoparasite in two instances. *A. elevatus* directly captured the prey, without participation of the host spider, once in an *Achaearanea* sp. web and a second time in a *L. geometricus* web. The preference of *A. elevatus* to steal host-captured prey in *Achaearanea* sp. webs could be related to the absence of hub in this species. We did not notice the 'feeding with host' strategy, which occur in webs of *Nephila clavipes*. These results, together with published data, point to a high capacity of *A. elevatus* to adjust its foraging tactics according to its actual host.
Supported by: FUNDAP

4.29 Predatory behaviour of *Zosis geniculata* (Oliver, 1789) (Uloboridae, araneae)

Tamelini PC[1], Huffenbaecher C[1], Kawamoto TH[1], Silveira MC[1] & Japyassú HF[1]
[1]Laboratório de Artrópodes, Instituto Butantan.

Introduction: There is not much knowledge about foraging among uloborid spiders. Studies of the predatory behaviour in Uloboridae could elucidate evolutive features of foraging in Orbiculariae because it is a basal taxa in this larger group. **Objectives:** We analised 20 predatory sequences in *Zosis geniculata* feeding tenebrio beetle larvae (*Tenebrio molitor*), videotaped in laboratory cages. **Methods:** Prey immobilization begins with wrapping, which occurs simultaneously with flexions of the first pair of legs (bouncing). Wrapping takes most of the time in the whole capture sequence and is intermittently interrupted by thread cutting. After immobilized, the prey is carried to the center of the web in the jaws. While carrying the prey the spider repair its web by connecting the damaged radius to the hub through a new line. At the hub the spider continues to wrap the prey. Next, the spider manipulates the prey while covering it with oral digestive fluid (beggining of the extracorporal digestion). **Results and Discussion:** The category bounce is very frequent in the predatory sequence of *Z. geniculata* and was not observed in any other orbweaver. Web repair during prey capture differs among *Z. geniculata* and *Theridion evexum* (Theridiidae), because *T. evexum* connects both extremities of the broken line and does not pay a new line while going back to the hub. The extensive wrapping time and the absence of biting attack in *Z. geniculata* could be associated respectively to the presence of a cribelum and the lack of poison glands in this species.
Supported by: FUNDAP

5. Animal care and veterinary diseases

5.01 Patterns of straw used in Central Bioterium

Rodrigues UP, Rocha da Silva AP, Mattaraia VGM, Valentini EJG, Barreto VM, Tavora MFCLF.
Divisão Biotério Central, Instituto Butantan, São Paulo, Brazil

Introduction: Straw used as laboratory animal bedding is considered one of the main contamination vectors that can influence experimental data and animal well-being. Despite its high importance little information exists in the national literature, nor in Good Manufacturing Practices. **Objective**: To report guidelines established by Central Bioterium Division for straw used in our colonies. **Methodology**: After several years of testing five fundamental straw characteristics were established: 1 – botanic identification by macro and microscopic analysis of anatomic xylem structure; 2 – moisture content (MC); 3 – water absorption capacity (WAC); 4 – verification of spotted and/or tainted fungi by macro and microscopic exams; 5 – granulometry/ particle dimensions measured by means of sifted sample. **Results**: Characteristic 1: the best wood was pinus (*Pinups sp*); Characteristic 2: acceptable content is from 10 to 13%; Characteristic 3: water absorption should be $\geq$ 300%; Characteristic 4: spotted and/or tainted fungi of 5% in maximum; Characteristic 5: specific values to particle dimension when sifted should be 0.0% in sieves with 28.6 mm of diameter, and from 0.1 to 0.6% in sieves of 22.2 mm. **Conclusion**: Straw with higher absorption power, to promote animal thermo equilibrium, higher intervals of bed changing, decreasing mortality of pre and post-weaning will contribute greatly to animal well-being.

5.02 Cytochemical analysis of blood from *Bothrops* and *Crotalus* species

Rameh de Albuquerque LC[1,2], Almeida CD[2], Sano-Martins IS[2] , Catão-Dias JL[1]
[1]Departamento de Patologia Experimental e Comparada-FMVZ/USP, [2] Laboratório de Fisiopatologia do Instituto Butantan

Introduction: The morphological characteristics of blood cells from reptiles are heterogeneous, although variations in populations and cell characteristics are evident even between species of the same order. Cytochemical stains are useful for characterizing different types of leucocytes. **Objectives:** This study evaluated cytochemical characteristics for leucocytes from the genus *Bothrops* and *Crotalus*, kept in captivity at the Herpetological Laboratory – Butantan Institute. **Methods:** Blood smears were obtained from 5 animals of each species, and reactions for Sudan Black B, Periodic Acid Schiff and Benzidine Peroxidase were recorded. **Results and Discussion:** The lymphocytes and basophils were negative for all stains in all species, except for *B. alternatus* where they presented a weak positivity for PAS. Heterophils were negative for SBB and BP and positive for PAS in all species, except for *B. moojeni,* which presented positivity for SBB and negativity for PAS. Degranulated heterophils showed a great variation between species, they were positive for SBB in *B. alternatus* and in some cells of *B. jararaca*; some degranulated heterophils presented positivity for BP in *B. moojeni*, *B. jararacussu* and *C. d. terrificus*; and for PAS in *B. moojeni*. Azurophils were variable for all stains in all species. **Conclusions:** The results of this study presented differences even between species of the same genus, showing these cytochemical results did not explain controversies on whether eosinophils exist in the blood of snakes. We think we have to perform more ultraestructural and imunocytochemical studies to resolve these problems.
Supported by: FAPESP

5.03 Hematological data of *Elaphe guttata* (Ophidia, Colubridae) born in captivity

Rameh de Albuquerque L C[1,2] , Zanotti A P[1,3] e-mail: cobra_znt@yahoo.com.br
[1]COBRA – Centro Operacional Brasileiro de Répteis e Aracnídeos – Criadouro Conservacionista, [2]Laboratório de Fisiopatologia- Instituto Butantan, [3] Lab. de Biologia Celular - Instituto Butantan

Introduction: *Elaphe guttata* (Corn snake) is a Colubridae snake distributed throughout the central-eastern portion of North America, inhabiting temperate zone. It is the most familiar snake kept in captivity in North America and an excellent first snake for beginners because they are easily available as captive-bred specimen. **Objectives**: Evaluation of hematological data is of fundamental importance for the analysis of the physical state of the animal and to diagnose diseases. Hematological values in American snakes are well documented in literature. Therefore, this study will complement knowledge about this specie. **Methodology**: This study was performed with 12 snakes (5 males, 7 females) ranging from 3 to 6 years old, born and kept in captivity at a conservationist breeding-colony registered at IBAMA. Blood samples were collected by venopuncture of the caudal vein and transferred to eppendorff tubes with heparin. Blood smears were obtained and stained with Rosenfeld for differential leucocyte count. For the red blood cell, white blood cell and thrombocyte cell count, 10 µl of blood were diluted in 2 ml of Natt and Herrick solution in a Neubauer chamber. Hemoglobin determination was performed with the comercial kit Labtest (Labtest diagnóstica). The packed cell volume was determinated by the microhematocrit method. **Results**: The mean results were 1283.73 x 10^9/L for RBCC; 9.77 x 10^9/L for WBCC; 39.9 x 109/L for TC; 8.38 g/dl for hemoglobin; 28.37% for hematocrit; 228.73 fl for MVC; 67.02 pg for MCH; 31.94% for MCHC. The mean results of differential counts were 13% of heterophils; 1.27% of basophils; 7% of azurophils and 79.54% of lymphocytes. **Discussion**: The results did not differ statistically between sexes. No degranulated heterophils were found in the group analized.

5.04 Equine Dermatophytosis in São Joaquim Farm, Butantan Institute, São Paulo

Almeida AL[1], Silva PMP[1], Freitas MG[1], Parra AC[2], Scalamandré PSB[2], Azevedo RF[1], Higashi HG[1], Marcelino JR[1], Araújo WP[2], Guidolin R[1], Távora JPF[1]
[1] Fazenda São Joaquim- Instituto Butantan; [2] FMVZ –USP.

Introduction and Objectives: Dermatophytosis is a cutaneous contagious disease, very common in equines, caused by *Trichophyton equinum*, *Trichophyton mentagrophytes*, *Microsporum gypseum* and *Microsporum canis*. The infection is self-limiting, but can seriously modify the functions of these animals; hence it is of zoonotic importance. It is spread by direct contact with the infected animals or by indirect contact through contaminated fomites or through the environment. The main predisposing factors are concurrent immunosuppressive disorders, inadequate nutrition, and stress due the overcrowding and senile animals. Also excessive environmental moisture, mechanical disruption of the protective skin barrier and hyperhidrosis appear to facilitate the penetration of the agent and infection. Symptoms are variable, including pain and pruritus. The characteristic lesions are circular alopecia with thick coats in the edge. Edema and hyperpigmentation can be observed. **Methodology and Results**: The microorganisms were identified and diagnosed by fungal and biopsy culture of the skin. Folliculitis, perifolliculitis and furunculosis, superficial dermatitis perivascular with parakeratosis were the histological findings. Immediate treatment was recommended to prevent dissemination. Several animals of the farm presented clinical symptoms and were treated with topical and systemic antifungal drugs. **Conclusion**: The animals were reintegrated to the flock in 45 days.

5.05 Occurrence of exsudative desmovilite in equine of the Farm São Joaquin, Butantan Institute, São Paulo.

Silva PMP[1], Almeida AL[1], Freitas MG[1], Parra AC[2], Scalamandré PSB[2], Azevedo RF[1], Higashi HG[1], Marcelino JR[1], Araújo WP[2], Guidolin R[1], Távora JPF[1]
[1] Fazenda São Joaquim - Instituto Butantan; [2] FMVZ –USP.

Introduction: The exudative desmovilite is a degenerative illness that involves frog, mainly the lateral, medial and central gap characterized by maceration, softening and destruction of the cornea layer with blackish necrotic secretion production and a putrid odor. The hooves with high coupon stubs hinder the contact of frog on the ground, favoring accumulation of excrements and fodder plants between the ridges, propitiating fermentative processes, which favor the proliferation of microorganisms. Many biological agents are involved in this process, the fusiforms, being the most important. The symptom most evident is the necrosis with blackish fetid secretion and the destruction of the anatomical structure of frog. Lameness rarely occurs, but when it exists, it reaches sensible structures, which it seriously compromises. **Methodology and Results:** Preventive treatment consists of the elimination of the predisposing conditions of the injury, with hygienic installations and elimination of muddy ground surfaces, allowing for adequate drainage of urine. The routine must be observed to allow for the contact of frog with the ground as well as the promoting cleanliness of the ridge central and sides of the frog. An adult animal with about 8 years of the bothropic group presented the aforementioned symptoms and its hoof was vigorously scrubbed with water and soap, necrotic fabric withdrawal of frog, then a an antiseptic solution of formol iodine 5%, dye 10% and later liquor of Villate was applied to the sole of the hoof. Had been applied chemotherapy by saw intravenous during 5 days. They had been the curative of hooves and waterproofed facts with Tar, in first the 7 days. **Conclusions:** After 45 days, it had reconstitution of frog and the animal was reintegrated to the breeding.

5.06 Equine Dermatophilosis ("rain scald") in the São Joaquim Farm, Butantan Institute, São Paulo.

Azevedo RF[1], Silva PMP[1]., Almeida AL[1], Freitas MG[1], Parra AC[2]., Scalamandré PSB [2], Higashi HG[1], Marcelino JR[1], Araújo WP[2], Guidolin R[1], Távora JPF[1]
[1] Fazenda São Joaquim- Instituto Butantan; [2] FMVZ –USP.

Introduction: Dermatophilosis is a cutaneous disease of bacterial origin occurring in equines and other large animals, caused by an *Actinomycetes*, gram positive, facultative anaerobic, *Dermatophilus congolensis*. The natural habitat of this microorganism is unknown, but it is assumed to exist in latent state in the carrier animals until transmission conditions are achieved. The development of lesions depends upon the moisture and presence of damaged skin in the animal. The skin can be injured by chronic maceration due to humidity, fly bites, prickly vegetation in the pasture or pruritic cutaneous diseases. The zoospores can remain viable in crusts in ambient temperature at 28 to 31°C for up to 42 months. The primary lesions are follicular become exudative and groups of hairs forming in a shape like a paintbrush. The lesions found in the back, gluteal region, face, neck and extremities. The animals seriously affected present fever, depression, lethargy, anorexia and regional lymphadenopathy. Dermatophilosis is diagnosed by the demonstration of the microorganism, in cytological examination of a crust or by histological evaluation through biopsy of the skin. The histological results include folicullitis, intraepidermic pustules, intracellular edema, alternated layers of hyperkeratosis, parakeratosis and orthokeratosis with remaining portions of leukocytes. **Methods and Results:** The adult equines of the bothropic group presenting this disease were treated with topical therapy, removed crusts using benzoila peroxide or clorexidine, applied with an absorbent sponge, daily, during 7 days, until lesions healed and afterwards, 2 times per week, until lesions disappeared. **Conclusions:** After 2 months, the animals were reintegrated to breeding.

6. Education and Science Diffusion

6.01 Inclusion on the data of the Acari Collection of the Butantan Institute (IBSP) in the SpeciesLink net, enabling the public use of scientific information

Arruda-Santos AD[1], Onofrio VC[1], Barros-Battesti DM[1]
[1]Laboratório de Parasitologia, Instituto Butantan, São Paulo, Brazil.

Introdução: The Acari collection of the Instituto Butantan (IBSP) includes mainly ectoparasites of wild animals. It is one of the largest and most important Latin American collections with many type specimens of Brazilian and foreign species. **Objective:** The aim of this work was to computerize the data of mites and ticks deposited in IBSP using speciesLink, a search engine concerning the biodiversity present in museums, herbarium and zoo collections. **Material and Methods:** Because the data are extensive it is being compiled little by little. However, most of the information regarding ticks has already been added to speciesLink. Data concerning each species and its geographic distribution is available on line and free. The material was in part obtained from collections supported either by FAPESP or CNPq. The remaining part was received by donations from other Institutions. Other projects, from other laboratories such as the Hepertology and Arthropod labs, as well as material from ransom fauna, and routine suppliers, also contributed to enlarge the collection. The data bank was based on files corresponding to the number of each lot and on the revision of the deposited material. **Results and Discussion:** In a period of eight months the data of 2,883 lots, including 42,681 specimens (41,582 tick and 1,099 mite), was posted online. Enabling the public access of scientific data using the internet contributes to the development of research programs and environmental conservation strategies.
Supported by: FAPESP

6.02 Phisalix and Brazil: the naturalist view and the discovery of serum specificity

Faria M [1], Fernandes SCG [1,2], Bochner R [3], Sant'Anna OA[1,4]
[1]Laboratório Especial de História da Ciência, [2]Museu Histórico, [3]Fundação Oswaldo Cruz, [4]Laboratório de Imunoquímica, Instituto Butantan

Introduction: The rise of the 20th Century matches the discoveries of serumtherapy and most importantly of its specificity. It is a common event in the history of scientific discoveries that they shall be independently enterprise simultaneously by different scientists or research groups. **Objectives**: Here we will compare the profile of three important characters in producing the facts that led these discoveries: Vital Brazil, Albert Calmette and Césaire Auguste Phisalix. **Results and Discussion**: The first lived in Brazil and founded the IB; the last two were living in France working respectively at Institute Pasteur and the National History Museum in Paris. By analyzing their scientific production and context along with personal motivations and interests as shown by articles and letters, we shall suggest witch were the key elements for the very diverse trajectories they adopted in their research. The fact that Calmette although exposed to the same facts as the other two could not postulate the specificity of serum therapy will be discussed under the light his formation and lack of background on snakes taxonomy and general physiology. We will show that Phisalix and Brazil, although not subject to the same facts had a common naturalistic reasoning that allowed them to draw similar conclusions from different observations. It shall be clear that besides strictly academic and professional differences, their social network was a decisive ingredient in crafting scientific bias.

6.03 From Serumtherapic Institute to Experimental Medicine Center: Butantan Institute in the 1930s

Fernandes SCG[1,2], Faria M[1], Ibañez N[1], Sant'Anna OA[1,3], Fan Hui Wen[1,4]
[1]Laboratório Especial de História da Ciência, [2]Museu Histórico, [3]Laboratório de Imunoquímica, [4]Hospital Vital Brazil

Introduction: The Instituto Butantan enters the 21st century as one of the main national organisms for biological drugs production and research. **Objectives**: Here we shall study a period during which the Institute undergoes deep transformations concerning its functions and organization. **Results and Conclusions:** In 1941 the institute becomes a Center for Experimental Medicine linked to the state secretary of public health and education. These formal changes are symptomatic of new attributions such as studies in human pathology and the distribution of drugs and vaccines. At the same time as the IB gains a new status and the state itself seems to be increasingly organized in a hierarchical structure, new economic and political demands come into the scene: the market economy gets stronger, the national drug industry is created along with the firs public universities and many foreigner scientists come to Brazil between wars. The Institute tries to adapt but new circumstances eventually bring about the first crisis. As much as the external factors, internal components had an important role in the etiology of the crisis; alternating directors with opposing strategic views become icons of how problematic is the construction of an institutional identity in a incipient state. Of importance are also the structural changes in state organization; the institute performance; the main actors inside the IB; the external facts as indexes of the relationship between state, scientific community, market and institutions.

6.04 Personal correspondences of Afrânio do Amaral: chronicling an academic career.

Fernandes SCG[1], Calleffo MEV[2]
[1]Museu Histórico e Laboratório Especial de História da Ciência, [2]Laboratório de Herpetologia

Introduction: The correspondence of Afrânio do Amaral included approximately 1,200 letters, received from diverse institutions and personalities of the time, scientists, politicians, fellow workers, among others as well as letters to institutions and private persons. These writings extend from 1920 to 1980 and are related to activities, contacts, and projects of this period. Graduating from the School of Medicine of Bahia with a doctorate from Harvard University, in Public Health, Amaral collaborated actively in the movements for the creation of São Paulo medical schools and participated in founding the São Paulo School of Medicine (1933) and of the University of São Paulo (1934-35). Directing Butantan Institute on various occasions, Amaral was responsible for reforming the Institute throughout the 20s and 30s when Butantan became a Laboratory of Experimental Medicine. **Methodology:** The letters are being catalogued for subjects and for dates, after donation to Instituto Butantan, then filed and thumbed. **Results and Discussion:** The letters were recently donated by the family to Dr. Paulo E. Vanzolini and are, primarily, not restricted to ophiological research, but include personal relations and academic contacts, prioritized at various moments of his career that mark his performances as a public administrator and scientist. Until now, approximately 1,000 letters have been catalogued. This documentation serves as the primary source for future research related to administrative, scientific history and politics of the Butantan Institute. This work has been guided for the Prof Dr. P.E. Vanzolini.

6.05 Portraits, cuttings, retrospects: iconography of the poisonous animal accidents at the Instituto Butantan.

Solosando A[1,2,3], Fan HW [1,3], Fernandes SCG[1,2].
[1]Laboratório Especial de História da Ciência do Instituto Butantan; [2]Museu Histórico do Instituto Butantan; [3]Hospital Vital Brazil do Instituto Butantan

Introduction and Objectives: The worries of the Instituto Butantan in rescuing its historical and scientific memory have made emerge many projects with the goal of promoting the rescue of the institutional collection, focusing on the cataloguing, restoration and the research on various sources, iconographic ones among them. Resulting from its mission as a research center in poisons and poisonous animals, the institution keeps and produces, since its founding on the beginning of the 20th century, records of poisonous animal accidents. Such records put into evidence the variety in the use of photographic images as medical illustration, but are also constitutive elements of the history of medical practices in Brazil. **Materials:** Examples of collections are: 1) Publications, like *Memórias do Instituto Butantan* (Memories of the Instituto Butantan) and *Coletânea de Trabalhos do Instituto Butantan* (Collections of Works of the Instituto Butantan); 2) The Section of Photographies of the Institute, containing images from since the end of 1930, and 3) The collection of the Hospital Vital Brazil, specialized on the assistance to people injured by poisonous animals, founded on 1945. This last collection probably represents the largest collection of this type, which has begun with Gastão Rosenfeld, hospital director between 1954 and 1966, having been maintained by the physicians until now. **Discussion:** These approaches, however, don't empty the analytical possibilities in the domain of History, in so far as the photograph ceases to be a mere illustrative element of the research to assume the status of a document and raw material in the production of knowledge.

6.06 Museum microbiology courses: pedagogical tools for biology and science teachers and evaluation of teacher expectations

Casadei K, Henrique BC, Imparato BA, Yoshida RY, Vieira JLA, Inglez, GC, De Franco MT
Museu de Microbiologia, Instituto Butantan.

Introduction: Understanding the needs of elementary and high school microbiology teachers, the Museum of Microbiology of Butantan Institute offers training courses for biology and science teachers from public and private schools. **Objective:** Provide distinct tools for teaching microbiology, performing simple and low costs practical activities, allowing teachers the creation of interesting microbiology classes as well as to evaluate the expectations of teachers enrolled in these courses. **Methodology:** Experiments, group presentations, games, films and text discussions are proposed and performed in courses, which have been offered yearly since 2003. At the end of each course, the teachers are invited to complete a questionnaire, with open and closed questions, to evaluate their satisfaction level. **Results:** The teachers participated actively in all activities proposed. The course contents fulfilled 95% of the participants' expectations. Moreover, 87% of the teachers considered the course Highly Gratifying, 10% Good and only 3% Sufficient. **Discussion:** The courses and activities performed in them fulfilled the expectation of most of the teachers who were very interested in applying these new tools in their microbiology classes.
Supported by: Butantan Foundation and FINEP

6.07 Program of voluntary monitors formation in the museum of microbiology of the Butantan Institute

Inglez GC, Casadei K, Miyamoto LF, De Franco MT
Museum of Microbiology of the Butantan Institute

Introduction: Concerning the improvement of services offered by the Museum of Microbiology of Butantan Institute, especially those services offered by its monitors team, the adoption of a training program for voluntary monitors candidates becomes crucial. This program aims at serving as a manual to promote periodic monitors substitution. **Objectives**: To structure the monitors training period that takes place during 3 months on average; to settle stages of learning, classifying the contents following logic based on the type and scope of monitors services; and to adopt an evaluation model of voluntary monitors. **Methodology:** In the first week of training, there's a Special Integration Programming for new monitors. Afterwards, with the orientation and supervision of a regular monitor, they are introduced to a Learning Programming. During all this time, there's a continuous assessment of individual performance and, at the end, they are submitted to both written and oral tests, when basic knowledge about the Museum is required. **Results:** The volunteers approved in this Program work as monitors for a period at least equal to their training duration. Until now, 35 voluntary monitors circulated in the Museum, and 10 of them were hired to substitute the regular monitors who have left the Museum. **Discussion:** We know that any activity without remuneration requires altruism and determination from people. Therefore, we value the attitude of Biology students who become voluntary monitors for a specific period. We expect that this Program of Voluntary Monitors Formation brings satisfactory professional growth and personal enrichment to students, beyond allowing the change of specialized monitors in the Museum.
Supported by: Butantan Foundation

6.08 The interaction between visitors and the museum of microbiology exhibition of Butantan Institute

Marques F[1], Inglez, GC[2,] De Franco MT[2], Bizerra AF[3]
[1]Estação Ciência, USP, [2]Museu de Microbiologia e [3]Museu Biológico, [2,3]Instituto Butantan

Introduction: The assessment of the existing relationship between visitors and the scientific museums exhibition has been considered as an important tool to study the process of scientific literacy. **Objective:** The present research aims to evaluate how groups of children and adults react to the scientific exhibits at the Butantan Institute Museum of Microbiology. Also, the research intends to obtain a profile of the visitors during the school holidays (December/January/February). **Methodology:** To this end, 49 direct observation and interviews were made with groups of six people each, collecting data on their origins, sex, age, schooling, profession, family relationship within the groups and their habitualness on visiting scientific museums. The number of total visitors during the mentioned months was also collected. The direct observation of the visitor reaction to the exhibits were based on the *trekking* and *timing* of one member of each group, and data were registered in *croquis*, together with his behavior at each station. After completing the visit, the subject was submitted to a semi-structured interview. **Results:** The most numerous visitors were children, at the age bracket 0-12 and adults aged 21-40 years old ($p<0,001$). From a total of 44 different exhibits, this population interacted with 18,6 of them, mainly microscopes. **Discussion:** The museum educational purpose should possibly be enhanced by the analysis of the interaction of the visitors with the devices and their relationship with the monitoring guides.

6.09 Diversification of classes given in the microbiology museum laboratory.

Vieira JLA, Inglez GC, Henrique BC, Pereira FF, Casadei K, Imparato BA, Yoshida RY, Will S, De Franco TM.
Museum of Microbiology of the Butantan Institute

Introduction: The classes (module I) carried out in the Microbiology Museum Laboratory are based on a "Kit of Experiences with Microorganisms". The success of these classes could be verified through the enthusiasm of teachers and pupils and the manifestation from them for the continuity of those practices. This success motivated us to prepare other four comprehensive modules to be offered as from March of 2006. **Objective:** Creation of four new thematic modules based on the "Kit of Experiences" aiming at the return of pupils who have already participated in module I and at increasing the learning of Microbiology concepts. **Methodology:** Through research carried out in books, sites and other sources, we have chosen and adapted some experiences to be realized in the Laboratory of the Museum and we grouped the experiences in five modules. **Modules:** Five thematic modules were developed as following: I-Introduction to Microbiology, II-Fungus, III-Action of physical and chemical agents on microorganisms, IV-Bacteria and V-DNA. **Expected Results:** We believe that our classes will improve the quality of education offered by teachers and will transform the working conditions in the matter of experimentation. We expect that the application of the modules will instigate the learning of Microbiology concepts through science practices and will motivate teachers to adopt this methodology in their schools.

Supported by: Butantan Foundation and FINEP

7. Cellular Biology

7.01 Evaluation of conformation of recombinant glycoprotein of rabies virus in *Drosophila melanogaster* (S2) cells using monoclonal and polyclonal antibodies.

Yokomizo AY[1], Astray RM[1], Ribeiro OG[2], Tordo N[3], Jallet C[3] and Pereira CA[1]
[1]Laboratório de Imunologia Viral, [2]Laboratório de Imunogenética, Instituto Butantan, São Paulo, Brazil; [3]Unité des Stratégies Antivirales, Institut Pasteur, Paris, France

Introduction: Rabies virus is a neurotropic virus which causes severe disease and death in great number of mammals, including humans. In our laboratory we obtained a recombinant *Drosophila melanogaster* cell line (S2) expressing glycoprotein of rabies virus for diagnostic and therapeutic use. **Objectives and Methods:** Methods to obtain recombinant glycoprotein (rGPV) from cells lysate and monoclonal (D1 specific for trimeric conformation) and polyclonal antibodies against glycoprotein of rabies virus (Institute Pasteur) have been used to evaluate the quality and quantity of rGPV. D1 anti GPV conjugated with fluorescein has been used to study cultures and subcultures of recombinant S2 selected by dilution or cytometry (FACS sorter) of cells. Different detergents and techniques to lyse cells (freezing, sonication, buffers containing Nonidet® or CHAPS) were evaluated by ELISA test using monoclonal and polyclonal antibodies with respective forms conjugated with peroxidase. Cytometry using D1 antibodies showed a population of 35% of cells expressing high levels of rGPV ($<10^1$) and a second one of 5% with higher level ($<10^2$) that can be sorted simultaneously. **Results and Discussion:** ELISA test with D1 antibodies reveled highest quantities of rGPV lysing cells with buffer containing 1% of CHAPS, which correspond 98% of total rGPV detected when polyclonal antibodies were used. This antibodies and methods have great importance once protein conformation is fundamental for biological activity.
Supported by: FAPESP, CNPq, CAPES and Butantan Foundation

7.02 Production of the G glycoprotein from rabies virus by transgenic *Drosophila melanogaster* S2 cells cultivated in animal protein-free media

Batista FRX[1], Pereira CA[2], Mendonça RZ[2], Moraes AM[1]
[1]Depto de Processos Biotecnológicos, Faculdade de Engenharia Química, UNICAMP, Campinas, SP, Brazil [2]Laboratório de Imunologia Viral, Instituto Butantan,São Paulo, SP, Brazil

Introduction: Insect cell culture has become increasingly useful for the production of heterologous proteins and several culture media can be employed for this purpose. **Objectives:** The goal of this work was to evaluate the G glycoprotein from rabies virus (GPV) production by transfected *Drosophila melanogaster* S2 cells using an animal protein free medium. **Methods:** The cells were cultivated in 100 mL spinner flasks (working volume of 60 mL) and incubated at 28 ºC and 100 rpm. IPL-41 medium supplemented with yeastolate (6 g/L), glucose (10 g/L), fructose (0.5 g/L), lactose (2 g/L), tyrosine (0.6 g/L), methionine (1.48 g/L), Pluronic F68 (0.05%) and lipid emulsion (1%) was used, and inoculated with 7.5 $x10^5$ viable cells/mL. Cell concentration and viability were determined by optical microscopy. Glucose and amino acid concentrations were measured using HPLC. Lactate was determined through a biochemical analyser. Ammonium was determined through an eletrode coupled to a potenciometer. Media osmolalities were analyzed using an osmometer and the GPV concentration, by an ELISA-type kit. **Results and Discussion:** Cultivation results showed that supplemented IPL-41 increased cell concentration (1.9 $x10^6$ cells/mL) to a level superior to that commonly verified for the commercially available serum free medium Sf900II (1.7 $x10^6$ cells/mL). However, GPV concentration in supplemented IPL-41 reached 1 ng/mL, while in Sf900II up to 3.5 ng/mL were detected, indicating the need of further optimisation studies to improve protein production.
Supported by: FAPESP and CAPES

7.03 Rabies virus glycoprotein (GPV) gene expression in *Drosophila melanogaster* S2 cells

Lemos MAN[1], Santos MAG[1], Santos AS[1], Jorge SAC[1], Pereira CA[1]
[1] Instituto Butantan, Laboratório de Imunologia Viral, São Paulo, Brasil

Introduction and Objectives: In this work we are comparing co-transfected cells with transfected Schneider 2 (S2) cells expressing a viral glycoprotein (GPV – rabies virus). **Materials:** We have constructed a gene vector (pMTGPV-Hygro) with both the viral protein gene (GPV) and the selection hygromycin gene (Hygro). This vector was constructed with the inserted selection gene under the control of drosophila copia promoter at the pMTGPV vector. A S2 cell culture was transfected with this vector or co-tranfected with pMTGPV + pCoHygro vectors using cellfectin and selected with hygromycin B. The selected S2MTGPV (MC3) and S2MTGPV-Hygro (MC2) cell populations were then cultivated and upon induction with 700 μM of $CuSO_4$ the expression of the recombinant protein was evaluated. The GPV was detected by cytofluorometry or ELISA. **Results and Discussion:** The data already obtained showed an expression of 15 ng/mL of GPV at S2MTGPV-Hygro (MC2) cells and we expect to optimize this production by preparing other transfections and/or by selecting subpopulations.
Supported by: FAPESP, CNPq and Butantan Foundation

7.04 Comparative study between the efficiency of co-transfection and transfection of vectors with the glycoprotein rabies virus (GPV) in *Drosophila melanogaster* S2 cells

Santos AS[1], Lemos MAN[1], Pereira CA[1], Jorge SAC[1]
[1]Instituto Butantan, Laboratório de Imunologia Viral, São Paulo, Brasil

Introduction: Insect cell cultures reach high cell densities with low costs and simple maintenance. In this work, we use *Drosophila melanogaster* S2 cells to study the expression of a viral protein (GPV – rabies virus). **Objectives:** To compare the efficiency of co-transfection with the vectors pAcGPV + pCoHygro with the tranfection with only one vector pAcGPVHygro in S2 cells. **Methods:** Stable transfected and co-transfected cell cultures were analyzed with immunoassays and a confocal microscopy. In addition, the viral protein GPV was detected by ELISA. **Results and Discussion:** The data obtained so far showed 76 ng/mL of GPV expression in the transfected cells and 15 ng/mL of GPV expression on the co-transfected cells, and we expect to optimize this production with another selection of subpopulations of our cultures to improve the production of viral protein GPV in a more homogeneous culture.
Supported by: FAPESP, CNPq and Butantan Foundation

7.05 Establishment of a real-time PCR protocol for the evaluation of heterologous GPV expression in S2 insect cells.

Astray RM[1], Mello IMVGC[1], Botosso VF[2], Pereira CA[1]
[1]Lab. Imunologia Viral, [2]Biotecnologia, Instituto Butantan, São Paulo, Brazil.

Introduction: The Schneider 2 (S2) *Drosophila melanogaster* cell is a heterologous expression system utilized to obtain proteins of biotechnological interest. We have established in our laboratory a transfected S2 cell line which produces the rabies virus glycoprotein (GPV) **Objective:** To study the correlation between the number of GPV genes (GPVDNA) and GPVmRNA transcribed per cell and their correlation with the final amount of GPV produced by the cultures. **Methodology**: Real-time PCR and RT-PCR for the quantification of nucleic acids (standardization steps: primers design, setup of the reaction, standard curve construction) and ELISA for the quantification of the GPV produced by the cultures. **Results**: We have obtained specific primers to the amplification of 177bp, 503bp and 683bp fragments of the GPVDNA or cDNA. The 503bp fragment which contains the real-time PCR target (the 105bp fragment) was cloned in a commercial vector for the standard curve construction. The same was done with a *Drosophila* housekeeping gene, which encodes the β-actin protein and resulted in a 105bp fragment that was also cloned for the same purpose. **Discussion**: The criteria for choosing the right gene segment and testing every step of the development have demonstrated good results and reproducibility. The cloning of the two fragments and the purification of their vectors are presently under development. We think that using this approach we will be able to better understand the pathway of gene to protein in our expression system.

7.06 Comparative Studies on the Internalization of Particles by Thrombocytes from *Bothrops jararaca* (Viperidae) and *Oxyrhopus guibei* (Colubridae) Snakes.

Almeida CD[1], Sampaio SC[1], Spadacci-Morena DD[1], Sano-Martins IS[1]*
[1]Laboratorio de Fisiopatologia, Instituto Butantan, (SP), Brazil *idasano@butantan.gov.br

Introduction: Thrombocytes are ellipsoid cells with central nucleus and are present at about 26.1±8.9 and 23.3±2.3 x10^9/L in circulating blood of *Bothrops jararaca* (*Bj*) and *Oxyrhops guibei* (*Og*) snakes respectively. Phagocytosis is a complex cellular event by which particles are recognized, engulfed and eliminated. **Objectives and Methodology**: The aim of the present work was: 1) to compare the ability of *Bj* and *Og* thrombocytes to uptake particles, such as opsonized zymosan particles via C3bi and *Candida albicans* via manose; 2) to investigate the formation of vacuoles or only open canalicular system (OCS) is involved in this process. *Bj* and *Og* thrombocytes (2x10^8/L) were incubated (30 min, at room temperature) with opsonized zymosan particles and live *C. albicans* suspensions (2x10^6particles). **Results and Discussion**: The quantification of internalized particles (Giemsa–stained preparations) showed drastic difference between *B. jararaca* (zymosan: 24,5 ± 1,9; *C.albicans*: 80,5 ± 7,5) and *Og*'s thrombocytes (zymosan:12,5±0,97; *C.albicans*: 3,7±0,3). Interaction with these particles was examined by confocal laser scanning microscope (CLSM) to observe the polymerization of F-actin using mouse rhodamine-phalloidin-labeled F-actin. Data obtained by CLSM observation suggest that *Og* thrombocytes presented alterations of the cell surface and cytoskeleton that are compatible with engulfment activity, suggesting true phagocytosis. On the contrary, uptake of the particles by *B. jararaca* thrombocytes seems that there is only OCS participation, covering the particles by spreading.

Supported by: CNPq

7.07 Crotamine nuclear localization sequences mediate gene delivery into cells.

Santos EJC[1], Kono K[2], Hayashi MAF[2], Mambelli LI[1], Yamane T[3], Pereira A[1], Stocco dos Santos RC[1], Kerkis A[4], Kerkis I[1]
[1]Lab de Genética, Instituto Butantan; [2]Centro de Toxinologia Aplicada (CAT-CEPID) - LETA, Instituto Butantan; [3]IPEN, USP; [4]Clínica e Centro de Pesquisa em Reprodução Humana "Roger Abdelmassih", São Paulo, Brasil.

Introduction: Crotamine is a new cell penetrating peptide (CPP), showing both cytoplasmic and nuclear localization in living cells. Crotamine can penetrate cells within few minutes, both in vitro and in vivo. **Objective**: In the present study we investigated the ability of the complex peptide-DNA to carry nucleic acids into cells. **Methodology:** Pepitide-DNA (3:1) condensates were prepared by combining and were allowed to cross-link for 30 min before being added in cell culture. The cells were incubated for 7-24 hours prior to testing for transgene expression. We used a complex DNA-lipofectamia 2000 as positive control. **Results**: We have shown that crotamine is capable of binding electrostatically to plasmid DNA forming DNA-peptide complexes, whose stabilities overcome the need of chemical conjugation for carrying nucleic acids into cells. Differently from others known CPPs, crotamine demonstrates cell specificity and a selective in vivo delivery of plasmid DNA into actively proliferating (AP) cells. **Discussion**: We suggest that these abilities are due to the presence of two nuclear localization sequences in crotamine molecule. These two sequences were synthesized and their ability to deliver plasmid DNA into actively proliferating cells was tested. The sequences are capable to transfect different cell lines used in our work, however only one sequence show the specificity to AP cells. The data qualified isolated sequences as new effective molecular carriers into the cells.
Supported by: FAPESP and CNPq

7.08 Crotamine, a peptide from a South American rattlesnake, mediates gene delivery into actively proliferating cells. Mechanism of crotamine uptake.

Nascimento FD[1], Hayashi MAF[2], Kerkis A[3], Oliveira V[4], Oliveira EB[5], Rádis-Baptista G[6], Nader HB[1], Yamane T[7], Tersariol ILS[8], Kerkis I[9]
[1]Depto.de Bioquímica, UNIFESP-EPM, SP; [2]CAT-CEPID - LETA, Instituto Butantan; [3]Clínica e Centro de Pesquisa em Reprodução Humana "Roger Abdelmassih", SP; [4]Lab. de Neurociências, UNICID, SP; [5]Depto de Bioq. e Imunologia,USP-RP; [6]Centro de Ciências Biológicas, UFPE, Pernambuc; [7]IPEN, USP; [8]Centro Interdisciplinar de Investigação Bioquímica (CIIB), UMC, SP; [9]Laboratório de Genética, Instituto Butantan, São Paulo, Brazil.

Introduction: Recently we have shown that crotamine belongs to the family of cell penetrating peptides (CPPs), which currently are extensively used as carriers of different molecules into the cells. Herein, we show that crotamine is capable of binding electrostatically to plasmid DNA forming DNA-peptide complexes, whose stabilities overcome the need of chemical conjugation for carrying nucleic acids into the cell. Differently from other known CPPs, crotamine demonstrates cell specificity and a selective *in vivo* delivery of plasmid DNA into actively proliferating (AP) cells. **Methodology:** The binding of crotamine to the plasmid DNA was evaluated by both agarose gel shift assay and circular dichroism. The involvement of heparan sulfate proteoglycans in uptake mechanism, followed by endocytosis and peptide accumulation within the lysosomal vesicles was also demonstrated. **Results and Discussion:** Similar uptake mechanisms were observed for both crotamine alone and crotamine-plasmid DNA complexes. Our data provide a strong support to stimulate the use of crotamine as an effective DNA carrier into different types of AP cells.
Supported by: FAPESP

7.09 Morphological study of the infralabial glands of the "goo-eater" snakes *Sibynomorphus mikanii*, *Atractus reticulatus* and *Dipsas indica bucephala*, and biochemical and pharmacological analysis in the secretion of *Sibynomorphus mikanii*.

Oliveira L[1], Antoniazzi MM[1], Lopes-Ferreira M[2], Lima C[2], Bruni FM[2], Pimenta DC[3], Conceição K[3], Prudente ALC[4], Zaher H[5], Jared C[1]
[1]Lab. Biologia Celular, [2]Lab. Imunopatologia, [3]Lab. Especial de Toxinologia Aplicada, Instituto Butantan, São Paulo; [4]Museu Paraense Emílio Goeldi; [5]Museu de Zoologia, USP-SP

Introduction: Despite infralabial glands being common among snakes, little is known about their morphology and function. They are generally homogeneous and mucous, but among dipsadines they are mainly serous and very developed. This fact is possibly related to the fact that some species of the group feed on soft and viscous invertebrates. **Objectives:** This work aims the comparative morphological study of the infralabial glands of *S. mikanii*, *A. reticulatus* and *D. indica bucephala*. It also aims the biochemical and pharmacological caracterization of the secretion of *S. mikanii* glands. **Methodology:** The glands were prepared for histology, histochemistry and electron microscopy. The biochemical analysis was made from tissue maceration and glandular tissue culture. After HPLC purification, the major fractions had their activity tested by zymography and intravital microscopy. **Results:** The glands of the three species are constituted in different proportions by seromucous and mucous cells. Only in *A. reticulatus* they are localized in the same acini. In *A. reticulatus*, it was observed a muscle connection with the glands. The biochemical study demonstrated the presence of a 25 KDa major protein that, showed genatinolitic activity and caused alteration in mice blood microcirculation. **Discussion:** A large morphological variation in the glands of the three species was observed. The analysis of *S. mikanii* secretion, indicate that it may present other functions besides food lubrication, as chemical prey imobilization.
Supported by: CAPES, FAPESP, CNPq and Butantan Foundation

7.10 Skin Morphology of the caecilian *Siphonops annulatus* during parental care

Marques-Porto R[1], Kupfer A[2], Antoniazzi MM[1], Wilkinson M[2], Jared C[1]
[1]Lab. Cell Biology, Instituto Butantan, São Paulo, Brazil; [2]Natural History Museum, London, UK.

Introduction: Parental care is very common among amphibians, especially in the order Gymnophiona (caecilians), including a variety of morphological, physiological and behavioural adaptations. Recently we have described a novel type of parental care in an oviparous African caecilian *Boulengerula taitanus* (*Bt*), in which the young feed on the female's skin that, during this period, secretes nutrients for their development. *S. annulatus* (*Sa*) is the most common oviparous caecilian species in South America. **Objectives:** We have studied the morphological modifications of female *Sa* skin during parental care comparing to *Bt*. **Material and Methods:** Skin samples from 3 different regions were prepared for routine histology and electron microscopy, and histochemistry. **Results:** In attending females, the epidermal cells increase in height but not in number, as compared to non-attending females. Large single vacuoles appear in the epidermal cell layers, increasing in size as they approach the outer layer. As seen by Sudan Black method, the vacuole's contents are of lipid nature. The outer cell layer is loose and peels off. The number and size of granular glands is much smaller than in non-attending females. **Discussion:** The epidermal lipid vacuoles are probably related to young nutrition by dermatophagy. Nutrients other than lipids must also be offered, probably in the cytoplasm and extracellular matrix of the peeling epidermis. The small number of granular glands is probably important to prevent intoxication of the young.
Supported by: CNPq and Butantan Foundation

7.11 Morphology and Histochemistry of the venom glands in *Stereocyclops incrassatus* (Anura, Microhylidae)

Jared SGS[1]., Hingst-Zaher E[2], Antoniazzi MM[1], Suzuki H[1], Zanotti AP[1], Jared C[1]
[1]Lab. Cell Biology, Instituto Butantan, São Paulo; [2]Museu de Zoologia USP, São Paulo, Brazil

Introduction: Among the anurans, microhylids are remarkable for the lack of information about their cutaneous morphology and toxinology. *Stereocyclops incrassatus* lives in the litter of Atlantic Rainforest, where it spends most of the time half-buried disguised as fallen leaves. When disturbed, presents a characteristic defensive behaviour, stiffening the legs and mimicking a leaf. If manipulated, it releases an abundant cutaneous secretion. The skin morphology of this species was studied and the results were analysed in the context of its natural history. **Methodology:** Samples from dorsal and ventral skin were prepared for routine histological and ultrastructural techniques. The sections were stained by HE, and submitted to Bromophenol Blue, PAS, Alcian Blue, and von Kossa histochemical methods. **Results:** The skin is very thick and presents an unusual amount of glands when compared to other anurans. The dorsal skin presents very large granular glands forming a continuous "wall", and is positive to Bromophenol Blue, indicating proteic content. There are two types of mucous glands, revealed by PAS and Alcian Blue. The calcified dermal layer is continuous and quite thick. Ventral skin shows the same characteristics, although with smaller number of glands. **Discussion:** The cryptic appearance of this frog may confer protection against visually oriented predators. On the other hand, the peculiar amount and arrangement of the glands may be related to the microhabitat of this species which is very rich in decomposing organic material, having an important role in protecting them against predators and microorganisms.
Supported by: CNPq, FAPESP and Butantan Foundation

7.12 Morphological changes of female dorsal skin of the amphibian *Pipa carvalhoi* (Anura: Pipidae) during egg implantation.

Fernandes TL[1], Antoniazzi MM[1], Sasso-Cerri E[2], Egami MI[3], Lima C[4], Miraglia SM[3], Jared C[1]
[1]Lab. Cell Biology and [4]Lab. Immunopathology, Instituto Butantan,São Paulo; [2]School of Odontology, UNESP, Araraquara; [3]Dept. Morphology, UNIFESP-EPM, São Paulo,

Introduction: *Pipa carvalhoi* is an aquatic anuran, exhibiting an elaborate courtship ritual. After egg laying and fertilization, the eggs rapidly penetrate into the dorsal skin, within which the embryos develop until the stage of tadpoles. The morphological and biochemical processes involved in egg penetration have not yet been investigated. **Objective**: This work aims a preliminary morphological approach of female dorsal skin changes following the first 24 hours after egg implantation. **Methodology**: Fragments of female dorsal skin were collected from virgins, specimens immediately post-egg laying, and specimens on the 1st day after egg implantation, and prepared for histology and electron microscopy (TEM). Ventral skin was used as a control. **Results**: The results indicate that both epidermis and dermis undergo profound changes beginning just after egg contact on the external epidermis. The skin is then induced to invaginate and the dermis passes through a stage of total reorganization. Morphometrical results indicate an increasing in the number of skin glands compared to the virgin skin. **Discussion**: The rapid and profound changes of the skin during egg implantation are probably intermediated by cellular signalling which may be trigged by the first egg contact on the skin. Hormonal and pheromonal intermediation of this process must also be involved. The increase in the number of glands indicates that they also participate of this process**.**
Supported by: CNPq and Butantan Foundation

7.13 Ultrastructural aspects of venom secretory cells of the venom glands of adult, newborn and embryo *Crotalus durissus terrificus*

Giannotti KC[1], Camargo GG[1], Fernandes W[2], Sant' Anna SS[2], Carneiro SM[1]
[1]Laboratório de Biologia Celular, [2]Laboratório de Herpetologia, Instituto Butantan, São Paulo.

Introduction: *Crotalus durissus terrificus* is a South American rattlesnake of great medical and pharmacological interest due to the neurological properties of its venom. However, venom gland studies are scanty and only related to adult snakes. The secretory cell ultrastructure differs from other viperid snakes in the presence of numerous electrondense intracisternal granules in the rough endoplasmic reticulum (RER). **Objective:** In this study we aim to compare the venom secretory cells of *C.d.terrificus* in the stages of embryo, newborn and adult. **Methodology:** Venom glands were excised after decapitation of sedated snakes, fixed and embedded in EPON resin. Ultrathin sections were observed in the transmission electron microscope. **Results:** At the final stage of embryo life, secretory cells present numerous apical secretory vesicles but lack intracisternal granules in the RER. In the newborn snakes, a few apical secretory vesicles are observed and rare small intracisternal granules occur in the RER of a few cells. In the adult, two kind of secretory vesicles are noticed, and numerous intracisternal granules of various sizes are present in almost all secretory cells. **Discussion:** The venom synthesis and secretion begin in the fetal life. Intracisternal granules first appear in the newborn; their origin and function are still unknown but they may be related to storage of secretory proteins or to misfolded protein accumulation.
Supported by: Butantan Foundation

7.14 Detection of microvesicles in the venom of *Crotalus durissus terrificus*

Carneiro SM[1], Fernandes W[2], Sant' Anna SS[2], Yamanouye N[3]
[1]Lab de Biologia Celular, [2]Lab.de Herpetologia, [3]Lab. de Farmacologia, Instituto Butantan, São Paulo.

Introduction: Different types of normal and pathological cells release membranous vesicles by different mechanisms. Many specific functions have been attributed to them, according to their origin, composition of their membrane and their content. Microvesicles 40-80 nm diameter with electrondense content are consistently observed by electron microscopy on the luminal face of secretory cells of venom glands of viperid snakes. **Objective:** we aimed to evaluate their presence in freshly collected venom of *C. d. terrificus*. **Methodology:** microvesicles were separated by ultracentrifugation of 5ml of cell-free venom and fixed and embedded in EPON resin. Ultrathin sections were examined in the transmission electron microscope. **Results:** Vesicles separated by ultracentrifugation are similar in size and structure to the microvesicles observed in the venom glands. A fine fuzzy coat surrounds each microvesicle. **Discussion:** the vesicles may result from fragmentation of apical microvilli; their function is still unknown but we discuss a possible contribution to inactivation of stored venom components or their activation after venom is released.
Supported by: Butantan Foundation

7.15 Transcription factor AP-1 is activated after venom extraction in the venom gland of Viperidae snake.

Luna MSA[1], Ferreira ZS[2], Yamanouye N[1]
[1]Laboratório de Farmacologia, Instituto Butantan, [2]Departamento de Fisiologia, ICB-USP, São Paulo, SP.

Introduction: Venom gland of viperid snakes has a central lumen where all venom produced is stored and a long venom production cycle that lasts around 30 to 50 days. Biting or manual extractions of venom reduce the amount of venom inside the lumen, and stimulates for a new cycle of venom synthesis. Both alpha and beta-adrenoceptor has an essential role in triggering venom production cycle by inducing the synthesis of proteins of the gland. We have shown that venom extraction increases the activation of NFκB in secretory cells of *Bothrops jararaca* venom gland. The aim of this study is to verify whether venom extraction could activate other potentially important transcription factors such as AP1. **Methods:** Nuclear extracts were obtained from male and female snake venom gland in quiescent stage and 30, 60 and 120 min after venom extraction. The activation AP1 was analyzed by electrophoretic mobility shift assay, using AP1 oligonucleotide labelled with γ^{32}P-ATP. **Results:** We detected AP1-DNA complex in quiescent cells. After venom extraction, an increase of AP1-DNA complex was observed. The highest increase in female and male venom gland occurs after 60 min (111.53 ± 27.27%, n=3) and 120 min (101.30 ± 61.25%, n=3) of venom extraction, respectively. **Discussion:** The data showed that AP1 is activated in quiescent venom gland and its activity increases after venom extraction. The data obtained until now suggest that activation of NFκB and AP-1 are relevant to induce the synthesis of proteins important for activation of secretory cells. Besides, the activation of AP1 presents a sexual dimorphism in venom gland.
Supported by: FAPESP, CNPq and Butantan Foundation

7.16 Effect of ovariectomy on antiapoptotic protein Bcl-2 expression in rat hippocampus.

Sales S[1], Porto CS[2], Abdalla FMF[1]
[1]Laboratório de Farmacologia, Instituto Butantan; [2]Depto. de Farmacologia, Setor Endocrinologia Experimental, UNIFESP-EPM .

Introduction: The effects of estrogens on neuroprotection have been shown, but the mechanisms involved remains unclear. Studies suggest that estrogen replacement in women can help to prevent or delay the development of Alzheimer's disease and reduce the severity of Alzheimer´s related dementia. Recent evidence suggests that some of the effects may be due, in part, to ability of estrogens to regulate members of the Bcl-2 family in specific regions of the basal forebrain. **Objectives:** The present study was designed to investigate the effect of ovariectomy on protein Bcl-2 expression in adult rat hippocampus. **Methods**: Hippocampus were obtained from rats in proestrus (control), ovariectomized for 15 days (C15) and ovariectomized for 21 days (C21). Protein levels of Bcl-2 were determined by Western blotting as previously described. Briefly, 50 µg of protein was electrophoresed on 12% SDS-PAGE, and then electrotransferred to nitrocellulose membrane. The membrane were blocked with 5% non-fat milk in PBS containing 0.2% Tween-20, 1 h, 4^{o}C and then incubated with primary antibody (1:100, Santa Cruz Biotechnology, CA, USA), 12 h, 4^{0}C, washed three times with PBS and incubated with a anti-rabbit secondary antibody (1:25,000), 1 h, 4^{o}C. Proteins were visualized on x-ray films after exposure to Luminol. b-actin levels were monitored on the same blot to ensure equal protein loading. **Results**: The expression of protein Bcl-2 decreased in hippocampus from C15 and C21 rats (respectively, 59.66 ± 25.86 %, n=3 and 45.69 ± 10.64 %, n=3) when compared with those obtained from control animals (100 %). This effect was similar in both period of ovariectomy. **Conclusion**: The results suggest the regulation by ovariectomy of the protein Bcl-2 expression in rat hippocampus.
Supported by: PRONEX and FAPESP

7.17 Norepinephrine and glutamate activates the nuclear factor NFκB in cultured rat pineal glands.

Villela DC[1], Scavone C[2], Lima L[2], Moutinho AA[1], Cipolla-Neto J[3], Afeche SC[1].
[1]Lab. Pharmacology, Butantan Institute, [2]Dept. Pharmacology and [3]Dept. Physiology, Institute of Biomedical Science, USP, São Paulo, Brazil.

Introduction: Melatonin synthesis in the pineal gland is stimulated at night by norepinephrine. Several amino acids and peptides modulate that synthesis, among them glutamate. It is localized in microvesicles, it is secreted by cholinergic activation and it acts paracrinally, inhibiting melatonin synthesis. In the central nervous system, glutamate can stimulate the nuclear factor NFκB and the NO synthase enzyme. **Objectives**: The aim of this work was to characterize the receptors and the transduction pathways involved in glutamate-induced melatonin synthesis inhibition. More specifically, we investigated the participation of the nuclear factor NFκB and the nitric oxide synthase. **Methodology**: Pineal glands were isolated from Wistar rats and maintained in culture for 48h, in BGJb medium. Then, they were stimulated by norepinephrine 1μM plus glutamate or AMPA. When glutamate was used, it was associated with one of the following drugs: MK-801 (NMDA receptor antagonist), MCPG (metabotropic antagonist), l-NAME (NO synthase inhibitor), PDTC (NFκB inhibitor). Melatonin synthesis was evaluated by HPLC with electrochemical detection. NFκB and NO synthase were analyzed, respectively, by eletrophoretic mobility gel shift and radiometric assays. **Results and Discussion**: Glutamate reduced melatonin synthesis in a concentration-dependent manner. The receptor involved in this response was of the AMPA-type, because this agonist reproduced the inhibitory effect of glutamate. MK-801, MCPG and l-NAME didn't show any effect on melatonin synthesis. Norepinephrine and glutamate, both activated the nuclear factor NFκB, and PDTC inhibited melatonin synthesis by 50%, indicating that NFκB has a fundamental role in melatonin synthesis.
Supported by: FAPESP and CNPq

7.18 Effect of the hidroalcoholic extract from pomegranate (*Punica granatum*, L.) at CACO-2 cells

Jardini FA[1], Mendonça RMZ[2], Pinto JR[2], Mancini DAP[2], Mancini-Filho J[1]
[1]Food and Experimental Nutrition Dept. of the Pharmaceutical Sciencies College of the São Paulo University, São Paulo, Brazil, [2]Virology Laboratory, Butantan Institute, São Paulo, Brazil

Introduction: The pomegranate (*Punica granatum,* L.) is a fruit with a strong antioxidant capacity. The Caco-2 cell line is originated from a human colon adenocarcinome. **Objective:** The purpose of this study was to evaluate the absorption and the protection of the compounds from the hidroalcoholic pomegranate´s extract at Caco-2 cells. **Methodology:** The 80% hidroalcoholic pomegranate´s pulp extract was obtained and filtered with a sterilizing membrane (45 μm). The Caco-2 cells were harvest in a 12 wells plates (5×10^5 cells/well), along 7 days. By these, the cells received the hidroalcoholic extract or a gallic acid solution (positive comparative control) at 50, 100 or 200 ppm concentration. After 10 days of incubation, the supernatant was collected and analysed by the Folin-Ciocalteau reagent method. The cells was detached and the viable ones was measured by the Trypan blue exclusion test. **Results:** The cells were morfologically like with the control ones. The cells treated with the hidroalcoholic extract at 200 ppm showed the higher absorption (63.25%) and had the lowest cellular missing content (4.16%) against 5.08% obtained from the control cells. The cells treated with 200 ppm of the gallic acid solution showed the absorption content about 72.54% and a cellular missing content about 5.08%. **Discussion:** These data suggest that the Caco-2 cells present a positive answer to the treatment with the hidroalcoholic pomegranate´s extract, being their antioxidant compounds absorbed and, thus, playing a protective role over the cells during their development.
Supported by: FAPESP; CAPES and CNPq

7.19 Optimized foreign protein expression in baculovirus/insect cell system promoted by proteins of *Lonomia obliqua* haemolymph.

Augusto EM[1], Sousa APB[1], Ribeiro B[3], Moraes RHP[2], Pereira CA[1], Mendonça RZ[1]
[1]Laboratório de Imunologia Viral, [2]Laboratório de Artrópodes, Instituto Butantan, São Paulo, Brasil. [3]Laboratório de Microscopia Eletrônica, Universidade de Brasília, Distrito Federal, Brasil.

Introduction: Insect cells used in conjunction with the baculovirus expression vector system (BEVS) are an important recombinant protein system. This combination is powerful due to a higher expression of recombinant proteins by baculovirus under the control of a polyhedrin promoter, allowing appropriate post-translational modifications. Many strategies have been used to improve upon the maximum recombinant protein production including medium supplementation. Many studies have reported the high production of recombinant proteins in insect cell-baculovirus system after medium supplementation with silkworm hemolymph. **Objectives and Methods**: In this work, hemolymph from *Lonomia obliqua* (1%, v/v) was used to enhance the production of β-galactosidase. This protein was obtained after infection of Sf-9 cells with 0.1MOI *Anticarsia gemmatalis* baculovirus recombinant expressing (AgNPV-βgal). **Results and Discussion**: Insect Cell cultures, infected and supplemented with hemolymph, produce twice as much recombinant protein than culture not treated with hemolymph. This protein was detected using ONPG. The protein recognized as promoting β-galactosidase expression was purified by exclusion liquid chromatography and identified as a low-density protein with approximately 14.4 Kda.
Supported by: FAPESP and Butantan Foundation

7.20 Ips/iFN-γ-Mediated protection of J774 macrophages from teniposide (VM26)-induced apoptosis is associated with repression of pro-apoptotic Bcl-2 members.

Marçal MS[1,3], Carneiro PS[2], De Franco M[2] , Amarante-Mendes GP[1,3]
[1]Department of Immunology, Institute of Biomedical Sciences, USP, [2]Lab. of Immunogenetics, Butantan Institute, SP, [3]Institute for Investigation in Immunology, Institutos do Milênio, Brazil.

Introduction: Activation of macrophage can be achieved by contact with others cells, cytokines and some components of foreign organisms. The contact with pathogens seems to interfere with the apoptotic machinery. **Objectives:** Herein, we evaluated if LPS, IFNγ or their combination could protect J774 cells against apoptosis induced by VM26. **Methodology:** J774 macrophage cell line were treated or not with LPS, IFNγ or both and then submitted to apoptogenic stimuli for 18h. Apoptosis was assessed by flow cytometry/cell cycle analysis of total DNA content. The mRNA expression levels of anti/pro-apoptotic genes were performing by quantitative real-time PCR. **Results:** The data obtained by flow cytometry revealed that only the treatment with the association of LPS + IFNγ protected J774 cells from VM26-induced apoptosis. Through the analysis the mRNA levels of some of the Bcl-2 family members, we observed that VM26 induced an upregulation of BAX and NOXA, but did not interfere with the expression of BAK. Moreover, VM26 induced a downregulation of the anti-apoptotic members Bcl-2, A1 and Bcl- X_L. Pre-treatment with LPS + IFNγ did not rescue the downregulation of these anti-apoptotic Bcl-2 members. However, LPS + IFNγ prevented VM26-induced upregulation of BAX and NOXA. **Discussion:** These data suggest that the treatment with LPS + IFNγ protects J774 cells from further apoptogenic insult by modulating the VM26-induced upregulation of certain pro-apoptotic Bcl-2 family members.
Supported by: CNPq and FAPESP

7.21 Phagocytosis modifies the activity of aminopeptidases (APs) in macrophages.

Olivo RA[1], Zuliani JP[1], Silveira PF[1], Teixeira CFP[1].
[1]Lab. Pharmacology, Instituto Butantan.

Introduction: APs constitute a family of enzymes relevant for cell adhesion and modulation of the activity of inflammatory mediators released from macrophages (Mϕ). **Objective:** In the this study we investigated the influence of phagocytosis (Ph) via C3b, Fcγ and β-glucan receptors on the activity of soluble (S) and membrane-bound (M) neutral AP (APN) and prolyl dipeptidyl aminopeptidase IV (DPPIV) in Mϕ. **Methodology:** Mϕs were obtained from Swiss mice peritoneal cavities 96 h after intraperitoneal injection of thioglycollate 3%. Ph via C3b, Fcγ and β-glucan receptors were stimulated with opsonized zymosan, opsonized sheep red blood cells or non-opsonized zymosan, respectively. The activities of APs were measured with a fluorogenic assay using naphthylamide-derivative substrates. **Results:** Activation of Ph via C3b, Fcγ or β-glucan receptors significantly reduced S-APN activity but did not modify M-APN activity. S-DPPIV activity was significantly increased during Ph via all the studied receptors and M-DPPIV was significantly increased only by Ph via C3b receptors. **Discussion:** These data demonstrate that activation of Ph in Mϕ modifies positive or negatively the activities of APs mainly those located in the S fraction of cells, suggesting a role for APs in a defense process of Mϕ.

Supported by: FAPESP.

7.22 Production of antibodies anti-immature stem cells of dental pulp (anti-IS CDP) in mice

Maranduba CMC[1], Carvalho LV[2], Monteiro BG[1], Cerruti Filho H[3], Kerkis A[4], Kerkis I[1], Sant' Anna OA[2].
[1]Lab. de Genética e [2]Lab. de Imunoquímica, do Instituto Butantan, [3]Clínica CERA, SP, Brasil, [4]Clínica e Centro de Pesquisa em Reprodução Humana Roger Abdelmassih, SP, Brasil.

Introduction: It is known that stem cells can be isolated from human dental pulp (hDP) and differentiate *in vivo* to bone, cartilage and dentine. Our group have isolated a population of stem cells from hPD that present rapid expansion and proliferation *in vitro* and, while immature, can express some mesenchymal stem cells (SH2, SH3 and SH4) markers, some specific-stage antigens and embryonic stem cells (SSEA-3, TRA-1-60, Oct-4 and Nanog) transcription factors. These ISCDP cells are immature, since they are capable of differentiation more easily than others previously described. **Objectives:** This work has two main goals: (1) to obtain a specific anti-ISCDP antibodies; (2) verify its efficiency in detection of ISCDP *in vitro* and engraftment present in various organs of mice after ISCDP inoculation. **Methods:** High responder mice [H_{III}] were ip injected with $1x10^5$ DPSCs/200µl PBS and after 30 and 60 days sera were collected and stored at -20ºC. The sera efficiency was tested in laminas containing ISCDP. As negative control, it was used a pre-immune serum. The ISCDP were marked with DiI Vybrant (red color) before administration in mice. The engraftments were detected using anti-ISCDP conjugated with FITC and counterstained with Dapi in tissue samples of mice. Analysis was done in a confocal microscopy. **Results:** The presence of fluorescence produced by dye (DiI Vybrant) and by the anti-ISCDP was evident. **Discussion:** These results corroborate that anti-ISCDP obtained in the H_{III} mice is specific for ISCDP *in vitro* and *in vivo.*

Supported by: FAPESP and CNPq.

7.23 Preparation of primary culture of embryonic cells from *Amblyomma cajennense* (Fabricius, 1787) (Acari: Ixodidae)

Giacomin FG[1], Máximo-Espíndola CP[1], Sousa APB[2], Souza SG[1], Mendonça RZ[2], Yoshinari NH[3], Barros-Battesti DM[1] e-mail: flaviagrecco@butantan.gov.br

[1]Lab. Parasitologia, Instituto Butantan, SP, Brazil; [2]Lab. Imunologia Viral, Instituto Butantan; [3]Lab. Lyme, Faculdade de Medicina da Universidade de São Paulo, São Paulo, SP, Brazil.

Introduction: Considering the emergency of tick-borne diseases in Brazil, and the difficulties for diagnosis and isolation of pathogens, primary culture of embryonic cells from *Amblyomma cajennense* was performed. In addition, the obtained cell lines will be used as source to microorganism growth. **Material and Methods:** Engorged females were washed with 70% ethanol, hypochlorite 1% and benzalkonium chloride, and they were put in sterile distilled water with antibiotics. They were dried with sterile gauze, individually placed in sterile Petri dishes and kept in BOD incubator at 27 $\pm$ 1°C and 95 $\pm$ 2% humidity in which they deposited fertile eggs. Between 23 to 27 days after, egg masses were transferred to sterile Petri dishes containing culture medium and they were gently crushed. The material was filtered to remove intact and shell eggs, and it was transferred to Falcon® 25ml flask. After resuspended in culture medium it was centrifuged. Tissue fragments were resuspended in culture medium and they were divided into 25cm^2 flasks that were incubated at 37ºC in CO_2 incubator, and given fresh medium weekly. **Results and Discussion:** The most critical factor in developing cultures of tick cells was to get cells to attach the surface of the culture flasks so readily, but they aggregate to tissue fragments that are important source of cells. Besides epithelial-like cells grow on the bottom of the flask attached each other and eventually form sheets of cells commonly observed in primary cultures, and they appear to emerge from tissue fragments.

7.24 The 20S proteasome as target for antitumoral compounds: screening for selective and reversible inhibitors

Felício AL[1], Pacheco AO[2], Piassa G[1], Helgueira L[2] and Demasi M[1]

[1]Laboratório de Bioquímica e Biofísica, Instituto Butantan, [2]Instituto de Química, USP

Introduction: The proteasome is a highly selective protease localized into cytosol and nucleus. Its role is to selectively degrade a multitude of proteins upon specific signals. The inhibition of its function can cause cell death by apoptosis. Tumoral cells are more susceptible to these effects, hence, proteasome has been proposed as a target for the therapy of certain types of tumor. **Objective:** Our group has been conducting the screening for selective and reversible proteasome inhibitors. Herein we show results obtained from the purification of two compounds extracted from the same natural source. **Methodology:** A pool of compounds obtained from a natural source was fractionated by successive thin-layer and column chromatographic steps. Fractions obtained in each step were tested for proteasome inhibition. Proteasome was isolated from horse erythrocytes and its catalytic activity determined by fluorimetric method. Active compounds were tested in three different cell lines. Tests comprised: viability assay and cell death characterization. **Results:** Two compounds showed inhibitory effect upon the proteasome. One of them showed reversible inhibitory effect upon two catalytic sites: Ic_{50} 40 μM. The other one showed irreversible effect upon the three catalytic sites: IC_{50} 30 μM. Both promoted cell death by apoptosis at 10 and 20 μM concentration. **Discussion:** Present findings revealed a new reversible inhibitor, not structurally related to any other known thus far. It might be a potential new drug for further investigation in tumor cell lines. **Supported by**: FAPESP

7.25 Production of human recombinant erythropoietin in mice milk

Mendes CM[1], Kerkis I[2], Martins EAL[3] and Visintin JA[11] Departamento de Reprodução Animal – FMVZ-USP, [2] Laboratório de Genética do Instituto Butantan, [3] Centro de Biotecnologia do Instituto Butantan

Introduction: Many recombinants proteins, such human insulin and growth hormone, can be produced in bacteria and yeast. However, animal cells are required to synthesize proteins that require post-translational modifications, such glycosylations. Transgenic animals are being used for this purpose. **Objective:** To establish techniques and conditions to produce a transgenic mouse, having as first model the human erythropoietin gene. **Methodology:** Genetic fragments containing bovine milk promoter, human erythropoietin gene without signal peptide and bovine milk protein coding region were amplified by PCR, and cloned in sequence in a vector containing neomycin resistance gene. The genetic construction is being introduced by electroporation in embryonic stem cells. The positive cells will be selected by resistence to neomycin and they will be aggregated to embryos flushed from superovulated mice. After 24 hours of in vitro culture, the well-developed embryos will be transferred to pseudopregnant recipients. **Results** All the stages described in the methodology are being pursued. The offspring (chimeras) are being breeded and its newborns will have their DNA analyzed to verify the presence of human erythropoietin gene. **Discussion:** The next step will be breed the positive animals (founders) and to have the borned females analyzed for human erythropoietin production in milk. It should complete the first phase of the appropriation of this important technology in our laboratory. Financial support: FAPESP/CAPES

8. Others

8.01 A collaborative study on the testing of equine antirabies serum by a virus neutralization test in BHK-21 cells

Moura WC[1], Mourão-Fuches RM[2], Frazatti-Gallina NM[2]
[1]Instituto Nacional de Controle de Qualidade em Saúde – INCQS, RJ; [2]Instituto Butantan, SP, Brazil

Introduction and Objectives: A collaborative study was realized to validate the virus neutralization test in cell culture to determine the potency of equine anti-rabies serum by the Instituto Nacional de Controle da Qualidade em Saúde (INCQS) of Brazil and the Instituto Butantan. **Methods and Results:** The test utilized was the virus neutralization in BHK-21 cells cultivated on 96 wells microplates. It was based on the recommendations of the fifth edition of the European Pharmacopoeia and the tests described by Trimarchi and Smith in Laboratory Techniques in Rabies. The medium effective dose (ED_{50}) and the potency (IU/mL) were calculated by the probit method. The accuracy, intra-assay precision, inter-assay precision and reproducibility (inter-laboratory precision) were evaluated in two batches produced at Instituto Butantan and in the 2nd International Standard for Rabies Immunoglobulin (IR). The geometric mean of ED_{50} obtained with the IR at both laboratories (1/39,39) was similar to that obtained by Trimarchi (ED_{100}=1/32), demonstrating the accuracy of the test. The intra-assay precision was observed when one batch of equine anti-rabies serum was tested in 10 replicates in a single assay and it was found a coefficient of variation (%CV) of 8,8%. The %CV of the ED_{50} of the batches tested presented variations of 12,4-19,8% in the INCQS and of 13,2-22,0% in the Instituto Butantan, showing the inter-assay precision. The %CV obtained with the two batches of equine anti-rabies serum and the IR tested at the two laboratories, between 12,8% to 16,3%, demonstrated the reproducibility. **Conclusions:** The study showed that the virus neutralization test in BHK-21 cell is suitable as a potency test of equine anti-rabies serum, presenting accuracy, repeatability, inter-assay precision and reproducibility, validating the test in these laboratories.

8.02 HPV: co-factor (*Pteridium aquilinum*) association in an exposed human population.

Encarnação TBC[1], Freitas AC[2,3], Trindade C[1], Ferraz OP[1], Lima AA[4], Teixeira LFM[4], Beçak W[1], Stocco dos Santos RC[1].
[1]Lab. de Genética, Instituto Butantan; [2]Depto. de Genética, UFPE; [3]LIKA, UFPE; [4]Escola de Farmácia, UFOP

Introduction: Human papillomaviruses are etiological related to cancer and several studies have been developed aiming control, prophylaxis and lesion therapy. Many co-factors have been discussed suggested to improve the virus action. **Objectives:** In this study, our aim was to evaluate the interaction between HPV and co-factor in a human population exposed to a specific agent: the chronicle consumption of the bracken fern, *Pteridium aquilinum*. **Methods and Results:** The material was obtained from cervical and blood samples in order to allow PCR and RFLP procedures to identify the virus DNA sequences and cytogenetic studies in short term peripheral blood. Clastogenic effect was verified: raising of chromosome aberration level. Different virus types could be identified, in blood and cervical samples. **Conclusions:** The correlation of virus type and level of chromosome aberration was not exactly clear, suggesting that the co-factor could act as promoter of virus action, but the virus could not be per se a clastogenic agent.
Supported by: CNPq, FAPESP and Butantan Foundation

8.03 Characterization of BPV positive cell line: histopathological, cytogenetic and molecular aspects.

Ferraz OP[1], Trindade C[1], Encarnação TBC[1], Góes LGB[1], Freitas, AC[2,3], Assaf SLMR[1], Beçak W[1], Stocco dos Santos RC[1]
[1]Lab. de Genética, Instituto Butantan; [2]Depto. de Genética, UFPE; [3]LIKA, UFPE

Introduction: Bovine papilomavírus (BPV) is a double strand DNA virus that can cause benign lesions, warts, in bovine epithelium. These lesions can be transformed in tumors when exposed to co-factors, leading to serious economic losses. Although the abundant literature relating BPV, there are few reports about cytogenetic characterization of these lesion cells or about the maintenance and distribution of virus sequences in the different wart cell layers. **Objectives:** The purpose of this study was to utilize this type of lesion and develop a primary cell culture, specific epithelial culture, evaluating presence of virus DNA sequence and kariotyping. **Methods and Results:** The culture was established from a wart biopsy obtained from a wart, submitting small pieces to RPMI medium supplied with 10% bovine fetal serum. PCR procedures were developed to identify the BPV types presenting in the sample and in the different stages of the cell line. The PCR products were identified as DNA sequences of types 1, 2 and 4. The cytogenetic studies showed karyotype comparable to normal bovine cell, with 60 chromosomes, and with detection of chromosome markers, mainly submetacentric, indicating centric fusions. **Conclusions:** The results strongly suggest that the cell line can keep the virus sequences and that present the same chromosome aberrations that were described in peripheral blood cells, which reinforce our hypothesis that the virus can remain in the blood and that chromosome aberrations are induced by virus action.

Supported by: CNPq, FAPESP and Butantan Foundation

8.04 Antioxidant extracts from spice with inhibitory effect on DNA virus, such as herpes virus, replication in cell culture

Mancini DAP[2], Torres RP[1], Pinto JR[2], Mancini Filho J[1]
[1]Food Science & Nutrition Dept., São Paulo University, [2]Virology Laboratory, Butantan Institute

Introduction: Antivirals from natural sources, used alone, as well as in combination with synthetic antivirals with synergistic action, have been reported. Anti-herpetic drugs such as acyclovir, in addition their competent action, are described as resistant-acyclovir strains of Herpes Simplex Virus (HSV). **Objectives:** This work aimed to evaluate antivirals in antioxidants from spices. **Methods and Results:** The antioxidant activity of a phenolic compound extracted from rosemary (*Rosmarinus officinallis, L*) by hot water was determined by spectrophotometry using β carotene/linoleic acid system. The rosemary extract was evaluated by antiviral assay of the HSV-1 replication in VERO cells in presence or absence of the spice. An amount of 10,000 $TCID_{50}$ /25 µl of the HSV-1 was kept for 2 h at 4°C in contact with 25µl of 300 p.p.m from rosemary, by itself, and with 25µl (v/v) of 100 p.p.m. from butyl hydroxy toluene (BHT). These samples were inoculated in VERO cells and incubated at 37° C in CO_2-5% for seven days. Daily cytopathic effect (CPE) was examined and the end point was based on 100% of CPE in virus control. The HSV-1 replication inhibition percentage (IP) formula measured the antiviral action from antioxidants, with reductions of the 82.0%, 82.5% on HSV replication, in presence of rosemary and rosemary+BHT, respectively. **Conclusions:** These results lead us to conclude that phenolic extracted from rosemary revealed antiviral action of HSV-1.The synergistic effect verified by the lipidic oxidation inhibition of these natural and synthetic antioxidants did not reinforce the rosemary anti-herpetic action.

Supported by: CNPq Proc. № 47093/03-3

8.05 Redescription of *Ixodes schulzei* Aragão & Fonseca, 1951 (Acari: Ixodidae) female, an endemic tick in Brazil.

Onofrio VC[1,3], Labruna MB[2], Faccini JLH[3], Barros-Battesti DM[1]
[1]Lab. Parasitologia, Instituto Butantan, São Paulo, SP, Brazil; [2]VPS, FMVZ/USP, São Paulo, SP, Brazil; [3]Depto Parasitologia Animal, UFRRJ, Seropédica, RJ, Brazil.

Introduction: The species *Ixodes schulzei* Aragão & Fonseca, 1951 was described based on an engorged female collected on wild rat from Teresópolis municipality, State of Rio de Janeiro that was deposited at the Instituto Oswaldo Cruz under the number IOC 481 (Holotype). Paratype females collected on *Nectomys squamipes* (Rodentia) from Nova Iguaçu, Rio de Janeiro State, and on wild rat from Brusque, State of Santa Catarina were also deposited at the same collection (IOC 455, IOC 456, respectively), as well. Previous studies placed *I. schulzei* into the "*luciae* group". Males of *I. schulzei* are unknown. After its description *I. schulzei* was only reported in 2003, from Santa Branca, State of São Paulo. **Methodology:** For this study we examined the types deposited at the FIOCRUZ and material from two other collections FMVZ/USP (Faculdade de Medicina Veterinária e Zootecnia da USP) and USNTC (United States National Tick Collection). The redescription of female was based on optical and scanning electron microscopy. Measurements are given in millimeters and they were obtained under Leica MZ12 stereomicroscope. One female was cleaned and prepared for scanning electron microscopy. **Results and Discussion:** In addition, the relationship of *I. schulzei* to other Neotropical *Ixodes* species is presented and discussed.
Supported by: CAPES **fellowship**

8.06 Revalidation and redescription of *Ornithonyssus brasiliensis* (Fonseca, 1939) (Acari: Macronyssidae)

Nieri-Bastos FA[1,2], Barros-Battesti DM[2], Labruna MB[1]
[1]Departamento de Medicina Veterinária Preventiva e Saúde Animal (VPS) – FMVZ-USP; [2] Laboratório de Parasitologia, Instituto Butantan

Introduction: The *Ornithonyssus* genus is composed of hematofagous ectoparasitic mites from birds, reptiles and mammals, including humans. Knowledge of this group's taxonomy is very important because it involves emergent diseases, such as rickttsioses. With the exception of rickettsial pox, scrub typhus, these mites are largely ignored as vectors of microorganisms to humans or animals. **Objective**: The current synonymy of *Ornithonyssus brasiliensis* (a species described from Brazil) with the cosmopolitan species *Ornithonyssus bacoti* is discussed. **Methodology**: The type specimens of *O. brasiliensis* and the original descriptions of both species were examined and the morphologic differences between them were observed. **Results and Discussion**: Considering their morphology and different geographical distribution, we are reasonably certain that both taxa are valid. While *O. bacoti* is considered a cosmopolitan specie, *O. brasiliensis* is known to only occur in Brazil. The redescriptions of *O. bacoti* and *O. brasilienis* females were based on optical microscopy. Based on the morphology and any records of *O. bacoti* for Brazil, we suggest they are valid species.

8.07 Establishment of comet assay in haemocytes of Biomphalaria glabrata (SAY, 1818).

Grazeffe VS[1],Tallarico LF[1],Murasaki NH[1], Suzuki MF[2], Okazaki K[2], Kawano T[1], Pereira CAB[3], Nakano E[1]

[1]Lab. de Parasitologia, Instituto Butantan, SP, Brasil; [2]Centro de Biologia Molecular, IPEN, SP, Brasil; [3]Depto de Estatística, Instituto de Matemática e Estatística, USP, SP, Brasil.

Introduction: The comet assay is a method developed to detect breaks in the DNA. The fragments of the damaged DNA show low molecular weight; on the electrophoresis they migrate first in relation to the weightier ones, acquiring the general aspect of a comet. This is a promising test for studies on genotoxicity, DNA repair, environmental and human monitoring. **Objective:** To standardize the comet assay in haemocytes of *Biomphalaria glabrata*. **Methodology:** Haemolymph of wild-type snails of the species *Biomphalaria glabrata* exposed to the Co-60 gamma radiation (12,5, 25, 50 e 100Gy) was mixed with low-melting point agarose and placed on the slide prepared with normal melting point agarose. The cells were lysed overnight, and there after exposed to an alkaline solution (pH>13) for 30 minutes. After the electrophoresis, the slides were neutralized with Tris solution, stained with ethidium bromide and analyzed in fluorescence microscope. **Results:** The control group did not form comets, but the exposed groups showed comets of several sizes and cells have suffered apoptosis. **Discussion:** The data has shown that how the bigger the dose of radiation, the greater the damage induced. The doses of 50 and 100Gy have shown citotoxic effect, with a high frequency of apoptotic cells. The obtained results have shown the sensitivity and capacity of this assay to detect the effects caused by Co-60 gamma radiation.

8.08 Description of larva and nymph of *Ixodes schulzei* Aragão & Fonseca, 1951 (Acari: Ixodidae), an endemic tick in Brazil

Barros-Battesti DM[1], Labruna MB[2], Onofrio VC[1,3], Arruda-Santos AD[1], Giacomin FG[1], Máximo-Espindola CP[1] e-mail: dbattesti@butantan.gov.br

[1]Lab de Parasitologia, Instituto Butantan,; [2]Depto de Medicina Veterinária Preventiva e Saúde Animal, FMVZ, USP; [3]Depto de Parasitologia Animal, Instituto de Veterinária, Universidade Federal Rural do Rio de Janeiro.

Introduction: The species *Ixodes schulzei* Aragão & Fonseca, 1951 is a tick endemic to Brazil, where nine other *Ixodes* species have been reported. Previous systematic studies place *I. schulzei* into the "*luciae* group", with at least three other species: *I. luciae* Senevet, *I. loricatus* Neumann, and *I. amarali* Fonseca. From these, males of *I. schulzei* and *I. amarali* are unknown, and descriptions of immature stages have been reported only for larva and nymph of *I. loricatus*, and for the larva of *I. amarali*. **Objectives:** In present study larvae and nymphs of *I. schulzei* were obtained from an engorged female collected on a free living water rat *Nectomys squamipes* (Brants) from Santa Branca municipality (23°23'S 45°52W), state of São Paulo. **Methods and Results:** The engorged female was held inside a desiccator under 90% RH and room temperature, in which it deposited fertile eggs. Batches of larvae resulting from this oviposition were fed on naïve laboratory rats (*Rattus norvegicus* Berkenholt) and naïve wild mice (*Calomys callosus* Rengger). Most of them successfully engorged and molted to nymphs. Unfed immature specimens were measured and the descriptions of both stages were based on optical and scanning electron microscopy as well as drawings for some features of the larva. In addition, the relationship of *I. schulzei* to other immature Neotropical *Ixodes* is presented and discussed.

Supported by the Project Biota/Fapesp no. 99/05446-8 to DMBB and CAPES **fellowship**

8.09 Geographical distribution of *Amblyomma parkeri* Fonseca & Aragão, 1952 (Acari: Ixodidae), a parasite of porcupine (Mammalia: Rodentia)

Onofrio VC[1], Labruna MB[2], Arzua M[3], Arruda-Santos AD[1], Giacomin FG[1] and Barros-Battesti DM[1]
[1]Lab. Parasitologia, Instituto Butantan, São Paulo, SP, Brazil; [2]VPS-FMVZ/USP, São Paulo, SP, Brazil; [3]Museu de História Natural Capão da Imbuia, Curitiba, PR, Brazil.

Introduction: The Brazilian endemic tick *Amblyomma parkeri* is closely related to the species *A. longirostre* and *A. geayi* which it has been confused since long time. The last ones are distributed in Central-South America and South America, respectively. Except for few accidental host records all of them are found on porcupines. **Objective:** We have revised the main Brazilian collections of ticks, to know the real geographic range of *A. parkeri*. **Material and Methods:** The followings collections were studied: IBSP (Acari Collection, Instituto Butantan, where the holotype female of *A. parkeri* is deposited), CNC-FMVZ/USP and MHNCI. In addition, some collected specimens from Biritiba-Mirim, State of São Paulo, not deposited yet were also examined. All data and geographic coordinates concerning *A. parkeri* were revised and the mapping was made by using the speciesMapper tools (CRIA, webpage). **Results and Discussion:** It was found 30 lots of *A. parkeri*, from which only five were identified correctly. The remainders *A. parkeri* specimens were erroneously considered either *A. longirostre* or *A. geayi*. In a total, 46 adult specimens of *A. parkeri* (29 males and 17 females) were deposited from 1933 to 2004, and those from Biritiba-Mirim included 15 specimens (10 males and 5 females) collected from 2004 to 2005. Therefore *A. parkeri* only occurs from Southeast to Southern regions, while *A. longirostre* is also found in North region, but *A. geayi* is just restricted to the North Region. **Supported by** FAPESP, CNPq and CAPES **fellowship**

8.10 Description of the larva of *Amblyomma pseudoconcolor*, Aragão, 1908 (Acari: Ixodidae) by optical and scanning electron microscopy

Barros-Battesti DM[1], Barbieri FS[2], Famadas KM[2], Arruda-Santos AD[1], Máximo-Espindola CP[1], Labruna MB[3], Faccini JLH[2]
[1]Laboratório de Parasitologia, Instituto Butantan, São Paulo, SP; [2] Depto. Parasitologia Animal IV, UFRRJ, Seropédica, RJ; [3]Faculdade Medicina Veterinária e Zootecnia, USP, São Paulo, SP

Introduction and Objectives: The description of the larva of *Amblyomma pseudoconcolor* Aragão, 1908 is based on optical and scanning electron microscopy. **Methods and Results:** Unfed larvae were obtained from a colony of *A. pseudoconcolor* originated from an engorged female collected on armadillo (*Euphractus sexcinctus*) from Mari municipality (7°3'36''S, 35° 19'10''W) State of Paraíba, Northeast Brazil. Several characters are presented including the chaetotaxy of idiosoma, palps and Haller's organ. **Conclusion:** In addition, topographical and numerical patterns of sensilla were presented using a new nomenclature proposed by the recent literature.

8.11 Redescription of *Amblyomma varium* Koch, 1844 (Acari: Ixodidae) based on light and scanning electron microscopy.

Onofrio VC[1,2], Barros-Battesti DM[1], Beati L[3], Faccini JLH[2] and Labruna MB[4]
[1]Lab. Parasitologia, Instituto Butantan, SP, Brazil; [2]Depto. Parasitologia Animal, UFRRJ, Seropédica, RJ, Brazil; [3]USNTC, Institute of Arthropodology and Parasitology, Georgia Southern University, Statesboro, GA, USA; [4]VPS, FMVZ, USP, SP, Brazil.

Introduction: *Amblyomma varium* Koch is commonly known in Brazil as the sloth's giant tick. It has been found almost exclusively on mammals of the Bradypodidae and Megalonychidae (Xenarthra) families. Its distribution range includes Costa Rica, Panama, Nicaragua, Guatemala, French Guiana, Venezuela, Colombia, Peru, Brazil and Argentina. Most male *A. varium* have a short spur on coxae IV. However, a few males from different localities have spurs three times longer. **Methodology:** All specimens from the Butantan Acari Collection as well as from other Acari Collections were examined. The redescription of adult specimens was based on optical and scanning electron microscopy. Five specimens of each sex were measured with a Leica MZ12 stereomicroscope, and all measurements are provided in millimeters. Two males (with short and long spurs on coxae IV) and two females were cleaned and prepared for scanning electron microscopy. Fragments of 12S rDNA mitochondrial gene sequences were obtained from males. **Results and Discussion:** When sequenced, the 12SrDNA genes of *A. varium* with both long and short spurs were found to be identical (GenBank accession number: AY974339) indicating that the length of the spurs is likely to be an intraspecifically polymorphic character.
Supported by: CAPES

8.12 Ectoparasite mites (Gamasida: LAELAPIDAE) of rodents from Brazilian Amazon

Barros-Battesti DM[1], Gettinger D[2], Nieri-Bastos FA[1], Arruda-Santos AD[1], Rebello VMM[1], Costa DB[1]
[1]Laboratório de Parasitologia, Instituto Butantan, São Paulo, SP, Brazil
[2]Department of Biology, University of Central Arkansas, Conway, AR; Laboratory of Parasitology, University of Nebraska, Lincoln, NE, USA

Introduction and Objectives: The family Laelapidae is cosmopolitan and composed of both predatory mites as well as parasites of vertebrates and invertebrates. Some laelapid mites maintain phoretic associations with insects and myriapods. Those that are ectoparasitic on vertebrates feed principally on blood or tissue secretions of their hosts. Because of their intimate relationship with rodents, they are considered important to public health and can be vectors of pathogens causing diseases in reptiles, birds, and mammals, including humans. The taxonomy of Laelapidae is complex, in part because of the high number of species as well as the paucity of critical studies on the Laelapidae group. **Results and Discussion**: The present work presents species of Laelapidae collected from rodents from the Brazilian Amazon with type data and collections.

8.13 Functional characterization of ET_{B1} and ET_{B2} endothelin receptors on *Bothrops jararaca* vasculature.

Frias FT[1], Mesquita L.S.M[1], Borgheresi RAMB[1]
[1]Lab. Pharmacology, Butantan Institute, Sao Paulo, Brazil

Introduction: Endothelin (ET-1, ET-2 and ET-3) are liberated physiologically in all vertebrates mammals and in some invertebrates. They are active isopeptides that interact with endothelin receptors to mediate a wide variety of functions, mainly control of vascular tonus. The endothelin receptors are classified as ET_A and ET_B. ET_A receptors are detected on vascular smooth muscle cells and mediate contraction, whereas ET_B receptors include ET_{B1} (endothelial) and ET_{B2} (muscular) subtypes, which mediate opposite effects on vascular tone. We have just characterized the ET_A receptor in the aorta of the snake *Bothrops jararaca* (Bj) (Exp Biol Med, 2006). **Objective:** We aimed to characterize functional ET_{B1} and ET_{B2} endothelin receptors in isolated of Bj. **Methods**: Concentration-response curves to IRL1620 and ET-3, both selective ET_B receptor agonists, were performed in normal and pre-contracted aorta isolated from Bj, in absence and presence of inhibitors of endothelium relaxation factors [L-NAME (NO sintetase inhibitor) and indomethacin (prostaglandins inhibitor)], and in presence of selective ET_B antagonist (IRL 1038). **Results:** IRL1620 and ET-3 induced contraction in control aortas [(EC_{50} =4.9x10^{-7}, pD_2=6.0±0.5, n=3) and (EC_{50} =1.10x10^{-7}, pD_2=6.90±0.25, n=13, respectively), and induced vasorelaxante endothelium dependent in norepinephrine pre-contracted aortas, that was abolished by IRL1038 (1μM) or by endothelium removal. **Discussion:** Our results reveled the presence of functional typical ET_B receptors both in the smooth muscle and endothelial cells, suggesting that in Bj, like in mammals, they are important in the control of basal tonus vasculature.

Supported by: FAPESP, FUNDAP and Butantan Foundation

8.14 Cysteinyl-based redox modification of the 20S proteasome from *S. cerevisiae* determines the rate of oxidized protein degradation

Silva GM[1,2], Netto LES[2] , Pimenta, DC[3], Demasi M[1]
[1] Departamento de Bioquímica e Biofísica, Instituto Butantan [2] Departamento de Genética e Biologia Evolutiva, Instituto de Biociências –USP [3]Centro Aplicado de Toxinologia /CEPID, Instituto Butantan

Introduction: The proteasome is a ubiquitous multisubunit proteolytic complex responsible for the breakdown of 80% intracellular protein. S-glutathionylation is a cysteinyl-based modification that alters proteasomal activity. **Objective**: In the present work our goal was to investigate the physiological meaning of proteasomal S-glutathionylation. **Methodology:** The 20 proteasome core was purified from yeast grown to stationary phase into glucose- and glycerol-containing media in a one-step affinity chromatography. Site-specific activities were measured by hydrolysis of fluorogenic peptides. S-glutationylation was evaluated by western blotting and colorimetric assay. Proteolysis rates were accessed by SDS-PAGE and MALDI-TOF mass spectrometry. **Results**: The S-glutathionylation of 20S proteasome was correlated to the redox state of the cell, *e.g* when cells grew into glucose medium, intracellular reductive ability was decreased together to increased proteasomal S-glutathionylation. Although glutathionylation promoted partial inhibition of chymotrypsin-like and post-acidic activity, it increased proteolysis rates in an ubiquitin-independent pathway. **Discussion**: These data suggest a physiological meaning for proteasome S-glutathionylation that positively correlates protein breakdown to increased pools of oxidized proteins when cells are under oxidative challenge.

Supported by: FAPESP

8.15 Post-proline dipeptidyl aminopeptidase IV, proline iminopeptidase and prolyl oligopeptidase activities in the hypothalamus and hippocampus of streptozotocin-diabetics rats

Zambotti-Villela L, Murena-Nunes C, Alponti RF, Marinho CE, Silveira PF
Lab. Pharmacology, Instituto Butantan, São Paulo, Brazil.

Introduction: Among proline-specific peptidases, aminopeptidases such as dipeptidyl aminopeptidase IV (DPPIV) and proline iminopeptidase (PIP), as well as endoproteases such as prolyl oligopeptidase (POP) play multifunctional roles in the central nervous system (CNS). **Objective:** To verify the effects of streptozotocin (STZ)-induced hyperglycemia on these peptidase activities in the hypothalamus (HT) and hippocampus (HC). **Methods:** (S)oluble and (M)embrane-bound fractions of HT and HC of (C)ontrol and STZ-treated rats were homogenized and ultracentrifugated to obtain S or M. Enzymatic activities were fluorometrically measured. **Results:** Hyperglycemia was detected 60-75 days after a single intraperitoneal injection of STZ 50 mg/kg (mg glucose/dL: STZ, 348±22, n=12 and C, 74±3, n=12). STZ-diabetic rats also presented a reduction of 34±2% of body wt in relation to C rats (n=12). The activity levels of DPPIV and PIP were similar in S from HT and HC between STZ and C, while those of POP increased 77±8% in the HT of STZ (n=6) in relation to C (n=9). In M, the activity levels of DPPIV from HT and HC and those of PIP from HT were similar between STZ and C, while those of PIP from HC were 228±63% higher in STZ (n=8) than in C (n=11). **Conclusions:** STZ-induced hyperglycemia generates a regional specific increase on the activity of M and S prolyl proteases in the CNS, which may change the metabolic control of their susceptible physiological peptide substrates in diabetes mellitus.

Supported by: FAPESP and CNPq

8.16 Prolyl, cystyl and pyroglutamyl aminopeptidase activities of hypothalamus and hippocampus from streptozotocin-diabetics rats

Alponti RF, Zambotti-Villela L, Marinho CE, Murena-Nunes C, Silveira PF
Lab. Pharmacology, Instituto Butantan, São Paulo, Brazil.

Introduction: We evaluated whether streptozotocin(STZ)-diabetes mellitus (DM) influences hippocampal (HC) and hypothalamic (HT) soluble (S) and membrane-bound (M) forms of prolyl (PIP)/cystyl (CAP) aminopeptidase activities, as well as the broad-spectrum pyroglutamyl aminopeptidase (PAP I) activity. **Method:** STZ treatment and fluorometric measurements of peptidase activities. **Results:** On days 60-75, after a single ip injection of STZ (50 mg/kg body wt), hyperglycemia reaches 348±22 mg glucose/dL. The activity levels of S PIP and S CAP were similar between STZ-DM and controls (C) in both areas. PIP in M of HT presented similar levels between DM and C. Compared to C, increased levels in DM were observed for M CAP (2.55 fold) in HT, and for M CAP (5.14-fold) and M PIP (3.28-fold) in HC. In both areas the levels of M and S PAP I were slightly lower (<0.8-fold) in DM than in C. **Discussion and Conclusions:** Our data, showing that DM rats presented altered CAP/PIP and PAP I catalytic activities in HT and/or HC, suggested that these peptidases are part of the common pathways that play a role in the interactions between DM-related pathologies and the functionality of their susceptible substrates, AVP, OT and TRH analogs. The comparison between the relative degrees to which these enzyme activities were affected in these different brain locations demonstrates their area-specific responses to DM condition.

Supported by: FAPESP and CNPq

8.17 Characterization of digestive carbohydrases from the spider *N.cruentata*

Genta FA[2], Ferreira C[2], Terra WR[2], Lopes AR[1] drilopes@butantan.gov.br
[1]Instituto Butantan, São Paulo; [2]Depto. Bioquímica, IQ, USP, São Paulo

Introduction: It is unknown whether digestive fluids are involved in spider toxicity. **Objectives:** We determined carbohydrases present in the digestive juice (Dj) and hepatopancreas (Hp) from the spider *N. cruentata*. **Methods and Results:** In Hp, the following enzyme specific activities (mU/mg) were determined with the indicated substrates: 1200 (starch); 4 (trehalose); 51 (4-methylumbelliferyl-β-N',N'',N'''-triacetylchitotrioside, MUC3); 13 (4-methylumbelliferyl-β-N'-acetylglucosamine, MUNAG); 0.4 (4-methyl-umbelliferyl-α-glucoside, MUαGlu); 1.3 (4-methyl-umbelliferyl-α-L-fucoside, MUαFuc). No activity was found using the following substrates: 4-MU-β-D-cellotrioside, 4-MU-β-D-cellobioside, 4-MU-β-D-xiloside, 4-MU-β-D-mannopiranoside, 4-MU-α-D-mannopiranoside, 4-MU-β-D-lactoside, 4-MU-β-D-galactoside, 4-MU-β-D-fucoside, 4-MU-β-D-glicoside, 4-MU-α-D-galactoside, 4-MU-α-L-arabinoside, and 4-MU-α-L-arabinofuranoside. A single activity against each of the substrates was found in eluates after ion-exchange chromatography and gel filtration. The enzymes present both in Dj and Hp samples have the same molecular mass, Km and optimum pH, indicating that the Hp is the site of their secretion. We found the following optimum pH, Km and molecular mass, respectively, using as substrate: starch (7, 0.4%, 40 kDa); trehalose (6, 1.8mM, 61 kDa); MUC3 (6, 1μM, 39 kDa); MUNAG (6, 0.3mM, 85 kDa); MUαGlu (5.5, 0.48mM, 67 kDa); MUαFuc (5.5, 12μM, not determined). **Conclusions:** All enzymes followed simple Michaelis Menten kinetics, except chitinase, which is strongly inhibited by high substrate concentrations.
Supported by: FAPESP and CNPq

8.18 Survey of proteinase inhibitor in porcine pulmonary extract

Dias SC[1], Sakauchi D[4], Netto SL[3], Raw I[1], Iourtov D[2], Kubrusly FS[1]
[1]Centro de Biotecnologia, [2]Lab. de Produção de Vacina Recombinante contra Hepatite B, [3]Lab. de Bioquímica e Biofísica, [4]Serviço de Controle de Qualidade – Instituto Butantan, SP

Introduction: The regulation of proteolytic enzymes in tissues by endogenous inhibitors is a critical requirement in the maintenance of homeostasis. Despite their life-giving functions, enzymes that breakdown proteins are damaging in living systems, so their activities need to be strictly regulated. **Objectives:** The survey of enzyme inhibitors in porcine pulmonary extracts was the main goal of this work. **Methods and Results:** To isolate these inhibitors we developed protocols using frozen pig lungs, saline extraction in triethanolamine buffer, extract clarification by CDR and centrifugation followed by TCA precipitation and centrifugation. One of the pulmonary extracts obtained had the pH adjusted to 7.8. The sample was concentrated by tangential flow filtration with a 30 kDa membrane. SDS-PAGE of the pulmonary extract and 30 kDa concentrate revealed bands among 60 and 8 kDa, 22 and 8 kDa for 30kDa filtrate. All the samples inhibited trypsin and elastase. The 30 kDa concentrate and the pulmonary extract showed bands of approximately 60, 20 and 14 kDa. Only one band above 60 kDa was detected in pulmonary extract in the reverse zymography technique. **Conclusions:** We believe that there are at least two proteinase inhibitors in the porcine pulmonary extract (active supernatant): approximately 60 (α-1 antitrypsin like) and 20 (SLEI or SLPI like) kDa bands. We are now carrying out new experiments to purify these inhibitors (60 and 20 kDa) in order to confirm their molecular masses and partial amino acid sequences.
Supported by: CAPES, CNPq, SADIA and Butantan Foundation

8.19 C-Terminus of murine S100A9 protein inhibits hyperalgesia induced by the activation of protease activated receptor 2 (PAR_2)

Dale CS[1], Cenac N[2], Britto LRG[3], Juliano MA[4], Juliano L[4], Vergnolle N[2], Giorgi R[1]
[1]Lab. Pathophysiology, Instituto Butantan; [2]Dep. Pharmacology and Therapeutics, U of Calgary, Calgary, Canadá; [3]Dep. of Physiology and Biophysics, ICB, USP, [4]Pharmacology Institute UNIFESP

Introduction: A peptide identical to the C-terminus of S100A9 protein (mS100A9p) induces antinociception in rodents evaluated in different experimental models of inflammatory pain. **Objective:** investigate the effects of mS100A9p on the hyperalgesia induced by the Protease-Activated Receptor-2 (PAR_2). **Methods:** effect of mS100A9p on mechanical and thermal hyperalgesia induced by a selective peptidic PAR_2 agonist (PAR_2AP) was evaluated in rats submitted to the paw pressure or plantar tests. Egr-1 expression was performed by immunohistochemistry. Calcium flux for human embryonic kidney (HEK) cells or Kirsten virus-transformed kidney cloned rat PAR_2 cells (KNRK-PAR_2) assay was evaluated by spectrometry and calcium flux on mice dorsal root ganglia neurons (DRG) or in N-, L-type calcium channel-transfected HEK cells was evaluated using a fluorescence microscope. **Results:** mS100A9p inhibited mechanical and thermal hyperalgesia induced by PAR_2AP. PAR_2AP enhanced Egr-1 expression and this effect was also inhibited by mS100A9p. mS100A9p inhibited calcium mobilization in DRG neurons in response to either trypsin or SLIGRL-NH_2, on the other hand, no effect on the calcium flux induced by trypsin or SLIGRL in HEK cells or KNRK-PAR_2 cells was observed. mS100A9p inhibited the calcium flux in N-type calcium channel-transfected HEK cells without modifying the response of L-type calcium channel-transfected HEK cells. **Discussion:** These data demonstrate that mS100A9p interferes with mechanisms involved in nociception and hyperalgesia, modulating directly on sensory neurons, PAR_2-induced nociceptive signal. Also the inhibitory effect induced by mS100A9p occurs by inhibiting N-type calcium channels.

Supported by: FAPESP, CAPES and CIHR

8.20 Study of SKI-1 activity in snake venom gland

Nascimento EB[1], Portaro FCV[2], Basak A[3], Carmona E[4], Borgheresi RAMB[1].
[1]Lab. Pharmacology, [2]LETA–CAT/CEPID, Butantan Institute, Sao Paulo, Brazil; [3]Ottawa Health Research Institute, Canada, and [4]Centre de Recherche Guy-Bernier, Montreal, Canada.

Introduction: We identified a new hypotensive peptide (DCPSDWSSYEGHCYKPFS) in the venom of *Bothrops jararaca* (Bj) snake. Its activity differs from bradykinin potentiating peptides from venom of Bj and hipotensina from *Crotalus atrox*, and it has homology to the N-terminal (100%) portion of a protein which binds and inhibits coagulation factors IX and X, isolated from *T. flavoviridis* (IX/X-bp). Probably this new peptide is originated by endoproteolitic cleavage of IX/X-bp through SKI-1. SKI-1 (subtilisin-kexin isoenzyme 1) was recently described in mammals, it is a calcium dependent protease and belongs to a pyrolysin group of subtilases. **Objective**: To identify SKI-1 activity in snake (Bj and Cdt) venom gland homogenates through the use of a fluorogenic substrate. **Methods**: Homogenates of venom glands (Tris-HCl buffer with sucrose) were centrifuged, and supernatants were ultra centrifuged and maintained at -80°C. The hydrolytic activity of this extract was detected, in the presence and absence of EDTA, by a spectrofluorometric assay using the fluorogenic substrate Abz-VGPRSFLL-EDDnp, which mimicks the thrombin cleavage site of the platelet receptor activated by protease 1 (PAR1), and would also be recognized by SKI-1. **Results:** Only Bj extract was able to hydrolyze this substrate and EDTA inhibited this activity. **Discussion**: We may suggest that Bj gland tissue may have SKI-1 but not Cdt gland. However, to check this hypothesis (results) we will use the SKI-1 specific substrate Abz-DIYISRRLLGTFT-Tyx-A over a purified enzyme from the Bj extract.

Supported by: Fapesp, Fundap and Butantan Foundation

8.21 Improving factor VIII purification by chromatographic methods

Jinzenji D[1,2], Carvalho RR[1], Raw I[1], Martins EAL[1,2], Cheng E[1,2]
[1] Centro de Biotecnologia, Instituto Butantan, São Paulo, Brasil; [2] Pós-graduação Interunidades em Biotecnologia – USP

Introduction: Coagulation Factor VIII (FVIII) is a plasmatic glycoprotein. Lack of FVIII activity causes the hemorrhagic disorder Hemophilia A. Treatment consists in infusions of FVIII purified from human or porcine plasma. FVIII concentrates are not produced in Brazil. **Objectives:** 1. Purification of FVIII employing solely chromatographic methods instead of separation of cryoprecipitate, avoiding centrifugation of large volumes of plasma and the need of cold rooms and 2. Implement the analysis of separation and stability of Factor VIII during purification steps by western blot. **Methodologies:** 1. Purification of FVIII by direct application of plasma in anion exchange column followed by gel filtration and 2. Production of recombinant fragments of FVIII for antibodies generation. **Results:** After anion exchange chromatography of human plasma, the FVIII containing fraction was applied to gel filtration. It was observed that the FVIII activity was present in the lower molecular weight fractions. In contrast, after direct purification of plasma in gel filtration column, the FVIII activity was observed mainly in the first fraction. **Discussion:** This result suggests that partial hydrolysis of the FVIII molecules occurred. Obtaining antibodies against recombinant fragments of FVIII, which are being produced in the lab, will help following the stability of FVIII during separations by western blot.
Supported by: Fapesp, CNPq and Butantan Foundation

8.22 Proliferative effects of casein peptides INKKI and YPVQPFTE on mice lymphocytes cell culture.

Azevedo RA, Maria DA, Lebrun I.
Biochemistry and Biophysics Laboratory, Butantan Institute, São Paulo, Brazil.

Introduction: Isolated INKKI and YPVQPFTE are peptides from the bovine casein hydrolysis. Previous studies had shown that these peptides presented bradykinin-potentiating activity, increase of the release of H_2O_2 for macrophages and increase of the phagocytic capacity in resident macrophages. **Objectives**: To evaluate the proliferative effect of the two synthetic peptides INKKI and YPVEPFTE in mice lymphocytes cell culture T **Methodology**: The cultures of lymphocytes T were obtained from lymphatic nodules from Balb-C mice, treated with concentrations ranging 0.015 to 8 μg/μl of each peptide, and the effect compared to a positive control (PHA 5 and 2.5 μg/μl). The proliferative effect was evaluated by the method of reduction of the MTT ("3-(4.5-dimethylthiazol-2-y1) 2.5-diphenyl tetrazolium bromide"), absorbance at 540nm was read on a Multiskan MS microplate reader. **Results:** Our results showed that INKKI with the concentration of 8 μg/μl induced a significantly increased proliferation activity when compared to control. Whereas, our results with YPVQPFTE peptide showed significantly increased in a dose dependent manner the proliferation when compared to control, PHA or even with the INKKI peptide ($p < 0.001$).**Discussion:** These results showed that octapeptide YPVQPFTE significantly increase the proliferative activity on lymphocytes T in vitro suggesting an immunomodulatory effect which could simulate the in vivo effect of the peptides derived from the proteolytic hydrolysis of the casein, and of this form to contribute to better comprehension diverse clinical situations of pathophysiological mechanisms related to dietary phenomena.
Supported by: FAPESP

8.23 Protein digestion in *Tityus serrulatus*

Fuzita FJ[1], Bertani R[2], Lopes AR[1]
[1]Lab. de Bioquímica e Biofísica – Instituto Butantan; [2] Lab. de Imunoquimica – Instituto Butantan

Introduction: Only two enzymes involved in scorpion prey digestion are known: amylase and lipase activity. **Objectives and Methods:** In order to characterize protein digestion in scorpions *T. serrulatus,* hepatopancreas (Hp) was isolated and homogenized. Protease activity was determined using carbobenzoxy-Phe-Arg-7amido4methyl coumarin, Z-FR-MCA, and casein-FITC as substrates. **Results and Discussion:** It was observed that the protease activity requires pre-activation in acidic medium (pH = 2.5) by kinetic activation assays. Protease activity measured after activation is 9.0 U/Hp, using Z-FR-MCA as substrate. This activity stability depends on the presence of 3.0 mM cysteine and 3.0 mM EDTA in the reaction medium. Experiments on the effect of pH on protease activity showed activities in pHs in the range of 2.5, 5.0 and 7.0, indicating the presence of proteases with different specificities. Chromatographic separation using an anion exchange column- HitrapQ-using homogenated, pre-activated, and non-activated Hp presented three different protease activities (P1, P2 and P3). P1 did not interact with the column and was active in acid pH and 100% inhibited by 10 μM pepstatin. P2 and P3 presented higher activity at pH 4.5 and were completely inhibited by 100 μM E-64. Results from 100 μM PMSF did not indicate inhibition. Chromatographic fractions were not active using casein-FITC as substrate.
Supported by: FAPESP

8.24 Changes in noradrenaline content of contralateral rat vas deferens after unilateral sympathetic denervation

Freitas TA[1,2], Garcez-do-Carmo L[1], Conceição IM[2]
[1]Lab. Pharmacology, UNIFESP, São Paulo, Brazil; [2]Lab. Pharmacology, Butantan Institute, São Paulo, Brazil.

Introduction: Noradrenaline (NA) content of vas deferens is largely decrease by unilateral surgical denervation (USD) of postganglionic sympathetic fibers. Consequences of changes in noradrenaline content are easily observed in functional studies of vas deferens and include reduced response to tiramine and electrical field stimulation. However, our recent observations have been pointed to alteration also in the counter lateral non manipulated vas deferens. **Objectives:** The aim of present study was to verify if these functional changes observed in counter lateral vas deferens are accompanied by alterations in content of NA. **Method and Results**: Male Wistar rats (2,5 months aged) were submitted to USD without manipulation of counter lateral vas deferens. After 4, 7, 14, 21, 28, 56 and 84 days (n=10/group), animals were killed and vas deferens were removed and processed to performing NA content measurements in a HPLC system with electrochemical detection. In comparison to control animals (NA content of 31,9±pmol/mg tissue), counter lateral vas deferens showed a statistically significant increase in the NA content in days 4, 7 and 56 after USD with elevations of 20%, 28% and 27%, respectively. **Conclusion:** USD can produced changes in the NA content of counter lateral vas deferens indicated a possible crossing adaptation to supply the lack of ipsilateral innervation.
Supported by: CAPES, FAPESP and Butantan Foundation

8.25 Characterization of inflammatory reaction in the knee synovial cavity induced by carrageenin injection

Dias RG[1], Sampaio SC[1], Picolo G[1], Cury Y[1]
[1]Laboratory of Pathophysiology, Butantan Institute, São Paulo, Brazil.

Introduction: Experimental animal models of joint lesions are an important tool to investigate the inflammatory reaction in the knee synovial cavity. **Objective**: The purpose of the present study was to characterize the inflammatory reaction in the knee synovial cavity induced by carrageenin injection by determinate of vascular permeability and leukocyte influx. **Methods**: Rats were injected with carrageenin (200µg/50µl) into the synovial cavity of the right knee joint. Control animals were injected with PBS. Animals were killed in different times after carrageenin injection and the cells within the knee articular space were harvested by washing the cavity with 60µl of PBS. Plasma extravasation and leukocyte migration induced by carrageenin injection were evaluated. Total and differential cell counts were performed. **Results**: Carrageenin induced a significant increase in leukocyte influx (1 and 4h) and drastic protein extravasation after 4h of carrageenin administration. **Discussion:** These findings show that intra-articular injection of carrageenin induces inflammatory reaction into the knee joint. This study is important to understand some mechanisms involved in the pathophysiology of articular cartilage lesions.
Supported by: CAPES

8.26 Characterization of a nociceptive model to evaluate arthritis induced by carrageenin: *Footprint* modified model

Dias RG[1], Picolo G[1], Sampaio SC[1], Cury Y[1]
[1]Laboratory of Pathophysiology, Butantan Institute, São Paulo, Brazil.

Introduction: Experimental animal models of joint lesions are an important tool to investigate the inflammatory reaction in the knee synovial cavity. The development of a new methodology is crucial to understand the nociception observed in inflammatory reaction in the knee synovial cavity. **Objective**: The aim of the present study was to characterize a new model to evaluate carrageenin-induced nociceptive reaction, using *Footprint* modified model. **Methods**: Rats were injected with carrageenin (200µg/50µl) into the right knee joint. Control animals received PBS injection. Nociceptive response was assessed after 3h of carregeenin injection, using the *Footprint* modified test. This test consisted in printing the hindpaws using ink. After this procedure, rats were placed in a plastic tube (1mx7cm) to walk on white paper. The parameters measured in this test were to count the total number of footprint, including partial and entire footprint, also a percentage of entire footprint was calculated after carrageenin injection. **Results:** Footprint analysis demonstrated that carrageenin-induced inflammatory reaction in the knee synovial cavity, increased the total number of footprint ($p<0.05$). On the other hand, this pathological process decreased ($p<0.01$) the percentage of entire footprint. **Discussion**: This new method provides a quantitative technique to perform limb function after carrageenin-induced arthritis.
Supported by: CAPES

8.27 Evaluation of the effect of soluble bound phenolic acid fraction obtained from olives (*Olea europeae*, L.) in HeLa cell culture.

Paes MD[1], Mendonça RMZ[2], Mancini DAP[2], Pinto JR[2], Mancini Filho J[1]
[1]Food Department and Experimental Nutrition of the Pharmaceutical Sciences College of the São Paulo University, SP, Brazil.[2] Virology Laboratory, Butantan Institute, São Paulo, Brazil.

Introduction: The olive (*Olea europeae,* L.) is a good source of phenolic compounds important in reducing oxidation in biological systems and plays a preventive role against certain chronic disease. **Objective:** To evaluate the antioxidant activity *in vitro* and verify its effect on HeLa cell live culture (derived from human cervix uterus tumors). **Methodology:** The olive (Ascolana variety) was triturated, lyophilized and defatted, to obtain the phenolic acid fraction (free, soluble and insoluble bound). The antioxidant activity was evaluated by the β-carotene/linoleic acid system and the radical 1.1´-diphenyl-2-picryl-hidrazil (DPPH•) test. The soluble bound phenolic acid fraction was filtered and added at two concentrations (5 and 20ppm) to the cell culture. After one week, the cells were removed and the pellet was separated for protein analysis and a viable cell count. **Results:** The soluble bound phenolic acid fraction reached 84% of protection, at 30ppm of concentration at β-carotene/linoleic acid system, and 94% at 20ppm in the DPPH test. The cells treated with the soluble bound phenolic acid fraction at 20ppm presented the highest amount of proteins (1.97 mg/mL) and viable cells (13.51%) compared with the 1.31mg/mL content of proteins and 2.89% of viable cells obtained from the control cells. **Discussion:** These results show that the antioxidant protection of the soluble fraction of the olive Ascolana variety influences HeLa cell culture development.
Supported by: CNPq

8.28 Anxiolytic and antinociceptive effects of extracts from *Erythrina velutina* in rats.

Simokomaki K, Barreto AS, Guinski-Chaguri LCA, Netto SL, Lebrun I
Laboratory of Biochemistry and Biophysics- Instituto Butantan, São Paulo, Brazil.

Introduction: In herbal medicine the plants are used to cure diseases related human being showing directions for discovery to new medicines. The *Erythrina* genus has antiviral, antibacterial to sedatives, analgesic, tranquilizing effects and relaxing properties. The *Erythrina mulungu* is used, to calm the agitation and other manifestations of the nervous system and possesses anxiolytic effect in defensive behavior related with the anxiety and panic. The *Erythrina velutina* studied is known as "mulungu" and has proprieties those sedative and neuromuscular blocking actions. **Objectives:** In the present research extracts obtained from seed of the *Erythrina velutina* were tested as capacity to neutralize the effects proved by the pain on hot plate and anxiety behavior with combination of the open field and the elevated plus-maze. **Methods and Results:** Tested animals (rats) with extracts of seed obtained with organic material demonstrate analgesic activity with hot plate the temperature from 48 to 50^{o}C, the animal supported the hot plate for approximately 10 minutes, indicating analgesic effect on the animal without motor effect. The animals also increased the numbers of locomotion on open field and even the numbers of entries and the time spent in the open arms of the elevated plus maze. These results demonstrated that lesser anxiety levels allow longer and more frequent exploration periods of the open arms, suggesting that *Erythrina velutina* has strong anxiolytic properties compared to the control anxiolytic diazepan (1mg/ng).

8.29 Use of Plackett-Burman statistical design for optimization of culture medium for monoclonal antibody production.

Marques FH, Borges DB, Inocencio AL, Targino, RC, Garbuio A, Rodrigues MTA, Moro AM
Laboratório Especial de Biofármacos em Célula Animal, Instituto Butantan

Introduction: In the production process for monoclonal antibodies (mAbs) by mammalian cells, the composition of culture medium plays a major role in cell growth and product yield. Due to the complex nutritional needs of cells and the growing concerns about the use of animal raw materials, its necessary to test several compounds in substitution of fetal calf serum (FCS) without prejudice of productivity and prohibitive cost increases. **Objective:** To test individual effects of 18 compounds in the production levels of anti-CD3 by hybridoma cells. **Methodology**: A Plackett-Burman experimental statistical design was used to formulate 24 different media options intended for the cultivation of the hybridoma in T-25 flasks. Basal medium was a commercial protein-free medium. The concentration of anti-CD3 was determined by a sandwich ELISA of the supernatant harvested at the end of the experiment. **Results**: Considering a significance level above 60%, nine compounds showed a positive effect: sodium piruvate, ITS, Yeastolate, glutamine, putrescine, α-tocoferol, ethanolamine, vitamins solution and lipids. Six compounds had a negative effect, while the others showed no significant effect. **Discussion**: The Plackett-Burman matrix design proved to be a useful tool for simultaneously evaluating the effect of several compounds. These results can be used for further determining the optimal level for each compound, in order to reach a significant increase in productivity in relation to the basal medium.
Supported by: FINEP and Butantan Foundation

8.30 The creation of a snake venom gland tissue bank

Hayashi MAF[1], Germano VJ[2], Serapicos EO[2], and Marques OAV[2]
[1] Center for Applied Toxinology (CAT/CEPID) and, [2] Laboratory of Herpetology, Instituto Butantan, São Paulo, Brazil.

Introduction: Butantan Institute has long been recognized as a reference and resource institution for maintenance and collection of snakes, as well as for venom extraction and anti-venom vaccine production. Aiming to conserve for future studies specimens that cannot be held captive for lengthy periods, snakes have been maintained long-term, immersed in formaldehyde solution. **Objective**: We report that several Institute members effectively trained the Institute community to modify collection procedures to assure maintenance of cataloged specimen genetic material. **Methodology**: Before the fixation procedure, the Harderian, venom and Duvernoy glands were extracted from the snakes, taking care not to damage the animal's scales, then the collected tissues were immediately frozen using liquid nitrogen and kept at -80°C. **Results**: Tissues from more than 300 specimens have been collected in approximately a three year period. Under the conservation conditions described above, the recovery of high quality RNA/DNA samples could be verified. **Discussion**: Considering the high scientific and economic interest in this material of inestimable value, a committee was created to monitor and assure fair access and the best use of it.
Supported by: FAPESP (CAT/CEPID)

8.31 Cloning and expression of human glucocerebrosidase (GCR) in *Escherichia coli* for the production of the anti-GCR antibodies

Novo JB[1,2], Oliveira MLS[1] and Ho PL[1,3]
[1]Centro de Biotecnologia, Instituto Butantan, São Paulo, SP; [2]Interunidades em Biotecnologia, Instituto de Ciências Biomédicas, USP, São Paulo, SP; [3]Instituto de Química, USP, São Paulo, SP.

Introduction: Glucocerebrosidase (GCR) is a lysossomal glycoprotein that catalyzes the breakdown of the glycolipid glucocerebroside in glucose and ceramide. The defective activity of the enzyme is the most common lysossomal storage disorder, know as Gaucher disease and leads to the accumulation of glycolipid in macrophages, particularly those in the liver, bone marrow, spleen and lung. In rare cases, there is neuronopathic involvement. Currently available treatment for Gaucher disease includes enzyme replacement therapy using a recombinant form of GCR expressed in mammalian cells and has been successful in alleviating many of the symptoms. **Objectives:** To produce anti-GCR specific antibodies for future analysis of expression of the recombinant protein in mammalian cells and possible clinic use for diagnostic. **Methods:** For this, the human GCR cDNA was amplified by PCR and cloned into the pAE vector which provides a high-level expression of heterologous proteins in fusion with six histidine residues (6Xhis-tag) in *E. coli.* Competent *E. coli* BL21 (SI) and *E. coli* BL21 (DE3) Star pLys were transformed. **Results and Discussion:** The presence of the expected 56 kDa protein band corresponding to the unglycosylated GCR was confirmed by 10% SDS-PAGE analysis in induced *E. coli* BL21 (SI) clones. Purification of the protein was performed with a nickel charged chromatography column. The purified GCR was inoculated in BALB/ c mice leading to the induction of anti-GCR IgG in the sera, with a titer of about 1:80.000 as evaluated by ELISA.
Supported by: FAPESP, CNPq and Fundação Butantan

8.32 *In silico* analysis of the putative pre-replication complex component Orc/Cdc6 of *Trypanosoma cruzi.*

Melo-Godoy PD[1], Mora AJC[2], Schenkman S[2], Elias MC[1]
[1]Lab. de Parasitologia, Instituto Butantan, SP; [2]Depto de Microbiologia, Imunologia e Parasitologia, UNIFESP, São Paulo, SP.

Introduction and Objectives: The pre-replication complex formation is conserved among eukaryotes. Orc (origin replication complex) a hexameric complex (Orc1-6) first binds origin DNA and then recruits Cdc6 and Cdt1. Together, these proteins act to load the Mcm2-7 helicase onto origin. In spite of DNA duplication is well established in eukaryotes, the molecular bases of replication in trypanosomes remain to be determined. **Methods and Results:** Analyzing *T. cruzi* genome database we found that it does not present genes coding to Orc1-6. Unlike, this parasite contains one open reading frame homologous to Orc1 and Cdc6. This fact suggests that Orc and Cdc6 proteins would be expressed in fusion as one protein, as also found in *Archaea.* ATP binding and hydrolysis are essential for pre-RC formation. Many proteins that participate in pre-RC assembly are members of the AAA+ family of ATP binding proteins. ATP binding by the Orc1 subunit is required for origin-specific DNA binding and can direct an initial round of Mcm 2-7 loading. ATP hydrolysis is required to allow ORC to participate in repeated rounds of Mcm2-7 loading. Phosphorylation of Cdc6 is essential to target this protein to degradation or to nuclear export, blocking a new round of replication in the same cell cycle. In fact, the predict structure of *T. cruzi* Orc1/Cdc6 putative protein showed an ATP/GTP binding domain, and phosphorylation sites, suggesting a functional role for this protein. We are expressing this protein in order to verify in vitro these activities. **Conclusions:** This work points that the replication process in trypanosomes is in part similar to *Archaea* and different from other eukaryotes.
Supported by: FAPESP

8.33 Modulatory effects of ATP and its metabolites on the tonic and phasic components in a contraction curve induced by high frequency electrical stimulation of rat vas deferens

Oliveira AB[1], Casasco BS[1], Conceição IM[1]
[1]Lab. Pharmacology Instituto Butantan, São Paulo, Brazil

Introduction: In VD, NE, and ATP are released from the same adrenergic terminal, characterizing the co-transmission process. **Objectives:** In this study, we intend to verify the role of ATP and its metabolites in the phasic and tonic components of HFEE-induced contraction in rat vas deferens. **Methods:** The vas deferens was submitted to continuous stimulatory trains for 10 s at frequency of 0.01 trains per second (with stimulus of 7 Hz, 0.1 ms and supramaximum voltage). After the maximum contraction stabilized (about 15 minutes), dose-response curves of ATP, AMP, ADP or ADN were performed and the effects were registered. **Results and Discussion:** We observed that all purines used decreased in HFEE-induced contraction. However, AMP and adenosine have most potent effect on the phasic component of the contraction curve; meanwhile, ADP more potently inhibits the tonic component. Our results showed that purines could affect both component's contraction in distinct ways, pointing to a deferential regulation ATP and NE releasing from sympathetic nerves in the RVD.

8.34 Promissory source of porcine SP-A for multiple purposes

Sakauchi D[1], Dias SC[2], Veiga LR[2], Toquetti RC[5], Horton DSPQ[1], Piazza RMF[4], Iourtov D[3], Raw I[2], Kubrusly FS[2]
[1]Serviço de Controle de Qualidade, [2]Centro de Biotecnologia, [3]Lab. de Prod. de Vacina Recomb. contra Hepatite B, [4]Lab. Bacteriologia - Instituto Butantan; [5]FMVZ-USP, Brasil

Introduction: Pulmonary surfactant protein A (SP-A) is a component of pulmonary surfactant and plays a role in surfactant biology and in the innate host defense and regulation of inflammatory processes. SP-A has four structural domains and these domains are involved in different aspects of SP-A function. Primates have two functional genes of SP-A, *SP-A1* and *SP-A2*. These genes are also differentially regulated during development. SP-A is a multimeric molecule consisting of six trimers. The size of human SP-A monomers ranges between 25 and 29 kDa, and the dimers ranges between 48 and 60 kDa. **Objectives:** We are purifying SP-A from porcine pulmonary extracts to provide it for important medicine research studies and/or therapeutics application. **Methods and Results:** One clarified and acidified pulmonary extract sample after nonreducing and reducing SDS-PAGE presented only the expected bands corresponding to monomers and dimers observed in pure human SP-A. The Western blotting results with an antiserum to human SP-A showed the same recognition patterns even regarding some immunoreative bands with lower molecular mass observed in monomers and dimers of human SP-A attributed to different glycosilation patterns due to the molecular complexity of the genes expression. An analyzed bovine bronchoalveolar lavage sample had extra bands not recognized by the same antiserum to human SP-A. **Conclusions:** These results permit us to consider this sample as a promissory source of porcine SP-A.
Supported by: CAPES, FAPESP, SADIA and Butantan Foundation

8.35 Valuation of dantrolen postnatal treatment in physical and behavioral development in newborn and adult rats

Pedicino CA[1], Dorce VAC[1], Nencioni ALA[1]
[1]Pharmacology Laboratory, Butantan Institute, São Paulo – Brazil

Introduction: Dantrolen was used as an inhibitor of intracellular calcium release because the TsTX toxin experimentally used unleashes its release. **Objective**: to verify whether dantrolen administration in newborn rats could change the physical and behavioral development and cellular integrity of SNC in newborns and adults. **Methodology**: Eight groups treated with 0 (saline), 5 (N=newborn 5; A=adult 5), 10 (N10; A10) and 15mg/Kg (N15; A15) of dantrolen i.p. on 2nd day of life. The physical and behavior development of N was observed. Four rats of each offspring were sacrificed on the 7th day of life for brain histological analysis. For A (2–3 months old), physical and behavioral development was evaluated before rats were sacrificed for brain histological analysis. **Results**: In N5: delay in eyes opening; weight increase of rat pups on the 8th, 14th and 20th day of life; in N10: a quickening of the hearing canal opening decrease on the time of geotaxis on the 8th and 12th; in N15: delay in eyes opening; weight increase of rat pups on the 8th; decrease of time of negative geotaxis on the 8th; decrease of time of general activities on the 14th and 18th. In adults, only the weight ($p<0.05$) increased or decreased. No significant alterations were observed for locomotor and total activity and forced swimming in all of groups. **Conclusion**: Dantrolen causes alteration in physical and behavioral development of pup rats, but causes alterations only in physical development of treated adult rats.
Supported by: Butantan Foundation

8.36 Application of biological assays in the evaluation of water quality in Rio Tietê

Tallarico LF[4], Suzuki MF[2], Borrely SI[1], Grazeffe VS[4], Ohlweiler FP[5], Okazaki K[2], Oikawa H[1], Barros GA[1], Hamada N[1], Kawano T[4], Pereira CA de B[3], Nakano E[4]
[1]Centro de Tecnologia das Irradiações – IPEN-CNEN/SP; [2]Centro de Biologia Molecular – IPEN-CNEN/SP; [3]Departamento de Estatística, IME – USP, SP; [4]Laboratório de Parasitologia/Malacologia – Instituto Butantan, SP; [5]Laboratório de Malacologia, SUCEN, SP.

Introduction and Objectives: Bioindicator organisms were used to evaluate the effects of Rio Tiete pollutants produced by inhabitants of the upper Tiete region. The river receives industrial and domestic waste, passing through the Waste Treatment Plant (WTP) (ETE/Suzano of the Companhia de Saneamento Ambiental do Estado de São Paulo-SABESP) in Suzano city, Sao Paulo state. **Methods and Results:** The micro crustacean *Daphnia similes*, the freshwater snail *Biomphalaria glabrata,* and the luminescent bacteria *Vibrio fischeri* were used for the evaluation of acute toxicity. A micronucleus assay in Chinese-hamster cells was used for the assessment of *in vitro* mutagenic potential. Two affluent and effluent water samples were collected and transported near the WTP by SABESP. The first affluent was 200 meters upstream from the WTP (P1) and the second was the affluent of the WTP (P2). There were two effluent points: the first was where the WTP discharged into the river (P3), whereas the last was 200 meters downstream from the discharge effluent (P4). The sample P1 and P4 were not toxic for all organisms tested, while the P2 was toxic for all organisms tested. The P3 was moderately toxic for *Biomphalaria glabrata* embryos. All samples were mutagenic for CHO cells, without S9. **Conclusions:** Therefore, the WTP biological treatment reduced the toxicity of effluents, as shown by the absence of toxicity of the P4 in all bioindicators.
Supported by: FAPESP, CNPq and Technical Support: SABESP/Suzano

8.37 Molluscicide effect of piperaceae extracts in schistosomiasis vector *Biomphalaria glabrata* (SAY, 1818)

Rapado LN[1], Kato MJ[2], Kawano T[1].
1- Lab Parasitology and Malacology, Instituto Butantan; 2- Lab. Chemistry, USP, São Paulo, Brazil.

Introduction: Schistosomiasis occurs in 54 countries and territories, mainly in the South America, Caribbean, Africa and east of the Mediterranean. In Brazil 5-6 million people are infected and 30 thousand are exposed to infection risk. Is a typical disease of poor region and is associated with the lack of basic sanitation and contaminated water in agriculture, housework and leisure. One of the methods more efficient to control this disease is the application of molluscicides that eliminates or reduces the snail population, intermediate host. The concern with the environmental preservation, the high cost and recurrent resistance of snail to the synthetic molluscicide has stimulated the study of molluscicides with plant origin. **Objectives:** In the present study the molluscicide action of the Piperaceae family was verified in adult snails *Biomphalaria glabrata*. **Materials:** Studies with 13 plant species were carried through, totalizing 15 extracts from stem, leaf and root. The snails had been submitted to the concentrations of 500ppm and 100ppm for choosen which one had the better molluscicidal potential. **Results and Discussion:** Rate mortality was 100% in eleven extracts with 100ppm of concentration and had been separated in order to get less lethal concentration. The leaf extracts of *Piper crassinervium, Piper hostmannianum, Piper diospyrifolium, Piper cuyabanum* and *Piper aduncum* presented good molluscicidal action with 100% of mortality of snail in concentrations from 10ppm to 60ppm.

8.38 Evaluation of Geometric Morphometrics for microevolutionary studies on *Aedes aegypti* and *Culex quinquefasciatus* (Diptera:Culicidae).

Suesdek-Rocha LS
Lab. Parasitologia, Instituto Butantan - São Paulo, Brazil.

Introduction: The mosquitoes *Aedes aegypti* and *Culex quinquefasciatus* are among the most medically important insects, since they are capable of transmitting etiological agents of several infections. Methods for controlling these mosquitoes frequently face a limiting factor: the *microevolution*, process through these insects develop resistance to insecticides and tolerance to polluted urban environments. Therefore, research on microevolution of Culicids is a central question in medical entomology. **Objective**: Since microevolutionary processes may be detected by morphology of wings, we tested the applicability of Geometric Morphometrics (GM) for detection of subtle variations in wing morphology. **Methodology**: We compared the wings of some populations of *Ae. aegypti* and *Cx. quinquefasciatus* from urban places submitted to different degrees of presence of pollution and insecticides. Comparisons were made through the following parameters of GM: Consensus, Centroid Size and Cannonical Variables. **Results**: The morphometric parameters showed bilateral assimetry and sexual dimorphism in some populations of both species. Non conspicuous similarities and dissimilarities among populations of the same species were also detected. **Discussion**: GM was here used firstly and appeared to be promising for studying the microevolution of mosquitoes, its correlation with ecological and epidemiological features and its implication on control methods for these disease vectors.
Supported by: FAPESP (06/0262-5)

8.39 Monitoring opposum scansorial behaviour (*Didelphis sp.*) in an experimental area simulating an electric substation

Vasconcellos TP[1], Suzuki H[1], Antoniazzi MM[1], Hingst-Zaher E[2], Marques-Porto R[1], Cavaleiro M[3], Oliveira AV[3], Jared C[1]
[1]Lab. Biologia Celular, Instituto Butantan, São Paulo; [2]Museu de Zoologia, USP, São Paulo; [3]Gerência de Meio Ambiente, Elektro, Campinas.

Introduction: Electric companies often face serious problems with accidents involving wild animals, which can cause short-circuits when entering energized areas, resulting in environmental and financial loss. Most accidents are caused by opossums at night, animals highly adapted in colonizing human-modified environments. These mammals climb trees or other vertical structures in a behaviour known as scansorial, using their tail as a fifth member. **Objective**: The scansorial behaviour of opossums was studied in an experimental area especially designed for this purpose inside Instituto Butantan. **Methodology**: Specimens of *Didelphis sp*. were collected in São Paulo State, and, after a period of adaptation in the experimental site, were individually monitored by 8 cameras in an 90 m^2 walled area, in the center of which was installed a structure 5 m high and 4 m wide, which is used by Elektro (Electrical utility company) as part of its electric substations. The animals were observed and filmed daily. **Results**: The opossums spend most part of their nocturnal active cycle on the structure, climbing and descending it several times, stimulated or not by the presence of food on its top, and indiscriminately using its different components. **Discussion**: Preliminary observations show that the opossums do not need any stimuli to climb the structure since they have the natural tendency of remaining most of the time on it. The observations justify the need for the use of physical barriers on all types of posts and cables.
Supported by: Elektro/Aneel

9. PIBIC program

9.01 Distribution and food habits of glass snake *Ophiodes* (Sauria: Anguidae) in the State of São Paulo

Rosa JHN[1,2], Calleffo MEV[2], Marques OAV[2]
Faculdade de Biologia, UMESP - SP[1]; Lab. de Herpetologia – Instituto Butantan, SP, Brasil[2].

Introduction: The genus *Ophiodes* WAGLER, 1828 occurs exclusively in neotropical region, distributing in South America. Data on natural history are scarce for genus *Ophiodes*. **Objectives:** Here we provide information on morphology, die activity, diet, feeding, and behavior from *Ophiodes* spp. recorded in São Paulo state. **Materials and Results:** We collected data on locality, snout-vent length, tail and head length, gut contents of specimens housed in collection of Instituto Butantan (IB) and Unicamp (ZUEC). Data on day activity and feeding behavior were obtained from captive lizards' behavior. Females ($\bar{x}$ SVL = 163.4mm) attained larger body than males ($\bar{x}$ SVL = 146.4mm), and mass did not differ significantly between sexes. Besides tail length males ($\bar{x}$ TL = 211 mm) were larger than females ($\bar{x}$ TL = 170.7mm) and; relative head length males ($\bar{x}$ HL = 15.2 mm) were significantly larger than females ($\bar{x}$ HL = 14.5 mm). Activity was diurnal and occurred mainly between 7:00 and 15:30 h. The diet included only Arthropod (about 90% insects and 10% spiders). The two species differed in manner of subjugation of spider: *Ophiodes fragilis* seizes spiders on the abdomen and compresses it against the ground; behavior not recorded in *O. striatus*. The larger tail in males was due to the presence of hemipênis and muscles retractors; larger body size in females conferred a considerable selective advantage for them, because fecundity was size-dependent. **Conclusions:** Apparently difference in feeding behavior is due lesser stoutness of *O. fragilis*.
Supported by: CNPq/PIBIC

9.02 Preliminary survey of the snake fauna from Cunha and São José do Barreiro, highlands of Atlantic Forest, southeastern Brazil

Franco FL[1], Serafim H[1,2], Rosa T da[1,3]
[1]Lab. Herpetologia, Instituto Butantan, SP; [2]Lab. Genética de Répteis e Artrópodes, Instituto de Biociências, USP, SP; [3]Curso de Ciências Biológicas, Uninove, SP, Brazil

Introduction: The Atlantic Forest is distributed along the Atlantic coast of Brazil from Rio Grande do Norte south to Rio Grande do Sul. The high pluviosity and temperature, associated with a complex topography contribute to the high diversity of species, which is considered one of the largest on the planet. **Objectives:** We prepared a preliminary list of snakes from Cunha (23°04´S 44°57´W) and São José do Barreiro (22°38´S 44°34´W) (SJB), SP, located at the Serra do Mar and Serra da Bocaina, respectively, in order to characterize the richness and the relative abundance of the species, as well as the seasonal incidence. **Methods and Results:** The herpetological analysis was associated with bibliographical research, voucher specimens in the collection (at Instituto Butantan, IBSP) as well as fieldworks. The 703 recorded specimens [Cunha (n=460) and SJB (n=243)] represent 40 species (33 from Cunha and 21 from SJB) of: Colubridae-30 (24 and 16); Viperidae-6 (5 and 5); Elapidae-2 (only from Cunha). We verified that *Bothrops jararaca* [n=172 (130 and 42)] and *Crotalus durissus terrificus* [n=240 (117 and 123)] were very abundant in both areas, with the highest incidence in summer. **Conclusion:** Viperid species were more prevalent than the colubrids, probably because the snake's suppliers select the poisonous animals, saving the non poisonous. We highlight that a species of *Clelia* from SJB, is probably unknown in the literature.
Supported by: FAPESP/BIOTA (99/08291-5), CNPq/PIBIC

9.03 Spiders of soil inventory in Estoril Park, São Bernardo do Campo, São Paulo, Brazil Araneae, Arachnida)

Távora BCLF[1], Brescovit AD[1], Knysak I [1], Guizze SG[1], Perroni GG [1]
[1]Lab. Arthropods, Instituto Butantan, São Paulo, Brazil.

Introduction: Brazil is responsible for approximately 20% of the spider fauna of the world. Nevertheless, knowledge on the ground dwelling species is poor and studies concerning this particular habitat in the state of São Paulo rare. **Objective**: Estimate and inventory the diversity of ground dwelling spiders of the Parque Estoril. **Methodology**: Six samplings, using pitfall traps, will be carried out every two months between February/2006 and January/2007. For each sampling period, one hundred traps, made out of plastic 500 ml cups, will be installed for 5 days. The contents of each cup will be considered an independent sample. The resulting material will be sorted and separated into morphotypes. **Results and Discussion**: To date, two samplings were carried out, one in the fall (IV/06) and one in winter (VI/06). Only 154 traps included spiders. A total of 482 spiders, 442 adults and 55 juveniles, were captured, averaging 2.43 spiders per trap. Species belonging to the families Sparassidae, Corinnidae, Theridiidae, Linyphiidae, Ochyroceratidae, Zoridae and Pholcidae were identified. Of these, Corinnidae, Zoridae and Sparassidae were represented solely by juvenile specimens. Linyphiidae is presently the most abundant family, accounting for almost 90% of the specimens. Although these are preliminary results, the abundance of Linyphiidae suggests that the area is quite altered and will present a low species diversity.
Supported by: CNPq/PIBIC

9.04 Formula for estimate of the biomass of spiders (Arachnida, araneae) on the basis of corporal measures

Cizauskas I[1,2], Brescovit AD[1]
[1]Laboratório de Artrópodes, Instituto Butantan, [2]Universidade Ibirapuera, São Paulo, SP.

Introduction: To date, dry weight is one of the most popular techniques to estimate arthropod biomass. This process is problematic when dealing with diversity, since the material is permanently destroyed and cannot be deposited in scientific collections to be made available for future studies. **Objective:** Develop a formula to estimate spider biomass using body measurements, instead of dry weight, thus avoiding the destruction of the material and enabling its preservation in scientific collections and its use in future studies. **Methodology:** The spiders were collected in the forest remnant of the Guarapiranga Reservoir located in Jardim Ângela, São Paulo from X/2004 to IV/2005. Four sampling methods were used: pitfall-traps, Winkler extractors, beating tray, and nocturnal manual collection. Total length (CT), cephalothorax width (LC) and height (AC) of 231 specimens were measured. These measurements together with the spider`s postmortem and dry weights (48hs to 65ºC) were used to develop the formula. **Results:** BM = CT*LC*0.0003*AC was the formula obtained to estimate the dry weight of spiders. Of the 1,800 collected spiders, only 897, with body dimensions ranging from 0.08 to 21mm were used. The estimated weight obtained by the measurement formula was 2.11g, which represented close to 99% of the weight value obtained from the same specimens. **Discussion:** The proposed formula is effective for all tested methodologies and prevents the destruction of the collected material. Approximately 40% of the material collected during this study is recorded for the first time in the area or is unknown to science and will be described in the near future.
Support by: CNPq/PIBIC and FAPESP (99/05446-8)

9.05 Does hunger in spiders influence the hypothesis of the evolution of predatory behavior?

Gonçalves, VP [1,2], Japyassú, HF[2] and Garcia, CRM[2]
[1]Universidade de Santo Amaro; [2] Laboratório de Artrópodes, Instituto Butantan, São Paulo, Brazil.

Introduction: Reeling is a predatory behavior in which the orb weavers pull a web radius with legs I and II, bringing to the hub a prey adhering to this radius. Previous studies suggest that this behavior first appeared as an exaptation in Uloboridae, to be then co opted for its actual predatory function among viscid thread orb weavers and, finally, transformed into a specialized adaptation within spiders that lost the orb web pattern. Nevertheless, this evolutionary hypothesis can be questioned on the basis that the occurrence of reeling in each taxon, and thus the scoring of this character on the cladistic matrix, could be a function of the hunger level of each spider tested. **Objectives and Materials:** To test for the effects of hunger on reeling, we used spiders of the species *Zosis geniculata* in the laboratory. Reeling was measured both in sated and hungry spiders, and we observed that hunger level did not influence this behavior, which occurred equally in both conditions. We expected hungry spiders to run towards the prey and not to reel the prey from the hub. Nevertheless, it seems that reeling is advantageous because in so doing, the spider remains in the hub all the time, checking the web for eventual predators or further prey. **Results and Discussion:** These are still preliminary results, but if indeed hunger does not influence the occurrence of reeling, we can trust the cladistic data matrix used to infer the evolution of this behavior. Thus, the present study lends support to the hypothesis that reeling has followed an evolutionary path from exaptation to cooptation and finally adaptation.
Supported by: CNPq/PIBIC

9.06 Proteomic analysis of *Bothrops* venoms.

Kitano ES, Paes Leme AF, Camargo ACM, Serrano SMT
Laboratório Especial de Toxinologia Aplicada-CAT-CEPID, Instituto Butantan–SP, Brasil.

Introduction: Snake venoms are complex mixtures of components, which have a diverse mode of action both on prey and human victims. The knowledge of snake venom proteomic composition and its variability is important both in basic research and in management of snakebite. **Objective:** To study the venom complexity by comparison of venom from eight species of *Bothrops* genus (*jararaca, cotiara, moojeni, jararacussu, bilineatus, erythromelas, insularis, neuwiedi*) in which the pharmacology, biochemistry, and mechanism of action of many of the toxins have been studied. **Methodology:** Venoms were examined by two-dimensional electrophoresis (2-DE) to analyze similarities and differences. The venoms were submitted to the first dimension isoelectric focusing on pH 3-10 strips followed by electrophoresis on 12% SDS-polyacrylamide gels and Coomassie blue staining. **Results:** The visual inspection of the gels indicated that there are notable differences between the observed 2-DE profiles of these eight *Bothrops* species. All venoms had protein dispersed across the p*I* and molecular mass range of the gels. The venom gel images showed well-stained spots at molecular mass between 50 kDa and 15 kDa and p*I* values ranging from 4 to 10. Using specific antibodies in Western blot analyses of 2-DE of the venoms we have examined subpopulations of proteins in these venoms including the serine proteinases, metalloproteinases and phospholipases A2. **Discussion:** These approaches have given rise to a more thorough understanding of the complexity of these venoms and provide insights to those who wish to focus on these venom subpopulations of proteins in future studies.
Supported by: CNPq/PIBIC and FAPESP.

9.07 Analysis of the fibrin(ogen)olytic activity of *Bothrops* protease A (BPA), a serine proteinase from the venom of *Bothrops jararaca.*

Bertholim L[1], Prezoto BC[2], Paes Leme AF[1], Yamashiro ET[1], Camargo ACM[1], Serrano SMT[1]
[1] Lab. Especial de Toxinologia Aplicada – CAT-CEPID, [2] Lab. de Farmacologia, Instituto Butantan.

Introduction*: Bothrops* protease A (BPA) is a serine proteinase isolated from *B. jararaca* venom. It is a heat-resistant, highly-glycosylated protein that migrates on SDS-PAGE as a single band of 67 kDa. Approximately 62% of BPA mol. mass assessed by SDS-PAGE is due to carbohydrate moieties. **Objective:** To analyze the fibrin(ogen)olytic activity of BPA and the role of its carbohydrate moiety. **Methodology**: Human fibrinogen and fibrin were incubated with BPA at various time intervals and the hydrolysis was analyzed by SDS-PAGE. Human plasma depleted of albumin was incubated with BPA for 2h followed by analysis by 2D-PAGE. **Results and Discussion**: Here we show that BPA is a potent fibrin(ogen)olytic enzyme. BPA rapidly degraded alpha and beta fibrinogen chains while the gamma chain was resistant to hydrolysis. On fibrin, BPA hydrolyzed the alpha chain, leaving the beta and gamma chains apparently untouched. Partial *N*-deglycosylation of BPA did not affect its ability to degrade these proteins. Incubation of human plasma depleted of albumin with BPA showed various changes in the protein profile visualized by 2D-PAGE indicating that plasma proteins were degraded by BPA. BPA also prevented thrombus formation in rats caused by both stasis in the vena cava and endothelial injury in the jugular vein. Taken together, these results suggest that BPA is a highly stable fibrin(ogen)olytic serine peptidase with potential therapeutical application.
Supported by: FAPESP and CNPq/PIBIC

9.08 Study of bioactive peptides isolated from *Bothrops jararaca* venom

Salomão, DF[1], Nascimento, EB [1], Borgheresi, RAMB[1].
[1]Laboratory of Pharmacology, Butantan Institute, Brazil

Introduction: Many biologically active components of venoms snakes are peptides (P), enzymatic and non-enzymatic proteins. Some active low-molecular-mass P components are well known, like BPPs from *Bothrops jararaca* (Bj), hypotensin from *C. atrox* and sarafotoxins (SRTXs) isolated only from venom of genus Atractaspis. **Objective:** Our purpose is the isolation of active P from the venom of the Brazilian snake Bj, in attempt those similar to SRTXs. **Methods:** Amounts of crude Bj venom were solubilized in water, 0,1M and 0,5M of acetic acid and semi purified by two filtration techniques (Sephadex G25 and Centriprep-YM3). A280nm monitored the filtrate fractions. The activity of each fraction or pooled fractions after lyophilization and adequate solubilization was detected on isolated rat aorta (IAR) or on arterial blood pressure (ABP). Active fractions were pooled to further purify by HPLC, using semi-preparative followed by analytical columns (both RP-C18). **Results:** The third pool obtained on Sephadex G-25 and the filtrate by centriprep YM3 that contain P, presented biphasic response on IAR (relaxant followed by contractile) and on ABP (hypotensive followed by hypertensive). Although the best protein yields were achieved with 0,5 M acetic acid solubilized amounts, their tamponated (pH=7.0) fractions cause alterations on baseline of the IAR and of the ABP assays. **Discussion:** We selected the pooled active fractions obtained from Sephadex G-25 (0,1 M acetic acid) to further purify by HPLC, because their buffered fractions have no effect on basal line of the used assays.
Supported by: FAPESP, FAPESP fellowship, CNPq/PIBIC, FUNDAP and Butantan Foundation

9.09 Partial Purification and Characterization of *Bothrops jararaca* (*Bj*) fibrinogen

Vieira CO, Fugiwara CY, Tanaka-Azevedo AM[1]
[1]Laboratório de Fisiopatologia, Instituto Butantan, São Paulo, Brasil.

Introduction: Fibrinogen is a plasma glycoprotein that acts in the end of coagulation cascade. This protein consists in two halves with two pairs of three no identical polypeptide chains Aα, Bβ and γ, interlinked by disulfide bonds. Thrombin releases fibrinopeptides A and B from Aα and Bβ fibrinogen chains, respectively, which results in the fibrinogen conversion to insoluble fibrin. This work presents the partial purification and characterization of *Bothrops jararaca (Bj)* plasma fibrinogen. **Material and methods:** The purification of *Bj* fibrinogen was done by ammonium sulphate precipitation, followed by gel filtration chromatography (Sephacryl S300 HR26/60). The protein concentration was determined by absorbance at 280 nm. Fibrinogen measurement was made according to Ratnoff & Menzie method (1951). **Results and discussion:** The fibrinogen purification and recovery were 9.3 fold and 22.3%, respectively. The molecular masses of fibrinogen chains were 115, 53 and 44 kDa, for Aα, Bβ and γ, respectively, by SDS-PAGE. The molecular mass of *Bj* fibrinogen in non reduced condition was about 428 kDa, this protein is bigger than bovine and human fibrinogen (340 kDa). Ratnoff OD & Menzie C [**J Lab Clin Med 37:** 316, 1951].
Supported by: CNPq/PIBIC and FAPESP (04/02224-4)

9.10 *Bothrops* venom induce autologous complement activation

Alves-Pessoa LR[1], Furtado MFD[2], Tambourgi DV[1],
[1]Laboratório de Imunoquímica, [2]Laboratório de Herpetologia, Instituto Butantan, São Paulo, Brazil.

Introduction: Bites by *Bothrops* snakes can induce severe clinical symptoms, including dermonecrosis, thrombosis, haemolysis and persistent inflammation. **Objectives**: In the present study we have investigated the action of venoms from 19 species of *Bothrops* genus on the complement system (C) and its regulators. **Methods**: Human erythrocytes (E) were incubated with venoms from 19 species of *Bothrops* snakes for 30 min at 37°C. Control samples were incubated with veronal buffer saline (VBS^{++}). The cells were washed, resuspended to the original volume in VBS^{2+}, analysed in a haemolysis assay and prepared for flow cytometry. **Results**: From the 19 *Bothrops* species tested only the venom from *B. pirajai* was able to render E susceptible to lysis by autologous C. To assess whether the increased susceptibility to human C was caused by interference of the venom toxins with membrane regulators of C, E were analysed for the expression of DAF, CR1, and CD59 by flow cytometry. No change in expression of any of the regulators was observed after incubation of E with the *B. pirajai* venom. Although DAF, CR1, and CD59 are powerful inhibitors of C, the E-membrane proteins known as glycophorins also contribute substantially to C resistance. E, incubated with *B. pirajai* venom were analysed for the expression of GPA and GPC by flow cytometry. A large reduction in the binding of anti-GPC antibodies was observed after treatment of E with venom. Although the venom of *B. billineatus* was able to induce a large reduction in the binding of anti-DAF and anti-CD59 antibodies, no increase in C-susceptibility was observed in these E samples. **Conclusion**: *B. pirajai* venom promotes autologous C activation on human erythrocytes by inducing removal of GPC from the cell surface.
Supported by: CNPq/PIBIC and FAPESP

9.11 Inhibitory effect of the crotoxin isolated from *Crotalus durissus terrificus* venom on the anti-human serum albumin antibody response when administered after immunization.

Ricardi R[1], Favoretto BC[1], Silva SR[1], Della-Casa MS[1], Baldo C[1], Jacysyn JF[2], Amarante Mendes JG[2], Faquim-Mauro EL[1].
[1]Lab. de Imunopatologia, Instituto Butantan, SP, [2]Lab. de Biologia Celular e Molecular, Instituto de Ciências Biomédicas, USP, SP, Brasil.

Introduction: Crotoxin isolated from *Crotalus durissus terrificus* venom (CdtV) has a suppressive effect on the humoral immune response against unrelated antigens when injected before the antigen immunization. **Objective:** To evaluate the effect of the crotoxin on the human serum albumin (HSA) antibody response when administrated after the immunization. **Methodology:** Male BALB/c mice were immunized s.c. with 100 μg/animal of HSA adsorbed in 1 mg/animal of $Al(OH)_3$ gel adjuvant, the crotoxin (5 μg) was injected (s.c.) 1 hour before or 24 hours after immunization. After 14 days of immunization the mice were bled, received the antigenic booster (100 μg/animal of HSA in PBS) and were bled again after 7 days. **Results:** The evaluation of anti-HSA IgG1 and IgG2a antibodies by ELISA showed that the crotoxin was able to suppress the primary and secondary antibody response even when administered after immunization. The analysis of the cells viability obtained at 6, 24, 48 or 72 h from mice immunized with HSA or HSA-immunized and injected with crotoxin showed that the inhibitory effect of the crotoxin is not mediated by the induction of cell death. **Discussion:** The crotoxin isolated from CdtV has a potent suppressive effect on humoral immune response when administered after the immunization by a mechanism independent of cellular death.
Supported by: CNPq/PIBIC

9.12 Pro-inflammatory cytokines released by Human Vascular Endothelial Cells (H UVEC) treated by Jararhagin.

Ramos-da-Silva BS, Tanjoni I, Baldo C, Della-Casa MS, Moura-da-Silva AM, Clissa PB
Laboratory Immunopathology – Butantan Institute – São Paulo

Introduction: Jararhagin is a multidomain SVMP from *Bothrops jararaca* venom comprising catalytic, disintegrin-like and cysteine-rich domains, which cause a local reaction manifested by acute inflammatory response. **Objectives:** In this study we analysed the toxic effect of jararhagin on cell cutures and the effect of jararhagin on cell adherence. The profile of pro-inflammatory cytokines and the receptors for these cytokines, produced by HUVECs treated with jararhagin was also analysed. **Results:** The exposure of HUVEC to jararhagin resulted in decrease of cell viability followed by loss of cell adhesivity. The jararhagin was incubated in the cell culture medium in the dose of 5 and 10 μg/ml with the cells, during 1 hour, after this period the medium was removed, and a new medium was replaced. We assayed the supernatant by ELISA to detect the following cytokines: IL-1α, IL-6 and TNF-α and the following cytokines receptors: IL-6sR and sTNF-R1. The antagonist of IL-1α, IL-1Ra was also assayed. All the experiments were carried out with a positive (LPS - 1μg/ml) and a negative control (PBS). Our results show that jararhagin is activating cells to produce detectable levels of IL-6 and sTNF-R1, however all the other cytokines and receptors were not detectable. **Conclusion:** Our results suggest that jararhagin is not toxic to HUVECs and is able to induce the release of IL-6 and sTNF-R1 in the cell culture.
Supported by: CNPq/PIBIC

9.13 Histopathological study of venom glands of *Crotalus durissus terrificus* with defective venom production or secretion

Camargo GG[1*], Giannotti KC [1], Grego K[2], Sant' Anna SS[2], Carneiro SM[1].
[1]Laboratório de Biologia Celular, [2]Laboratório de Herpetologia, Instituto Butantan, São Paulo.

Introduction: Venomous snakes are kept in captivity in the Laboratory of Herpetology, with a range of venom extraction used in snake antivenom production and for research purposes. The animals are milked once a month but sometimes certain snakes fail to produce venom or present a hemorrhagic or purulent secretion, even after appropriate treatment. **Objective:** a histopathological study of the venom glands of 5 *C.d. terrificus* with defective venom production or secretion was done to analyze possible histological alterations and causes of impaired venom production. **Methodology:** Specimens were decapitated after preliminary sedation with carbonic gas. Venom glands were excised, fixed and embedded in paraffin. Sections were stained with H.E. or Mallory trichrome. Histochemical reactions were PAS, Alcian Blue and Bromophenol Blue. **Results:** Most of the secretory tubules presented a disorganized structure, with obstruction of the tubular lumina by proliferating transformed hyperplasic epithelial or basal cells; intense heterophilic infiltration was observed in two of the glands; multinucleated cells in the tubular epithelia and mononuclear cell infiltration in the connective tissue occurred in other glands. **Discussion:** Two possible causes of the pathological changes were considered: ascendent bacteria via oral infections and leakage of the venom into the secretory parenchyma while pressing the glands. Extended studies electron microscopy are in progress.
Supported by: CNPq /PIBIC scholarship

9.14 Phospholipases A2 (PLA2) isolated from Brazilian coral snake (*Micrurus lemniscatus)*: behavioral, eletroncephalographic and histopathological effects

Oliveira DA[1], Casais e Silva LL[3], Lebrun I[2], Sandoval MRL[1]
[1]Laboratory of Pharmacology and [2]Laboratory of Biochemistry and Biophysics, Butantan Institute; [3]Laboratory of Animais Peçonhentos, Federal University of Bahia, Brazil.

Introduction: Brazilian snakes of the genus *Micrurus* (coral snakes, family Elapidae) induce severe envenomation in humans, characterized by neurotoxic effects of rapid evolution which may be fatal. Some toxic components have been isolated from coral snake venoms as PLA2 pre- and postsynaptic neurotoxins but their central effects have been poorly investigated. The aim of this work is to investigate behavioral, EEG and histological effects of two PLA2s, MI-8 and ML-9, isolated from the venom of *Micrurus lemniscatus*. **Method**: Cannula and electrodes were implanted in the CA1 hippocampus (Hpc) 4 days prior the MI-8, MI-9 or phosphate buffer injection into the Hpc. EEG and behaviour were recorded during 8 hours. Seven days after, the brains were processed for histological analyses PLA2 activity was previously determined. **Results:** MI-8 (2.1μg/μl), MI-9 (2.4 ug/ul) induced circling behavior, jumping, intense scratching, forelimb clonus and the EEG record showed spikes and epileptiform discharges. Lesions on CA1 region of the Hpc were also observed. **Conclusion:** MI-8 and MI-9 caused behavioral and EEG seizures and hippocampal neuronal death. The toxicity might be associated with the binding of these toxins with specific brain site (Lambeau et al, *J Biol Chem* 1989, 264, 11503) and/or a β-BuTx –like effect at the pre-synaptic terminal. These PLA2 can be a useful tool for exploring the death- signaling pathways of neurotoxicity.
Supported by: CNPq/PIBIC , FAPESP and Butantan Foundation

9.15 Study of anticonvulsants on the seizures and neuronal injury induced by TSII toxin isolated from *Tityus serrulatus* scorpion venom

Oliveira DA[1], Lebrun I[2], Sandoval MRL[1]
[1] Lab. of Pharmacology; [2] Laboratory of Biochemistry and Biophysic - Butantan Institute SP, Brazil.

Introduction: TsII, a β-toxin bind to receptors of Na+ channels, induces seizures and hippocampal lesions. In the current study we studied anticonvulsants on the neurotoxicity induced by TsII. **Methods:** Four days after cannula and electrodes implanted in the CA1 hippocampus (Hpc), rats were injected i.p. with Diazepam, Dz 7,5 mg/Kg (n=6), Phenobarbitone, Pb 12, 25, 50 or 60mg/Kg, Carbamazepine, Cz 50 mg/Kg or saline 15, 30 or 60 min before TsII 2ug/ul injection into the Hpc, respectively. EEG and animal behavior were recorded four 8 hours. After 7 days, the brains were processed for morphological analyses. **Results:** DZ pre-treatment prevented the behavioral and EEG seizures but it was not able to inhibit the hippocampal injury. Pb 12.0 and 25.0 mg/Kg were not able to block behavioral and EEG seizures (spikes and epileptiform discharges) induced by TsII. Pb 50.0 and 60.0 mg/kg inhibited the behavioral and EEG seizures. All doses assayed were not able to prevent the neuronal damage. Cz 50.0mg/Kg did not blocked behavioral and EEG seizures and neuronal loss. **Discussion**: Cz acts decreasing sodium conductance and Pb and Dz act increasing GABA levels in the brain may be interfering with the neuronal ability to generation and spread convulsions. Previously we showed that TsII increased the hippocampal glutamate levels. It is know that an increase in Glutamate release induces excitotoxicity. The partial blockage of neuronal lesions and seizures could be due to the involvement of others neurotransmitters, such as glutamate in TsII effects.
Supported by: FAPESP; and CNPq/PIBIC and Butantan Foundation

9.16 Characterization of the amylase activity from *Tityus serrulatus.*

Filietaz CFT[1], Bertani R[2], Lopes AR[1]
[1]Laboratório de Bioquímica e Biofísica and [2]Laboratório de Imunoquímica, Instituto Butantan

Introduction: Insects, the principal scorpion´s prey, present high concentrations of glycogen as energy storage. **Objectives:** Thus, it is expected that scorpion present high α-amylase (EC 3.2.1.1) activity in order to use glycogen as a source of sugar in their diet. **Methods and Results:** An antibody developed against *Tenebio molitor* digestive α-amylase was used in dot-blooting assays indicating the presence of α-amylase in the hepatopancreas (Hp) from *Tityus serrulatus*. *Tityus serrulatus* adult female were dissected and their Hp were isolated and homogenized in cold water. Hp homogenate presented an activity of 123 U/Hp using starch as substrate and a specific activity of 1,5 U/mg. Chromatographic separation from Hp homogenate using an anionic exchange column, HitrapQ (GE-Healthcare Bio-Sciences), indicated the presence of at least two α-amylase activities. **Conclusions:** Properties of these different amylase activities, as molecular mass, optimum pH, thermal inactivation and substrate specificity (Km, Vmax and chloride activation) will be determined, as well as, their isolations.
Supported by: CNPq/PIBIC and FAPESP

9.17 Effects of the venom of *Tityus bahiensis* on the neuronal integrity of the offspring from of dames treated on the prenatal period.

Petrini ASQ, Dorce VAC, Nencioni ALA
Pharmacology Laboratory, Butantan Institute, São Paulo - Brazil.

Introduction: The venom of *Tityus bahiensis* is known for its high toxicity, but there are no studies about its effects on the offspring of dames which received it experimentally or accidentally. **Objective**: To check the effects on the central neuronal integrity on the offspring of rats that received the venom during pregnancy. **Methodology**: Pregnant dames were separated into 2 groups: control (C) and experimental, and were injected with saline or with the venom 2.5 mg/kg on the 10^{th} day (E10). The adult pup rats were anesthetized with CO_2 and perfused. The brains were taken out, dissected, blocked, cut and afterwards colored with cresyl violet. A counting of the intact cellular bodies was made on CA1, CA3 and CA4 hippocampal areas. **Results**: On C group, six animals were evaluated, none presented total lesion, one animal had partial lesion on CA4 on the right side and the five animals left were normal. On E10 group, seven animals were evaluated, four of them suffered total lesion, one had a lesion on CA3 and CA4 on the right side, other suffered a lesion on CA3 and CA4 on the left side, other suffered a lesion on CA4 on the right side and another one suffered a lesion on CA1, CA3 and CA4 on the left side, none of the animals had partial lesions, and the three animals left were normals. **Discussion**: A moderate envenomation causes hippocampal lesion on the offspring of rats, although it does not happen in all the animals, what is compatible with the effect "All or Nothing" described to the venom.
Supported by: CNPq/PIBIC and Butantan Foundation.

9.18 Purification and characterization of proteases and hyaluronidases of *Loxosceles gaucho* venom.

Rangel DM, Lira MS and Barbaro KC
Laboratory of Imunopathology, Butantan Institute, São Paulo, Brazil

Introduction: *Loxosceles sp.* spiders are described as the most medically important species in Brazil. Loxoscelism is characterized by a dermonecrotic lesion at the site of bite, sometimes evolving to a systemic reaction that may be severe or even fatal in some patients. **Objective:** The aim of this study was to purify and characterize proteases and hyaluronidases from *Loxosceles gaucho* venom. **Methodology:** After chromatografy in mono S HR 5/5 column, 22 fractions were obtained and assayed for protease and hyaluronidase activities. To perform the enzymatic assay, casein (2 mg/ml), gelatin (2 mg/ml) or hyaluronic acid (170 µg/ml) were incorporated as substrates in a SDS-PAGE (12%). **Results:** By zymography, a large array of proteases and a considerable number of hyaluronidases were shown in *L. gaucho* venom. Considering caseinolityc activity, the vast majority of protein bands were located between 80-19 kDa. Gelatinolytic activity showed major protein bands around 50-19 kDa. Five fractions of hyaluronidase activity were observed next to the region of 45 kDa indicating the presence of more than one component with this activity. **Discussion:** Our results showed that *L. gaucho* venom possesses many components with enzymatic activity. Since these enzymes can damage extracellular matrix, they likely participate in the pathogenesis of *Loxosceles* envenomation.
Supported by: CNPq/PIBIC and FAPESP

9.19 Protein digestion in *Nephilengys cruentata* (Araneae).

Bastos AF[1], Genta FA[2], Ferreira C[2], Terra WR[2], Lopes AR[1]
[1]Lab. Bioquímica e Biofísica, Instituto Butantan, [2]Depto. Bioquímica, IQ, USP, São Paulo, SP.

Introduction: Comparison of proteolytic enzymes between different insect orders had provided information about specificity and evolution. A comparison of evolutionary trends of Arthropoda peptidases is not possible due to the lack of data mainly for Arachnida enzymes. **Objectives:** In order to understand protein digestion in spiders the digestive juice (DJ) and the hepatopancreas (HP) from *N.cruentata* (giant spider) were characterized. **Methodology and results:** The following enzymes were measured in both samples, HP and DJ, respectively: aminopeptidase (LpNa; 2,4 mU/mg; 3,0 mU/mg), dipeptidase (Gly-Leu, 1,4 mU/mg; 2,0 mU/mg); carboxypeptidase (Z-GlyPhe 3,0mU/mg; 0,3 mU/mg), endopeptidase (Carbobenzoxy-PheArg-7-amido-4-methyl-coumarin 11U/mg; 6,7 U/mg). Cysteine-proteinase has an optimum pH of 5.9. Inhibition assays indicated that the major endopeptidase activity is a cysteine proteinase in both samples (60% of inhibition by E-64 HP and 100% of inhibition on DJ. PMSF had no effect on this activity). Western and dot blotting experiments also indicated the presence of cysteine proteinase in *N. cruentata*. Both samples were submitted to gel filtration and the cysteine proteinase activity from both DJ and HP presented the same molecular mass (57kDa, determined by gel filtration). Western and dot blotting experiments confirmed the presence of aminopeptidase, which presented optimum pH of 7.3 (HP) and 7.9 (DJ). **Discussion:** Ion-exchange chromatographies indicated that carboxypeptidase and dipeptidase do not differ between both samples, suggesting that the hepatopancreas is the site of their secretion. On the other hand, cysteine proteinase activity presented one activity in digestive juice and at least two activities in hepatopancreas.
Supported by: CNPq/PIBIC and FAPESP

9.20 Electrophysilogical recordings from the frog neuromuscular junction and its use in toxinology.

Ujikawa GY[1], Kini RM[2], Troncone LPR[1]
[1]Lab. Farmacologia, Instituto Butantan, SP, Brasil; [2]Dep. Biol. Sci. Nat. University of Singapore.

Introduction: The neuromuscular junction electrophysiology (NMJ) has been employed to study chemical neurotransmission and neuroactive drugs and toxins that interfere with neuromuscular transmission. **Objective:** We intended to implement the method of electrophysiological recordings employing NMJ of the frog *Rana catesbeiana* and by this mean evaluate the actions of three purified toxins from the venom of the snake *Bungarus candidus*. **Methods:** The bungatoxins were named after their molecular weight: 7442, 7207 and 7275. Parameters to be evaluated were the amplitude of the end plate potential (EPP) obtained under low calcium conditions and electrical stimulation of the nerve and observing miniature end-plate potentials (MEPP). **Results:** As a control we employed the calcium channel blocker PF3, a toxin obtained from the spider *P. nigriventer* venom and observed the typical reversible decrease in EPP amplitude without changes to MEPP. Bungarus toxins 7442 and 7207 were devoid of any effects in this preparation but toxin 7275 showed a progressive decrease in EPP amplitude and also on MEPP amplitude. Washing the muscle with low calcium Ringer did not reverse the effect but normal calcium Ringer partially reversed the effect of this toxin. **Discussion:** The electrophysiological recordings of frog NMJ were performed successfully. Both EPP and MEPP signals were quantified appropriately and allowed for the study of bungatoxins. Toxins 7442 and 7207 were without effect but bungatoxin 7275 performed a post-synaptic blockade resembling the alpha-bungarotoxin isolated from the venom of *B. multicinctus*.
Supported by: CNPq/PIBIC and FAPESP

9.21 Characterization of the effects of catfish *Pseudoplatystoma fasciatum* venom in the microcirculation and muscle fibers: an intravital microscopic study

Erlichman C[1], Conceição K[2], Pimenta DC[2], Lopes-Ferreira M[1]
[1]Lab. Imunopatologia, [2]LETA-CAT/CEPID, Instituto Butantan, São Paulo.

Introduction: One of the most abundant venomous species of catfish found in the North of Brazil is *Pseudoplatystoma fasciatum* (surubim). The accidents are characterized by local effects as intense pain and edema. **Objective:** The aim of this study was characterize the effects of the venom on the microcirculatory net and on muscle fibers, using intravital microscopy. In addition, eletrophoretical and cromatography profile of the venom was determined. **Methodology**: The cremaster muscle of Swiss mice was displayed and different doses of the venom (5, 15, 30, and 60 µg) were topically applied. Control experiments were performed by applying 30 µl PBS under otherwise identical conditions. Five minutes of observation were recorded before application of the venom to analyze the dynamics in control tissue. Experiments were carried out for up to 30 min. **Results**: The higher dose of venom (60 µg) induced hypercontraction in muscle fibers, a significant increase of leukocytes rolling, and a blood clot formation in venules. After application of 30 µg the same alterations were observed but in later periods. Using 15 µg it was observed only hypercontraction in fibers. No alterations were seen after 5 µg application. The electrophoretic profile (SDS-PAGE) showed intense bands at 18, 25, and 66 kDa. Subsequently it was carried out chromatographic profile using HPLC that showed 3 hidrofilic peaks and 2 hidrofobic peaks. **Discussion:** These data suggest that the *P. fasciatum* venom induce an important inflammatory activity characterized by alterations in the microcirculation and muscle fibers, and these modifications could be related with toxins present in the venom.
Supported by: CNPq/PIBIC and FAPESP

9.22 Evaluation of delayed hyperalgesia bt crotalphine, an opioid analgesic obtained from *Crotalus durissus terrificus* snake venom

Pereira LM[1], Picolo G [1], Brigatte P [1], Cury Y[1]
[1]Laboratory of Pathophysiology, Butantan Institute, SP, Brazil

Introduction: Crotalphine (CRP), a synthesized analgesic peptide based on the natural analgesic obtained from CdtV, induces potent and long-lasting antinociception mediated by κ and δ opioid receptors. A side-effect induced by opioid treatment is the development of delayed hyperalgesia. **Objective:** To investigate the possible development of hyperalgesia after treatment with CRP. **Methods:** Pain threshold was assessed using the rat paw pressure test before and at different times after treatments. CRP (p.o.) or morphine (MOR, s.c.) were administered in subanalgesic (CRP=8 pg/kg or MOR=1 µg/kg) or analgesic (CRP=20 ng or 10 µg/kg; MOR=1 or 5 mg/kg) doses. Naloxone (NAL, 1 mg/kg, s.c.) was used to evaluate the involvement of opioid receptors in both the analgesic and hyperalgesic effects. U50488 (4,-.5; 9 to 18 µmol/kg, s.c.) and DPDPE (250, 500 e 1500 ug/kg, s.c.) were used to investigate the hyperalgesia induced by other opioid drugs. **Results:** Antinociception was observed 3 and 5 h after CRP (20 ng and 10 µg/kg) or 1 h after MOR administration. Hyperalgesia was detected 96-144 h after MOR (5 mg/kg) treatment. NAL blocked both the analgesic and hyperalgesic effects induced by MOR. Delayed hyperalgesia was not observed after U50488 or DPDPE treatments. **Conclusions:** CRP is a powerful analgesic substance that does not induce delayed hyperalgesia, thus being an important candidate for a new opioid drug for pain therapy.
Supported by: CNPq/PIBIC, COINFAR Pesquisa e Desenvolvimento

9.23 Detection of virulence genes in atypical EPEC strains

Porangaba TM[1,2], Franzolin MR[1] and Sircili MP[1]
[1]Laboratório de Bacteriologia, Instituto Butantan, São Paulo, Brasil; [2]Ciências Biológicas Modalidade Médica, 4º ano, Universidade Estadual Paulista - Júlio de Mesquita Filho.

Introduction: The term atypical enteropathogenic *Escherichia coli* (EPEC) was created in 1995 to define diarrheagenic *E. coli* strains witch differs from typical EPEC by the absence of EPEC adherence factor (EAF) plasmid ,and from enterohemorrhagic *E. coli* (EHEC) by not producing the Stx toxin. Until this moment it was not described any exclusive virulence factor to atypical EPEC strains, on the opposite, every one of them that was found has been common to others pathotypes, even among the distant ones. **Objective and Methodology**: In this study, it was made two multiplex PCR reactions to detect *efa1/lifA*, *pic*, *pet*, *astA,* and *hly* genes among 89 strains atypical EPEC isolated from patients of Salvador (BA) and 25 strains of the Bacteriology's laboratory culture collection, Butantan Institute. **Results**: More than 50% of the studied strains showed positive results for at least one of the five genes studied. The *astA* gene was the most detected and *hly* gene showed the lower frequency among the studied strains. **Discussion**: Based on the results we can say that some strains of atypical EPEC have virulence factors common to others diarrheagenic *E. coli* pathotypes confirming the theory that atypical EPEC are part of a quite heterogeneous group.
Supported by: CNPq/PIBIC and FAPESP

9.24 Adherence of different atypical enteropathogenic *E. coli* serotypes to intestinal epithelial cells.

Zarpelão E, Soares ES, Góes AC, Ruiz RC
Lab. Bacteriologia, Institute Butantan, São Paulo, Brazil

Introduction The mechanism of EPEC pathogenesis is a lesion A/E, which is characterized by microvilli destruction, adherence of bacteria to the intestinal epithelial, pedestal formation, and aggregation of actin at sites of bacterial attachment. Typical EPEC (TE) produce a characteristic adherence pattern called localized adherence (LA).This phenomenon is associated with the presence of EAF plasmid, but atypical EPEC (AE) does not posses this plasmid and may show the localized like adherence pattern (LAL), diffuse adherence (DA), aggregative adherence (AA), or no adherence (NA).These patterns were always defined in HEp-2 cells, from larynx **Objective** To verify if the adhesion patterns of different AE serotypes, defined in HEp-2, had their adhesion pattern altered intestinal cells **Methodology** The Caco-2 and HEp-2 cells adhesion assay was performed with AE: LAL 2103 (O26:H11), 4147(O55:H7); 320(O55:H7), 3137(O119:H2), 289 (O119:H15); AA 92(O2:H16), 268(O49:H6); DA 84(O86:H11), 895 (O153:H19),LA 3970(O55:H-), 558(O111:H40), 2791 (O119:H19),3414 (ONT:H6) or NA 251(O11:H10); 4013(O88:H-). **Results** The adhesion phenotype observed in the Caco-2 was the same as in the control group **Discussion** Up to this time there has been very few studies on the different AE adhesion patterns and none on the absence of adhesion in some of the serotypes. Our results show that, even in polarized intestinal cells, the natural ambient for the induction of diarrhea, the patterns previously described in HEp-2 are maintained, suggesting that the use of adhesins specific to these patterns present a conserved mechanism.
Supported by: FAPESP, FUNDAP and CNPq

9.25 Atypical enteropathogenic *Escherichia coli* - macrophages interaction

Melo, KCM, Ruiz, RC
Lab. Bacteriologia, Instituto Butantan, São Paulo, Brazil

Introduction: Macrophages can be viewed as either an opportunity or an obstacle for microbial pathogens. Several virulent bacteria have evolved mechanisms to avoid and prevent phagocytosis. Typical EPEC interaction with phagocytic cells inhibits its uptake. This requires a functional type III secretion system, including components of the translocation machinery EspA, EspB and EspD and occurs via the inhibition of PI 3 kinase dependent F actin rearrangements, but is Tir-independent. **Objective:** To study the interaction of atypical EPEC with macrophages, because Esp B and Esp D molecules and intimin can be different when compared to typical EPEC. **Methodology:** macrophages interaction assays were performed with atypical EPEC: 7(O55:H7); 1(O125ac:H6); 268(O49:H6); 320(O55:H7); 84(O86:H11); 251(O11:H10) and typical EPEC control 28(O55:H6) for 10, 30, 60 min infection pulses. The cultures were treated with gentamicin and the number of intracellular bacteria was determined. **Results:** Sample 7 was less phagocyted after 10 and 30 min pulses, with 6,9% and 16% of infection, respectively, while the 268 sample infected 40,6% and 60,3%, respectively the macrophages, at the same times. These results were similar to the others, including the control sample. **Discussion:** Our results suggest that sample 7 may present a different anti-phagocytosis mechanism, since in typical EPEC, the antiphagocytic capacity is triggered lately, 2h after phagocytosis, with the bacteria necessarily adhered. This strategy might delay the immune response, contributing to the intestinal colonization.

Supported by: CNPq/PIBIC and FAPESP

9.26 Detection of LT-I and ST-I toxins produced by enterotoxigenic *Escherichia coli* (ETEC)

Imamura SY[1], Menezes C A[1], Menezes MA[1], Beutin L[2], Elias W P[1], Piazza RMF[1]
[1]Lab. of Bacteriology, Instituto Butantan, São Paulo, SP, Brazil, [2]National Reference Laboratory for *E. coli*, Federal Institute for Risk Assessment (BfR), Berlin, Germany

Introduction: ST and LT toxins are the main virulence factors of ETEC strains, which can express ST, LT, or both. These toxins are different in structure and pathophysiology. LT is an 86 kDa AB_5 toxin and ST is a small peptide of 2 kDa. Since the characterization of ETEC is based on detection of both enterotoxins, monoclonal antibodies against these toxins have been raised in our laboratory. **Objectives:** In this study two monoclonal antibodies were characterized and their uses in diagnosis were evaluated. **Methods and Results:** After purification an IgG2b anti-LT recognized by immunoblotting both subunits of the toxin with a dissociation constant of 2.2×10^{-8} M. The IgG1 anti-ST-I recognized the peptide with a dissociation constant of 1×10^{-10} M. Detection of both toxins in diarrheagenic *E. coli* by immuno-dot showed a sensitivity of 60% and 77% of specificity when the IgG1 anti-ST-I was employed. On the other hand, a sensitivity of 100% and 76% of specificity was observed when the IgG2b anti-LT was used. **Conclusions:** Due to the variability in sensitivity and specificity showed by both monoclonal antibodies in detecting LT-I and ST-I toxins in ETEC isolates an improvement of these assays is necessary. Therefore, the cloning of the ScFv encoding regions of these hybridoms in an expressing vector are under progress for the *in vitro* production of highly specific monoclonal antibodies against ST-I and LT-I.

Supported by: FAPESP and FINEP - SYI is CNPq/PIBIC fellow

9.27 Detection of seric antibodies against SA-11 rotavirus (serotype G3) in healthy Brazilian adults

Ferreira, TL, Macedo SMR, Carbonare SB, Tino De Franco, M.
Laboratório de Imunogenética, Instituto Butantan, São Paulo, SP.

Introduction: Rotavirus is the leading etiologic agent of infectious gastroenteritis in infants and young children, but less is known about its role in adult gastroenteritis. Some studies have correlated seric antibodies against rotavirus in adults with protection and the probability of infection and illness. **Objective:** Detection of serum IgA and IgG anti-rotavirus in 30 serum adult samples**. Methodology:** The SA-11 rotavirus strain was propagated in MA-104 cells, and rotavirus antigen was obtained by ultracentrifugation. The detection of anti-rotavirus antibodies was performed by ELISA with purified antigen, using rabbit anti-SA-11 serum and a human serum pool as positive controls. The titer was determined as the reciprocal of the dilution that provided and absorbance of 0.5. The final titer is reported in percentage of the pool. **Results:** We expect variable levels of antibody titers indicating fluctuation in the degree of individual exposure to rotavirus**. Discussion:** This study is a starting point for further studies concerning antibody subclasses, cross-reaction between serotypes and their protective role.
Supported by: CNPq/PIBIC and FAPESP

9.28 Effect of crotalic venoms on humoral immune response in mice immunized with human serum albumin emulsified in Complete Freund Adjuvant.

Favoretto BC[1], Ricardi R[1], Silva SR, Jacysyn JF[2], Faquim-Mauro EL[1].
[1]Laboratório de Imunopatologia, Instituto Butantan, São Paulo, [2]Laboratório de Biologia Celular e Molecular, Instituto de Ciências Biomédicas - USP, São Paulo, Brasil.

Introduction: Adjuvants have the ability to stimulate and modulate the immune response to different antigens. In contrast, it was demonstrated that crotalic venoms has suppressive effect on the humoral response to proteic antigen adsorbed in $Al(OH)_3$ gel, when administered before the immunization. **Objective:** The aim of this work was to evaluate the effect of venoms from *Crotalus durissus terrificus* (CdtV), *C. d. collilineatus* (CdcolliV) and *C.d. cascavella* (CdcascV) snakes on the humoral response in mice immunized with human albumin (HSA) emulsified in Complete Freund Adjuvant (CFA). **Methodology:** BALB/c mice were immunized s.c. with 100 µg of HSA in CFA only or also injected s.c. with 5 µg of CdtV, CdcolliV or CdcascaV 1h before or 24 h after the immunization. After 14 days the mice were bled, received 50 µg of HSA and were bled again after 7 days. **Results:** The analysis of anti-HSA antibodies showed that CdtV and CdtcolliV suppressed the primary IgG1 and IgG2a antibody responses and this suppression was more intense when mice were injected before immunization. The CdcascV inhibited the primary response only when injected before immunization. The secondary response was suppressed only in mice that received the CdtV before the immunization. **Discussion:** The crotalic venoms administered before immunization are able to suppress the humoral immune response induced by antigen even in the presence of a potent adjuvant as CFA.
Supported by: CNPq/PIBIC.

9.29 Immuno-identification of a protein in the rat brain using polyclonal antibody anti–precursor protein of snake Bradykinin-Pontentiating Peptides (BPPs)

Campeiro JD, Ramos MLS, Camargo ACM, Assakura MT, and Hayashi MA.
LETA-CAT/CEPID, Instituto Butantan, São Paulo, Brazil.

Introduction: The bradykinin-potentiating peptides (BPPs) are potent natural inhibitors of the angiotensin-converting enzime (ACE). This property was very important to demonstrate both the role of the ACE in blood pressure regulation and for the development of anti-hipertensive drugs, e.g. Captopril. **Objective:** In the present study, we described the expression of the recombinant BPP precursor protein from *Bothrops jararaca* venom gland and its use to produce specific polyclonal antibody able to recognize specifically the BPPs and/or their precursor, aiming the identification endogenous counterpart of BPPs in mammals. **Methodology:** The BPPs precursor protein was obtained by expression in *E. coli* codon plus strain, and was purified using affinity columns. This recombinant protein was used to produce antibodies by immunizations of BALB/c mice. The obtained antiserum was used for Western Blot analysis for detection of protein bands in the cytosol of rat brain and the titers were determined by ELISA. **Results:** A recombinant BPP precursor protein corresponding to a band of about 17 kDa was obtained. The SDS-Page analysis of rat brain cytosol showed a number of protein bands, but just one of them was recognized by the antibody anti-BPPs obtained. **Discussion:** This specific recognition suggests the existence of a homologous protein or an endogenous protein with the same immunological characteristics of the snake BPP precursor protein. Therefore, many efforts are been done to purify and characterize this protein.

Supported by: CNPq/PIBIC and FAPESP (CAT/CEPID)

9.30 Murine antibodies against outer membrane proteins (OMP) from *Neisseria meningitidis* B

Rivera HCB[1*], Gonçalves BI[1*], Santos S[1*], Baruque-Ramos J[2], Gebara VCBC[1], Ferreira VRF[1], Tanizaki MM[1], Schenkman RPF[1]
[1]Centro de Biotecnologia, Instituto Butantan; [2]Escola de Artes, Ciências e Humanidades, USP.

Introduction: Meningococcal disease is one of the most frequent causes of death due to infection in childhood. Mortality resulting from meningococcal septicemia may be as high as 20-50%. Exposure to *Neisseria meningitidis* results in asymptomatic nasopharyngeal carriage. Meningococcus has an outer membrane that contains polysaccharide, lipopolysaccharide and various outer membrane proteins (OMP). Defense against disease caused by *N. meningitidis* involves both innate and acquired immune mechanisms that recognize these bacterial surface structures. Serogroup B surface exposed proteins are important for development of natural immunity. **Objective:** The aim of this study was the production of polyclonal antibodies against OMP´s to be used as tools for further immunological studies. **Methodology:** *N. meningitidis* released outer membrane vesicles (OMV) in culture. OMV were obtained from 14 hours culture in bioreactor followed for centrifugation and ultracentrifugation. OMP were isolated from OMV by SDS-PAGE. Mice were immunized with three surface proteins: NadA a high molecular weight invasin, Tbp1 an iron regulated protein, and PorA a cation porin. Sera were tested by ELISA. **Results:** High antibody titers ($3X10^{-3}$) were observed against NadA followed by anti-PorA ($2X10^{-2}$). Antibodies against Tbp1 were not detected. Protein denaturation allows antibodies to recognize NadA and PorA but not Tbp1.

Supported by: *CNPq/PIBIC and Butantan Foundation

9.31 A murine monoclonal antibody against rabies virus as an important tool in diagnosis.

Aievoli E[1], Silveira CRF[1], Santos NM[1], Fang FLW[2], Faquim-Mauro EL[1], Frazatti-Gallina NM[2], Mourão-Fuches RM[2], Pereira CA[3], Takehara HA[1] and Fernandes I[1].
[1]Lab. Imunopatologia, [2]Seção de Raiva, [3]Lab. Imunologia Viral, Instituto Butantan, São Paulo, Brazil.

Introduction: Rabies is invariably a fatal encephalomyelitis that is considered to be a serious public health problem and diagnosis must be rapid and conclusive. Detection and quantification of anti rabies antibodies is used for assessment of the effectiveness of rabies vaccines. **Objective:** To obtain a monoclonal antibody (MoAb) against rabies virus able to be used in diagnosis and to predict serum antibody titres after vaccination. **Methodology:** Spleen cells from BALB/c mice immunized with rabies antigen cultured in Vero cells (PV/Vero) were fused to SP2-0 for hybridoma production. Screening of hybridomas was realized by ELISA and Immunofluorescence. **Results:** Five MoAb were obtained from which one of IgM isotype (2G10) reacted with Challenge Standard Virus by Immunofluorescence and with rabies glycoprotein from Platelia Kit. **Discussion:** This MoAb can be produced at large scale to ensure sufficient availability and batch-to-batch consistency fundamental to standardize diagnostic procedures and quantification of anti rabies antibodies after vaccination by techniques such as Rapid Fluorescent Focus Inhibition Test.
Supported by: FAPESP, FUNDAP and CNPq/PIBIC.

9.32 Cloning, expression, purification and evaluation of two conserved hypothetical lipoproteins identified in the genome of *Leptospira interrogans.*

Watanabe MM[1,2], Nascimento ALTO[1] , Vasconcellos SA[3], Morais ZM[3] and Abreu PAE[1]
[1]Centro de Biotecnologia, Instituto Butantan; [2]Faculdade de Ciências Farmacêuticas, USP; [3]Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, São Paulo, Brasil.

Introduction: Leptospirosis is caused by pathogenic spirochetes belonging to the genus *Leptospira*. Leptospiral serovar diversity comprises more than 250 variants. Transmission to humans occurs through the contact of contaminated urine from chronically infected animals. The bacteria enter the body, via skin or mucosa, and multiply in blood and tissues; they can spread to any tissue, but particularly affects liver and kidney. The colonization and survival of leptospires in the host require several types of surface-exposed proteins, such as lipoproteins, porins, adhesins and others (Levett, 2001). **Objectives:** The proteins chosen for this study are putative membrane lipoproteins identified in the *Leptospira* genome (Nascimento et al., J. Bacteriol. 186, 2164, 2004) that might be involved in pathogenesis. **Methods and Results:** The DNA sequences coding for LIC10507 and LIC10009 were amplified from genomic DNA by PCR and cloned into pAE, an *E.coli* expression vector. The recombinant 6xHis tagged proteins were expressed in the soluble form and purified by nickel affinity chromatography. Analysis of structural integrity of the purified proteins by circular dichroism spectroscopy revealed high alpha helix content. Mouse polyclonal antiserum against the recombinant proteins were produced with a high antibody level detected by ELISA and recognized related antigens in a panel of *Leptospira* sorovars extracts by western blotting experiments. **Conclusions:** These proteins will be further evaluated for their reactivity against sera from patients diagnosed with leptospirosis.
Supported by: CNPq/PIBIC, FAPESP and Butantan Foundation

9.33 'Shotgun' cloning studies of molecular compositionof *Phyllomedusa hypochondrialis* skin

Vargas DC[1], Jared C[2], Conceição K[1], Souza KS[1], Assakura MT[1], Camargo ACM[1], Antoniazzi M[2], and Hayashi MA[1]
[1]Center for Applied Toxinology (CAT/CEPID) and [2]Lab. Biologia Celular, Instituto Butantan, Brazil.

Introduction: In response to stress or predator attack, the amphibians use to secrete a complex cocktail of biologically active compounds. The studies of molecular composition of the amphibian skin have provided the discovery of a number of new biologically active components, especially anti-microbial peptides and opioid peptides. **Objective**: In the present work, the molecular composition of the skin of a monkey tree frog, *Phyllomedusa hypochondrialis*, was studied. **Methodology**: The skin of the frog was collected and a cDNA library was constructed in λZAP phage. The obtained clones were randomly excised and only those containing cDNA inserts longer than 500 bp were sequenced. A homology search was performed by using the BLAST ("Basic Local Alignments Search Tool") program. **Results**: Databases searching revealed a number of sequences not described up to date, some of them showing homology with biologically active peptides like protease inhibitors, dermorphin, preprotryptophilin, between others. **Discussion**: The transcriptome is undoubtedly a powerful tool to access the molecular composition of complex biological materials. This is clearly the case of amphibians skin where a number of therapeutically useful molecules have been found in distinct species.
Supported by: CNPq /PIBIC and FAPESP (CAT/CEPID).

9.34 Repetitive DNA analysis in basal cell carcinomas.

Capitanio JS[1], Martinez M[2], Ruiz IRG[1].
[1]Laboratório de Genética- Butantan Institute, [2]School of Medicine, University of Sao Paulo, Brazil.

Introduction: Basal cell carcinoma (BCC) is the most common skin cancer; alterations in the PATCH 1 gene are usually involved on its development. Several repetitive sequences, scattered all around the genome, are subject to unrepaired mutations that often affect gene function in cancer cells. **Objective**: To analyze different repetitive sequences close to the PATCH 1 gene and in other positions of the genome in BCCs. **Methodology**: 34 BCCs, and correspondent controls (leukocytes and adjacent skin), from 19 patients were analyzed. PCRs for microsatellites D9S50, D9S180, D9S287 and D9S196, and RAPDs for primers OPA-2 and OPA-7 were performed. **Results**: The RAPD primers OPA-2 and OPA-7 were altered in 45 % and 91,6% tumors, respectively. Microsatellite primers showed alterations in 29,5 % (D9S180); 23 % (D9S196); 15,8 % (D9S287); and 5 % (D9S50) tumors. **Discussion**: No clear correlation was found between the I-III severity groups of BCCs and the alterations observed. Microsatellites D9S196, D9S287 and D9S180, closer to the PATCH gene than D9S50, were more altered. This is in accordance to the assumption that microsatellites closer to this gene would be more affected in BCC cells. The great percentage of alterations in RAPD patterns corroborates the high genomic instability often present in cancerous cells.
Supported by: CNPq/PIBIC and FAPESP.

9.35 Glutamate modulation of melatonin synthesis in the rat pineal gland.

Moutinho AA[1], Villela DC[1], Scavone C[2], Abrahão MV[1], Afeche SC[1].
[1]Lab. Pharmacology, Butantan Institute and [2]Dept. Pharmacology, Institute of Biomedical Science, USP. São Paulo, Brazil.

Introduction: Glutamate is an amino acid that acts paracrinally on rat pinealocytes and inhibits melatonin synthesis. Acetylcholine is the stimulus for the secretion of glutamate, inducing its exocytosis from the microvesicles present in the cytosol. The action of glutamate seems to be mediated by metabotropic and ionotropic receptors on pinealocytes membranes. However, there are evidences for the existence of glutamate receptors on glial cells in the rat pineal gland. **Objectives**: To study the role of glial cells on the glutamate-induced melatonin synthesis inhibition we compare the effects of glutamate on two experimental models: organotipic culture of pineal glands and isolated pinealocytes. This last preparation had an accentuated reduction in astrocytes number. **Methodology**: Pineal glands isolated from Wistar rats were cultured by 48h, in BGJb medium, at 37°C. Then, they were stimulated by norepinephrine1μM, in the absence or in the presence of glutamate (100, 200, 400 e 600μM). Pinealocytes were obtained by papain dissociation, and maintained in culture, in DMEM medium, by 24h, when the supernatant containing the pinealocytes in suspension was transferred for another culture flask. After 1h, the pinealocytes were stimulated by norepinephrine 1μM with or without glutamate (100, 200 e 600μM). Melatonin was quantified by HPLC with electrochemical detection. **Results and Discussion**: Melatonin was significantly reduced by glutamate ($p<0.05$) in the cultured pineal glands, whereas in isolated pinealocytes glutamate had no effect. This result pointed to a role of glial glutamatergic receptors in the inhibitory effect of glutamate on the melatonin synthesis.
Supported by: CNPq/PIBIC and FAPESP

9.36 *Ascaris suum* extract affect lung eotaxin production and the exit of mature eosinophils from the bone marrow

Kadura AM[1], Boletini-Santos D[1], Macedo MS[2], Lima C[1].
[1]Lab. Imunopatologia, Instituto Butantan, [2]Dep. Imunologia, ICB/USP, São Paulo.

Introduction: We demonstrated that *Ascaris suum* extract (ASC) inhibits airway hyper responsiveness associated with eosinophilic hypersensitivity. **Objective**: To investigate the effect of ASC on mature eosinphil recruitment to the lungs, peripheral blood and bone marrow as well as the production of eotaxin. **Methods**: a fragment of heat-coagulated hen's egg-white alone (EWI) or mixed with ASC (EWI+ASC) was implanted s.c. in C57BL/6 mice, and 14 days later, was exposed to 1%-aerosolized ovalbumin for 3 days. At 0, 1, 2, 3, or 5 days after challenge, the animals were bled and sacrificed. The airway space was washed for BAL and lung homogenates were prepared. Bone marrow (BM) cells were also collected from a femoral bone at several time-points after challenge. **Results**: Significant amounts of eotaxin were only detected in lung tissue from EWI-sensitized mice at 6 and 48 h and in bronchoalveolar lavage fluid at 48 h compared with non-immunized mice. After challenge of EWI+ASC immunized mice, inhibition of eotaxin production in BAL and lung tissue was observed. The levels of MCP-1, TNF-α, and KC were the same among the three groups. The absolute numbers of eosinophils in BAL increased 20-fold at 48 h compared to the control group, which represented 75% of all cells in BAL. The suppressive effect of ASC on this compartment was followed by a low number of eosinophils (3%) found in EWI+ASC-immunized mice in peripheral blood or in bone marrow. **Discussion**: These results indicate that inhibition of the exit of mature eosinophils from the BM to the lung might be related to inhibited eotaxin production in the lungs. This inhibition results from the suppressive effect of ASC on airway inflammation.
Supported by: CNPq/PIBIC and FAPESP

9.37 Ectoparasitic mites on wild rodents of two biomes from State of Paraná, Brazil

Barros-Battesti DM[1], Arzua M[2], Costa NF[1], Fávero D[1], Nieri-Bastos FA[1,3]
[1]Instituto Butantan – Laboratório de Parasitologia [2]Museu de História Natural Capão do Imbuia
[3]Departamento de Medicina Veterinária Preventiva e Saúde Animal (VPS) – FMVZ/USP

Introduction: Several studies about ectoparasites on wild land mammals were conducted in Brazil during last Century. **Objective:** The present work aims to contribute with knowledge about ectoparasitic mites on wild mammals from two different biomes within Paraná State. **Material and Methods:** The studied areas are composed by Atlantic Forest (Adrianópolis municipality, 24º43'S e 48º41'W) and Seasonal Semideciduous Florest (Parque Estadual Mata dos Godoy, Londrina municipality, 23º27'S, 51º15'W). After anesthetizing with ether, mites were recovered from the hosts by combing and brushing. The mites were identified using the original descriptions as well as by comparison of the material with types deposited at the Acari Collection from Butantan Institute (IBSP). **Results and Discussion:** The following species of land mammal (N= 100), were trapped: Muridae - *Akodon* sp., *Bolomys* sp., *Nectomys squamipes*, *Oligoryzomys* sp., *O. flavescens*, *O. nigripes*, *Oryzomys russatus*, *Oximycterus judex* e *Thaptomys nigrita*. Of these, 2.174 specimens were removed by 13 species of Acari identified as: Laelapidae – *Androlaelaps fahrenholzi*, *Eubrachylaelaps rotundus*, *Eulaelaps vitzthumi*, *Gigantolaelaps gilmorei*, *G. goyanensis*, *G. oudemansi*, *G. wolffsohni*, *Laelaps acuminata*, *L. castroi*, *L. paulistanensis, Mysolaelaps parvispinosus*; Macronyssidae – *Ornithonyssus* sp. and Listrophoridae. All these species are first records for both municipalities although some of them are known to be occurring in these biomes. However the species *G. goyanensis*, *G. gilmorei* and *G. oudemansi*, as well as *L. acuminata* and *L. castroi* were found only in Atlantic Forest biome and they are first records to Paraná.

Author Index

D

E

F

G

H

I

J

K

L

M

N

O

P

R

S

T