XII Congress of the Brazilian Society for Cell Biology

and

IX Ibero-American Congress of Cell Biology

Abstracts

The Royal Palm Plaza Hotel
Campinas SP, Brazil
July 15-18\textsuperscript{th}, 2004
XII Congress of the Brazilian Society for Cell Biology and IX Ibero-American Congress of Cell Biology

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The STAT (Signal Transducers and Activators of Transcription) proteins are implicated in stimulating gene transcription in response to many different cytokines, growth factors and peptide hormones that activate a very broad array of receptor tyrosine kinases. Recent attention has focused on negative regulators of STAT activation or STAT activity. Using Drosophila we have found negative regulators to have a profound effect on STAT contributions to various developmental events. Other recent work has linked the STATs, particularly STATs 3 and 5, to regulated growth. STAT3 is a required participant in v-src oncogenesis in cells and can in a mutant constitutively active form act as an oncogene. The role of STAT3 in oncogenesis is to negate apoptotic signals and persistently active STAT3 acts to promote survival of many human cancer cells. On the biochemical side, collaboration with the J. Kuriyan laboratory defined the structure of the NH2 terminal (1-130) and "core" (130-712) regions of STAT1. Knowing the structure has greatly facilitated detailed studies on the functional domains of the STAT molecule and on proteins that interact with various STATs. Finally, we have begun to study the assembly of collections of nuclear proteins on both STAT3 and STAT1 dependent enhanceosomes. The 2-M macroglobulin gene is activated through a STAT3 dependent enhanceosome containing c-Jun/c-Fos and OCT-1 constitutively and STAT3 and the glucocorticoid receptor inducibly. Only after STAT3 recruitment attendant to IL-6 treatment is transcription increased. Interfaces of protein:protein interaction in this and other STAT3 dependent enhanceosomes may furnish good targets for inhibition of STAT3 to produce anti-cancer effects.
the stereocilia, lateral wall, and tight junction complex contribute to the sensory–motor functions of OHCs. Recent studies from our laboratory demonstrate that: 1) an actin molecular treadmill and accessory myosins maintain the functional architecture and self-renewal of stereocilia; 2) prestin and associated proteins organized in a mosaic of regularly structured membrane patches or rafts form the molecular basis for OHC electromotility; and 3) Claudins 9 and 14, which are hair cell specific tight junction proteins, segregate into distinct tight junction strands forming a unique permeability barrier and adhesive complex.

THE ROLE OF ENZYMES INTERACTIONS IN HEPARAN SULFATE BIOSYNTHESIS

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Profa Titular da Disciplina de Bioquímica da Faculdade de Medicina - Fundação ABC

Heparan sulfated proteoglycans are located on the cell surface and in the extracellular matrix, where they play important roles in cell adhesion, differentiation, and growth. To a large extent, these biological activities depend on the specific heparan sulfate chains attached to the core protein. The formation of heparan sulfate occurs within the lumen of the endoplasmic reticulum (ER) and Golgi network by the concerted action of glycosyltransferases, an epimerase, and multiple sulfotransferases. Many of these enzymes are type II membrane proteins thought to reside in the Golgi. However, the precise distribution of these enzymes within the Golgi or other subcellular compartments are unknown. In this report, we have examined the location and interaction of tagged forms of five of the biosynthetic enzymes: galactosyltransferase I and glucuronosyltransferase I, required for the formation of the linkage region, and glucosaminyl-N-deacetylase/N-sulfotransferase 1, uronosyl-5-epimerase, and uronosyl-2-O-sulfotransferase, the first five enzymes involved in the modification of the heparan sulfate chains. All of the enzymes co-localized with the medial-Golgi marker -manosidase II. To study whether any of these enzymes interacted with each other, they were relocated to ER by replacing their cytoplasmic N-terminal tails with an ER retention signal derived from the cytoplasmic domain of human invariant chain (p33). Relocating either galactosyltransferase I or glucuronosyltransferase I had no effect on the other's enzymes location or activity. However, relocating the epimerase to the ER caused a parallel redistribution of the 2-O-sulfotransferase. Transfected epimerase was also located in the ER in the mutant lacking the 2-O-sulfotransferase, but moved to the Golgi when the cells were transfected with 2-O-sulfotransferase cDNA. Epimerase activity was depressed in the mutant, but increased upon restoration of 2-O-sulfotransferase, suggesting that their physical association was required for both epimerase stability and translocation to the Golgi. These findings provide in vivo evidence for the formation of complexes among enzymes involved in heparan sulfate biosynthesis. The functional significance of these complexes may relate to rapidity and specificity of heparan sulfate formation.
In trypanosomes most of the genome is constitutively transcribed and gene expression is mainly controlled at the post-transcriptional level. Exceptions are the ribosomal genes which are transcribed by RNA polymerase I, and the splice leader genes that are transcribed by a RNA polymerase sensitive to α-amanitin, all having defined promoters and transcription factors. A major feature of trypanosomes is that the 5’-end of the splice leader transcript is capped and trans-spliced to all pre-messenger RNA, usually synthesized as long polycistronic RNAs. Concomitant with the trans-splicing reaction, the pre-mRNAs are polyadenilated and exported to the cytoplasm. Therefore, we propose that transcription and RNA processing events might have a simplified spatial organization when compared to other eukaryotes, which present a complex pattern of gene expression control. To provide evidences for this hypothesis we decided to investigate the localization of the replication and transcription machineries in the nucleus of proliferating epimastigote forms of *Trypanosoma cruzi*.

We found that replication as seen by bromodeoxy-uridine incorporation is located mainly at the nuclear periphery. The chromosomes detected by satellite DNA labeling move to the nuclear periphery sites to replicate. We also found that the replication machinery, detected with an anti-*T. cruzi* proliferating cell nuclear antigen (PCNA) is formed in two opposed nuclear domains closed to the nucleolus. When replication starts, the PCNA labeling moves toward the periphery and disperses only when the cells divide. In contrast, the transcription machinery, as observed by using an antibody prepared against the carboxy-terminus of the large sub-unit of RNA polymerase II, was always found more centrally in the nucleus. In addition to a more diffuse distribution, we detected a strong labeling in a small area, close to the nucleolus. This labeling coincided with the localization of the splice leader genes detected by fluorescent in situ hybridization and was sensitive to the presence of actinomycin D and partially to α-amanitin, suggesting that it corresponds to actively transcribing RNA Pol II. This transcription pattern was confirmed by incorporation of bromo-uridine in lysolecithin permeable cells, with a more intense labeling close to the RNA Pol II sites. We conclude that although being a highly dynamic structure, the nucleus of *T. cruzi* has defined domains for replication and transcription. While replication of DNA occurs mainly at the nuclear periphery, the transcription events are more centrally distributed with a concentrated transcription of the splice leader genes close to nucleolus. This unique distribution may be helpful to understand the mechanism that controls the nuclear organization in eukaryotic cells, as well as to provide insights about the transcription and replication apparatus of trypanosomes.

Supported by FAPESP and CNPq
PROTEOLYSIS AND PEPTIDASES
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Extralysosomal proteolysis by multicatalytic complexes such as the 26S proteasome produces large amounts of peptides in the cytosol, mitochondria and nuclei of eukaryotic cells, and there is increasing evidence that the resulting free intracellular peptides can modulate specific protein interactions. The demonstration that free peptides added to the intracellular milieu can regulate cellular functions mediated by protein interactions suggests new putative roles for these molecules in gene regulation, metabolism, cell signaling and protein targeting. Such interactions frequently involve specific consensus amino acid sequences that can be predicted based on similarities in domain composition. We have recently developed a new strategy for identifying novel natural peptides, the sequences of which correspond to fragments of intracellular proteins and contain putative post-translational modification sites. In this presentation, we show evidences that intracellular peptides released by proteasomes may be involved in regulating protein interactions. In particular, the cell biology of endopeptidase 24.15 (thimet oligopeptidase; EC 3.4.24.15) will be discussed in detail since this enzyme has been implicated in both extracellular and intracellular peptide metabolism.

CELL BIOLOGY OF T CELL MIGRATION: COMBINED ROLE OF CHEMOKINES AND EXTRACELLULAR MATRIX
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Cell migration is crucial in lymphocyte biology and the cellular interactions involved now begin to be unraveled; with chemokines, extracellular matrix (ECM) proteins and their corresponding receptors being relevant in lymphocyte oriented movement. In the thymus, microenvironmental cells produce both groups of molecules, whereas developing thymocytes express chemokine and ECM receptors. Importantly, although chemokines and ECM proteins can drive thymocyte migration per se, a combined role of these molecules probably concurs for the resulting migration patterns of thymocytes in their various differentiation stages. In this respect, we found synergy between fibronectin or laminin with some chemokines, particularly CXCL12. Importantly, similar data were obtained in peripheral T lymphocytes. Moreover, changes in ECM/Chemokine-driven migratory response of developing and mature T cells were found in some pathological conditions including chagas disease. In conclusion, the fine dissection of the mechanisms governing T cell migration will provide clues for designing therapeutic strategies targeting developing and mature T cells.

The work was partially supported by grants from Fiocruz, CNPq, and Capes (Brazil).

CORTICAL CODES FOR PERCEPTUAL DECISIONS
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The problem of neural coding has stimulated a large amount of research in neuroscience. The underlying belief is that unraveling the neural representations of sensory stimuli from the periphery to early stages of
cortical processing is one of the keys for addressing brain function, be it local or distributed. Early investigations have demonstrated how neural activity represents the physical parameters of sensory stimuli both in the periphery and in the cerebral cortex. These results have paved the way for new questions that are more closely related to cognitive processing. For instance, are these neural representations of sensory stimuli sufficient for perception? What attributes of the observed neural responses are relevant for downstream networks (for example, on what timescale is spike timing important), and how are these responses related to cognition and to decision-making? To understand the neuronal dynamics of decision-making we need to know how the quantities upon which the decision is based are encoded; this is the crucial link. Recent studies combining psychophysical and neurophysiological experiments in behaving monkeys have provided new insights regarding this problem. In particular, we have explored how neural codes are related to perception and decision-making in the somatosensory system. Here, I review the recent literature on this problem and contrast these observations with those made in other sensory modalities.

Round Tables

CELL CYCLE
Coord.: João Viola (INCA-RJ)

*Molecular regulation of cell cycle during lymphocyte activation*
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Upon antigen stimulation, lymphocytes enter in cell cycle and proliferate, differentiate and most of the activated T cells die by apoptosis. Many of the proteins that regulate lymphocyte activation are under the control of transcription factors belonging to the NFAT family. NFAT activation is regulated by the serine-threonine phosphatase calcineurin, which is inhibited by the immunosuppressive drugs, such as cyclosporin A. Here, we evaluate the role of NFAT1 in regulating lymphocyte proliferation and its involvement in the control of cell cycle progression during lymphocyte activation. Cell cycle analysis after antigen stimulation showed that NFAT1-/- cultures contained more cycling cells when compared with NFAT1+/+ cultures, which is related to a shortening in time of cell division upon activation. Furthermore, hyperproliferation of NFAT1-/- lymphocytes is correlated to an overexpression of cyclins A2, B1, E, and F. Further analysis of cyclins mRNA expression by RNAse protection assay demonstrated that cyclosporin A inhibit the expression of cyclins A2 and E. Preliminary data using a luciferase reporter gene assay demonstrated a putative binding site for NFAT transcription factor on the cyclin A2 proximal promoter. These results suggest that NFAT1 transcription factor may directly play a role in the regulation of expression of some cyclin
genes and is central to control of cell cycle progression during lymphocyte cell activation.
Supported by: INCA/FAF, CNPq and Furnas Centrais Elétricas S.A.

Molecular mechanisms of cell cycle control in mouse cell lines
Érico Costa, Fábio Forti, Paula Asprino, Tatiana Matos, Kátia Rocha, Miriam Moraes & Hugo Armelin
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Y1 is an ACTH-responsive, steroid secreting, adrenal, tumorogenic cell line, that retains a stringent dependency of the ERK1/2 pathway on mitogens stimulation, accounting for the remarkable cell cycle control displayed by these cells. However, chronic c-KiRas-GTP high levels, found in Y1 cells, constitutively activate the PI3K/AKT pathway. Attenuation of the high c-KiRas-GTP levels, by conditional expression of the dominant negative mutant HaRasN17, renders the c-KiRas/PI3K/AKT pathway dependent on mitogens and reduces Y1 tumorogenicity in Balb/nude mice.

FGF2 triggers a robust mitogenic response in G0/G1-arrested Y1 cells. But, FGF2 also triggers a cell death program dependent on constitutively high c-KiRas-GTP levels. This FGF2 death effect might be a natural unsuspected mechanism for restraining oncogene induced proliferation. Recently, we demonstrated in Y1 cells that ACTHReceptors, via the cAMP/PKA pathway, elicit concerted anti-mitogenic mechanisms involving: a) Akt deactivation, b) c-Myc protein destabilization and c) CDK-inhibitor p27Kip1 induction. However, in Y1 cells carrying low c-KiRas-GTP levels, ACTH does not inhibit: a) Akt transient activation, b) c-Myc protein expression and c) DNA synthesis stimulation by FGF2. In conclusion, ACTH exerts a tumor suppressor activity rather than anti-mitogenic effects in Y1 cells.
Supported by FAPESP and CNPq.

Gap junctions regulate life and death of hematopoietic cells in the bone marrow
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Normal and leukemic blood cell progenitors depend upon the bone marrow stroma with which they communicate through soluble and membrane-anchored mediators, adhesive interactions and gap junctions (GJ). Regarding hematopoiesis, it is believed that it can be influenced by connexin expression, but the exact role of GJ in cell death and proliferation is not clear. Using flow cytometry, we monitored the division rate of leukemic cell lines, communicating and not with stromal cell line through GJ. We found that GJ-coupled cells (i) did not proliferate; (ii) were kept in G0; and (iii) were protected from drug-induced apoptosis when compared to either total or uncoupled cell population. We conclude that GJ-coupling between stroma and leukemic lymphoblasts prevents proliferation, keeping cells in a quiescent state, increasing thus their resistance to antimitotic drugs. Since GJ are particularly abundant in the sub-endosteal environment, which harbors blood stem cells, we also asked which cells within the normal human bone marrow communicate with stroma. Using a primary bone marrow stroma cell culture, our results show that 80% of CD34+ progenitors communicate through GJ. We
propose that blood cell progenitors might be retained in the low-cycling state by GJ-mediated communication with the hematopoietic stroma. In agreement with the above hypothesis, initial studies using stroma from Cx43 deficient mice show decreased numbers of Coupledstone Forming Units when compared to normal bone marrow stroma. Radiation quimeras are being developed in order to study the role of GJ in the maintenance of immature hematopoietic progenitors in vivo.

**INTRACELLULAR FUNCTIONS FOR MYOSINS**

Coord.: Roy Edward Larson (USP, Ribeirão Preto)

*Actin and myosin motors at the ER/Golgi interface protein transport*

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It is well established the dependence on microtubules of the Golgi complex morphology and Golgi-derived transport events. However, up to the present time, the role of actin filaments in the secretory pathway remained unclear. This was caused in part by the limited availability of anti-actin tools. In my laboratory, we have recently demonstrated that, like microtubules, microfilaments are also essential in the Golgi morphology and function. In contrast to the Golgi fragmentation and subsequent cytoplasmic dispersion of ministacks produced by nocodazole, the disruption of microfilaments with different anti-actin drugs (cytochalasin, latrunculins, botulinum toxins, etc) induces an unusually compact Golgi morphology. Concomitantly to this Golgi collapse, the ER/Golgi interface transport is perturbed but only in its retrograde (Golgi-to-ER) pathway. We have also shown that the locomotion of retrograde transport intermediates occurs along actin tracks and not actin comets. Thus, we next examined which actin motor(s) could mediate in this pathway and observed a significant role of the nonmuscle myosin II. We also studied the putative role of Rho GTPases as master regulators of the cellular actin organization. Unlike Rho and Rac, Cdc42 is located in Golgi membranes. Interestingly, the Cdc42 activation (GTP-bound form) leads to its enrichment in the lateral rims of cis/middle-Golgi cisternae and to negatively regulate retrograde protein transport. The latter is mediated by N-WASP and Arp2/3, which are both recruited to the Golgi in a Cdc42-dependent manner. We conclude that actin and the actin assembly and polymerisation mediated by Cdc42/N-WASp/Arp regulate secretory pathway trafficking.

**Cytoplasmic transport of RNA upon cellular stress in oligodendrocytes.**

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As many other polarized cells, oligodendrocytes shows an asymmetric distribution of mRNAs, being myelin-targeted messengers present in granules at the cytoplasmic processes. Staufen is a conserved double-stranded RNA-binding protein required for microtubule and microfilament-dependent localization of granules of mRNAs in Drosophila oocytes and embryos. We investigate the relevance of the mammalian homologs Staufen 1 and Staufen 2 in mRNA subcellular distribution in oligodendrocytes. We found that Staufen 1 and Staufen 2 are present in two independent sets of RNA granules that contain ribosome subunits.
and associate with microfilaments and microtubules. The distribution of Staufen 1 and Staufen 2 granules dramatically changes upon heat shock or oxidative stress induction. These two proteins are recruited into stress granules (SG), which are stress-induced organelles containing transiently silenced messengers. Staufen recruitment into perinuclear SG is paralleled by a similar change in the localization of total polyadenylated RNA. In contrast, the cytoplasmic transport of newly synthesized mRNA molecules to the distal oligodendrocytes processes appears to occur by a Staufen-independent pathway. Our results suggest a role for Staufen 1 and Staufen 2 RNPs in the relocation of polysomal mRNAs in response to different stimuli and -as an extreme example- their coalescence into perinuclear SG upon cellular stress. As microtubule and microfilament-disrupting drugs display distinct effects on SG assembly, the participation of myosins and unknown microtubule-dependent motors during SG aggregation is speculated. A working hypothesis is that Staufen may mediate the recruitment of the molecular motors required for SG aggregation.

Nuclear localization of site-specific phosphorylated myosin Va
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Myosin Va (MVa) is an actin-based molecular motor composed of an amino-terminal motor domain with binding sites for actin and ATP, a regulatory neck domain composed of six tandem IQ motifs that bind calmodulin and light chains, and a tail domain, structurally distinct within the myosin superfamily, thought to be involved in cargo binding and/or other protein-protein interactions within the cell. MVa is a binding partner and phospho-substrate for calmodulin-dependent protein kinase II (CaMKII). The binding of CaMKII to the proximal/medial tail domain of MVa results in its autophosphorylation and subsequent phosphorylation of MVa on Ser\textsuperscript{1650} (mouse sequence) located in the globular tail domain. Cell-cycle dependent phosphorylation of MVa at this site was recently shown to regulate MVa binding to and motility of melanosomes in Xenopus laevis melanophores (Rogers et al., 1999; Karcher et al., 2001). We have produced a monoclonal antibody against the sequence containing the phosphorylated Ser\textsuperscript{1650} and are using it as a tool for the study of in vivo phosphorylation of MVa. Immunocytofluorescence studies on several cell types, including the neuroblastoma N2A line, cells from a primary culture from cerebellum, mouse melanoma cell lines B16 (wild-type for MVa) and S91-6 (null mutant of MVa), with or without hormonal stimulation (MSH), demonstrate the nuclear localization of phosphorylated MVa in structures resembling speckles, which are regions active in RNA processing. Also, strong diffuse labeling was observed at several stages of mitosis. We hypothesize that punctually phosphorylated MVa may participate in energy-dependent RNA localization within the nucleus.

Financial support from FAPESP.
The role of class V myosins in vesicle and organelle trafficking is well known. However, several lines of evidence also implicate these myosins in the anchorage, precise distribution and regulated delivery of signaling proteins and mRNA to specific functions within the cell. For instance, it has recently been proposed that myosin-Va sequesters the proapoptotic Bcl2-modifying factor, Bmf, to the actin cytoskeleton through its interaction with dynein light chain-2 (DLC2), and damage signals, such as loss of cell attachment and F-actin depolimerization induce the release of Bmf triggering apoptosis (Puthalakath et al., Science, 293:1829, 2001). These findings suggest a role for myosin-V in anoikis, a specific type of apoptosis induced by loss of cell attachment. Since anoikis is considered one of the barriers to metastasis, we investigated the expression and function of myosin-Va in human melanoma cell lines, which are amongst the most therapy resistant tumors. We found an increase of myosin-Va mRNA and protein levels in two metastatic cell lines when compared with their parental cell lines from the primary tumor. Searching for an association between high expression of myosin-Va and possible features involved in tumor progression, we found a positive correlation with the proliferation rate, resistance to anoikis, and the ability to easily attach and detach from the substrate. Additionally, we transfected B16-F10 melanoma cells with a myosin-Va fragment that is part of the characterized DLC2 binding domain (fused with GFP or RFP). Interestingly, we found a dramatic decrease of viability in the transfected cells, with a highly active membrane blebbing and nuclear condensation, as well as loss of the mitochondrial membrane potential, based on DiOC staining. Co-transfection with Bcl2 partially protected from cell death. The bacterially expressed myosin-Va fragment was sufficient for in vitro interaction with DLC2. The results shown here suggest a specific role for the recombinant peptide in competing for DLC2/Bmf, releasing Bmf from its anchorage sites, although additional studies will be required in order to pinpoint the molecular pathway involved in the apoptosis triggered. In conclusion, this work identifies a powerful tool to induce cell death in one metastatic melanoma cell line, but further efforts will be necessary towards determining the spectrum of action of this new molecular tool.

Financial Support: FAPESP, CNPq, CAPES, FAEPA
the host organism). Today this concept is revived by the idea that DNA is imperious to the effects of organismic experience (= “molecular Weismannism”). In fact there are two types of genes: \( \text{Gene-P} = \) defined with respect to phenotype (descriptor, preformationist, instrumental, explicit, context-independent); \( \text{Gene-D} = \) defined with respect to DNA sequence but indeterminate with respect to phenotype (constructed, epigenetic, context-dependent). Then, the cells have the ability to act as genetic engineers, resulting the DNA configurations of an **intelligent cytoplasmic-chromosome system**. Cellular states inform genome structural and symbolic change so as to actualize morphological attractors (teleomorphic recursivity). **Second (practical role):** to apply advanced microscopies (confocal, two-photons, scanning probe and electron microscopies and others) to explain the “cytoplasmic intelligence”, the nuclear organization, to define the morphological attractors and 3-D architecture (organizational morphological phenotype). The sequenced genes and proteins can be localized in the cells and in the tissues (Histogenomic and Histoproteomic) using PCR and hybridization in situ, immnuhistology and tissue microarray. The topographic expression of genes and proteins can be precisely discriminated by Laser Tissue Microdissection and now is possible to perform MALDI mass spectrometry imaging and optical microscopy on the same section. Then, technological and conceptual advances allow the Morphology and Pathology to deeply contribute to the understanding of genomic, transcriptomic and proteomic tissue expression and organization.

**Antisense intronic noncoding rna transcripts correlated to prostate cancer**

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Microarray technology has permitted a refined high-throughput mapping of transcriptional activity in the human genome, revealing a two– to ten–fold excess of transcripts along chromosome 22 than originally predicted by mapping of known genes. Using microarrays that probed the genomic DNA along all the extension of chromosomes 21 and 22, recent work in the literature has detected comparable fractions of transcriptional activity within exons or introns of annotated genes, and nearly half of these intronic transcripts were expressed antisense to their respective well-characterized introns. Nevertheless, large-scale transcription profiling has so far been performed with commercial exon arrays, which rely on annotation of the human genome to choose which genomic protein-coding regions to sample. Our goal was to study the transcriptional profile of intronic messages in cancer, using prostate tumors with different degrees of differentiation as a model. Therefore, we constructed a 4,000-element cDNA microarray enriched with transcripts that map to intronic segments of known genes. We used a bioinformatics approach to select these from nearly one million ESTs generated by the Human Cancer Genome Project with the ORESTES technique from 24 distinct types of cancer and normal adjacent tissues. The resulting intron array was used for measuring the expression profile of 27 prostate tumor samples with different degrees of differentiation (Gleason score, GS 5 to 10). Our results show that a considerable fraction of tumor-correlated genes are intronic antisense noncoding transcripts, pointing to the biological relevance of these messages in complex diseases such as cancer. Supported by FAPESP, CNPq and CAPES
Perform a precise morphologic diagnosis is essential in oncology in order to establish histogenesis, tumor differentiation, and to evaluate the biological behaviour. Most of the classifications of the tumors are based in morphological findings only. It is necessary to approach the old tumor classifications in order to include the new molecular findings. The role of the pathologist is not only performing diagnosis, but understands physiopathology. The skills necessary to do diagnosis should be used to understand the tumors and its clinical consequences. Advances in molecular biology of the tumors are immense, and the role of the pathologist in molecular oncology should include: to provide the right diagnosis; to provide the right material; to provide the right leads; to suggest the right experiments, and to evaluate the consequences of the molecular finding in diagnosis and prognosis of the neoplasms. The use of appropriate techniques to evaluate the gene expression, as immunohistochemistry and tissue microarray, might bring advances in the comprehension of the tumoral biology. Only the integration between molecular biologists, pathologists, clinicians, surgeons, epidemiologists and other professionals could lead the investigation on oncopathology to practical results in benefits of the patients.

ADHESION AND CYTOSKELETON

Coord.: Manoel L. Costa (UFRJ - RJ)

Adhesion proteins in the zebrafish (danio rerio) myogenesis.
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The two main models for the role of adhesion centers in development are in vivo cell cultures and human diseases. If there is a lot of information on the in vitro binding properties of the purified proteins, cell cultures are simplified two-dimensional systems, while cells actually develop in a much more complex three-dimensional environment. On the other hand, human development is not amenable for experimentation, only for observation. We are interested in the cell adhesion structures because they are nucleating centers for myofibrillogenesis, the main event in muscle differentiation. Because embryos are more difficult to manipulate than cell cultures, this differentiation process has scarcely been studied in avian and mammalian embryos in situ. Therefore, we used immunofluorescence microscopy and biochemical techniques to study the optically clear and easy to handle embryos of the zebrafish Danio rerio. We already studied the main sarcomeric proteins in the zebrafish myogenesis. The adhesion proteins integrin, dystrophin, paxillin and vinculin were distributed along both sides of the connective tissue septa between the somites, where the tips of the myoblasts are inserted. Those proteins were not confined to small patches as their in vitro counterparts, and they were not present anywhere else in the myoblasts. On the other hand, cell-cell contacts, important for muscle fusion and differentiation, were not
observed in the zebrafish muscle cells using cadherin or catenin antibodies. We propose that the patchy adhesion structures described in vitro are distant remnants of quite large adhesion sites that actually exist in situ. Support: FAPERJ, CNPq, FUJB

The contribution of muscle protein analysis in the diagnosis of genetic myopathies and in the understanding of the organization and maintenance of muscle fiber integrity.
Mariz Vainzof, Human Genome Research Center, Dept of Biology, IB, University of Sao Paulo, Sao Paulo, SP, Brasil

Neuromuscular disorders are a heterogeneous group of genetic diseases, characterized by progressive muscle degeneration resulting in muscle weakness and consequent loss of the motor ability. In the last decade, mutations in several genes have been identified, resulting in the deficiency or loss of function of different important proteins. Complementary biochemical and immunohistological analyses have localized these proteins in several compartments of the muscle fiber, such as the sarcolemmal muscle membrane (dystrophin, sarcoglycans, dysferlin, caveolin 3), the extracellular matrix (α2-laminin, collagen VI), in the sarcomere (telethonin, myotilin, titin), in the muscle cytosol (calpain 3, FRPR, TRIM32), and in the nucleus (emerin, lamin A/C, SMN protein). Some of the diseases associated to alterations in these proteins are the progressive muscular dystrophies and the congenital myopathies. In the muscular dystrophies group, the allelic X-linked Duchenne and Becker forms are caused by mutations in the dystrophin gene. Among the Limbgirdle forms, six autosomal dominant (LGMD 1A to LGMD1F) are responsible for about 10% of the cases, and ten autosomal recessive (LGMD2A-2J) forms have already been described in the other 90% of the patients. Additionally, some congenital forms are also recognized, being the structural forms associated to different alterations within the muscle fibers.
Genotype-phenotype correlation through the analysis of the effect of different mutations on protein expression and on phenotypic variability can contribute significantly for the elucidation of the physiopathology of each genetic disorder involved and can help to understand gene function. FAPESP-CEPID, CNPq, PRONEX

Effects of cholesterol depletion in myogenic differentiation
Cláudia Mermelstein (Dept. Histologia e Embriologia – ICB - UFRJ)

The formation of a skeletal muscle fiber begins with the withdraw of committed mononucleated precursors from the cell cycle. These myoblasts elongate while aligning to each other, guided by the recognition between their membranes. This step is followed by cell fusion and the formation of long and striated multinucleated myotubes. We used the chemical methyl-β-cyclodextrin (MCD) in primary cultured chick skeletal muscle cells, to deplete membrane cholesterol and investigate its role during myogenesis. MCD enhanced myoblast fusion and induced the formation of large multinucleated myotubes with nuclei centrally clustered and not aligned in the cell periphery. MCD-myotubes were perfectly striated, as indicated by sarcomeric α-actinin staining, and microtubules and desmin filaments distribution was not altered. Pre-fusion MCD-treated myoblasts form large aggregates, with cadherin and β-catenin accumulated in cell-cell adhesion contacts. We also showed that the membrane microdomain marker GM1 was not visualized as clusters in
the membrane of MCD-treated myoblasts. Our data demonstrate that cholesterol is involved in the early steps of skeletal muscle differentiation.

**EXTRACELLULAR MATRIX**

Coord.: Telma M T Zorn (USP – São Paulo)

**Galectin-3 acts as a matricellular protein.**

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Expression of galectin-3, a β-galactoside binding lectin, is associated with sarcoma tumor progression and metastasis. Intracellular galectin-3 may play a role in cell survival, conferring resistance to anoikis. However, galectin-3 is also secreted and may play a role in cell:matrix interactions in a carbohydrate dependent way. Here we present evidence that galectin-3 acts as a matricellular protein, modulating integrin function. Upon transfection with EJ-ras, murine fibroblasts shifted from a stationary to a more migratory phenotype associated with an increase in both α6β1 integrin and galectin-3 expression. Galectin-3 was found in the leading edge of migrating cells, colocalizing with polyllactosamine containing glycoconjugates. Transformed cells displayed galectin-3 on the cell surface, as determined by flow cytometric analysis. The haptotatic response of transformed cells to laminin, which was mediated by α6β1 integrins, was inhibitable by lactose, suggesting a role for galectins in cell migration. We further evaluated the migratory response of cells derived from galectin-3 null mice. Embryonic mesenchymal cells from both wild-type and galectin-3 null mice were analyzed regarding both integrin expression and the migratory response to laminin. Despite having similar amounts of α6β1 integrins on the cell surface, galectin-3 null cells were less migratory than control cells. We have also established cell lines from methylcholanthrene-induced sarcomas from both wild type and galectin-3 null mice. In this system, galectin-3 null cells were also less migratory than control cells in laminin. Finally, when galectin-3 was transiently expressed in galectin-3 null sarcoma cells, it inhibited cell adhesion to laminin and stimulated the migratory response to laminin. Mechanistically, extracellular galectin-3 disrupted the focal adhesion plaque, as defined by the loss of phosphorylated FAK in the ventral surface of migrating cells. Although it is clear that the α6β1 integrin is the actual mediator of fibroblast migration towards laminin, galectin-3 acts as a positive modulator of this process. Supported by FAPESP (97/13100-9 and 99/13013-4) and TWAS.

**Elastic system in cutaneous tissue repair**

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The elastic system is a major extracellular matrix component, present in large amount in normal skin that endows tissues passive recoil without energy input. In pathological conditions, such as fibrosis, elastic fibers may be present in large amount. The two major structural components of elastic fibers are fibrillin-rich microfibrils and elastin. Fibrillin-rich microfibrils may be observed alone, but elastin is always associated with preformed template of microfibrils. Three types of fibers form the elastic system: oxytalan, elaunin and elastic fibers. In normal skin, elastic fibers
form a network continuum throughout the dermis comprising thick fibers within the deep dermis. Elaunin fibers form a plexus that interconnects oxytalan fibers with elastic fibers. Oxytalan fibers are found mainly in superficial dermis where they insert in epidermal basement membrane forming a typical candelabra-like pattern. The elastic system fibers are rarely considered in cutaneous wound healing studies, and contradictory data about their distribution during skin wound healing are observed, probably due to different technical approaches used. Our group showed that in hypertrophic scars the clinical improvements occur concomitantly with the rearrangement of elastic system components, and that fibrillin-1 and elastin are differently expressed in hypertrophic scars and keloids presenting two patterns one for normal scars and another for excessive scars.

Decidualization in rodents. the role of the small leucin-rich proteoglycans in the collagen fibrillogenesis.
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Embryo implantation and development demands a complex sequence of cellular and molecular modifications in the uterus. Preceding the implantation, a wave of cell proliferation occurs in the endometrium followed by a transdifferentiation of endometrial fibroblasts into decidual cells. These new cells form a new temporary uterine structure called decidua, which plays essential role in maintaining normal pregnancies and proper embryo development. Decidualization is characterized by an extensive remodeling of the endometrial stroma including synthesis and degradation of extracellular molecules and a surge of very thick collagen fibrils in the decidualized areas of the endometrium. Studies performed in our laboratory and in another have shown differences between the pre-implantation and post-implantation molecular profiles of the endometrial extracellular matrix. This finding suggests that remodeling is probably necessary for embryo implantation and development. Our immunocytochemical studies showed that the expression and distribution of SLRPs in the uterus of pregnant mice varies in different regions of the uterus and along the pregnancy. Before the embryo implantation the proteoglycans decorin and lumican where abundant in the endometrium. Curiously, following embryo implantation, the quantities of these molecules in the endometrium decrease currently with the appearance of very thick collagen fibrils. Contrary, biglycan was highly expressed in the decidualized endometrium and was associated with thick collagen fibrils. These results strongly indicate that biglycan plays a role in collagen fibrillogenesis and probably participate in the determination of collagen fibril thickness in the mouse decidua confirming the role of small rich-leucin proteoglycans (SRLPs) as important regulators of collagen fibril growth. They also indicate that morphological and molecular changes observed during the pre-implantation period, together with the redifferentiation of endometrial fibroblasts into decidual cells, comprise a new selective genetic program characterized by sequential and coordinate down and up regulation of various proteoglycans and glycosaminoglycans. Supported by FAPESP (01/01443-6; 01/12283-0)
PHAGOCYTOSIS
Coord.: Maria Isabel Colombo (Universidad Nacional de Cuyo - Argentina)

Autophagy is an innate immunity mechanism inhibiting mycobacterium tuberculosis survival in infected macrophages
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Mycobacterium tuberculosis is an intracellular pathogen that persists within phagosomes by interfering with membrane trafficking pathways which govern normal acidification and phagosomal maturation. Here we show that stimulation of autophagy in macrophages causes mycobacterial phagosomes to become acidic and mature into phagolysosomes. Physiological induction of autophagy by starvation, or its pharmacological stimulation by rapamycin, caused mycobacterial phagosomes to colocalize with proteins of the autophagic pathway such as LC3, a factor involved in autophagosome formation, and Beclin-1, a subunit of the phosphatidylinositol 3-kinase hVPS34. hVPS34 is necessary for autophagy in general, and is a target for mycobacterial factors causing the mycobacterium phagosome maturation arrest. Interestingly, induction of autophagy suppressed mycobacterial survival in macrophages. These findings demonstrate that autophagic pathways can overcome the trafficking block imposed by M. tuberculosis, and indicate that autophagy, which is a hormonally and developmentally regulated physiological process, represents a previously unappreciated innate defense mechanism for control of intracellular pathogens.

Intracellular trafficking of coxiella burnetii.
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Coxiella burnetii is an intracellular microorganism that replicates in the host cell generating large vacuoles, likely phagolysosomes. However, very little is known about the trafficking and the survival of C. burnetii within this harsh environment. To understand the mechanism controlling the trafficking of this pathogen we have studied the localization of Rab5 and Rab7, markers of early and late endosomes, respectively. Hela cells at 48h post infection were infected with recombinant virus to overexpress GFP-Rab proteins. Our results indicate that parasitophorous vacuoles containing C. burnetii interact with late compartments of the endocytic pathway and localize in large vacuoles labeled with Rab7. These vacuoles were also labeled by monodansylcadaverine, a specific autophagosomal marker and colocalized with the autophagic protein LC3 suggesting a connection between the C. burnetii-replicative vacuole and the autophagic pathway. In order to characterize the role of cytoskeleton in the formation of the parasitophorous vacuole, CHO cells were infected with C. burnetii and then treated with cytoskeleton disturbing agents. Untreated cells showed numerous bacteria-containing vacuoles decorated with actin. When infected cells were treated with microfilament depolimerizing agents, vacuole number decreases significantly. A marked inhibitory effect was also observed in cell treated with a myosin ATPase inhibitor. These results suggest that both actin and myosin play a critical role in the biogenesis of the parasitophorous vacuole.
SPOT CELL BIOLOGY

Coord.: Ricardo Louro (UFRJ)

Comparative ultrastructural study of spores germination in red algae. 
Zenilda Laurita Bouzon (UFSC – Florianópolis)

Spores germination, be they tetraspores or carpospores, is known to follow well-established patterns of cell division at the order or even family level in the Rhodophyta. The present investigation adopted a comparative approach, using transmission electron microscopy, to study the changes at the subcellular level, during the initial processes of spore germination in five different patterns of red algae spores germination. The spores are released naked, surrounded by a mucilage envelope which provides a primary attachment of the spore to substrate. Numerous tubular invaginations linked to the plasma membrane become conspicuous after spore's release and seem to be connected with the endoplasmic reticulum, which may have a role in the transport of cell material. The small fibrillar vesicles, abundant in the spores are no longer seen in the sporelings, suggesting that these vesicles contain the precursors of cell wall material which accumulates during sporogenesis. Soon after attachment the cell wall is deposited as a thin layer over the spore surface. In the species regarded as the most primitive, P. var. amplifolia and N. helminthoides, the spores exhibited a single, stellate chloroplast without peripheral circular thylakoid. The spores of the other species presented numerous chloroplasts, elongated and elliptical, with a peripheral circular thylakoid. Three processes were observed in relation to the initial behavior of the spore: 1. formation of the germ tube (P. spiralis var. amplifolia, N. helminthoides and G. floridanum), 2. cell polarization (L. arbuscula) and 3. division by binary fission or equal division (H. musciformis).

Cell aspects in the apomictic reproduction
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Apomixis is the asexual reproduction through seeds, which leads to a progeny genetically identical to the mother plant through two mechanisms. The sporophytic apomixis consists on somatic cells of the ovule (embryogenic initials-EI) directly originating an embryo, adventitious embryony. Otherwise, cells can undergo the gametophytic apomixis (apospory or diplospory) forming an unreduced female gametophyte (apomeiosis) with the autonomous development of the embryo (parthenogenesis) from the unreduced egg cell. Endosperm development can be autonomously as in Hieracium spp. or after fertilisation as in the pseudogamous Brachiaria brizantha. Apomixis in these genera is of aposporic type because the initial cells of the unreduced aposporous embryo sac (aposporous initials - AI) are somatic cells of the ovule. EI and AI position within the ovule and differentiation timing are described for many species. AI ultrastructure was described for Brachiaria spp. However, EI and AI identity is still a challenge due to the limited cell markers distinguishing somatic cells that changes from sporophytic to gametophytic program during the apomictic pathway. Differences in callose deposition in the walls of the cells initiating sexual and apomictic reproductive pathways were the early clues distinguishing them in some
but not all apomictics. Some available sexual developmental cell markers were used in *Hieracium* spp. and showed that the apomictics seems to be under a modified sexual reproduction program. The early activation of the unreduced egg cell is considered an important, but not the only means to avoid fertilisation as in *B. brizantha*, where rates of precocious embryony vary among accessions.

**INFLAMMATORY PROTEINS**

Coord.: Sônia Oliani (UNESP – São José do Rio Preto)

**Hormonal regulation of allergic lung inflammation in rats**

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Asthma is an inflammatory lung disease characterized by cell (neutrophils, eosinophils and lymphocytes) migration, epithelial damage, and bronchial hyperresponsiveness. Inflammatory mediators released by antigen-activated mast cells display a pivotal role on asthmatic response. Asthmatic women exhibit a deterioration of pulmonary function during pre-menstrual period, indicating that hormonal fluctuations during menstrual cycle correlate with the worsening of asthma symptoms. We demonstrated in a rat model of allergic lung inflammation that oophorectomy (OVx) reduced the recruitment of inflammatory cells into the lungs, caused leukocytosis and bronchial hyperresponsiveness, and increased the number of cells in bone marrow. There was lowered release of TNF-\(\alpha\) and \(H_2O_2\) by cultured pulmonary phagocytes from these animals. Treatment of OVx allergic rats with estradiol and with a corticosterone synthesis blocking agent reestablished lung inflammation, whereas progesterone accentuated the lung anti-inflammatory effects of OVx. Conversely, bronchial hyperresponsiveness was prevented by progesterone. Our data argues in favour of the existence of an optimal balance between the levels of estradiol, progesterone and corticosterone, which, once disrupted by OVx, changes the severity of allergic lung inflammation and bronchial responsiveness. As a conclusion, variations in such hormones along the menstrual cycle may relate strongly to the clinical picture defined as “premenstrual asthma”.

**Galectins, inflammation and tumor escape**

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Recent evidence has implicated galectins as novel regulators of immune cell homeostasis. Whereas some members of this family behave as amplifiers of the inflammatory cascade, others trigger homeostatic signals to shut off T-cell effector functions. These carbohydrate-binding proteins, identified by shared consensus amino acid sequences and affinity for beta-galactoside-containing sugars, participate in the homeostasis of the inflammatory response, either by regulating cell survival and signaling, influencing cell growth and chemotaxis, interfering with cytokine secretion, mediating cell-cell and cell-matrix interactions or influencing tumor progression and metastasis. During this presentation, we will summarize the state-of-the-art of the effects of galectins in inflammatory and immunomodulatory processes. In particular we will highlight the
effects of galectin-1 in the modulation of chronic inflammatory processes and its novel role in immune privilege and tumor-immune escape. The current wealth of new information promises a future scenario in which individual members of the galectin family or their specific ligands will be used as powerful anti-inflammatory mediators and selective modulators of innate and adaptive immune responses.

Action of the annexin 1 protein on the inflammatory process
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Inflammation is a dynamic response that functions to achieve homeostasis by balancing adaptation to the environment with integrity of the organism. Tilting of the local fine regulation at the tissue level can be detrimental, as exemplified by many complex inflammation-associated diseases which result from disconnect between responding to a stimulus and returning to homeostasis. Increasing evidence supports the notion that in the neutrophil, resolution is an active reprogrammed fate. Annexin 1 (AnxA1) is an endogenous protein highly expressed in certain cell types including circulating neutrophil. In particular, granulocytes contain between 2 - 4% of total cytosolic proteins as AnxA1. This protein acts as an inhibitor of the leukocyte emigration and its importance is also highlighted by the fact that its synthesis is at least under partial control by glucocorticoid hormones. AnxA1 is one of several endogenous anti-inflammatory mediators that operate in the body to ensure the transient profile of the inflammatory reaction, i.e. to prevent it from over-shooting thereby causing damage to the host. Using a combination of pharmacological and morphological tools, and genetically modified animals, it has been possible to highlight the negative role that this protein exerts on the process of neutrophil recruitment. Endogenous AnxA1 is rapidly mobilized specifically at the step of neutrophil adhesion to the endothelial wall. The patho-physiological role of the AnxA1 system can be evinced by the study of AnxA1 null mice, recently generated by Hannon and Flower. Finally, AnxA1 null mice have been generated inserting a reporter gene (LacZ gene) within the construct, such to monitor gene promoter activation in vivo. Administration of a non-lethal dose of LPS was lethal in AnxA1 deficient mice, with 60% mortality within 24 hours. This was associated with strong gene activation in trachea, mesentery and lung. Within the mesentery, endothelium of post-capilllary venules showed transient staining maximal at 24 hours post-LPS. In conclusion, understanding how anti-inflammation is brought about can lead to innovative drug discovery.

Key words: anti-inflammation - neutrophils – electron microscopy
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CELLULAR AND MOLECULAR MECHANISM UNDERLYING CELL MIGRATION
Coord.: Marinilce F Santos (USP - São Paulo)
DEVELOPMENTAL BIOLOGY
Coord.: Chao Yun Irene Yan (USP – São Paulo)

Developmental control of asymmetry in the embryonic zebrafish brain.

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Animals show behavioural, cognitive and neuroanatomical asymmetries but the genetic pathways regulating these asymmetries are not clearly understood. In the recent years, we have shown that initial establishment of asymmetry in the zebrafish brain is controlled by independent mechanisms that operate in tandem. A primary, genetically based mechanism, ensures that structural asymmetries of the diencephalic habenular nuclei and the photoreceptive pineal complex are established in the brain and involves competitive interactions by which one side of the brain inhibits the ability of the contralateral side to develop as 'left'. A second genetic mechanism of CNS development ensures that laterality of asymmetry is consistently biased to the same - left - side of the brain and involves a genetic pathway with a conserved role in the control of asymmetry of the heart and viscera in vertebrates. Activation of Nodal signaling in left-sided precursors of the habenula and pineal complex acts to ensure that laterality of asymmetries in these brain structures is always directed to the left side within the population. Recent work in our lab is focused on the mechanisms that integrate the control of asymmetry and laterality within in the brain, and the genetic players of asymmetric morphogenesis.

Msx1 function and regulation during mice development

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Developmental genes present multiple functions during development, requiring a spatial-temporal orchestrated regulation, which relies upon interactions of several signaling pathways. Mice msx1 gene is a homeobox gene that is related to several tissues formation. In general this gene is responsible in maintaining cells undifferentiated and in a proliferative state. This process culminates with structures growing where the gene is expressed. In mice and man this gene belongs to a gene family composed by three members: msx1, msx2 and msx3. Their pattern of expression suggest for msx1 and msx2 important roles in cranio-facial structures organogenesis, including all bones of the skull, maxilla, mandible and odontogenesis. Besides, it seems that these genes are important to neural tube patterning, limbs growing, muscle differentiation and valves of the heart formation. Otherwise, the pattern of expression of msx3 suggests only a role in neural tube formation. Although this wide pattern of expression during development, knockout mice for this gene has only demonstrated cranio-facial defects and lack of some teeth. Our group has been performing analysis in these knockout mice in different background, and the results we have accumulated suggest that these animals present abnormalities in the cardiac valve formation, similar to those found in Fallot’s tetralogy. Defects in blood cells maturation have also been identified as well as anomalies in the bone marrow, suggesting a role for msx1 in the proliferative capacity of the mesenchymal stem cell. Studies of the msx1 regulation were also performed using transgenesis and in vitro analysis, indicating the presence of several regulatory
elements. Two of them are responsive to BMP4, answering to the activation of the gene basal promoter and promoting an enhanced activity of the gene. Finally, our transgenesis studies, as well as in silico analysis, have demonstrated the presence of a responsive element to a bicoid like protein, which seems to have a fundamental role in the activation of the gene in head.

Proteolysis of the bmp antagonist short gastrulation produces a bmp activity gradient during drosophila oogenesis
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During dorsal-ventral patterning of the Drosophila embryo, the BMP family member decapentaplegic (dpp) and the BMP antagonist short gastrulation (sog) play a well established role in defining neural versus non-neural territories. Maternal expression of these genes during mid-oogenesis also affects DV patterning, by altering the level of the IκB homologue Cactus and consequently releasing the NFκB homologue Dorsal for nuclear translocation (Araujo and Bier (2000), Dev. 127: 3631-3644). We have investigated whether the protein products of sog and dpp, produced during oogenesis, are transferred to the embryo and exert their role during early embryogenesis. Using antibodies produced against different regions of the Sog molecule we show that, indeed, Sog protein is produced by the follicle cells during mid-oogenesis and is transferred to the periviteline space while the vitelline membrane is being formed. Using overexpression of different Sog fragments we find support for a model in which an activity gradient of maternal Sog isoforms could generate asymmetry reflecting both on embryonic DV patterning and chorion formation. Our results indicate that asymmetric expression of Tolloid and Tolkin metalloproteases in follicle cells may generate an asymmetric distribution of Sog fragments with differential activities that results in a BMP activity gradient along the DV axis of the pre-blastoderm embryo.

Pluripotency and self-renewal capacity of cephalic and trunk neural crest progenitors
Andrea Trentin (UFSC - Florianópolis)

Mesencephalic NC cells are heterogeneous in proliferation and differentiation capacities, comprising highly pluripotent stem-like cells able to give rise to neurons, glial cells, melanocytes and cartilage as well as diverse developmentally restricted oligopotent precursors and committed cells. In addition to pluripotency, stem cells are defined by self-renewal capacity, producing both differentiated cells and new stem cells through asymmetrical cell divisions. Therefore, we were interested in the mechanisms generating cell diversification in the neural crest (NC). In addition, we address the question as to whether quail NC progenitors are capable of self-renewal. We identified by in vitro serial clonal cultures of quail NC cells from cephalic and trunk regions, the presence of highly pluripotent cells able to give rise to neurons, glial cells, melanocytes and miofibroblasts and precursors common to glial cells, melanocytes, miofibroblasts and cartilage. In addition, the glial-melanocyte and glial-miofibroblast bipotent precursors are able to self-renew during several rounds of successive cell cloning. Endothelin-3 increases clone growth and subcloning abilities of the glial-melanocyte precursors but not glial-miofibroblast ones. These findings confirm the existence of neural-mesectodermal precursors that
can be considered as NC stem cells. Such highly pluripotent cells can, during migration and proliferation, generate precursors of more restricted developmental options and finally yield unipotent committed precursors in the sites of differentiation.

Financial Support: CAPES, CNRS

HOST CELL-PARASITE INTERACTIONS

Coord.: Marlene Benchimol (Universidade Santa Úrsula - RJ)

Early events of the interaction between tachyzoites of Toxoplasma gondii and phagocytic cells

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It has been shown that the infective tachyzoite forms of the parasitic protozoan T. gondii developed the ability to attach to the cell surface and to penetrate into all nucleated cells of warm blooded animals tested up to now. Penetration may take place by at least two mechanisms: first, stimuli leading to parasite motility in a process involving the participation of actin and myosin, and exocytosis of specialized parasite organelles, leading to parasite invasion and the formation of a parasitophorous vacuole (PV) within the host cell cytoplasm; the second mechanism involves the participation of cytoskeletal structures of the host cell, especially actin filaments, leading also to the formation of the PV. We have analyzed basic aspects of this interaction process using phagocytic (macrophages, neutrophils and eosinophils) and non-phagocytic cells. Our observations show that binding of the protozoan to the host cell surface lead to activation of protein kinases. Indeed, previous incubation of the host cells in the presence of staurosporine, a wide range inhibitor of protein kinases, tyrphostin and genistein, specific inhibitors of tyrosine kinases, significantly interfered with the process of interaction. Confocal laser scanning microscopy of cells labeled with antibodies recognizing phospho-aminoacids showed labeling at the parasite host cell interface region and in the flagellar pocket. We also obtained evidence for the participation of surface-exposed phosphatidylserine on the process of parasite internalization with subsequent secretion of TGF-beta, Smad phosphorylation, iNOS degradation, actin depolymerization and lack of nuclear factor-kB.

Acknowledgments: Pronex, CNPq and FAPERJ

Trafficking of Trypanosoma cruzi trypomastigotes in vero cells persistently infected with coxiella burnetii: invasion, transference, growth, and escape.

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In order to complete its life cycle, trypomastigotes forms of Trypanosoma cruzi, the protozoan parasite that causes Chagas' disease in humans, must invade cells, escape from the parasitophorous vacuole and once free in the cytoplasm, transform into amastigotes to grow. We have studied the intracellular fate of different T. cruzi trypomastigote forms after invading Vero cells persistently colonized with Coxiella burnetii, the
ethiological agent of Q fever and obligate intracellular bacterium that resides within acidified vacuoles with secondary lysosomal characteristics. It has been shown that the bacterium per se influenced some trafficking properties of *T. cruzi* trypomastigotes that is affected by the target organelle pH. We have now seen that in Vero cells transfected with LAMP-1-EGFP, trypomastigotes promptly incorporate the lysosomal marker and, release unusual trails following parasite migration throughout the cytoplasm. We also noticed that acidification of the parasitophorous vacuole, detected by Lysotraker incorporation is not concomitant to LAMP-1 recruitment, and vacuoles unexpectedly lose the acidic marker hours before escaping. Transferred trypomastigotes differentiate into amastigotes that may grow in the *Coxiella* vacuole and live and fixed-cell images indicate that these forms may escape the vacuole.

Financial support: FAPESP, CAPES, and CNPq.

**Interaction between malarial parasites and the mosquito vector: study of the midgut and salivary gland invasion.**

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Malaria affects 300-500 million people with 2.5 million deaths. Malaria is a serious public health problem in tropical and sub-tropical countries including Brazil. Important factors influence the vectorial competence of mosquitoes. The life cycle of *Plasmodium* parasites needs to be completed inmosquito vectors with the invasion of two target organs: the midgut and the salivary gland. In this study we investigate the invasion of these organs. We used *Plasmodium berghei* and *Plasmodium gallinaceum* in three mosquitoes: *Anopheles stephensi*, An. *aquasalis* and *Aedes aegypti*. The results were analysed by scanning laser confocal and transmission electron microscopies. The invasion of the organs is intracellular. The mechanism of midgut epithelial repair differs according to the parasite-vector pair. The damage cells were detached from the epithelium by actin-mediated displacement and the space occupied by neighbouring cells. Also, there were epithelial responses to the invasion. After escape from the midgut to the hemocelle, the parasites invade the salivary gland. Initially the anterior portion of the parasite forces its entrance in the salivary cell. Tens of thousands of parasites invade the same secretory cell. Fifteen days after the infective blood meal, the parasites completely occupy the secretory cavity of the invaded cells. Some salivary cells appear to be more susceptible to parasite invasion. Parasites form groups within the salivary gland developing special cellular junctions in order congregate. These junctions appear to protect them from saliva substances. Finally, a few parasites invade the salivary gland duct and are ready to be injected in the new vertebrate host.

**Interação de Trichomonas vaginalis com células vaginais epiteliais (vecs)**

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Trichomonas vaginalis is the causative agent of human trichomonosis, one of the most common parasitic infections in humans. They are flagellate protozoa which interact with the squamous epithelium lining the human urogenital cavities. Despite the high incidence, little is known about the pathogenesis of trichomoniasis. This parasite adheres to the epithelial cells often forming interdigitations. It has been suggested that epithelial cell damage may be produced by substances released by the parasite or through direct cell contact. Previous studies have used mainly epithelial monolayer models, such as MDCK, HeLa cell, CHO cells to study the cytopathic effect of T. vaginalis. In the report of González-Robles et al. (1995) the authors demonstrate that damage to MDCK cells occurs initially through adhesion and clumping of parasites which induce changes in the plasma membrane of target cells followed by cell death. These authors also presented one form of adhesion where formation of surface microchannels takes place. Alderete et al. (1995) claimed that the specific cytoadherence is responsible for the cell cytotoxicity and that the extent of host cell killing is related to the levels of parasite cytoadherence.

In the present report we decided to use a specific cell lineage obtained from biopsies of healthy woman vagina and also using Caco cells, and we also developed obtain a new cell lineage obtained from human vagina. The transepithelial electric resistance was compared in epithelial cells before and after parasite interaction. Several antibodies anti proteins present in junctional areas, such as ocludins, were used in order to compare modifications induced by parasite interactions, and the behavior of both lineages were compared.

Acknowledgements: PRONEX, AUSU, CNPq, CAPES, FAPERJ.

CELL NUCLEUS

Coord.: Luis Felipe Jiménez Garcia (Universidad Nacional Autónoma de México, Mexico)

Calcium signalling in the nucleus

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Free Ca²⁺ within the nucleus regulates such important functions as protein transport across the nuclear envelope, transcription of certain genes and others. However, there is controversy regarding the mechanism by which free Ca²⁺ within the nucleus is controlled. We identified a novel reticular network of nuclear Ca²⁺ stores that is continuous with the endoplasmic reticulum and the nuclear envelope. This network expresses inositol 1,4,5-trisphosphate (InsP3) receptors, and the nuclear component of InsP3-mediated Ca²⁺ signals begins in the region of this network. We also found that chelation of nuclear but not cytosolic Ca²⁺ increases the activity of TEAD transcription factor. Finally, localized release of Ca²⁺ within the nucleus causes nuclear protein kinase C (PKC) to translocate to the region of the nuclear envelope, while release of Ca²⁺ within the cytosol instead induces translocation of cytosolic PKC to the plasma membrane. These findings demonstrate for the first time that the nucleus contains a nucleoplasmic reticulum with the capacity to regulate Ca²⁺ signals in localized subnuclear regions. The presence of such machinery provides a potential mechanism by which Ca²⁺ can simultaneously regulate multiple independent processes within the nucleus.
**Atomic force microscopy of the cell nucleolus**


The nucleolus is the site of pre-rRNA synthesis and processing and ribosome sub-unit assembly. It is a ribonucleoprotein organelle composed of many molecules including proteins, rRNA and its precursors, UsnoRNAs, and rDNA genes. These components are organized ultrastructurally into three domains, i.e. fibrilar center (FC), dense fibrillar (DFC) and granular component (G), each being involved in different steps of ribosome biogenesis. We have been working in exploring the possibilities of using the atomic force microscopy to study nuclear (1,2) and nucleolar morphology at high resolution. This step would allow us to perform those studies in solution. Nucleoli from different sources as the plant *Lacandonia*, which were prepared as for electron microscopy, were cut and mounted in glass slides. Some samples were stained with toluidine blue. Atomic force microscopy (Bioscope) operating in contact mode and equipped with silicon nitride tips was used. Correlative light and electron microscopy show authentic nucleolar structures where DFC and FC are recognizable. In addition, nuclear bodies associated to nucleoli have also been observed. We are now trying to visualize the granular component to try to observe individual ribosomal particles. Supported by DGAPA-UNAM IN-221202.


**Chromatin insulator, chromatin domain formation and nuclear organization**

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Eukaryotic genome is organized into discrete chromatin domains (1, 2). It has been established that intergenic sequences are critical for tissue and time specific gene expression. Such regions known as chromatin domains possess a more open configuration which favors the following transcriptional regulatory events. We believe that chromatin domain formation represents one of the first regulatory levels in specific gene expression. In addition, cytological and biochemical studies support the idea that each domain has well defined limits and contributes to nuclear organization (3). In recent years and consistent with this notion, chromatin boundaries also known as insulator elements, have been discovered and suggested to be responsible for the particular establishment and maintenance of a chromatin domain. In our laboratories we are interested in the study of chromatin domain formation in a way to better understand critical regulatory processes and controlled modifications of chromatin structure and nuclear organization.
Processes involved in homologous chromosome recognition
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The nuclei of guinea pig spermatogonia and spermatocytes were studied by means of quantitative autoradiography, as high resolution cytochemistry, immunocytochemistry, and in situ hybridization. Our results reveal, in the nucleus of spermatogonia type B, small lampbrush structures of extended chromatin not found in other cell types. In meiotic interphase I, pairs of parallel lampbrush structures become associated by numerous filaments. The formation of the synaptonemal complex is simultaneous with the extension of chromosomal axes in a continuous leptotene-zygotene stage. The immunocytochemical localization of Dmc1 and Rad51 supports that these proteins are not involved in homology search and final pairing. Immunolocalization of DNA, RNA polymerase II, hnRNPs, snRNPs, and the trimethyl-guanosin cap of snRNAs suggests that the chromatin of lampbrush structures transcribe hnRNA and that splicing is scarce. The results of quantitative autoradiography after 3H-uridine labeling, demonstrate an intense transcription accompanied by a very slow export of RNA. In situ hybridization shows the presence of RNA in the regions of homology recognition and pairing. These results lead us to propose that the RNA synthesized in the lampbrush structures is involved in the process of homology searching and recognition.

Cell Biology Education I
Coord.: Hugo Gonzales Figueroa (Universidad Ricardo Palma, Peru)
Learning some of bioinformatics into molecular cell biology course
Gonzales-Figueroa, Hugo
Development Biology Laboratory. Faculty of Biological Sciences Ricardo Palma University. Lima, Perú hgonzales@mail.urp.edu.pe

Current knowledge of structure and cell function is due to technological innovation of resolution power, this has allowed that available tools for the investigation are more effective, making that some old concepts, sometimes, be totally reformulated. This fact, made possible the fusion of concepts and experimental techniques drawn from biochemistry, genetics and molecular biology with those of classic cell biology so that the central dogma of modern biology has become in the pivot for a dynamic conception of the cellular life.

At the present time, bioinformatics represents the interphase between traditional and computacional biology, it’s a key driver that allow scientific exploration and exploitation of previously uncharted interdisciplinary and can be axis main very well that influences in the cell biologists because their major challenge, in the next years, will be to analyze the molecular basis of integrated functions in whole organisms including learning, behavior and aging.

Regarding, it is necessary to introduce a bioinformatics segment into molecular cell biology course for all undergraduate students.
In this case, it will be include design exercises that will allow the students to use bioinformatics tools to carry out computer searches of nucleic acid and protein sequence and structure data bases. Nucleic acid sequences corresponding to specific organisms will be provided to students, and specific problems requiring analysis and comparison of these sequences will be posed. Students will learn how to access and use web-based bioinformatics programs to carry out these exercises.

**New paradigms for the teaching of biology**

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The amount of information freely available in data bases and the modern tools for retrieval present in the web have profoundly widened the spectrum of biological information to which the students can access. Concomitantly, young students have “computer based culture” preferring to read on the screen and not on the books and a tendency to zapping. The international programmes for genomics of human, animals and plants provide an enormous amount of information and tools for retrieval and analysis that have influenced Genetics, Medicine, Agriculture and Basic Biology. In particular, Phylogenetics can be more precisely elaborated because many more characters are available to analyze the evolution and homology of organisms. The everyday exposure to biotechnology news in magazines, journals and TV affecting daily life (health, food, environment) has popularized many aspects of biology. Taken together these facts lead to a profound change in the paradigm for teaching Biology. On the one hand, the huge amount of information that is obtained continuously requires an unmerciful selection of the topics to be included in the syllabus covering the traditional principles of Biology and the modern concepts recently discovered and accepted. And on the other hand, the dynamism of the teaching techniques should be able to keep the attention of the students for the longest possible time (a few minutes) to comply with their mental attitude.

**CELLBIO-INSPIRATION FOR BUILDING NEW MATERIALS**

**Coord.: José Luis Arias (Universidad de Chile, Chile)**

**EXTRACELLULAR MATRIX MACROMOLECLES FOR CONTROLLING CRYSTAL GROWTH**

José Luis Arias  
**Facultad de Ciencias Veterinarias, Universidad de Chile y Centro para la Investigación Interdisciplinaria Avanzada en Ciencias de los Materiales (CIMAT), Santiago, Chile**

Biomineralization of egg- and seashells is controlled by an intimate association of inorganic materials with extracellular matrix macromolecules. Among them, proteoglycans have been implicated in the calcification of these biominerals. The localization and distribution of particular proteoglycans in egg- and seashells, and the influence of glycosaminoglycans in the in vitro crystallization of calcium carbonate is reviewed here. The sulfate and carboxylate distribution and the configuration of the polymers affect crystal morphology. The action of these natural biopolymers could be a source of inspiration for the fabrication of novel materials with desired properties.
Bio-inspired synthesis of materials through self-assembly
Carlos Semino
Massachusetts Institute of Technology - USA

A class of self-assembling peptide nanofiber scaffolds with more than 99% water content has been shown to be an excellent biological material for cell culture and tissue constructs in vitro. Recently, we developed a new generation of material scaffolds consisting in the functionalization of these self-assembling peptides. One of them, RAD16-I (AcN-RADARADARADARADA-CONH2), has been functionalized by direct solid phase synthesis extension at the amino terminal with three short peptide motifs. These motifs are present in two major protein components of the basement membrane, laminin 1 (YIGSR, RYVVLPR) and collagen IV (TAGSCLRKFSTM). These motifs have been previously shown to promote specific biological activities such as endothelial cell adhesion, spreading, and tubular formation. Therefore, the generic functionalized peptide obtained was AcN-X-GG-RADARADARADARADA-CONH2 with each motif represented by "X". We demonstrated that these tailor-made scaffolds increased the proliferation rate and survival of human aortic endothelial cells (HAEC) as well as the formation of functional cell monolayers. In addition, these scaffolds promoted the formation of human keratinocyte proliferation and multilayer formation. Moreover, additional assays performed to evaluate endothelial cell function included LDL uptake, nitric oxide release, and production of laminin 1 and collagen IV. These experiments indicated that HAEC cultured on these scaffolds enhanced endothelial activity and promoted basement membrane deposition. This biological material would have a broad spectrum of applications for biomedical research, cancer biology and regenerative medicine.

STRESS, ANXIETY AND FEEDING BEHAVIOR: MISMATCHES AND OVERLAPS OF PATHWAYS AND PEPTIDES

Coord.: Jackson Bittencourt (USP – São Paulo)

Teneurin c-terminus associated peptide: an anxiolytic ligand with structural similarity to corticotropin-releasing factor and calcitonin
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The teneurins (ten-m, odz) are a family of four 2800-residue multidomain type II transmembrane proteins that are highly conserved in all vertebrates. The terminal 44 residues of these proteins encode for a peptide-like sequence that possesses a prohormone convertase-like cleavage site at the amino terminus, and a cleavage/amidation motif at the carboxy terminus. We have termed this sequence 'teneurin C-terminal associated peptide' (TCAP). There is about 80% sequence identity among all four versions of TCAP. The TCAP amino acid sequence possesses about 20% sequence identity to the corticotropin-releasing factor family of peptides. The amino terminal portion of the peptide aligns with the carboxy terminal region of the cryptic peptide of calcitonin prohormone whereas the carboxy terminal region aligns with the middle portion of the calcitonin peptide sequence. A detailed in situ hybridization analysis indicates that teneurin-1 gene expressing the TCAP portion is found throughout the limbic system in rats. A synthetic version of this peptide modulates cAMP accumulation in hypothalamic cell lines, and regulates proliferation. Moreover, TCAP can regulate the expression of
the teneurin protein \textit{in vitro}. Administration of the synthetic peptide into the basolateral amygdala reduces the level of the acoustic startle response in high emotionality rats, but increases the acoustic startle response in low emotionality rats. Intracerebroventricular administration of TCAP of 30 pM each day for 5 days inhibits the acoustic startle response for up to 3 weeks afterward in these animals. Moreover, it inhibits the anxiogenic actions of i.c.v.-injected CRF. Systemic injections of TCAP can also modulate locomotory and exploratory behaviours, although does not affect the HPA axis. The TCAP sequence may represent a distant paralogous lineage to the CRF and calcitonin peptide families and retain the ability to regulate stress and emotionality in vertebrates.

\textit{Unraveling the cns pathways regulating body weight homeostasis}

\textit{Joel K. Elmquist}

\textit{Division of Endocrinology, Harvard Medical School, Boston, Massachusetts, USA}

Despite the dramatic increase in the standard of living over the past century, obesity, eating disorders and cachexia continue to endanger the lives of people worldwide. Fortunately, during the last decade, there has been rapid and substantial progress towards uncovering the molecular and neural mechanisms by which these extremes of energy balance develop. Central to this research has been the identification and characterization of certain peripheral metabolic signals, including leptin and ghrelin, which serve as fundamental indices of energy sufficiency. In this talk, we will outline examples of central mechanisms that underlie the adaptive responses that occur in states of energy abundance or insufficiency. We will focus much of our attention on the hormones leptin and ghrelin, both of which act as crucial signals at either end of the energy spectrum. Finally, we will offer a model in which leptin and ghrelin interact with similar CNS circuits, including several hypothalamic and brainstem nuclei, setting into motion an integrated, coordinated and adaptive response to the particular state of energy balance and food availability. Our model predicts that over- or under-stimulation of these pathways result in obesity, anorexia or cachexia. Such responses have undoubtedly been operational throughout evolution but are now being increasingly elaborated in the world we now live.

\textit{Hypothalamic regulation of sleep and circadian rhythms}

\textit{Clifford B. Saper}

\textit{Dept. of Neurology, Harvard Medical School, Boston, Massachusetts, USA.}

The hypothalamus contains a biological clock, the suprachiasmatic nucleus (SCN), but the pattern of daily behaviors in animals may vary widely, from diurnal to nocturnal patterns, to crepuscular activity (awake in the morning and evenings), and these patterns may shift over the course of the year based upon the availability of food and external temperature. We have examined the circuitry downstream of the SCN that is responsible for organizing these behavioral patterns. The main output of the SCN is to the subparaventricular zone (SPZ), a region just dorsal to the SCN and curving back into the anterior hypothalamus, just below the paraventricular nucleus. Cell specific ibotenic acid lesions of the ventral SPZ eliminates the circadian rhythm of sleep and wakefulness and locomotor activity, but has less effect on body temperature. Lesions of the dorsal SPZ primarily eliminate the circadian
rhythms of body temperature. However, the ventral SPZ has few outputs to cell groups concerned with sleep regulation. Its main target is the dorsomedial nucleus. Lesions of the dorsomedial nucleus nearly eliminate the circadian rhythms of sleep and wakefulness, locomotor activity, feeding, and corticosteroid secretion, but not of body temperature and melatonin secretion. The dorsomedial nucleus sends glutamatergic and TRH-containing outputs to the wake-active lateral hypothalamus, including the orexin neurons, but GABAergic outputs to the sleep-active ventrolateral preoptic nucleus. The dorsomedial nucleus also receives inputs from the food regulatory system and other sites that may be important in sculpting circadian behavioral patterns. Our data indicate that the dorsomedial nucleus may be the main output site for organizing a wide range of circadian behavioral and physiological rhythms.

**CELL BIOLOGY EDUCATION II**

Coord.: Shirlei M R Pimentel (UNICAMP - Campinas)

**MITOCHONDRIA**

Coord.: Anibal Vercesi (Unicamp – Campinas)

*Molecular mechanisms of dna repair in mammalian mitochondria*

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Mammalian mitochondria contain a small circular genome that encodes for 13 polypeptides, all components of the electron transport chain. The mitochondrial DNA (mtDNA) accumulates high levels of DNA damage and mutates at significant higher rate than the nuclear DNA. These characteristics, associated with the observation that mitochondria do not remove UV-induced damage, led to the belief that mitochondria lack DNA repair mechanisms. In the past 15 years, we and others have clearly demonstrated that mammalian mitochondria remove several types of DNA damage from their genome very efficiently. We have shown that mouse and human mitochondria contain an active base excision repair pathway, which is responsible for the repair of oxidized bases, and this pathway have been reconstituted *in vitro* utilizing mitochondrial extracts. Surprisingly, mitochondria lacking mtDNA retain all the activities necessary for BER, suggesting that this pathway is constitutively active. Using a knockout mouse for the enzyme 8-oxoguanine DNA glycosylase (OGG1), which repairs the abundant base damage 8-oxoguanine, we demonstrated that mitochondria have no backup system for the repair of this lesion, and accumulate much higher levels of 8-oxoG than the nuclear DNA in these animals. However, despite the high levels of mtDNA damage, liver and heart mitochondria from these animals do not show any bioenergetic deficit, as show by oxygen consumption rates and complex II/IV activity. In order to understand how the mitochondrial isoform of OGG1 functions we generated several mutants with aminoacid substitutions in the αO helix, a domain present in the nuclear but absent in the mitochondrial isoform. Our results suggest that this domain is essential for DNA binding and catalysis, and that other aminoacids are necessary for OGG1 activity than previously established by crystallography. Lastly, we have recently identified a DNA mismatch binding protein in human mitochondrial extracts. This protein binds preferentially to G/T mismatches and small insertion/deletion loops.
These results, together with the ability of rat mitochondrial extracts to correct mismatches in a mutation reverse assay (Mason et al., 2003) provide the first evidence that mammalian mitochondria contain an active mismatch repair system.

**Role of mitochondrial ATP-sensitive k+ channels in ischemic protection and preconditioning**
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Mitochondrial ATP-sensitive K+ channel (mitoKATP) agonists are effective in the prevention of cardiac and neural damage caused by ischemia/reperfusion. Our group has focussed on the understanding of the physiological function of these channels and the mechanisms through which they promote ischemic protection. We found that activation of mitochondrial K+ transport prevents the loss of high energy phosphates during ischemia. MitoKATP opening also protects against excessive mitochondrial Ca2+ uptake and the generation of reactive oxygen species. Finally, we have determined the pathway through which mitoKATP activity is spontaneously increased during ischemic preconditioning.

**How mitochondrial complex II inhibition promotes neuronal damage**
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Changes in mitochondrial integrity, reactive oxygen species release and Ca²⁺ handling are proposed to be involved in the pathogenesis of many neurological disorders including methylmalonic acidemia and Huntington’s disease, which exhibit partial mitochondrial respiratory inhibition. In this report, we studied the mechanisms by which the respiratory chain complex II inhibitors malonate, methylmalonate and 3-nitropropionate affect rat brain mitochondrial function and neuronal survival. All three compounds, at concentrations which inhibit respiration by 50%, induced mitochondrial inner membrane permeabilization when in the presence of micromolar Ca²⁺ concentrations. ADP, cyclosporin A and catalase prevented or delayed this effect, indicating it is mediated by reactive oxygen species and mitochondrial permeability transition (PT). PT induced by malonate, methylmalonate and 3-nitropropionate was also present in mitochondria isolated from liver and kidney, but required more significant respiratory inhibition. In brain, PT promoted by complex II inhibitors was stimulated by increasing Ca²⁺ cycling and absent when mitochondria were pre-loaded with Ca²⁺ or when Ca²⁺ uptake was prevented. In addition to isolated mitochondria, we determined the effect of methylmalonate on cultured PC12 cells and freshly prepared rat brain
slices. Methylmalonate promoted cell death in striatal slices and PC12 cells, in a manner attenuated by cyclosporin A and bongkrekate, and unrelated to impairment of energy metabolism. We propose that under conditions in which mitochondrial complex II is partially inhibited in the CNS, neuronal cell death involves the induction of PT.

Supported by: FAPESP and CNPq.

**Mitochondrial dysfunction in genetically dyslipidemic mice**

Aníbal E. Vercesi

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Introduction: High plasma lipid levels are common features in arteriosclerosis. Since mitochondria have been implicated in cell death under a variety of metabolic disorders, we examined liver mitochondrial functions in two types of genetic dyslipidemias, hypertriglyceridemia (HyperTG) due to over-expression of apolipoprotein CIII and hypercholesterolemia (Hyperchol) due to LDL receptor gene knockout. Results: Liver mitochondria isolated from HyperTG mice presented lower respiratory control as compared to control mice (3.22±0.37 vs. 4.45±0.46, respectively). This effect resulted from faster resting respiration in HyperTG mitochondria and was not related to UCPs content and activity. Mitochondria from Hyperchol mice presented respiratory control and phosphorylation efficiency similar to wild type mitochondria. After calcium loading, mitochondria from both HyperTG and Hyperchol mice did not retain their electrochemical proton membrane potential. These effects were abolished by cyclosporin A, characterizing mitochondrial permeability transition (MPT). Increased reactive oxygen species and oxidized NADP were found in Hyperchol mitochondria. In vitro, reduction of NADP by isocitrate reversed the higher susceptibility of Hyperchol mitochondria to develop MPT. In vivo, ciprofibrate treatment reduced mice plasma TG levels and prevented MPT in HyperTG mitochondria. Conclusion: Hypertriglyceridemia increased resting respiration and predisposed to mitochondrial permeability transition. Ciprofibrate therapy normalized respiration and prevented MPT. Mitochondrial lower reducing power in hypercholesterolemia explains both increased reactive oxygen species content and higher susceptibility to MPT in these conditions. Financial Support: FAPESP, CNPq/Pronex.

**CELL-EXTRACELLULAR MATRIX INTERACTIONS**

Coord.: Luiz Eurico Nasciutti (UFRJ – RJ)

**Cell-extracellular matrix interactions modulate thymic physiology and pathology: the thymic nurse cell model.**

Déa Maria Serra Villa-Verde

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Thymocyte differentiation occurs in the context of the thymic microenvironment where they interact with non-lymphoid cells and extracellular matrix components (ECM). Cell migration is an important aspect in this process, as differentiating thymocytes migrate from thymic cortex to medulla. Migration of immature thymocytes within the thymic epithelial cell context can be investigated in vitro by studying thymic nurse cells (TNC), lymphoepithelial complexes in which one cortical epithelial cell harbors variable numbers of differentiating thymocytes. TNC have
been described as a special niche in thymocyte physiology. When settled in culture, TNC spontaneously release thymocytes and TNC-derived epithelial cells can reconstitute lymphoepithelial complexes after co-culture with fetal thymocytes. We showed that ECM components and receptors (VLA antigens) modulate TNC behavior in physiological and pathological conditions. For example, TNC derived from *Trypanosoma cruzi* infected mice showed increased expression of ECM molecules and were able to release thymocytes in a faster ratio than those isolated from control animals. In the case of non-obese diabetic (NOD) mice, a model of type I diabetes, a defect of fibronectin receptor (VLA-5) was accompanied by changes in TNC behavior *in vitro*. Cell interactions within TNC were also modulated by soluble proteins such as galectin-3, that interacts with -galactosides presented on the cell surface and ECM glycoproteins. We showed that this lectin can influence TNC behavior in normal and *T. cruzi* infected mice. Taken together, these data place TNC as a model to further investigate the mechanisms governing thymocyte migration in the thymic epithelial context, both in normal and pathological situations.

Financial support: CAPES, CNPq, FAPERJ, FIOCRUZ (Brazil), CNRS, Inserm, (France).

*Extracellular factors modulate neuronal migration during cerebellar development.*  
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*Departamento de Bioquímica*  
UNIFESP

During post-natal cerebellar development, granule cell precursors (GCPs), the main type of cerebellar neuron, proliferate in the external granule layer, and migrate inwards to form the internal granule layer. These events occur during the first 2 weeks post-natal in rodents, and 18 months in humans. Defects during cerebellar neuronal migration give rise to abnormal phenotypes, such as motor problems, lack of motor coordination, and balance problems. Understanding the mechanisms of neuronal migration is fundamental to understand, and eventually treat, neurological diseases caused by defects in neuronal migration. Our interest is to understand how extracellular factors control and direct neuronal migration. These factors can be soluble, such as the neurotrophin BDNF (brain-derived neurotrophic factor), or extracellular matrix (ECM) components, such as laminin and reelin. Our results show that BDNF-induced migration of GCPs *in vitro* can occur in the absence of glial cells, but requires laminin. Analysis of sulfated glycosaminoglycans expression by neurons and glia obtained from mice cerebella shows that GCPs express more heparan sulfate (HS) with different degrees of sulfation than chondroitin sulfate (CS), and that HS:CS ratio varies with age. On the other hand, glial cells express the same amount of HS and CS, apparently without changes during development. Changes in expression of extracellular components, soluble or deposited in the ECM, may regulate neuronal microenvironment, thus controlling migration.

Supported by CNPq, CAPES, FAPESP
The role of galectin-3 in the interaction between hematopoietic cells and the inflammatory microenvironment in mice infected by schistosoma mansoni

El-Cheikh, MC., Oliveira, FL., Frazão, P., Chammas, R., Takiya, C., and Borojevic, R

Galectin-3 is a multifunctional animal lectin, found in both intra- and extracellular environments. Extracellular galectin-3 modulates cell migration and adhesion in several physiological and pathological processes. Intracellular galectin-3 may interfere with proliferation and gene expression through its interaction with transcription factors. Galectin-3 is highly expressed in activated macrophages and specific subsets of lymphocytes. Here we have investigated wild type and galectin-3 deficient mice, which were infected with Schistosoma mansoni. S. mansoni elicits a well characterized macrophage-dependent granulomatous inflammatory reaction induced by soluble egg antigens. While in wild type animals, the monocyte to macrophage ratio within granulomas indicated a clear accumulation of the more differentiated cells, this ratio was inverted in galectin-3 deficient mice. Differently from the granulomatous reaction in wild type mice, the S. mansoni-induced granulomas from galectin-3 null mice displayed a diffuse fibrosis, with centrifugal collagenous fibers invading the hepatic parenchyma, where both atypical foci of lymphocytes and myeloid cells were found. Besides the local reaction to the egg antigens, i.e. the hepatic granulomas, other lymphoid tissues from the peritoneal cavity, such as spleen and mesenteric ganglia, are continuously stimulated by circulating worm antigens. Cells from spleen, mesenteric ganglia, peritoneal cavity from either infected or control wild type and galectin-3 null mice were then harvested and had their phenotype analyzed. Remarkable changes were observed both in the differentiation process of monocytes and B cells. As observed in the granulomas, there was a decrease in the number of macrophages in the periphery. On the other hand, there was a clear decrease in the number of B cells accompanied by an increase in the number of plasma cells in spleen and in the mesenteric ganglia of galectin-3 null mice. This increase in plasma cells was associated with higher concentrations of plasma IgG and IgE in the same animals. The accumulation of plasma cells in the periphery led us to investigate the mobilization of B cell precursors from the bone marrow. The compartment of c-kit+B220lowCD19low cells (B cell precursors) was significantly increased in galectin-3 null mice in the bone marrow. Preliminary results showed that survival of bone marrow derived B220+ cells from galectin-3 null animals was impaired when compared to those derived from wild type animals in in vitro assay. Galectin-3 seems to be associated with delayed maturation of macrophages and accelerated differentiation of B cells to plasma cells in peripheral sites. Further studies, would indicate if the increase of B precursor cells in the bone marrow is associated with an increase of the production or a delay in the differentiation of this population in the central hematopoietic tissue.

CELLULAR MODIFICATIONS INDUCED BY CARCINOGENS

Coord.: Maria Luiza S Mello (UNICAMP - Campinas)

Hepatic cellular changes induced by exposure to genotoxic and non-genotoxic carcinogens.

Luis Fernando Barbisan
Liver carcinogenesis chemically-induced in rodents is preceded by a variety of metabolic and molecular changes, some of which have been related to the development of preneoplastic lesions (i.e., altered foci of hepatocytes -AFH) that precede benign and malignant hepatic neoplasms. Besides rodents, AFH has been also detected in non-human primates and human liver after exposure to different hepatocarcinogenic agents (chemicals, radiation and virus). Different types of AFH have been distinguished in rats based on carcinogen-induced changes in cytoplasmatic components, such as glycogen, the endoplasmatic reticulum, ribosomes, peroxisomes and enzymatic markers. In fact, AFH represent parts of three preneoplastic hepatocellular lineages: glycogenotic-basophilic, xenomorphic-tigroid and the amphophilic-basophilic cell lineages that develop after exposure to genotoxic (DNA reactive) or non-genotoxic (non-DNA reactive) hepatocarcinogens. In special, non-genotoxic carcinogens (enzymatic inducers, peroxisome proliferators, toxicants etc.,) can promote the selective growth of different populations of spontaneously or chemically-induced AFH. Finally, hepatic preneoplasia have been used as end-point in different medium-term liver carcinogenesis models and recognized as additional evidence for carcinogenicity. Future approach in chemical hepatocarcinogenesis will focalize about specific global gene expression prolifes carcinogen-dependent (microarray technology and laser capture microdissection) associated to development and progression of AFH

Changes in nuclear phenotypes of human breast epithelial cells following treatment with benzo[a]pyrene and 17β-estradiol

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The immortalized human breast epithelial cell line MCF-10F is an important tool for studies on experimental tumorigenesis. Many relevant molecular biology data as associated with cell proliferating activity and invasiveness have been established for MCF-10F cells treated by drugs and hormones or transfected with oncogenes. MCF-10F cells treated with benzo[a]pyrene (BP) and 17β-estradiol (E-2) at equal doses have been reported to develop similar neoplastic characteristics such as anchorage-independent growth, colony formation in agar methocel, and loss of heterozygosity in several chromosomes. In the present study we demonstrate similar changes in nuclear phenotypes, defined in terms of their geometry, densitometry and textural characteristics, as assessed by image analysis, in Feulgen-stained MCF-10F cells which had been treated twice a week for two weeks with BP and E-2 at the doses of 0.007 nM and 3.6 µM. These results are attributed to similar metabolic pathways pursued by BP and E-2. Indeed, endogenous E-2 is metabolized by the cytochrome P450 enzyme isoforms, which also activate BP. (CNPq; NIH R01 CA67238, PHS, DAMD 17-00-1-0229/17-00-1-0249; FAPESP)
Thyroid stromal cells modulate FRTL-5 epithelial cells activity
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To approach the question of the functional role of stromal cells in the thyroid epithelial cells activity, we established and characterized a homogeneous stromal cell population of rat thyroid gland (TS7 cells). These fibroblastoid cells presented many short processes and expressed the cytoskeleton proteins α-smooth muscle actin and vimentin, and the extracellular matrix components laminin, fibronectin, type IV collagen, decorin, chondroitin sulfate and heparin sulfate. Co-culture assays were performed with FRTL5 thyroid epithelial cells cultivated on TS7 cells monolayer. They formed follicular-like structures of different sizes and proliferated more than cells plated on glass coverslips, skin fibroblasts (SF), bone marrow stromal cell line (S17 cells) and fetal liver stromal cell line (AFTO24 cells). Then, iodine uptake activity was analyzed, and, interestingly, it was observed that TS7 stromal cells induced a negative iodine uptake by FRTL5 cells. In conclusion, interactions between TS7 stromal cells were required for the morphology organization, proliferation, iodine uptake and probably thyroglobulin secretion of FRTL5 cells, and can be very useful to study the intercellular communication and the effects of ECM components and cytokines/growth factors on the modulation of thyroid secretory cells.

Support: Millennium Institute for Tissue Bioengineering, CNPq, FUJB, FAPERJ.

Detection of early response genes in rat adrenal stimulated by acth and FGF2 using infusion in situ system.
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In order to obtain further information on the trophic mechanism of action of ACTH (adrenocorticotropin) and FGF2 (fibroblast growth factor) in an architectural-preserved intact gland system, we used an in situ isolated rat adrenal infusion technique. We analyzed early response genes expression, fos, jun and myc families of proto-oncogenes related with G0-G1-S transition of cell cycle to insight the composition of complex AP-1. Sprague-Dawley male rats were anesthetized and the left adrenal gland was isolated with clamps and the abdominal aorta cannulated. The gland was provided with DMEM/heparin (30min) and then treated (2h) with 10^{-7} M ACTH(A) or/and 20ng/ml FGF2(F) using an infusion pump (200µl/min). The glands were fixed, embedded in paraffin, cut at 6µm and the protein expression was determined by immunohistochemistry. Without stimulation Fra-2 and c-Jun proteins were detected in medulla but not in the adrenal cortex of control rats. Glands treated with ACTH
showed Fra-2 and c-Jun expression in ZF and ZR but not in ZG while FGF2 treatments showed c-Jun protein expression also in ZG. A+F combination showed increase in the c-Jun levels in ZR and medulla but down regulation in ZF. In conclusion ACTH and FGF2 are capable of differentially regulating c-Jun protein and this situation could result in change in the ratio of protein forming the AP-1 complex. Support by CAPES, FAPESP, UNIDERP E FMB.

**STI1 binding to cellular prion protein modulates glioma proliferation**

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Malignant gliomas are aggressive and highly invasive. In glioblastoma, median survival ranges from 9 to 12 months. Mechanisms of tumorigenesis include disregulation of both proliferation and programmed cell death, and therefore the identification of trophic systems with action upon tumor cells may be of importance. Previous studies identified stress-inducible protein 1 (STI1), a co-chaperonin, as a cellular prion protein (PrPc) ligand, that triggers neuroprotection in retinal explants. The present work examines the effect of STI1 upon the proliferation of a human glioblastoma cell line (A172). STI1 expression was detected by Western blot analysis and the presence of PrPc at the cell surface of this tumor cell was detected by cytofluorometry. Conditioned culture media assayed by Western blot showed that STI1 is also secreted. To investigate the effect of STI1 upon tumor proliferation we assayed radioactive thymidine uptake in cell cultures treated for 24 hours either with or without mouse recombinant STI1 protein. These experiments showed an increased thymidine uptake when treated cells were compared with those untreated. The effect of STI1 was abolished by simultaneous treatment with an anti-PrPc antibody. These results support the hypothesis that the increase in cell proliferation induced by STI1 depends on endogenous PrPc. In conclusion, our data show that STI1 modulates the proliferation of a human glioblastoma, upon engagement of PrPc, possibly through an autocrine/paracrine mechanism.

Financial support: CNPq; FAPERJ; FAPESP; FUJB.

**SHP-2 is a potential modulator of FAK activity by mechanical stress in cardiac myocytes.**

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We have previously demonstrated that focal adhesion kinase (Fak) is rapidly activated and coordinate the initial activation of hypertrophic genetic program in cardiac myocytes in response to mechanical stress. Here, we examined whether the tyrosine-phosphatase SHP-2 interacts with FAK in the myocardium and in cultured neonatal rat ventricular myocytes (NRVM). Fak was highly expressed, but barely phosphorylated at Tyr-397 (activity) at baseline in myocardium or NRVM. Co-immunoprecipitation assay showed that FAK is highly associated with SHP-2 at baseline in both models. Mechanical stress, represented by aortic constriction or cyclic stretch of NRVM promptly activated Fak, as detected by phosphospecific antibody against Tyr397. This was paralleled by a reduction of FAK/SHP-2 association.
Immunohistochemistry and confocal microscopy analysis showed that both FAK and SHP-2 were located most at sarcomeric A-band in both models. Mechanical stimuli led FAK to be re-located to sites such as costameres, Z discs and nuclei, while SHP-2 remained localized at the A-Band. Thus, the results of the present study indicate that in cardiac myocytes inactive FAK is associated with SHP-2 while FAK activation by mechanical stimuli is accompanied by a reduction in this association. These data indicate that inactivation of SHP-2 might be an important mechanism for FAK activation by mechanical stress in cardiac myocytes.

Modulation of rab5a during endocytosis inhibition by okadaic acid involves erk 1/2 activation in hct-116 cells.
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The functioning of the endocytic pathway is regulated by the activity of several kinases, phosphatases and GTPases. Among them, the small GTPase Rab5 plays key roles in this process. Rab5 was showed to be regulated by Erk 1/2 through GDI phosphorylation and to be phosphorylated in vitro by Erk 1/2. Okadaic acid (OA), a well known inhibitor of protein phosphateses 1 and 2A was previously observed to inhibit both endocytosis and activate Erk 1/2. Thus, the aim of this study was to evaluate OA effects on endocytosis, Erk 1/2 activation and the function of Rab5A. We observed that OA caused inhibition of vesicle fusion and HRP ingestion by HCT-116 cells using enzymatic cytochemistry and HRP enzymatic assay. This drug caused translocation of Rab5A from membrane to cytosolic fractions as observed by subcellular fractioning and immunoblotting and promoted activation of Erk 1/2 as revealed by pErk 1/2 immunoblots. Treatment with the Mek-1 inhibitor PD98059 prevented OA effects on Rab5A translocation. Furthermore, alterations at Rab5A phosphorylation state due to OA treatment were found. Finally, we propose a mechanism of signaling transduction in which okadaic acid performs its effects on endocytosis by Rab5A modulation, in an Erk 1/2 dependent manner.

Human colostral phagocytes are able to ingest and kill Giardia lamblia trophozoites
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Giardiasis is a worldwide distributed parasitosis and it is more prevalent in children up to four years of age, especially in collective environments. Breast-feeding, provide the newborn several immunological active components involved in anti-infectious protection. The objective of this study was to determine the phagocytical activity of polymorphonuclear (PMN) and mononuclear (MN) cells present in the human colostrum and verify the influence of opsonins in the adherence, ingestion and killing of trophozoites of Giardia lamblia. PMN and MN phagocytes were incubated with trophozoites of G. lamblia, in the presence or absence of supernatant of human colostrum (opsonins source) for 120 minutes. The trophozoites/phagocytes rate was 1:1, and the percentage of trophozoites
phagocytosed was determined by microscopic examination of acridine orange stained cells. The MN phagocytes presented greater functional activity more effective than PMN. The largest indexes of ingestion (68.90 ± 5.50) and kill (48.50 ± 4.85) were obtained with incubation of MN in the presence of colostrum supernatants. The phagocytes of the human colostrum are able to ingest trophozoites of *G. lambia* and presented microbicidal activity *in vitro*, suggesting that these phagocytes could act as an additional mechanism of protection against infant giardiasis through breast-feeding.

**CELL DEATH**
Coord.: Rafael Linden (UFRJ – RJ)

**PLASTICIDAD NEURONAL: DEL DESARROLLO A LA PATOLOGÍA.**
Coord.: Luis Barbeito (Instituto Clemente Estable – Uruguai) y Vivaldo Moura Neto (UFRJ RJ)

*Microtubule-associated protein 1b function in axonal growth, neuronal migration and axonal guidance.*
Christian Gonzalez
*Instituto Milenio de Estudios Avanzados, CBB, Universidad de Chile*

Microtubule-associated protein 1B is the first MAP to be specifically expressed during development of the nervous system. In order to ascertain the in vivo roles of such a protein, we have analyzed a MAP1B loss-of-function obtained through the gene trapping approach. MAP1B deficient mice die perinatally due to several brain malformations. Neurons derived from mutant animals display shorter axons as compared with their control littermates. Additionally, less microtubules and disruptio of the balance between dynamic and stable microtubules was found as well. The differences in the morphology of neurons and the consequences upon brain architecture lead us to analyze the consequences of MAP1B disruption in neuronal migration and axonal guidance. We have set our efforts in analyze how Reelin and Netrin-1, two canonical signals for neuronal migration and axonal guidance respectively can alter MAP1B function. Thus, both signals were able to induce phosphorylation of MAP1B, mostly dependent on GSK3 function. Moreover, both the reeler and Netrin-1 mutants showed decreased levels of MAP1B phosphorylation. We will discuss about functional consequences for MAP1B loss-of-function and how MAP1B phosphorylation could be important for both neuronal migration and axonal guidance.

**CELLULAR THERAPY**
Coord.: Sara T. O. Saad (UNICAMP - Campinas)
BIOLOGY OF REPRODUCTION

Coord.: Luiz Renato França (UFMG – Belo Horizonte)

**Molecular mechanisms involved in mammalian sperm-egg fusion.**

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Rat epididymal protein, a member of the CRISP (Cysteine-Rich Secretory Protein) family, associates to the sperm surface during maturation, migrates to the equatorial segment with the acrosome reaction, and participates in sperm-egg fusion through complementary sites on the egg surface. Results from our group indicate that both the epididymal mouse (AEG-1) and human (ARP) homologues of DE also participate in gamete fusion through specific binding sites localized on the fusogenic area of the corresponding eggs.

To gain insights into the molecular mechanisms involved in the interaction of DE with its egg binding sites, rat DE was expressed in a bacterial system. Comparison between the native and recombinant protein (recDE) indicated that while carbohydrates would not have a role in DE-mediated gamete fusion, disulfide bridges are required for the conformation and full biological activity of the protein. To identify the active domain of DE, different fragments of the protein were bacterially expressed and examined for their biological activity. Results indicated that the domain responsible for the interaction of DE with the egg would be located in the region spanning from aminoacids 62 to 158. Further studies are being currently undertaken to identify the minimum domain containing the biological activity of DE. Recent evidence indicates that while DE would not be involved in the subsequent step of egg activation, it might have a role in the previous stage of sperm-zona pellucida binding. These results will provide important information for both a better understanding of the molecular mechanisms involved in sperm-egg interaction and the potential use of epididymal proteins for contraceptive development.

**Regulation of the onset of postnatal Leydig cell differentiation by thyroid and anti-Müllerian hormones**

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Androgens are important for the general health and reproductive functions of the male mammal and Leydig cells in the testis interstitium are their main source. The establishment of the Leydig cell population in the postnatal testis begins during the prepubertal life and it is established that these Leydig cells, i.e. the adult population of Leydig cells, are differentiated primarily from the peritubular mesenchymal cells in the testis interstitium. In the Leydig cell lineage, five distinct cell stages are identified, namely, the mesenchymal cells (i.e. stem cells), progenitor cells, newly formed adult Leydig cells, immature Leydig cells and mature Leydig cells. At the onset of the process of postnatal Leydig cell differentiation, a mesenchymal cell which is a non-steroidogenic and a spindled-shaped cell differentiates into a progenitor cell which is still spindle-shaped in configuration but has a limited potential for steroidogenesis; to date, it is not clear what triggers this process. Recent research has revealed that this process is arrested under hypothyroid conditions and accelerated with hyperthyroid conditions, suggesting that
thyroid hormones have a positive regulatory role in this process. Nevertheless, anti-Mullerian hormone (AMH) secreted by the immature Sertoli cells is considered to be a negative regulator of this process. Based on these facts, we hypothesized that thyroid hormones and AMH could act directly on the mesenchymal precursor cells to trigger and inhibit, respectively, their differentiation. As thyroid hormones act on Sertoli cells to induce maturation, it is also a possibility that AMH production by the Sertoli cells is inhibited with their maturation. If this is the case, withdrawal of the inhibitory effect of AMH could possibly trigger the onset of mesenchymal cell differentiation. In this presentation, these concepts will be addressed using findings from rat studies.
Poster Session
Abstracts
A-001 LECTINS KM+ AND CONCANAVALIN A INDUCE RECRUITMENT OF MAST CELLS FROM THE BONE MARROW TO THE MESENTERY IN RATS
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Mast cell precursors migrate from bone marrow to repopulate the peritoneal cavity after mast cell depletion with distilled water. This study investigated the ability of Concanavalin A (ConA) and KM+ to recruit mast cells from bone marrow to the mesentery. Rats were injected intraperitoneally (IP) with ConA (10µg/4ml PBS) or KM+ (10µg/4ml PBS) 24h after mast cell depletion with distilled water. Mast cells were identified either by Toluidine blue staining or by immunomagnetic isolation with the mast cell specific antibody AA4. Seven days after injection of ConA into non-depleted animals there were twice as many immature mast cells near mesenteric blood vessels as in control animals. At 7 days, depleted rats injected with ConA had half of the number of immature mast cells in the mesentery as compared to non-depleted rats. 7 days after IP injection of ConA in both depleted rats and non-depleted rats, there was an increase in the number of AA4+ cells in bone marrow. After 7 days, depleted rats injected with KM+ showed a 5X increase in the number of immature mesenteric mast cells and the number of bone marrow AA4+ cells was doubled. These results suggest that ConA and KM+ induced the recruitment of immature mast cells from the bone marrow to the mesentery.

Financial support: FAPESP

A-002 LECTINS KM+, CON A AND WGA INDUCE AN INCREASE IN MAST CELLS IN THE BONE MARROW AFTER PERITONEAL CAVITY DEPLETION
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Mast cells originate from bone marrow and are thought to migrate to peripheral tissues through the blood stream. The mechanisms by which they are recruited from the bone marrow to other sites remain unclear. Wistar rats were injected intraperitoneally (IP) with distilled water to deplete the peritoneal cavity of mast cells. Two days later they were injected IP with 10µg/4ml ConA, WGA or KM+ in PBS. Controls received either PBS or distilled water. Eight days later, femoral bone marrow and peritoneal lavages were collected. Pure populations of mast cells were obtained by immunomagnetic isolation using the mast cell specific monoclonal antibody AA4 conjugated to magnetic beads. Isolated mast cells were quantified using a hemocytometer. Cells from the peritoneal lavage were stained with Toluidine blue and the mast cells were quantified. Peritoneal lavages of rats injected with ConA and WGA after depletion showed a very low percentage of mast cells when compared to the controls, but KM+ resulted in 106%. All of the lectins induced an increase in AA4+ cells in the bone marrow, but only KM+ was effective in causing mast cell migration into the peritoneal cavity. Perhaps ConA and WGA require interaction with peritoneal mast cells releasing mediators that recruit bone marrow mast cells.

Financial support: FAPESP

A-003 ENDOGENOUS EXPRESSION OF GALECTIN-1 AND –3 IN NEUTROPHIL DURING RECRUITMENT PROCESS
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Galectins are a growing family of β-galactoside-binding lectins with immunoregulatory functions, modulating cell adhesion, survival and pro-inflammatory cytokine secretion. In the present study we monitored galectin-1 (Gal-1) and -3 (Gal-3) in human and rat neutrophils (NPs) using a combination of in vitro (transmigration assay induced by IL-8) and in vivo models (rat peritonitis induced by carrageenin - 4 hours). Human NPs and fragments of rat mesentery were fixed (4% paraformaldehyde and 0.5% glutaraldehyde) and embedded in LRGold. For immunocytochemical studies, human NPs and rat tissues were incubated with rabbit serum anti-Gal-1 or -3. Both models demonstrated a modulation of Gal-1 and -3 expressions in NPs during recruitment process. An intense Gal-3 immunoreactivity was detected to non-transmigrated human NPs. In contrast, a decrease in Gal-1 was observed in transmigrated cells. Similarly, NPs adherent to the postcapillary endothelium in the inflamed mesentery presented a significant increase in endogenous Gal-3, whereas a reduction in Gal-1 was detected in transmigrated NPs. Probably the increase in endogenous Gal-3 may be important to activate NPs that could release Gal-1 to inhibit leukocyte rolling and extravasation. In conclusion, endogenous regulation of galectins in NPs suggests an anti-inflammatory role for Gal-1 and pro-inflammatory for Gal-3.

Financial Support: FAPESP, FAMERP.

A-004 GANGLIOSIDES MODULATE CELL MIGRATION AND CELL SURVIVAL
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Upon tumor progression, melanoma cells accumulate disialoganglioside derivatives. The function of these molecules in tumor progression remains unknown. To address possible functions of the disialoganglioside GD3, the murine melanocyte cell line, Melan-a, was transfected with the GD3 synthase gene, LacCer-ve)/GM3 (+ve) cells, suggesting that GM3 plays a migratory role of GD3. Therefore, we propose that GD3 plays a negative role in cell migration. We next examined the effect of exogenously added gangliosides. Addition of GD3 to Melan-a cells also inhibited cell migration; whereas exogenously added GD3 promoted it. Taken together, our results suggest that the ratio between GM3 and GD3 in melanocytes modulates migration. We have also uncovered a pro-apoptotic function of GD3 in murine melanomas, as we failed to achieve stable expression of GD3 in melanoma cells due to their increased sensitivity to cell death inducing agents. Therefore, we propose that persistent GD3 expression may select cells resistant to cell death, which in turn acquire a more migratory phenotype.

Support: FAPESP, CNPq and TWAS.
The skin covering the body and fins of fish is composed of the epidermis (stratified epithelium) and dermis (conjunctive tissue). When cut, the fins of the teleostei quickly regenerate. On the first day of regeneration, the cells of the epidermis start migrating to cover the amputated extremity, forming a thin epidermal layer. On the second day more epidermal cells are added, resulting in an increase in the layers and consequent thickening of the epidermis, leading to the formation of the epidermal cap. In this study, the regeneration of the epidermis was studied in young carp maintained at a temperature of 26°C (control) and in young carp maintained at temperatures of 18 and 14°C. In the control fish regeneration occurred normally as described above. However in the animals kept at 18 and 14°C there was a considerable delay in cellular migration at the beginning of regeneration (1st and 2nd days) and only after the 4th day of regeneration was the epidermal cap formed. This delay in epidermal regeneration showed that the low temperature affected cellular migration and consequently the normal regeneration of the carp fin epidermis. These findings are in agreement with the studies of Johnson & Weston (1996) who showed slower growth in fish during the winter season.

Cell-cell adhesion loss is a crucial step in carcinoma development, enabling cells to detach and migrate, contributing to invasion and metastasis process. Adherens junctions (AJ), which are responsible for homophilic calcium-dependent contact between adjacent cells, and tight junctions (TJ), which are responsible for paracellular permeability regulation and maintaining of cell domains and polarity, are part of junctional complex. AJ and TJ participates of many signaling pathways. The objective in this study was access the intracellular signaling alterations related to the loss of cell-cell adhesion using normal and tumoral human colorectal tissue samples. A loss of cell-cell contact was observed in the tumoral tissue by transmission electron microscopy. E-cadherin, the main adherens junction molecule, had its expression diminished in the colorectal carcinomas, whereas claudins-1, -3 and -4, which are tight junction proteins, were overexpressed in tumoral tissue, by immunoblotting analysis. These alterations were concomitant with an overall increase in PI3K and src expression in the colorectal carcinoma, but no alteration were observed in MAPK expression, as well as on its phosphotyrosine contents. These results indicates a possible role of PI3K and src in the loss of cell adhesion in this cancer type. These results also indicate that AJ and TJ are modulated by different signaling pathways.

A-005 EFFECT OF TEMPERATURE ON THE REGENERATION OF CARP FIN EPIDERMIS
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A-006 SIGNALING PATHWAYS INVOLVED IN CELL-CELL ADHESION LOSS IN COLORECTAL CARCINOMA
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A-007 EFFECT OF BOTHROPS JARARACA (BJV) SNAKE VENOM ON THE ACTIVITY OF MACROPHAGE (MØ) PEPTIDASES. IN VITRO STUDIES.
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Aminopeptidases (APs) are a family of enzymes relevant for modulation of the activity of several inflammatory mediators released from MØ. In this study the in vitro effect of BJV on MØ soluble and membrane-bound peptidases APN/CD13 (APN), APB and DPPIV was evaluated. Peritoneal MØs were collected 96h after injection of 3% thioglycollate into peritoneum of male Swiss mice. 4x10⁶ MØ/mL were then incubated with BJV (5µg/mL) or PBS (control) for 1h. The APs activities were measured by fluorogenic assay using naphthylamide-derivative substrates. Results showed that BJV did not modify the activities of APN and APB in both cell fractions, in comparison to controls. However, this venom significantly reduced the membrane-bound DPPIV activity, and increased that activity founded in the soluble fraction. It is concluded that BJV can directly modulate the activity of MØ DPPIV but not those of APN and APB. BJV-induced effect on DPPIV is related to the enzyme cellular location. Since BJV induce a prominent inflammatory reaction, and DPPIV regulates cell adhesion processes and inactivates IFN-γ, this enzyme may play a role in the inflammatory effect induced by the venom.

Financial support: FAPESP

A-008 INHIBITION OF NEUTROPHIL MIGRATION IN SYSTEMIC INFLAMMATORY RESPONSE SYNDROME
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Sepsis is a systemic inflammatory response commonly caused by bacterial infection. In severe sepsis induced by endotoxemia and by cecal ligation and puncture (CLP) a failure of neutrophil (NΦ) migration to the inflammatory site was observed. NΦ migration is extremely important in the control of bacterial growth by preventing bacterial dissemination. In this study, we investigated in the peritoneal lavage and mesentery whether the failure of NΦ migration occurs during murine septic shock induced by CLP. The sublethal (SL-CLP) and lethal (L-CLP) groups consisted of animals with 3 and 12 punctures in the cecum, respectively. The intra- and extravascular numbers of NΦ were analyzed 4 h after CLP. The mesentery fragments and peritoneal cells were fixed (4% paraformaldehyde; 0.5% glutaraldehyde), embedded in LRGold and stained with Diff Quick. In the L-CLP there was a significant reduction in the NΦ suggesting a reduction in cell adhesion mechanisms. In the SL-CLP, there was a massive NΦ migration. Consequently these animals exhibited efficient bacterial clearance, infection control, and a high survival rate. In conclusion, we used pharmacological and morphological techniques to demonstrate that failure of NΦ migration is an important event in the evolution of CLP sepsis.

Financial support: FAPESP

A-009 INHIBITION OF NEUTROPHIL MIGRATION IN ACUTE PERITONITIS USING POLYMORPHONUCLEAR GRANULOCYTE (PMN) PHAGOCYTOSIS IN VITRO STUDIES.
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P. aeruginosa is a Gram-negative bacillus that produces a number of virulence factors which enable it to colonize and cause disease. It is a major problem in hospitals, in patients suffering from cystic fibrosis, or who are immunocompromised. In particular, infection with P. aeruginosa in the lungs of patients with cystic fibrosis results in severe pulmonary disease with increased mortality. In this study, we investigated the mechanisms responsible for the decreased migration of PMN in the presence of P. aeruginosa. We found that P. aeruginosa was able to inhibit the migration of PMN in a dosedependent manner. This inhibition was reversed by the addition of phagocytosis stimuli, such as opsonins or chemotactic factors. The results of this study suggest that P. aeruginosa is able to inhibit PMN migration through a mechanism involving phagocytosis.
A-009 BETA 2 INTEGRIN EXPRESSION DURING CARDIOGENESIS
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Adhesion receptors play a key role in a variety of functions during embryonic development. Among these functions are processes involving cellular migration, differentiation and morphogenesis. Integrins are the major family of surface receptors that mediate attachment of cells to the extracellular matrix. Integrin receptors are heterodimers composed of alpha and beta transmembrane subunits that link to intracellular cytoskeletal and signal transduction complexes. Within the integrin family is a subfamily which contains beta 2 integrin (CD18) in conjunction with one of several different types of alpha subunits. This subfamily has been reported to be primarily expressed on the surface of leukocytes, where it mediates adhesion to endothelial cells. The gene for CD18 is encoded on chromosome 21. Individuals with Down Syndrome, in which there is an extra copy of chromosome 21, display a reduction in the number of mature lymphocytes, which may involve high expression of CD18 on thymocytes. In addition, approximately 40% of Down Syndrome patients have congenital heart defects, most commonly atrioventricular valve malformations. Since there have been no studies on CD18 expression in the embryonic heart, we used immunofluorescence procedures to determine its distribution pattern. We observed CD18 expression in the endothelial and mesenchymal cells of the atrioventricular valve during its formation. This suggests that an overexpression of CD18 during heart formation may be associated with the congenital heart defects observed in Down Syndrome.

A-010 EFFECT OF CYTOKINES AND CHEOKINES UPON SICKLE NEUTROPHIL ADHESION TO FIBRONECTIN
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A role for leukocytes in sickle cell vasooclusion is becoming increasingly recognised. Neutrophil counts are higher in sickle patients and neutrophils from these patients demonstrate increased adhesion to endothelial monolayers. The effects of the cytokines IL-6, G-CSF and IL-8 upon the adhesion mechanisms of normal neutrophils and neutrophils from sickle cell anaemia patients (SCA neutrophils) were investigated. Neutrophils were separated from the blood of homozygous (HbSS) SCA patients and healthy controls. Following pre-incubation (25 min, 37°C) of the cells with cytokines, the adhesion of the cells to fibronectin (FN)-coated plates (20 µg/ml) was determined (60 min, 37°C, 5 % CO2). Basal adhesion of normal and SCA neutrophils to FN was not statistically different (4.8 ± 0.6 % and 4.7 ± 0.6 %, respectively). Pre-treatment of normal neutrophils with either IL-6 (10-100 pg/ml), GCSF (1-10 ng/ml) or IL-8 (1-100 ng/ml) had no significant effect upon their adhesion to FN. In contrast, SCA neutrophil adhesion to FN was increased significantly by 62.9 ±24.7 % (P < 0.001) following pre-incubation with IL-6 (100 pg/ml), by 37.7 ± 18.3 % with G-CSF (1 ng/ml, P < 0.01) and by 90.3 ± 36.3 % with IL-8 (10 ng/ml, P < 0.01). In conclusion, SCA neutrophil adhesion may increase in the presence of certain cytokines, in vivo, and this activation may contribute to the pathophysiology of the disease.

A-011 INCREASED ADHESIVE MECHANISMS IN SICKLE EOSINOPHILS
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Vaso occlusion of blood vessels is the hallmark of sickle cell anemia (SCA). A role for leukocytes in vasoocclusion is becoming increasingly recognized. Here we investigate a possible role for the eosinophil in SCA. Analysis of whole blood samples of SCA demonstrated that absolute eosinophil numbers were significantly elevated in these individuals vs. healthy individuals. Furthermore, eosinophils isolated from a random selection of these SCA patients demonstrated a significantly greater adhesion to fibronectin (FN) than normal eosinophils in static adhesion assays. Co-incubation of eosinophils with integrin-blocking monoclonal antibodies in adhesion assays showed that an association of the VLA-4, LFA-1 and Mac-1 integrins mediate the adhesion of SCA eosinophils to FN. Incubation of eosinophils with cytokine GM-CSF further increased SCA eosinophils adhesion to FN, but not control eosinophil adhesion, indicating that inflammation process may further stimulate eosinophil adhesion in these patients. Flow cytometry demonstrated that the expression of these integrins, however, was unaltered on surface of SCD eosinophils, suggesting that the increased SCA eosinophil adhesion is a consequence of increased integrin affinity or avidity. In conclusion, we report evidence to may indicate a role for the eosinophil in vasoocclusive process.

A-012 JARRAHAGIN STIMULATES EPITHELIAL CELL MIGRATION, PROMOTES ACTIN POLYMERIZATION, FAK PHOSPHORYLATION AND THE RECRUITMENT OF SPECIFIC INTEGRINS TO FOCAL CONTACTS.
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Objectives: Jararhagin (JG) is a toxin from the venom of Bothrops jararaca that has different domains such as the metalloprotease and disintegrin. In this study we investigated the effects of JG on epithelial cell adhesion and migration in vitro, using an epithelial restitution experimental model. Methods and results: F-actin arrangement and the distribution of laminin, fibronectin, several integrins and phosphorylated FAK were studied using rhodamine-plaoidid and immunofluorescence. Maximum stimulation of migration (about 100%) was obtained with 5 µg/ml JG, with about 38% inhibition of cellular adhesion. In migratory cells the toxin stimulated the formation of filopodia, lamellipodia and stress fibers. The pericellular fibronectin matrix was lost in migrating cells, while laminin was less affected. The toxin stimulated FAK phosphorylation and the recruitment of αv-containing integrins to focal contacts, whereas integrins containing the α2 subunit were reduced in these junctions. Inactivation of the toxin with 1,10 phentanolidine showed that the catalytic activity is important for the effect of JG on cell migration, FAK phosphorylation and for the recruitment of αv, but not as much for the anti-adhesive effect. Conclusion: Jararhagin stimulates the migration of epithelial cells in vitro through a mechanism that involves its proteolytic activity, qualitative changes in cellular adhesion and the formation of actin-rich cellular processes.

Financial support : FAPESP, CNPq.
A-013 ANALYSIS OF THE RELATIONSHIP BETWEEN MYOSIN Va AND CELLULAR MIGRATION.
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Myosin Va (MVA) is a actin-based molecular motor involved in various processes, including membrane trafficking and vesicular transport. We have studied the involvement of MVA with cellular migration in mouse melanoma cell lines B16-F10 (wild-type for MVA) and S91-6-Cloudman (null mutant of MVA). The cell lines were transfected with MVA tail domains from mouse brain (C2) or melanocytes (M2) as well as chicken full-length myosin-Va construct (FL-MVa), all fused to EGFP. The analysis of cellular migration up until 72hs showed that non-transfected B16 cells, cells transfected with EGFP, or cells transfected with complete MVA showed higher values when compared to B16 cells transfected with C2 and M2. The distance migrated by M2 transfected B16 cells was lower than all the other transfected B16-F10 cells. Analysis of non-transfected S91 cells indicated a lower migration than non-transfected B16 cells while the transfection of S91 cells with FL-MVA was partially able to restore the migration. The transfection of S91 with C2 and M2 showed a decrease in migration in comparison with the others. The decrease of cell migration in C2 and M2 transfected B16 cells and the increase of S91 migration capacity after the transfection with FL-MVA indicate involvement of MVA in the process of cell migration. Financial support from FAPESP, CNPq, FAPEPA.

A-014 OSTEOSTMIS ADHESION TO FIBRONECTIN-COATED SURFACES IS MODULATED BY THE PH IN WHICH THE PROTEIN WAS ADSORBED
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Taking on account that the pH of a solution where fibronectin (FN) is found may directly determine its conformation, we decided to study the adhesion of human osteoblasts to substrates coated with FN at different pH values: pH 4.5, in which the glycoprotein aggregates; pH 7.2, a value in which FN acquires a globular conformation; and pH 11.0, in which FN acquires an extended conformation. The interaction between osteoblasts and FN-coated substrates resulted in similar adhesion rate profiles for each condition tested, although the dynamics of the phenomenon was quite different. After 30 minutes of cell-FN interaction, most cells were spread onto FN adsorbed at pH 4.5 or 11.0 but many osteoblasts were still round when FN was adsorbed at physiological pH. Focal adhesion parameters, such as stress fiber formation, were also more developed in cells adhered to FN adsorbed at pH 4.5, followed by those seen in cells adhered to FN adsorbed at pH 11.0. Even more, FN aggregation at pH 4.5 appears to stimulate an increased cell spreading after 30 minutes of cell-FN interaction, while a higher rate of cellular protrusion development was observed in cells adhered to FN adsorbed at pH 11.0 than those adsorbed at physiological pH. An unusual actin structure, resembling microfilament irradiation centers, was also observed during human osteoblasts adhesion to FN-coated substrates.

A-005 JARARHAGIN PROMOTES ACTIN POLYMERIZATION AND INCREASES LEUKOCYTE ADHESION TO EXTRACELLULAR MATRIX AND ENDOTHELIAL CELLS.
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Objectives: The snake venom metalloproteinase-disintegrin Jararhagin (JG) has no chemotactic activity but stimulates the migration of epithelial cells in vitro and neutrophils in vivo, through a mechanism still unclear. In this study we analyzed the effects of JG on leukocyte adhesion to several substrata, as well as their filamentous actin (F-actin) organization. Methods and results: Blood samples were collected from Wistar rats and the leukocytes were isolated using a discontinuous gradient of Percoll®. Neutrophils-enriched fractions (98%) were treated with different concentrations of JG (0,31 to 10µg/ml) and submitted to adhesion assays using treated dishes, basement membrane extract (Matrigel®) and non-stimulated murine endothelial cells (tEND) as substrata. Controls consisted of unstimulated cells and cells treated with 1 µM n-formyl-methionyl-leucyl-phenylalanine (fMLP). The number of adherent cells was evaluated through myeloperoxidase activity, whereas rodamine-phalloidin was used for F-actin staining. The toxin increased cellular adhesion to treated dishes and tEND in concentrations above 0,31µg/ml after 30 and 90 minutes, respectively, but not in a concentration-related manner. Adhesion to Matrigel was increased after treatment with 2,5µg/ml JG during 30 minutes. Under the same conditions, JG clearly stimulated cortical actin polymerization in adherent neutrophils. Conclusions: JG promotes actin polymerization and stimulates the adhesion of neutrophils to different substrata. Financial support: FAPESP, CNPq.

B-001 HEMATOLOGICAL ALTERATIONS IN THE GESTATION OF MICE 2ª PROGENY INDIRECTLY EXPOSED TO THE ASSOCIATION OF THE 2,4 DICHLOROPHENOXYACETIC ACID HERBICIDES AND GLIFOSATE (COMMERCIAL FORMULATIONS)
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Hematological alterations in the gestational period represent a factor of great importance in normal embryonic and fetal development. Anaemia, hemorrhage and immunosuppression are some alterations that can occur in this period, that can cause anoxia, infections and reabsorption of fetus. The objective of this study was to evaluate the hematological alterations caused by the indirect exposition of pregnant mice to an association of the 2,4-D herbicides and Glifosate. The mixture was administered in the concentrations of 15mg/kg, 75mg/kg and 150mg/kg. Control group was treated with clean tap water. Samples of total blood were obtained by puncture from the abdominal artery and then treated with EDTA. Hemograms were performed on the 6th, 12th and 18th gestational day. The analysis of the results indicates that the exposition to the mixture of these herbicides led to a hematopoetic aggression represented by a low anemia; dose-dependent leucopenia, characterized by absolute and relative lumphocytopenia and neutrofilia. The results show both cellular and humoral (antibody) immune response decrement reduction count of lymphocytes and disorganization of innate immune response had rise neutrophil population.
B-002 EFFECT PRE AND POST NATAL OF THE 3rd PROGENY OF MICE EXPOSED OF 2,4-D (COMMERCIAL FORMULATION) AND ROUNDUP® IN ASSOCIATION

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The success of the embryonic and fetal development depends on the uterine environment and the adjusted genetic heritage. In previous experiments made by Sachetti et al. (2002) with embryos of the 1st progeny, different concentrations (15mg/Kg, 75mg/Kg, 150mg/Kg) of a mixture of herbicides were established and different hemorrhagic patterns were verified. In the present study we investigate the embryotoxic effect of the combination of the herbicides 2,4-D and Roundup in mice of 3rd progeny. For that, small sites of embryonic and fetal implantation in 6th, 8th, 10th, 12th, 15th, 18th day of gestation were collected and analyzed for the number of atrophic and hemorrhagic sites, and for further microscopic analysis. The hemorrhage and atrophy observed after the use of the different concentrations were considered significant when compared to the controls. However, there was no significant difference between the experimental groups. In the microscopic analysis, the hemorrhages were observed both in the mesometrial and antimesometrial regions, and inflammatory cells were constantly seen. Our data suggest the presence of aggressive components in the herbicides, which can penetrate the embryonic cell, and act direct or indirectly in the blood vessels, causing hemorrhages and fetal death, that ultimately lead to a reduction in the offspring.

B-003 SPERM ULTRASTRUCTURE: CONTRIBUTION TO DISCERN PHYLOGENETIC RELATIONSHIPS AMONG ANNUAL FISHES

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Annual fishes show high plasticity to an extremely variable environment and they are a special model to study aspects of genetic and developmental biology. During the dry season, when ponds disappear, adults die and the species have escape mechanism. They also can stop their development at different stages (diapauses) and in the next rainy season they hatch a few hours after the pond is filled. The genus Cynolebias is one of the most important annual fishes genus in South America. A multidisciplinary team focused on different aspects about phylogenetic relationships between sperms are at tail morphology. The aspect and variety of lateral fins found in C. charria are in agreement with its former inclusion in the controversial adloffi complex. These findings have oriented our analysis in order to establish the sperm ultrastructure of two related species (C. bellotti and C. reichertii) attempting to contribute to elucidate this question.

B-004 OOGENESIS AND CHORION ULTRASTRUCTURE IN ANNUAL FISHES Cynolebias.

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Populations of annual fishes survive the dry season, when ponds dry out, through live embryos that are buried in the bottom mud. Some Cynolebias (Cyprinodontiformes: Rivilididae) habit in a geographic area declared Reserve of Biosphere (UNESCO,1976). It is of special interest to know the biodiversity of this area and to learn more about the reproductive and developmental biology of species living in those places in order to improve its conservation and management. We are interested in investigate different aspects of the sex-determination and differentiation of this South American annual fishes genus. Briefly, we are characterizing the type and timing of gonadal differentiation during embryonic development. In order to reach this goal it is necessary to know the organization and fine structure of adult gonads and germinal cells. In this poster we present the oogenesis and the ultrastructure of chorion in Cynolebias species. Samples were processed for light and electron microscopy analysis. We recognize four kinds of ovarian follicles during oogenesis. Chorion SEM images reveals a rough outer surface ornamented by two kinds of hairlike filaments that differ in thickness. Under TEM it appears formed by three zones distinguishable by its electron-density and organization. Results are discussed in relation to the special reproductive strategies of these species.

B-005 REPRODUCTIVE EFFECTS OF ANABOLIC STEROID DECA-DURABOLIN ON THE MORPHOLOGY OF THE OVARIAN OF FEMALE RATS SUBMITTED TO THE PHYSICAL EFFORT.

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In the last decades, the general use of androgenic anabolic steroids (AAS) has been common between young and adults, athletes or not. The goal of this study was to analyze the morphologic structure of the ovaries of adult female rats submitted or not to the physical effort (forced swimming), treated with AAS. Wistar females were housed in standard environment and randomly distributed in four experimental groups (n= 5/group): control (physiologic solution, submitted or not to the physical effort) and treated (Deca-Durabolin, 6mg/kg, submitted or not to the physical effort). The drug and the physiologic solution were administered by a single intraperitoneal injection in the week, during 4 consecutive weeks. The swimming was chosen as model of physical effort (20 minutes/day during 5 days/week, in 4 weeks of treatment). After this period, the female was sacrificed and the ovaries were collected and prepared by usual histological routine. In the ovaries of controls, antral follicles and well developed corpus luteum were evidenciated. In the treated group, large areas of cortex were occupied for interstitial glands, being these apparently more frequent in the female rats submitted to the forced swimming. The female rats treated with Deca-Durabolin presented aggressiveness and oestral aciclicity. The use of AAS associated to the physical effort, affects the morphological pattern of the ovaries.
The long-term alcohol treatment causes negative changes in the male reproductive system. It’s known that prostatic stroma is involved in the pathophysiology of the prostate. Thus, the aim of this study was to analyze histochemical and immunohistochemical alterations of the prostatic stroma and epithelium related to glandular pathologies of rats submitted to chronic alcohol ingestion and alcoholic abstinence. Thirty rats (10 Wistar and 20 UChB) were divided in three experimental groups: the control received tap water; the alcoholic received ethanol diluted to 10º G.L. and the abstaining received the same liquid diet as the alcoholic group up to 120 days of treatment, and only tap water for 30 days thereafter at the end of 150 days of treatment. All of the animals were sacrificed and the ventral lobe of the prostate was removed and processed for histochemistry and immunohistochemistry techniques. Also, the plasmatic testosterone level was measured. The results showed prostatic intraepithelial neoplasia, infolding of the epithelium towards stroma, stromal hypertrophy and occurrence of inflammatory cells in both alcoholic and abstaining animals. Then, it can be concluded that the ethanol is a great trigger of incidence of pathologic processes.

Diseases such as cancer and benign prostatic hyperplasia are related to disruption in the mechanism regulating the balance between both processes of cell proliferation and apoptosis in prostatic cells. As castration and vasectomy might alter that balance, this study evaluated the cell proliferation and apoptosis in both distal and intermediate regions of the ducal system of the rat ventral prostate 48h and 7 days after castration and vasectomy. The histological sections were immunohistochemically stained using the antibodies anti-PCNA and anti-Ki67 for detection of cell proliferation and cytchemically stained using Fuelpgen reaction for detection of apoptosis. It was observed that the cell-proliferation indices decreased significantly in both regions of the ductal system 48h and 7 days after castration compared to the sham-operated group. The apoptotic-cell index increased significantly after 48h, declining 7 days after castration. The cell-proliferation indices did not differ after 48h significantly however they increased 7 days after vasectomy on the ductal system. The apoptotic-cell index did not differ after 48h significantly in either study moment after vasectomy. Thus, castration caused imbalance between the processes of cell proliferation and apoptosis in favor of apoptosis, whereas castration caused imbalance in favor of cell proliferation.

The Anura, Lysapsus limellus reproduces through out the year in ponds and flooded areas at Base de Estudos do Pantanal - UFMS (19º34’S, 57º00’W). Spermatogenesis of this Hylidae species is described on the basis of aspects observed with light microscopy. The germ cells include primary spermatogonia, large cells usually with a bilobed nucleus; secondary spermatogonia, smaller cells, with darkened cytoplasm and a nucleus with loose, radially arranged chromatin; primary and secondary spermatocytes that can be differentiated according to the distinct nuclear characteristics that determine each stage of meiotic cell division. Spermiogenesis, a process of morphologic differentiation shows: rounded spermatids that are much smaller, with compacting chromatin and reduction of their cytoplasm; elongated spermatids are generally arranged in parallel forming well defined sheaths, with the anterior region directed toward the seminiferous locule periphery and the tail toward the lumen. Spermatooza occur freely in the lumen of the seminiferous locule. All the cells are found in organized cysts, which are supported by abundant Sertoli cells (in testes collected in the month of May). Spermatogenesis of L. limellus has many similarities with that of other Anura, such as the germ cell organization, the innumerable free Sertoli cells in the testis lumen, the extensive cytoplasmic prolongations of the melanomacrophage cells, which contain pigments and surround the seminiferous locule. Support: CNPq (150007/03-5).

The objective of present study was determine the duration of spermatogenesis in two lines of rats (UchA and UchB), selected to free-choice alcohol intake and exhibit low and high levels of alcohol consumptions, respectively. Based on the development of the acrosomic system and changes in the nuclear morphology, XIV stages or cellular associations were characterized in seminiferous epithelium in rats. The duration of seminiferous epithelium cycle (SEC) was estimated through 3H-thymidine and the relative frequency of the stages. The relative frequency of each stage varies during the SEC and was directly proportional to the duration of each stage. Each spermatogenic cycle in UchA rats lasted 12.07 days. A similar value was found in UchB rats (12.3). Considering that the entire spermatogenic process takes approximately 4.5 cycles to be completed, its total duration in UchA and UchB rats was estimated to last 54.3 and 55.3 days, respectively. The results suggest that the both lines genetically selected to voluntary consumption of ethanol showed difference compared to original line (13.3). In this regard, this species is a potential model to study of chronic alcoholism on reproduction.
B-010 ULTRASTRUCTURAL FEATURES OF THE PRINCIPAL CELLS OF VAS DEFERENS IN DOMESTIC QUAIL

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Introduction: Principal cells in birds had been related to phase fluid endocytosis and other physiological roles as adsorptive endocytosis and spermiophagy. The morphological features this cells in the vas deferens of quail were investigated in the four seasons of the year for describing possible seasonal variability.

Material And Methods: Fragments of the vas deferens of 12 adult domestic quails were collected during the seasons: autumn, winter, spring and summer. The tissues were processed for studies in Transmission Electron Microscopy. The investigations were made in the most expressive months of the seasons: middle of April, June-July, October and January-February. Results: In spring the principal cells showed rough endoplasmic reticulum (RER) very developed presenting lamellar and vesicular cisterns and an active endocytotic apparatus: coated vesicles, endosomes, multivesicular bodies (MVBs) and lysosomes. In summer, was noted RER lamellar less developed and some vesicles. In autumn the principal cells showed a cytoplasmic system of small channels related to protein secretion. Other lysosomal activity such as phagocytosis was verified in principal cells. Tall columnar dark cells of corpus showed a cytoplasmic system of small channels formed by coalescence of variable vesicles, perhaps directly related with absorption of water.

FAPESP-Proc.02/04127-0

B-011 ULTRASTRUCTURE OF THE EPITHELIUM LINING THE EPIDIDYMIS OF GERBILL

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Introduction: The epithelium epididymidis of mammals was related to some functions such as absorption, endocytosis and secretion of several compounds with direct influence on spermatogenesis and spermiophagy. Material And Methods: selected fragments for studies in Transmission Electron Microscopy. The investigations were made in the most expressive months of the seasons: middle of April, June-July, October and January-February. Results: In spring the principal cells showed rough endoplasmic reticulum (RER) very developed presenting lamellar and vesicular cisterns and an active endocytotic apparatus: coated vesicles, endosomes, multivesicular bodies (MVBs) and lysosomes. In summer, was noted RER lamellar less developed and some vesicles. In autumn were observed few organelles, small cisterns of RER, vesicles and lysosomes. Was observed a degenerative process of apical cytoplasm, great and pale vacuoles invading the lumen. In winter presented several mitochondria, RER developed, pale MVBs and lysosomes, cytoplasmic expansions and luminal vesicles. Was concluded that the vas deferens of quail present a cyclic pattern with an active phase (spring), a regressive phase (summer), a marked quiescent phase (autumn) and a recrudescence phase (winter).

B-012 ULTRASTRUCTURAL Spermatozoa STUDY IN THE Cetopsis coecutiens SPECIES (SILURIFORMES: CETOPSIDAE)

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The Cetopsidae family is the sister group to all Siluriformes except Diplomystidae. Actually as spermatozoa morphological features are phylogenetically related, the description of Cetopsidae spermatozoa can be useful in the phylogenetic analysis. Four adult males of Cetopsis coecutiens were collected from the Araguaia River, Araracuara, Goiás, Brazil. Tests was fixed in 2% glutaraldehyde and 4% paraformaldehyde in 0.1 M Sorensen phosphate buffer, pH 7.4. The fragments followed the usual routine process for analysis in TEM. The C. coecutiens spermatozoa have a semi-ovoid head; a symmetric midpiece of short length; and two flagella medial to the nucleus. The acrosomal vesicle is absent. The semi-ovoid nucleus contains highly condensed heterogeneous chromatin with some electron-lucent areas. The nuclear fossa has a moderate depth and the centrioles, which are lateral and parallel to each other, are inside it. The midpiece contains few rounded mitochondria, several vesicles and a cytoplasmic channel of short length. Mitochondria are found peripherally arranged to the nucleus and the vesicles are grouped mainly at the basal region of the midpiece. In this region, the interconnected vesicles are forming a membranous compartment. Mitochondria are separated from the flagellum by vesicles and cytoplasmic channel. The flagella have a classic axoneme (9+2) and no flagellar lateral projections or fins are present.

B-013 ULTRASTRUCTURAL CHARACTERISTICS OF THE Anodus elongatus SPERMATOOZA (CHARACIFORMES, HEMIODONTIDAE)

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Anodus elongatus, member of the family Hemiodontidae, occur in most rivers and basins of northern South América and also in the South in the Paraná-Paraguay basin. A description of A. elongatus spermatozoa ultrastructure may provide several characters that can be used with other ones (somatic and molecular) in future phylogenetic analyses of the Hemiodontidae fish group. Adults males of Anodus elongatus were collected from the rio Acre, Rio Branco, AC/Brazil (10°03.320’S; 67°51.450’W). Fragments of tests were fixed overnight in 2% glutaraldehyde and 4% paraformaldehyde in 0.1M Sorensen phosphate buffer, pH 7.4 and processed to transmission electron microscope. A. elongatus spermatozoa have a head, a midpiece and a flagellum. The nucleus is spherical, 2.05 μm in diameter, surrounded by a narrow strip of cytoplasm with no organelles. It is occupied by thin fibers of highly condensed chromatin with electron-lucent areas; the shallow nuclear fossa show excentric position in relation to the nucleus base and is penetrated only by the proximal centriole. The midpiece is located in the nucleus base. There are some irregular mitochondria and many elongate vesicles concentrate in different sides of the midpiece. Both the structures are separated from the flagella by the cytoplasmic channel. The flagellum contains the classical 9+2 axoneme with no central par in the initial segment. The Anodus elongatus spermatozoa show several characters that are observed in other characiform spermatozoa.
B-014 ULTRASTRUCTURE OF SPERMATOZOA OF
Achroia grisella in FEMALE GENITAL TRACT
(LEPIDOPTERA, PIRALIDAE)
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The moth Achroia grisella is a bee wax pest. As in other Lepidoptera the spermatogenesis is dichotomous, producing eupyrene (nucleate) and apyrene (anucleate) spermatozoa. Both types of spermatozoa are transferred to female genital tract during mating. The fate and function of apyrene spermatozoa in female is still not clear yet. Therefore in order to contribute for advancing this knowledge the genital tract of imago's of mated females A. grisella reared in special diet in the Campus Biotery of UNESP were used for ultrastructural studies. The female genital tract was fixed in Karnovsky fixative for Transmission Electron Microscopy, processed routinely. Both types of spermatozoa were found in female reproductive tract. The eupyrene spermatozoon show up features different from that present in male tract. They have acquired an outer coat inside of wich an amorphous material accumulates around the spermatozomal structures. Eupyrene spermatozoa can be observed in large quantity along several parts of female tract, both intact and showing characteristics desorganization, however apyrene spermatozoa are few and apparently unchanged. The eupyrene spermatozoa apparent desorganization is charaterized by the loss of external coat and amorphous material. This discharged material fill the spermatoeal lumen almost completely, while the naked eupyrene cells lie in this mass periphery near the spermatoeal epithelium. The naked spermatozoa is the fecundating cell, therefore the signification is unknown.

B-015 PROSTATIC PATHOGENESIS IN THE CHRONIC NICOTINE USE.
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This study intend to correlate nicotine, a harmful drug to the male reproductive system, and prostate, due to the high incidence of pathologies that occur in this gland. Particularly in the prostate, the stroma-epithelium interaction is fundamental for the glandular homeostasis and disturbances in this relationship are appointed as the main factor in the advancement of malignancy. Therefore, this study aimed to detail possible stromal alterations in the ventral prostatic lobe after using nicotine for long time. Twenty rats were divided into two experimental groups: the nicotine group received nicotine daily (0.125 mg/100gr of body weight, Sigma, St Louis, USA) and the control group received saline solution; both were subcutaneous administered for 80 days. After this period, the animals were sacrificed and samples of the ventral prostatic lobe collected and processed for histochemical and stereological analyses. The results showed stromal disorganization characterized by increasing of smooth muscle cells and fibrilar compounds. Also, infolding of the basement membrane was common. The stromal compartment occupied 68% and the glandular mucosa 32%, characterizing the stromal hypertrophy. These results indicate that nicotine cause disequilibrium in the stroma-epithelium interactions, which may interfere in the reproductive process and be considered a primary agent in development and advancement of prostatic diseases.

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In insects, the fat body distributed throughout the whole body, play a role in filling empty spaces. It is composed by adipocytes, to which two other types of cells, the oenocytes and the urocytes, are associated. The present work had the object of performing a comparative study of the fat body of mediumsized workers of the ants Atta laevigata and Acromyrmex disciger, through the use of histological techniques. The results revealed that the fat body of both species is located mainly around the digestive tract, ovaries, and adjacent to the integument. The cells of the fat body were grouped into lobules and the main cellular type found was the adipocyte, which appeared mononucleated and polygonal. In Acromyrmex disciger these cells were smaller and had a higher number of vacuoles in their cytoplasm; these vacuoles were probably of a lipid nature and had varying shapes and sizes. The oenocytes were less numerous, more strongly stained, exhibited a rounded or ovoid shape, an evident rounded nuclei and, a homogeneous cytoplasm without inclusions. In Atta laevigata the oenocytes were smaller and more numerous. Supported by CAPES.

B-017 DETERMINATION OF THE PHASES OF THE ESTROUS CYCLE IN MICE THROUGH VAGINAL LAVAGE AND/OR SMEAR: A COMPARATIVE ANALYSIS.
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Vaginal cytology is widely used to monitor the estrus cycle in many mammal species. In this study, it was used a total of 20 female mice to determine the estrous cycle by using the two techniques: the vaginal smear and the vaginal lavage. Vaginal smear was performed using a cotton swab to remove epithelial cells from the vaginal wall and to attach them into a glass slide for further fixation, staining and light microscopic observation. Vaginal lavage was carried out using a pipette to inject 50 µL saline into the vaginal cavity and, soon after, gathering it and depositing it on a slide for direct observation without staining. Both methods allowed the identification of cell types and determination of the estrous cycle phases. However, the use of either technique during estrus induced the condition of pseudopregnancy. These animals remained in diestrus from 8 to 14 days, before returning to the estrus. Therefore, the use of either method is adequate to classify the stage of the estrous cycle however, to monitor consecutive estrous cycles avoiding pseudopregnancy, both techniques were not successful.
The diabetes causes alterations in various organic systems, encompassing the male accessory sex glands. The prostate is very important in the male reproductive process and it is a frequent target of malignant changes. The aim of this work was to demonstrate the histochecmistry and ultrastructural alterations in the prostate of the animals submitted to experimental diabetes. It was attempted to associate this results to cellular events involved in prostatic diseases. Three groups of animals were utilized: control, non-obese diabetic positive mice (NOD+) and non-obese diabetic negative mice (NOD-). The diabetic status was characterized using Bio Bras reagents strips. Twelve days after the characterization of diabetic status the ventral lobe was collected, fixed in Karnovsky solution and paraformaldehyde and processed for histochecmistry and TEM associated to stereology. The results showed reduction of the epithelial and luminal areas and increasing of the stromal area with muscular layer hypertrophy and collagen and reticular fibers in the prostatic gland. Also, it was characterized development of prostatic intraepithelial neoplasia, inflammatory process and dilatation of the organelles involved in the secretory process. NOD- demonstrated alterations similar to NOD+, denoting that diabetes predisposition is a relevant factor to the occurrence of changes in prostatic stromal microenvironment. Then, it was concluded that the diabetes besides damaging the reproductive process, affect the glandular homeostasis favoring the development of prostatic pathologies.

B-019 EPIDIDYMAI CHANGES INDUCED BY 45 DAYS-ANTIANDROGEN THERAPIES
Marcela Cristina Correa de Freitas; Fernanda de Mattos Egydio; Sebastião Roberto Taboga; Rejane Maira Góes. Departamento de Biologia, IBILCE - UNESP. 15054-000

This experiment was designed to investigate the influence of steroidal (ciproterone acetate) and non-steroidal (flutamide) antiandrogens on epididymis of adult Mongolian gerbils. The drugs were subcutaneously administered (10mg/ Kg b.w.) for 45 days at three days time intervals. Examination of PAS staining tissue sections revealed marked differences along the epididymal duct of control gerbils concerning the epithelium and luminal areas and increasing of the stromal area, with muscular layer hypertrophy and collagen and reticular fibers in the prostatic gland. Also, it was characterized development of prostatic intraepithelial neoplasia, inflammatory process and dilatation of the organelles involved in the secretory process. NOD- demonstrated alterations similar to NOD+, denoting that diabetes predisposition is a relevant factor to the occurrence of changes in prostatic stromal microenvironment. Then, it was concluded that the diabetes besides damaging the reproductive process, affect the glandular homeostasis favoring the development of prostatic pathologies.

B-020 EFFECT OF TNF-α ON THE MOUSE PRE-IMPLANTATION EMBRYO DEVELOPMENT IN VITRO
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During the development, embryos are under the effects of different cytokines, growth factors and other regulatory molecules present in the female tract. Particularly interesting is the presence of TNF-α at physiological doses in these sites and, at high concentrations in inflammatory processes. Studies focusing the embryonic susceptibility to the TNF-α have been done in different species, but most of these were directed to the blastocyst stage. In this study, mice embryos were collected at 4-8 cells phase, cultivated for 24 h in the presence or absence of different concentrations of TNF-α, and morphologically analyzed. In the presence of TNF-α the embryos had less blastomeres, modified nuclear morphology and most of them were blocked in the morula phase when compared with the control group. Great part of the TNF-α-treated blocked embryos was positive for caspase-8 and reactive for anexina-V indicating the entrance in apoptosis process. Similar but less frequent responses were also found in the control group. The use of impermeable fluorescent dyes and Hoechst was also important for characterizing the nuclear integrity of plasma membrane and nuclear morphological alterations in the different experimental groups. These findings suggest that TNF-α may act on the cleavage phase by blocking the course of embryo development, leading to cellular death by apoptosis, adding new information on the embryos susceptibility in the phase of pre-implantation.

B-021 ULTRASTRUCTURAL CYTOCHEMISTRY OF OOCYTE PRIMARY AND SECONDARY GROWTHS IN Serrasalmus spilopleura (TELEOSTEI, CHARACIFORMES, SERRASALMINAE) USING EDTA METHOD
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Teleost female gametogenesis consists of oogenesis and oocyte primary and secondary growth. During primary growth, the membranous organelles proliferate, and RNA synthesis is intense. RNA associate with proteins and is transferred to the cytoplasm where accumulates forming the nuage. Secondary growth includes the formation of cortical alveoli, vitellogenesis, culminating with ovulation. To detected cellular components that contain ribonucleoproteins (RNPs), in the oocyte, S. spilopleura females were monthly collected during 2003, from the Jurumirim Reservoir. The specimen ovaries were removed, fixed, sectioned, submitted to EDTA method (Bernhard, 1969), and analyzed under a TEM. S. spilopleura previtellogenic oocytes show the reaction product as electron-dense points, certainly ribosomes scattered in the cytoplasm or ribosomes joined to the endoplasmatic reticulum. In the nuclei, the nucleoli appear electron-dense with some faintly areas. Moreover, some electron-dense areas can be seen into the nuclei and in the perinuclear cytoplasm too, maybe RNPs clusters being transferred from the nucleus to the cytoplasm. In the vitellogenic oocytes, the cytoplasm is filled with electron-dense points, the scattered ribosomes and ribosomes of the endoplasmatic reticulum. Curiously, the cortical alveoli exhibit the reaction product as electron-dense fine points, which suggest the presence of RNPs in these organelles. Similar results were obtained with oocytes of other animal species.

B-018 PROSTATIC STROMAL MICROENVIRONMENT AND EXPERIMENTAL DIABETES.
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This experiment was designed to investigate the influence of steroidal (ciproterone acetate) and non-steroidal (flutamide) antiandrogens on epididymis of adult Mongolian gerbils. The drugs were subcutaneously administered (10mg/ Kg b.w.) for 45 days at three days time intervals. Examination of PAS staining tissue sections revealed marked differences along the epididymal duct of control gerbils concerning the epithelium and luminal areas and increasing of the stromal area, with muscular layer hypertrophy and collagen and reticular fibers in the prostatic gland. Also, it was characterized development of prostatic intraepithelial neoplasia, inflammatory process and dilatation of the organelles involved in the secretory process. NOD- demonstrated alterations similar to NOD+, denoting that diabetes predisposition is a relevant factor to the occurrence of changes in prostatic stromal microenvironment. Then, it was concluded that the diabetes besides damaging the reproductive process, affect the glandular homeostasis favoring the development of prostatic pathologies.
The quantitative behavior of gerbil’s prostate secretory cells at three phases of its post-natal development, as well as, the occurrence of spontaneous neoplasias consequent of the aging were evaluated. Prostates were removed from youngs (1-2 months), adults (3-6) and aged animals (12-24), prepared to be embedded in historesin and stained with Hematoxylin-eosin and Feulgen Reaction. Four animals were used for each age group. Morphometric analyses were accomplished using the analysis system Image Pro-Plus and the obtained data were submitted to Tukey's statistical test (p<0,05, significant). The young group showed a rudimentary prostate by the ending of organogenesis, with increased relative frequency of prostate epithelial cells when compared to adult and old group (p<0,00* and 0,01*, respectively) and its epithelium height was larger than in adults (p<0,00*), however, smaller in relation to the old ones (p=0,00*). In the young epithelium the area nuclei were wider than that observed in adults (p<0,00*) and lesser than the olds (p, no significant), There were spontaneous proliferation sites in the old animals, where the nuclear areas were smaller than those with a no-altered epithelium (p<0,00*). The morphology and homeostasis of prostate epithelium, along the development, are maintained by endocrine factors, which can enter in unbalance during the senescence and contribute to the appearance of epithelial proliferation disorders.

The structure of germ cells forms one of the initial points for the study of reproductive biology in fish and is important for the practical application of the cultivation of these animals. Histological analysis through the inclusion in historesin was used for the description of the morphological characteristics of the female germ cells as well as for the realization of measures of the diameters of the follicles in the different phases during one year. All the stages of oogenesis were characterized and the morphometry was realized in pre vitelogenic oocytes, in primary and secondary growth. All the stages of the oocytes were observed during all the analyzed year, but varied in their frequencies of occurrence. It was observed that pre vitelogenic oocytes had their diameters varying from 75 to 103µm, primary growth oocytes varied between 150 and 277µm and both were present in the gonads during all the studied period; early secondary growth oocytes varied between 267 and 378µm and late secondary growth oocytes varied between 525 and 1064µm and occurred in larger frequency in the gonads, from August to December. It was possible to characterize all the stages of maturation of the oocytes including the oogonium showing morphological and peculiar morphometrical characteristics that allow describing the morphological pattern of the oogenesis found in this teleost fish.

It has been demonstrated that the synthetic agonist fenofibrate (F) for the nuclear receptor peroxisome proliferator-activated receptor α (PPARα), increase the expression of fatty acid oxidation enzymes. This study investigated its effect on hepatic steatosis induced by the administration of 1% orotic acid (OA) (a pyrimidine nucleotide precursor), in the diet. The rats were divided in 5 groups and treated with the following diets: control (C; standard diet), high carbohydrate (HC), (HC) + (OA), (HC) + (F), (HC) + (OA-F). Fragments of liver were processed for standard light (LM) and electron microscope (EM) studies. The morphometric analysis were done by measuring 30 hepatocytes nuclei and the area occupied by the lipids droplet of 3-5 slides of each group. The morphometric data were collected by image analyses software FS 400 (Zeiss). The results showed that rats with HC + OA diet had an increase of 304% and 413% compared to ones feed with HC and the controls. The groups OA and HC treated with fenofibrate showed a lipid content reduction of 69% and 24%, respectively. The cytoplasm volume of the hepatocytes of the OA group increased in 35% and 11% if compared with the HC and C, respectively. The results suggest that the fenofibrate treatment was efficient to decrease the hepatic lipid accumulation in OA treated rats.

The female of Trachelyopterus galeatus ('cangati'), an internal South American fertilizer fish species, is able to keep sperm deposited within its genital tract for a large period of time - an unique reproductive family trait among the catfishes. This work investigates the ultrastructure of cangati’s spermatozoa since it possibly bears an acrosomal vesicle. Testis and gravid ovarian informations was processed for the nuclear receptor peroxisome proliferator-activated receptor α (PPARα), increase the expression of fatty acid oxidation enzymes. This study investigated its effect on hepatic steatosis induced by the administration of 1% orotic acid (OA) (a pyrimidine nucleotide precursor), in the diet. The rats were divided in 5 groups and treated with the following diets: control (C; standard diet), high carbohydrate (HC), (HC) + (OA), (HC) + (F), (HC) + (OA-F). Fragments of liver were processed for standard light (LM) and electron microscope (EM) studies. The morphometric analysis were done by measuring 30 hepatocytes nuclei and the area occupied by the lipids droplet of 3-5 slides of each group. The morphometric data were collected by image analyses software FS 400 (Zeiss). The results showed that rats with HC + OA diet had an increase of 304% and 413% compared to ones feed with HC and the controls. The groups OA and HC treated with fenofibrate showed a lipid content reduction of 69% and 24%, respectively. The cytoplasm volume of the hepatocytes of the OA group increased in 35% and 11% if compared with the HC and C, respectively. The results suggest that the fenofibrate treatment was efficient to decrease the hepatic lipid accumulation in OA treated rats.

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The study of the Leydig cells is important for understand and establish the pattern of spermatogenesis. Degeneration of the Leydig cells causes total disorganization of spermatogenesis in seminiferous tubule surrounding it. Testis of fifth young adult male was analyzed. All animals were killed after pithing. Testes were fixed in glutaraldehyde 2.5% in sodium phosphate buffer 0.1M, pH 7.4 and included in glicolmetacrilate. Three-µm sections stained with toluidin blue/sodium borate 1% were analyzed. Nuclear diameters of 30 Leydig cells/animal were measured, using image analyser. The volumetric proportions between connective tissue/Leydig cell and nucleus/cytoplasm was obtained using the grid lines. Calculations were as follows: nuclear volume was obtained by sphere volume, the cytoplasmatic volume was estimated by nucleoplasmatic relation, and cellular volume by adding both volumes. Leydig cells showed ovoid or round nuclei (5.97 µm of nuclear diameter) with variable chromatin density; the nucleolus was sometimes visible. These cells were observed isolated or in-groups near to blood vessels and totalized 27.33% of interstitial tissue. Blood and lymphatic vessels, cells of the connective tissue and other elements represented 72.67% of interstitial volume. The nucleoplasmatic relationship was 76.22% for nuclei and 23.78% of cytoplasm representing volumes of 85.85 µm³ and 26.78 µm³, respectively. Total cellular volume was 112,63 µm³. The population of Leydig cells per testis and per gram of testis was estimated as 14,14 x 10⁷ and 33,91 x 10⁷, respectively.

During the spermatogenesis of several vertebrates it has evidences of the cellular synthesis of RNA and, simultaneously, appears a characteristic cytoplasmic organelle, the "chromatoid body", that presents typical lobated form. Most interesting of the proposed functions of this corpuscle is its participation in the RNA metabolism of the spermatogenic cells. The aim of the present paper was to get given data about the cytochemical structure of nucleolar cycle and the distribution of RNAs cytoplasmics in the seminiferous tubules cells of Meriones unguiculatus. The testis had been fixed in Bouin and Karnovsky for the conventional histological analysis (Hematoxilin-Eosin) and for cytochemical study that included: Feulgen Reaction; Silver-Ion Impregnation, Toluidine Blue and a Variant Method of Critical Electrolyte Concentration (CEC). All the used techniques had brought evidences of the persistence of nucleolar material at the meiosis. Therefore, considering the persistence of DNA and nucleolar proteins (RNPs), during the spermatogenesis of Meriones unguiculatus, our findings corroborate that these molecular complexes are very important in the spermiogenesis phases. It can be suggested that these corpuscles of ribonucleoproteins ("chromatoid bodies") have a function in the successive serie of the events that occurs in the formation of the spermatozoa, as the condensation of the chromatin and the formation of the acrosome.

**B-028 THE BULLFROG (Rana catesbeiana, Shaw 1802) LEYDIG CELLS BY MORPHOMETRIC ANALYSIS**

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Financial support: Fapemig, CNPq.

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**B-029 CYTOCHEMICAL STUDY OF NUCLEOLAR CYCLE AT SPERMATOGENESIS OF Meriones unguiculatus**

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Financial support: CNPq/CAPES.
Macrophages are found in the testis and epididymis of several mammalian species; however, their presence in birds’ excurrent ducts is not well defined. Although it has been suggested that these cells may be involved in the regulation of spermatogenesis and phagocytosis of spermatozoa, the role of macrophages in reproduction is still obscure. Aiming to investigate the occurrence of macrophages in the epididymal region of the drake and to characterize these cells, histological, ultrastructural, histochemical and vital staining studies were performed. The epididymal region of the drake presents the intratesticular, intracapsular and extratesticular rete testis, efferent ductules and epididymid duct. Differing from the other segments of the male genital tract investigated, a great number of cells, characterized morphologically as macrophages, were identified within the lumen of the extratesticular rete testis. These cells were found isolated or in clusters, free or associated to the epithelium. Macrophages showed PAS-positive granules (identified as lysosomes) and clear vacuoles scattered in the cytoplasm. The cytoplasmatic vacuoles contain lipidic material, as shown by the osmiophily and positivity to Sudan Black B and Sudan III stain. The presence of phagocytized spermatozoa was frequent. The occurrence of numerous macrophages restricted to the rete testis region suggests that these cells may play a role in the regulation of local functions, such as spermiophagy.

Suported by: CNPq, FAPEMIG

B-032 REPRODUCTIVE EFFECTS OF DIFFERENT DOSES OF STEROID ANABOLIC DEA-CURABOLIN ON THE HISTOLOGY OF THE TESTICLE AND FERTILITY OF ADULT MALE RATS.

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The Decanoate Nandrolona (Deca-Durabolin) is an androgenic steroid with intense anabolic properties. The objective of the study was to analyze the effect of Deca-Durabolin (DD) on reproductive aspects of sexually mature rats, treated with 2, 4 or 6 mgDD/Kg per kilo. The animals from the control group received physiological solution. The substances were administered in the scheme of 1, 2 and 3 doses, respectively in the 1st, 2nd and 3rd week. By the end of this period, the fertility index was obtained and the gonads in the male rats were collected and prepared to the analyses in the light microscopy. The doses of 4 and 6 mgDD/Kg reduced the fertility of animals and promoted alteration in the testis structure. The results suggest that had a dose-dependent effect of the drug on the testicular histology and fertility of adult male rats.

B-031 CARBOHYDRATE CHARACTERIZATION AND DETECTION DURING OOGENESIS IN THE FISH STRONGYLURA MARINA, BAY OF PINHEIROS, COAST OF THE PARANÁ

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This study objective is to characterize the oogenesis process in Strongylura marina, thus contributing for the knowledge on the biology of estuary fish of the Parana coast. Specimens were collected in February on the Bay of Pinheiros, Parana coast. Gonads were removed and prepared through routine histology procedures for the confection of microscopic slides, being fixed in Alfac for 20 hours and embedded in paraffin, stained with: Hematoxylin-eosin, Periodic acid-Schiff, and Alcian Blue. Six phases were characterized: Phase I, oogonia, small cells with scarce cytoplasm, big nucleus and a unique nucleolus. Phase II, oocyte with basophilic cytoplasm and a layer of acidophilus filaments covered by follicular cells fit closely together. These filaments grow in thickness during the subsequent phases, and the follicular cells remain closely together until the end of the oogenesis process. Phase III, lost of cytoplasmic basophil and emergence of cytoplasmic vesicles and a vitellin membrane. Phase IV, cytoplasm with vesicles and vitellin granules. Phase V, great amount of vitellin granules on the cytoplasm. Phase VI (exclusive for marine fish), vitellin granules fuse together because of the water entrance on the oocyte. Neutral and acid sugars quantity increase on the oocyte during its development. These filaments are probably related with the egg fixation on the substratum and have only neutral sugar.

B-030 OCCURRENCE AND MORPHOLOGICAL CHARACTERIZATION OF MACROPHAGES IN THE RETE TESTIS OF THE DRAKE (Anas platyrhynchos)

1André Gustavo Oliveira; 1Luiz Telles; 1Irina Geraldo; 1Germán Mahecha; 1Cleida Oliveira (cleida@ich.ufmg.br), 1Dep. Morfologia, UFMG

The B-030 occurrence and morphological characterization of macrophages in the rete testis of the drake (Anas platyrhynchos) was performed. The study was based on two experiments. In the experiment I, male rats from 90 days of age were distributed in two groups of mating: a single dose of cyclophosphamide. In the experiment II, 10 pairs of Wistar rats were distributed in two groups of mating: (A) control and (B) treated with cyclophosphamide. The drug was administered in the dose of 30mg/kg. The progeny was collected and prepared to the analyses in the light microscopy. The cyclophosphamide is largely used in chemotherapeutic and immunosuppressive treatments. The goal of this study was to analyze the gonad histology of male rats treated direct and indirectly with cyclophosphamide, their fertility and pregnancy outcomes. The study was based on two experiments. In the experiment I, male rats from 90 days of age were distributed in two experimental groups: (A) control and (B) treated with cyclophosphamide. In the experiment II, 10 pairs of Wistar rats were distributed in two groups of mating: (A1) control and (B1) treated with cyclophosphamide. The drug was administered in the dose of 30mg/kg. The progeny was sacrificed at 90 days of age. At the first experiment, it was observed that the animals treated with the drug had their fertility reduced. The animals of the second experiment did not show an alteration on the fertility index. The drug did not promote alteration in the testis morphology on the animals of the two experiments. At the experimental conditions, the drug did not affect the gonad structure in the progeny. The morphometric results demonstrated that had significant differences in the seminiferous tubular diameter of animals, in each experiment.
B-034 MELANOMACROPHAGES IN THE TESTES OF Physalaemus Nattereri (ANURA)

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The testes in the anurans are paired ovoid organs constituted by seminiferous structures surrounded by the fibrous connective tissue, commonly unprovided of pigments. The germ tissue, which constitutes the testicular parenchyma, has different cellular types: spermatogonia in the epithelium boundary; spermatocytes and spermatids following the sequence of the cellular differentiation; and spermatooza in the lumen. This study tried to analyze the histological characteristics of the testes, as well as to describe cellular characteristics of rare and conspicuous pigment-containing cells. Five males originated from the São José do Rio Preto city (Brazil), were used. Testicular fragments were submitted to the histological routine and coloration with H/E. A rare peculiarity was the presence of numerous pigment-containing cells (melanomacrophages) randomly distributed in the albuginea tunic and testicular interstitium, giving the testes a dark brown coloration. This unusual characteristic has been rarely observed or described in other species (P. cuvieri, P. fuscomaculatus, Bombina bombina and Xenopus laevis). In other lower vertebrates, the pigmented cells can be found in different organs, constituting an extracutaneous pigmentary system of unknown function. Further, it was identified a conspicuous variation, as to presence and distribution pattern due to possible species-specific aspects. The inter-locular tissue is relatively scarce, presenting melanomacrophages in all specimens analyzed. This work was supported by FAPESP (Grant nº 02/08016-9).

B-035 EXTRACUTANEOUS PIGMENTARY SYSTEM AND STRUCTURAL ANALYSES IN THE TESTES OF PHYSALAEMUS FUSCOMACULATUS (ANURA, LEPTODACTYLIDAE).

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In amphibians, great, plain and irregular cells can be found in different organs, constituting an extracutaneous pigmentary system, called melanomacrophages (MMs), which has unknown function. Associated to the reproductive organs of some species, MMs also are observed in the albuginea tunic and testicular interstitium, and they give to the testes a dark brown coloration. This study describes the occurrence and the distribution pattern of the pigmented cells in the organs and morphology of the testes in Physalaemus fuscomaculatus. Ten male samples, originating from the Nova Itapirema (São Paulo State, Brazil), were used. After anatomic analyses and obtainment of the testicular fragments, the material was submitted to the histological routine and subsequent documentation. MMs are presents in various organs with typical cellular population between the structures. The testes are paired ovoid and pigmented organs, with the germ tissue arranged in seminiferous locules. Germ tissue has different cellular types (spermatogonia, spermatocytes, spermatids and spermatooza) surrounded by Sertoli Cells which give a cistic arrangement. Interlocular tissue are composed by Leydig interstitial cells, fibroblasts, blood vessels, efferent ductules and great amount of melanomacrophages. The results indicate that the extracutaneous pigmentary system has specific-specie characteristic, however, they can contribute for important taxonomic and filogenetic application. Proc. FAPESP nº 20/08016-9

B-036 SPERMATOGENESIS OF Hyla Minuta (ANURA, HYLIDAE)

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This study has as an objective morphologically describe Hyla minuta verifying the stages of cellular differentiation from the spermatogenetic lineage. In São José do Rio Preto City (São Paulo State, Brazil), nine samples had been captured during their reproductive period. After anatomic analyses and obtainment of the testicular fragments, the material was submitted to the histological routine and subsequent documentation. The testes are paired organs, smaller measurement (2 mm of diameter), and rounded, whitish and located neighboring kidneys. The cells were differentiated and identified according to cystic morphology and arrangement. Near to locular periphery are found spermatogonia I, which are the biggest lineage's cells and present multilobular nucleus with chromatinic granulations. Because of consecutive differentiation and proliferation, the spermatogonia I originate the rest of the spermatogenensis lineage cells: spermatogonia II, spermatocytes I and II, spermatids I and II, and finally the spermatooza. Cysts formation in the germinative tissue is caused by the association of Sertoli cells, which supplies sustentation and nutrition, with germinative cells, grouping them in the same cellular differentiation phase. Although the spermatogenesis characterization is well-known in other animal groups, it was verified some peculiarities in these cellular kinds from this Hylidae, important information by virtue of few studies in neotropical anurans. This work was supported by FAPESP (Grant nº 02/08016-9).

B-037 EFFECTS OF AROMATASE INHIBITION ON THE GERBIL FEMALE PROSTATE

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The letrozole has been indicated as endocrine therapy for the treatment of breast cancer because it impedes the peripheral tissues aromatization of testosterone to estrogen. As this enzymatic inhibitor interferes in the systemic hormonal balance it can cause alterations in the female prostate physiology which is regulated by steroids hormones. To evaluate the effects of this therapy on the adult gerbil female prostate, the animals were treated by letrozole (1mg/kg/day) for 3, 7 and 14 days, and their organs were processed for light microscopy followed by morphologic and stereological analyses. In addition, serum testosterone, estrogen and prostate specific antigen (PSA) were determined. The serologic data indicate that the letrozole treatment caused a progressive and significant increase (p≤0.05) in estrogen and testosterone levels. Furthermore, high estrogen levels were associated with low PSA values. Morphologically, it was observed an increase of the epithelial cell proliferation and gland secretory activity. Stromal and intraluminal inflammatory focuses and neoplasia intraepithelial were observed at all time intervals of therapy. Benign prostate hyperplasia was observed at the 14 days of the treatment. These data demonstrate that female prostate is sensitive to the hormonal alterations caused by the short term letrozole administration, besides long periods of this treatment can lead to prostatic lesions.
The post-natal development of RVP was analyzed during the first 12 weeks post-natal using morphologic-stereological analyses to characterize the behavior of the tissue compartments. The relative and absolute volumes of the RVP tissue compartments were determined by stereology. The relative weight of the organ demonstrates an initial growth within the first three weeks, a resting pre-pubertal period, in which growth only reflects overall body weight increase. At the 7th week the RVP starts to grow steadily up to the twelfth week. This second growth phase coincides with beginning of the seric testosterone concentration. The luminal compartment formation occurs within the initial three post-natal weeks, besides an increase during puberty and a final raise at the 12th week. It also showed a decrease in the relative volume of the non-muscular stroma and of the smooth muscle cells with growth. Absolute volume of the lumen showed three phases of growth (1-3; 6-9; 11-12th weeks). On the other hand, the increase in the epithelium absolute volume was steady up to the 8th week and then showed an increase up the 10th week, being observed as an epithelial infolding and budding, and decreased slightly thereafter. The results suggest the post-natal growth of the RVP alternate between phases of epithelial proliferation and synthesis/accumulation of the luminal secretion.

Female prostate secretory cells of the gerbil Meriones unguiculatus are low cube and synthesize glycoproteins. In males, the androgens maintain the physiology of these cells. However, the mechanisms that maintain the functionality of the female prostate are unknown. Thus, to evaluate the function of the androgens in the activity of the secretory cells in the adult female gland, the gerbils were treated with testosterone cipionate (1mg/Kg/48hours) for 3, 7, 14 and 21 days, processed for light microscopy and submitted to morphometric analyses in Image-Pro-Plus software. The morphometric parameters were: secretory cells height; ratio area nucleus/cytoplasm; and area; perimeter and nuclear form factor. The androgenic administration caused a significant increase (p≤0,001) in the thickness of the epithelium which became tall prismatic (14,38±3,94 control; 23,35µm±4,80 testosterone treatment). Besides, cytoplasmic area increased proportionally to the nuclear area. The nucleus exhibited larger volume (22,98µm±5,38 control; 30,29µm±6,06 testosterone treatment; p≤0,0001) and higher nucleoli number. These facts indicate that the testosterone promoted an increase in nuclear activity, probably related to the stimulatory effects on the synthesis and cellular secretion. In conclusion, these results suggest that the female prostate cells are sensitive to the androgenic action and normal activity of these cells can be subordinated to the low androgens levels existent in the female organism.

Cellular differentiation and proliferation of the prostatic epithelium have been long considered to be mediated by androgens. However, estrogens can induce a marked proliferative alteration of prostatic epithelium. The objective of this work was to evaluate the histopathological alterations in the epithelium of the ventral gerbil prostate after hormonal supplementation. It were used 20 adult male gerbils divided in 4 groups: Control (C); Testosterone (T); Estradiol (E) and Testosterone + Estradiol (T+E). The animals received the hormones in alternate days, for 21 days. After the treatment, the animals were killed and blood was collected to hormonal dosages (LH, FSH and prolactin). The ventral prostate was removed, immediately fixed and embedded in historesin; H&E sections were analyzed. The morphometrics analyses were accomplished at Image-Pro-Plus analyzer. The data were submitted to Tukkey’s statistical test. Hormonal treatments provoked increase in the epithelium height (µm), p≤0,05 (C=9,07±2,13; T=17,80±4,18; E=25,30±4,80; T+E=30,15±6,11). Prostatitis and focal PIN (Prostatic Intraepithelial Neoplasia) were evidenced in T. In E and T+E, epithelial dysplasia and hyperplasia were clearly observed. Apoptotic cells were observed in all treatments, principally in hyperplasic regions of the E and T+E. These data prove that hormonal supplementation alters the homeostasis of secretory cells of the gerbil prostate.

To compare the effects of non-steroidal and steroidal antiandrogens on testicular structure of the gerbil Meriones unguiculatus, the animals were subcutaneously injected with pharmacological vehicle only (C), ciproterone acetate (CA) and flutamide (F). These androgen receptor antagonists were administered in doses of 10mg/ Kg body weight, each three days time intervals, for 45 days. The body weight progressively decrease to 7, 13 and 15% of control values at 15, 30 and 45 days after the beginning of flutamide treatment but the testicular weight remained unchanged. Both antiandrogens caused very similar effects on testicular structure. A slight reduction in seminiferous tubule diameter following antiandrogen treatments (C- 284,6µm±22,2; F -272,3 ±17,2 and CA- 272,5 ±21,9; p ≤0,001) was concomitant with the increase in germinative epithelium relative volume (C- 79,9%±3,4; F- 82,8±3,1; CA- 84,4±3,2). Additionally, the decrease observed to the interstitial tissue after medium-term antiandrogen therapies was due to a reduction in Leydig cell relative volume of 46,3% in F and 59,7% in CA-treated gerbils (p ≤0,001). No signals of epithelial lesions and germ cell detachment were observed by light microscope evaluation. These findings constitute evidence that independent of antiandrogen chemical nature its prolonged use affects Leydig cell rather than seminiferous tissue.

**B-038** ALTERNATING PROLIFERATIVE AND SECRETORY ACTIVITIES DURING THE RAT VENTRAL PROSTATE (RVP) GROWTH

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**B-039** MORMETRIC EVALUATION OF TESTOSTERONE EFFECTS ON THE PROSTATIC SECRETORY CELLS OF FEMALE GERBIL


**B-040** HISTOPATHOLOGICAL ASPECTS OF THE ADULT GERBIL PROSTATE AFTER HORMONAL SUPPLEMENTATION

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**B-041** TESTICULAR CHANGES IN ADULT GERBIL AFTER FLUTAMIDE AND CIPROTERONE ACETATE TREATMENT

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B-042 MAST CELL BEHAVIOR IN THE PROSTATE OF CASTRATED RATS
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Marked changes take place in the prostate gland after androgen ablation. One such change is an increase of relative volume of the stroma. The stroma is characterized by different cell types and a number of extracellular matrix components. Mast cells are found in the prostatic stroma, but their involvement in the stromal alterations following castration has not been addressed before. This work was undertaken to characterize the variation in mast cell morphology and content in the prostate of castrated rats, using stereological procedures. The ventral prostate of control and castrated rats were removed, fixed and carefully processed for random sampling and serial sections obtained and stained with toluidine blue. The Weibel’s method and the optical dissector method were employed to estimate mast cell number. Cell volume was calculated by measuring smaller and larger diameters and using the volume of the ellipse. Mast cell volume was significantly smaller castrated rats. They occupy a larger volume of the organ, but showed a reduced absolute volume, indicating that the total number of cell reduced, in spite of considering the cellular volume reduction. The use of the optical dissector demonstrated an increased volume density, but also demonstrated reduced total volume. The results demonstrate that mast cells were lost (they leave the organ, degranulated or died), but the remaining cells are concentrated in the stroma.

B-043 POST-NATAL DEVELOPMENT OF THE RAT SEMINAL VESICLE
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The male accessory glands are greatly dependent on the androgens for development, growth and activity. While the prostate originates from the urogenital mesenchyme, the seminal vesicle (SV) develops from the Wolffian duct. These different embryonic origins may have influences on their developmental behavior. In this work we have examined the post-natal development of the seminal vesicle, using morphological and stereological analysis to follow the dynamics of the different tissue compartments. The SV weight showed a marked increment after the 6th post-natal week, following the increase in circulating testosterone. This increase was steady up to the 10th week and then declined. The epithelial and stromal compartments contribute equally to this overall increase of the organ weight. At the microscopic level, there was an intense epithelial branching and stromal invasion in the first week. By the third week, one could observe a prominent folding of the epithelium. By the 5th week, the smooth muscle cells could be distinguished in the stroma. The functional activation of the organ also occurred following the testosterone raising, as observed by the increase in the lumen and the accumulation of secretory material. In spite of the intense epithelial activity in the first three weeks, there was not an increment in relative and absolute weight of the SV. This is the main difference between the SV and the ventral prostate.

B-044 AN ULTRASTRUCTURAL STUDY OF THE SPERMATOZOA OF COLOSTETHUS STEPHENI (ANURA – DENDROBATIDAE)
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In this work, light microscopy and transmission electron microscopy were used to described the spermatozoa of C. stepheni. For light microscopy a suspension of spermatozoa was prepared in cacodylate buffer and fixed in this buffer containing paraformaldehyde-glutaraldehyde. The slides were stained with DAPI and examined with an Olympus BX60 microscope. For transmission electron microscopy, fragments of the testes were fixed in the same fixative solution, post-fixed in OsO4 and potassium ferricyanide in the same buffer, dehydrated in acetone and embedded in Epon. Sections were stained with uranyl acetate and lead citrate and examined with a Leo 906 transmission electron microscope. The main characteristic of the spermatozoa was the presence of only one complete flagellum, whereas other Colostethus species studied so far have biflagellate spermatozoa. The acrosomal vesicle was conical and subacrosomal cone covered the tip of the nucleus, as in most neobatrachian species. In the midpiece region, a mitochondrion collar surrounded the initial portion of the flagellum. In the posterior region, mitochondria were also seen in the undulating membrane. In the distal region of the flagellum, no axial sheath and the undulating membrane were seen, with only the axonemata persisting. These findings may be useful for elucidating the intragenic relationships in dendrobatids.

Support: FAPESP, CAPES.

B-045 ULTRASTRUCTURAL CHARACTERISTICS OF SPERM IN HYLOIDINAE SPECIES (ANURA, LEPTODACTYLIDAE) AND THEIR RELEVANCE TO TAXONOMIC RELATIONSHIPS OF THIS GROUP
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Hyliodinae leptodactylids form a group of diurnal frogs thought to be the basal group from which the dendrobatids arose. This assumption is based on classic analysis of morphological traits. In this work, we describe ultrastructural characteristics of sperm from three hyliodine species (Hyloides phylloides, Crossodactylus sp. n. and Megaelosia massarti), aiming to reassess the intergeneric relationships within this group, as well as the possible Hyliodinae/Dendrobatidae relationship. Sperm ultrastructure was very similar among the three species examined here, and indicated the conserved nature of these gametes within the Hyliodinae. The structure of the acrosomal complex was very similar to that of other leptodactylid species, to most of the remaining species included in the Bufonidae and dendrobatid species examined so far, and so contributes little to our understanding of Hyliodinae/Dendrobatidae relationships since it has been considered as a plesiomorphic trait. The flagellar apparatus of Crossodactylus sp. n. was very similar to that of most leptodactylids. Megaelosia massarti and Hyloides phylloides sperm shared a distinctive condition in their axial and juxta-axonomal fibers, which may serve to reinforce the affinities between these two genera. This distinctive flagellar condition expands the already well-known variability in sperm structure within the Leptodactylidae. Financial support: FAPESP.
D-046 DYNAMICS OF OOCYTE SECONDARY GROWTH IN PLATY, Xiphophorus maculatus (TELEOSTEI, POECILIIDAE)

B. solisianus. The platy, an ovoviviparous ornamental freshwater fish, presents internal fertilization, sperm storage in the ovary and intrafollicular gestation. Considering the peculiarities of this reproductive biology, we have detailed here the oocyte secondary growth. Adult females were anesthetized with benzocaine, and the ovaries prepared by routine electron microscopy methods. Oocyte cytoplasmic vesicles are electron-lucent, electron-dense or contain an electron-dense granule. Mitochondria, rough endoplasmic reticulum and Golgi complex are scattered in the ooplasm. An electron-dense material, deposited between the oocyte and follicular cells/FC, surrounds the microvilli, forming the zona radiata/ZR. The oolemma reveals deep infoldings reflected in the FC arrangement, making these cells are either squamous or cubic. The plasma membrane of the oocyte and the FC continue to fold, so that the microvilli increase in number and become highly interdigitated. Later oocytes contain yolk globules/YG that are deposited first at the periphery. Cyttoplasmic organelles are dislocated to the periphery; lipid droplets fuse, increasing in size. As the oolemma infoldings decrease, the FC become cubic. In this phase, the ZR is double layered. The oocyte in late vitellogenesis presents YG that fuse into a central, compact mass. The ZR becomes linear and thicker. The FC are uniformly cubic at this final stage and show distinct intercellular spaces, maintaining contact only by their interdigitated lateral surfaces.

Acknowledgements: CAPES/PICDT; FAPESP Proc. 01/07888-0.

B-047 MORPHOLOGY OF THE SPERMATOZOA OF Brachidontes darwiniatus AND B. solisianus (BIVALVIA, MYTILIDIAE) FROM THE SOUTHERN BRAZILIAN COAST

Numerous investigations have demonstrated the usefulness of sperm morphology for evaluating molluscan phylogeny. In this work, we used transmission and scanning electron microscopy to study the structure of mature spermatozoa from two bivalves. Brachidontes darwiniatus occurs from the state of Rio de Janeiro, Brazil, to Patagônia, in Argentina, whereas B. solisianus is distributed from Mexico to Uruguay. The spermatozoa of both species were of the primitive type. In both species, the spermatozoan head contained a spheroidal nucleus capped by a conical acrosome with an anterior extension. The nuclei contained electron-lucent regions formed by invaginations of the nuclear envelope. These invaginations were detected by E-PTA staining for glycoproteins. The mid-piece region consisted of five spherical mitochondria grouped in a ring around a pair of centrioles. The flagellum exhibited the typical 9+2 microtubule structure. The only difference in the morphology of spermatozoa from these two species was the longer anterior extension of the acrosomal vesicle in B. solisianus. This elongated acrosome may facilitate penetration of the jelly coat and cytoplasm of large oocytes and could increase the efficiency of fertilization. The resulting enhanced reproductive success could account for the wider geographic distribution of B. solisianus.

B-048 QUANTITATIVE EVALUATION IN THE EPITHELIUM AND STROMAL COMPARTMENTS DURING DEVELOPMENT OF THE GERBIL FEMALE PROSTATE.

The mechanisms that control the development and secretory activity of prostate in females are unclear. Previous observations have shown that prostate of female rodent Meriones unguiculatus is a good model to investigate the factors involved in regulation of gland morphology. The objective of the study is to describe the gerbil female prostate during postnatal development. The glands of the young (1-2 months), adult (3-11) and old (since 12) gerbils were processed for histological examination followed by morphometric and statistics analyses. Unlike young male prostate, the young female gland already showed a differentiated morphological state and the secretory activity remained intense until senescence. A discrete and progressive increase in the height (µm) of the secretory epithelium was detected throughout aging (young: 13.62±0.32; adult: 15.30±0.35; old: 17.60±0.43) with an apparent increase in epithelial proliferation in the old ones. The muscular layer sourrounding the alveoli showed a significant (p<0.032) thickness increase from 11.83±0.325 in the young to 13.33±0.484 in the old animals. The female prostate morphogenesis shows significant differences in relation to male gland, mainly by concerning their possible premature activity. Although the female prostate has been considered a rudimentary and immature organ, the morphological evidence of secretory activity observed here suggests a probable functional contribution to the female reproductive tract. (FAPESP, CNPq)

B-049 CULTIVE AND CARACTERIZATION OF FETAL AND ADULT FIBROBLASTS ISOLATED OF NELORE BOVINE FOR NUCLEAR TRANSFER.

Cloning by nuclear transfer is a technology that has been used to make major advances in reproductive sciences. It allows a more efficient method of making transgenic embryos, fetuses and offspring and multiplication of genetically superior adult animals. Basic studies on nuclear transfer have contributed to understanding how genomic activation and cell cycle synchrony affect nucleus reprogramming and cloning efficiencies. For this, it is important the characterization of donor cells to improve the production of viable offspring, that remain very low. The aim of this study was the establishment of bovine skin adult and fetal stable primary fibroblasts cell culture for embryo reconstruction. Cultures were characterized by morphological aspects, immunocytochemical and ultrastructural analysis. The light microscope results show typical allogatedt aspects of normal fibroblastic cell; with rounded nuclei, prominent nucleoli and free ribosomes predominante in the cytoplasm. Dense and organized network occurred in adult and fetal fibroblasts culture and presented positive immunomarker to vimentin in 5, 10 and 15 subcultures. Ultrastructural analysis shows cytoplasm typical of cells actively engaged in macromolecular synthesis. Large numbers of granular and agranular endoplasmic reticulum cisterna are present in addition to well-developed Golgi complexes, mitochondria and lysosomes.
This study aimed to evaluate in vitro and in vivo viability of bovine nuclear transferred embryos from fetal and adult Nelore fibroblasts. After serum starvation, each fibroblast was inserted under the zona pellucida of each enucleated oocyte and the COCs electrofused and activated (2 pulses of 4KV/cm for 20µs). The activated reconstructed embryos as well as IVF embryos (control) were co-cultured with granulosa cells in TCM 199 + 10% FCS for 7 – 9 days. After electrofusion, 212 (fetal cells) and 181 (adult cells) embryos fused and 32 (15.1%) and 30 (16.6%) reached blastocyst stage, respectively. After coculture, a group of embryos was fixed in order to evaluate the number of cells per blastocyst. Another group of embryos was transferred into recipients. The mean number of cells of cloned blastocyst from fetal, adult and IVF embryos were 129.3; 101.3 and 114.3, respectively. No significant difference among these groups was verified. After transferring 18 (fetal) and 21 (adult) blastocysts, pregnancy rates at day 90 were 16.7% (3) and 19% (4), respectively. No significant difference among pregnancy rates was verified. Pregnancies from fetal and adult cells delivered a healthy male and female calf at day 290, respectively. These results indicate that fetal and adult fibroblasts can be used as nuclei donors, with similar rates of in vitro and in vivo development.

The objective of this study was to evaluate the in vitro maturation of bovine oocytes after blocking Germinal Vesicle (GV) for 24 hours by action of inhibitors of M phase promotion factor (MPF) Butyrolactone I and Roscovitine. Oocytes of slaughterhouse ovaries were divided into 4 groups of maturation: group 1 (0h Control), group 2 (50µM of Roscovitine - 24h), group 3 (150µM of Butyrolactone I - 24h blocking and 24h maturation) and group 4 (24h Control). For accomplishment of FIV, oocytes of group 1 after 24h maturation and oocytes of group 2 and 3, after 24h blocking and 24h maturation, were in vitro fertilized and cultivated for 9 days. Data were analyzed by Qui-square ($\chi^2$), (P<0.05). The embryos of groups 1 and 3 showed similar rates of blastocyst (22.02% and 17.74%) and hatching after 9 days of co-cultive (15.18% and 12.36%), however group 2 showed lower rates of blastocyst (6.32%) and hatching (1.15%). Butyrolactone I showed to be efficient for embryos development after oocytes blocking in GV.

Peritubular myoid cells surround the seminiferous tubules in the testis. These cells contain a contractile cytoskeleton with an isotype of smooth muscle myosin II (T-myosin) which is not assembled in filaments. In order to determine whether T-myosin is able to assemble in vitro, T-myosin of rat myoid cells was purified and the salt-dependent solubility was assessed in a buffer containing different concentrations of NaCl. 200 µg/ml of myosin in phosphate-buffered 350 mM NaCl were dialyzed for 12 h at 4 ºC, against 0-350 mM NaCl. The samples where centrifuged, the pellets and supernatants analyzed by 7.5% SDS-PAGE, and the fraction of assembled myosin quantified by comparison of staining intensity of the bands. The assay showed that only 5% of the myosin in 0-350 mM NaCl was assembled in filaments. These results indicate that T-myosin is unable to assemble in vitro showing a particular characteristic among the rest of smooth muscle myosin II.
B-054 MALE REPRODUCTIVE TOXICITY OF INSECTICIDE FENVALERATE IN RATS.

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Studies have shown that pyrethroid insecticides, such as fenvalerate, can have estrogenic activity, acting as endocrine disruptors. The objective of this study was to investigate the effects of fenvalerate on the reproductive system and fertility of adult male rats. Fenvalerate (40 mg/kg/day, gavage, 30 days) dissolved in corn oil was administered to male adult rats. The following parameters were analyzed: body weight, absolute and relative weight of reproductive organs, germ cell counts, sexual behavior and fertility after natural mating and artificial insemination in utero. The treatment with fenvalerate decreased the absolute weight of the testis, however, when the relative weight of this organ was calculated the difference no longer existed. Treated animals presented a reduction in the following parameters: number of spermatids in the testis (control: 222.81±14.52; 40 mg/kg: 156.87±9.09* x10^6/organ), daily sperm production (control: 36.85±2.51; 40 mg/kg: 25.72±1.49* x10^6/testis/day), number of sperm in the caput/corpus epididymis (control: 128.76±8.76; 40 mg/kg: 79.78±3.79* x10^6/organ), number of sperm in the cauda epididymis (control: 194.29±14.88; 40 mg/kg: 131.60±9.64* x10^6/organ)(*p<0.05). Although the sperm concentration has existed. Treated animals presented a reduction in the results of the present study showed that rats exposed to fenvalerate exhibit reduction in the sperm number.

Financial Support: CAPES, FAPESP, CNPq

B-055 MALE REPRODUCTIVE TOXICITY OF DIURON IN ADULT RATS.

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There are few studies on the toxic effects of Diuron, a ureic herbicide, especially on the reproductive system. The objective of this study was to evaluate the toxicity of Diuron on the reproductive organs and sperm production of adult male rats. 30 rats were divided into 3 experimental groups of 10 rats each. Treated groups received Diuron (Sigma, 98% of purity) dissolved in corn oil, by gavage, in the doses of 125 and 250 mg/Kg/day for 30 days. Control rats received the vehicle. At the end of this period the rats were killed and had the testis, epididymis, seminal vesicle, ventral prostate, liver and kidneys removed and weighed. There was a significant increase in absolute and relative weights of the liver. The number (mean ± SEM) of homogenization-resistant spermatids significantly decreased in the testis of the rats that received 125 mg/Kg (162.18 ± 7.73) compared to the control group (183.76 ± 4.48), but the difference no longer existed when the concentration of sperm per gram of testis and the daily sperm production were calculated. Although the experiment is still in development, the results obtained so far showed that while Diuron was toxic to the organism, as evidenced by the increase in the liver weight, did not alter the male reproductive parameters investigated.

Financial support: CNPq

B-056 SENSORIAL STRUCTURES ASSOCIATED TO THE PARS STRIDENS IN Gryllus assimilis (ORTHOPTERA, GRYLLIDAE).

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The calling sound in Gryllus is a fundamental characteristic for the interspecific recognition, being essential to reproductive success in this species. This calling sound is also one of the principal character for the taxonomy of this group; it’s produced by pars stridens, placed in the wing, composed of small teeth. Several authors have quoted the presence of sensorial structures associated to the pars stridens in several species of crickets, pointing out the importance of the study of these structures, once the opening of the wings and the composition of the syllables of the produced sound depend on the integrity of these structures. Adults’ males of Gryllus assimilis were collected in Rio Claro, SP and histological analyses as well as scanning electronic microscopy were realized. The histological analysis presented the wing formed by two layers of chitin, were the superior was thicker, and between them, an epithelium. The scanning, as well as the histological sections detected three patterns of structures, with different diameter, morphology and sizes. The smaller one typifies a bristle; the other two are similar to a typical structure of trichoidea sensillum, whose function would be mechanoreception. The results achieved seems to confirm the presence of sensorial structures associated to pars stridens in Gryllus assimilis, contesting some authors who believed that these structures where simply bristles.

B-057 EFFECT OF Bothrops jararaca ENVENOMATION IN PREGNANT MICE

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Since snakes of the Bothrops genus account for most of the envenomation cases in Brazil, this study aimed to provide data on the embryo-fetotoxic potential of Bothrops jararaca venom (BjV) as well as to correlate it with the morphology of decidual. BjV or saline was administrated to mice on the 8th day of pregnancy (dp). One group of animals was sacrificed on the 9th dp for morphological examination of decidua; another group was sacrificed on the 18th dp to evaluate the number of resorptions and external malformations of fetuses and to examine their skeleton. In envenomed mice we observed many disorganized mature decidual cells, close to the embryo, scattered among healthy decidual cells. These cells presented pyknotic nuclei, acidophilic cytoplasm and reduced cytoplasmic area. At the maternal-fetal interface, as well as within capillary lumens, a great number of polymorphonuclear cells were observed. Trophoblastic giant cell exhibited an unusual round form. The exposure to BjV during organogenesis period of prenatal development induced an increase in reabsorbed fetuses. A higher incidence of signs of external malformation was also noticed in fetuses although the skeletal survey of the remained fetuses was unremarkable. There was an increase in number of stunted fetuses. No adverse effects were seen in saline group.
B-058 SPERM TRANSFER IN THREE SPECIES OF DUGEIIDAE (PLATYHELMINTHES, TRICLADIDA, PALUDICOLA)
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Freshwater triclads are hermaphroditic and perform mutual copulation. There are reports about the formation of a spermatophore for sperm transfer in some Paludicola, but there are no experimental researches. In the present study, aiming at enhancing the knowledge on the reproductive biology of freshwater planarians, we analyze three species of Dugesiae: G. tigrina (Girard, 1850), G. schubarti (Marcus, 1946) and G. biapertura Sluys, 1997. The specimens were fixed after copulation in neutral formaldehyde and embedded in Paraplast. Serial sections were stained with trichrome methods interposed with following histochemical reactions: Alcian Blue/ Acid Periodic Schiff for detection of mucosubstances and Bromphenol Blue, Ninhydrin and DMAB, for protein. We verified that, in the three species, sperm are transferred inside a spermatophore which shows an oval elongated body and a long and thin tail. The body is constituted of three layers and the tail represents mainly an extension of the outer layer. Associated with sperm there are glycoproteic or proteic granules. The inner layer shows a mucopolysaccharidic constitution. The median layer is composed of glycoprotein. The outer layer is constituted of glycosaminoglicans or neutral mucopolysaccharides. During the copula, the spermatophore is transferred to the partner and after several hours it is decomposed, settling sperm free to move anteriorly along the oviducts towards the ovaries.

B-059 CHARACTERIZATION OF A NEW ANTIGEN INVOLVED IN THE SPERMATOZOA PREPARATION LEVEL DURING FERTILIZATION
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Fertilization depends on a sequence of events that culminates in the activation of the egg by the spermatozoa. The complete differentiation of the testicular germ cells in cells with fertilization ability involves the testsis, epididymis, vas deferens and female reproductive organs. Several proteins synthesized and secreted by the epididymal epithelial cells can be further located on the spermatozoa membrane surface or inside acrosomal vesicle. The antigen recognized by the monoclonal antibody (mAb) TRA 54 is exclusively located in spermatoocytes and spermatids of the seminiferous tubules and in the epithelial cells of the epididymis of C57 BL/6 mice. The aim of this work was to obtain additional information about the antigen in the epididymal epithelial cells for biochemical characteristics and expression regulation. The results of immunohistochemistry and immunoblotting studies revealed that the antigen could be secreted by the epithelial epididymal cells and further adhered to luminal spermatozoon. The expression of the antigen in the epididymis and testis are independent events and epididymal synthesis seems to be regulated by testicular androgens. Different isoforms appears to be produced in each organ. Preliminary results show the antigen presence in testsis, epididymis and ejaculated spermatozoa of swiss mouse, rat and human, indicating that this antigen must play an important role in testicular spermatogenesis and subsequent epididymal maturation and fertilization.

B-060 INFLUENCE OF ESTROGEN AND TAMOXIFEN CITRATE ON THE TESTICULAR DYNAMICS OF IMMATURE RATS
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The short- (1 week) and medium-term (7 weeks) effects of single dose (35mg/Kg of body weight) of β-estradiol and anti-estrogen tamoxifen on the testis of young rats (5 weeks age) were evaluated. The histological and quantitative analyses showed that, one week after estrogen administration, the testicular structure as well as the spermatogenesis were drastically affected. Thus, a significant decrease of Testicular-Somatic-Index, seminiferous tubule diameter and volume of Leydig cells were accomplished by appearance of structural lesions in the seminiferous epithelium, degeneration and apoptosis of germ cells and disappearance of elongated spermatids. Some of these changes persisted seven weeks after estrogenic treatment. The estradiol-induced changes observed here might to be explained by irreversible suppression of androgen receptors in the testes of developing rat, as previously reported. A disorganization of the germinative epithelium was detected one week after tamoxifen injection, and became more evident after seven weeks, when a slight but significant decrease in seminiferous tubule diameter was observed. The tamoxifen not influenced germ cell apoptosis and apparently not interfered in the final phase of spermatogenic differentiation at both time intervals. It may be concluded that the transient exposure of young rats to the antineoplastic agent tamoxifen causes less qualitative defects in spermatogenesis in comparison with estrogen.

B-061 MORPHOLOGY OF THE SPERMATOZOA OF IPORANGAIA PUSTULOSA (ARACHNIDA: OPILIONES)
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The existing morphological studies of the male reproductive tract and the spermatozoa in harvestman are from the decade of ‘70; they are incomplete and the photographic plates are not satisfactory. A description of the male reproductive tract as well as the spermatozoa of the harvestman, Iporangaia pustulosa, was undertaken. Adult males, collected in Atibaia/SP, were dissected removing their reproductive tract and processing for transmission and scanning electron microscopy. The male reproductive tract of I. pustulosa is made up of a tubular U-shaped testis connected to two efferent tubules, converging to a deferent tubule that extends to the seminal vesicle. There follows a propulsive organ and a retractable penis. All the spermatogenesis stages occur in compartments found along the testis. In the seminal vesicle only dispersed spermatozoa occur. The spermatozoa are oval-shaped with thin extremities. With SEM, two sperm types were observed: a large thick one and a small, thin and smooth one. The large sperm are more frequent than the small one. With TEM, the two types present the same organelles, and differ only as to the presence of extracellular projections on the large sperm. The meaning of this sperm dichotomy and the composition and function of the projections are unclear. Because the spermatozoa are devoid of flagella, these projections may be involved in the locomotion of the large sperm.
B-062 ULTRASTRUCTURAL IMMUNOCYTOCHEMICAL EVIDENCE FOR ACTIN IN THE ACROSOMAL COMPLEX DURING SPERMIOGENESIS OF THE LIZARD, Tropidurus Itambere.
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In lizards, the origin of structures that contribute to the acrosomal complex has received little attention and some discord exists in relation to the perforatorium and the subacrosomal cone. In this study, early developmental stages of the acrosomal complex have been analyzed and new suggestions made as to the origin of these structures during spermio genesis of Tropidurus itambere. We detected actin in the acrosomal granule and pro-acrosomal vesicle of spermatids, with ultrastructural immunocytochemistry, using an antibody against α-smooth muscle actin (monoclonal α-SMA). Intense marking also identified actin in two regions of the spermatozoan acrosomal complex, the subacrosomal cone and the perforatorium. The acrosomal and subacrosomal granules appear to be responsible for the formation of the perforatorium, while the pro-acrosomal vesicle is responsible for the formation of the subacrosomal cone. This conclusion, reached by various authors, was based on the location of these structures during spermio genesis and on their electron density. Our results corroborate the proposed origin, considering cytochemical properties such as the presence of actin and the strong staining verified with E-PTA for basic protein, which is very similar for these structures. The results endorse the view that, in lizards, the perforatorium and subacrosomal cone are homologous, in composition and probably also in function. Support: FAPESP (01/06744-4), CNPq (150007/03-5).

B-063 ARRANGEMENT OF THE SPERMATOZOA OF THE COFFEE LEAF-MINER (LEPIDOPTERA)
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The Lepidopteran order (butterflies and moths) presents a dichotomic spermogenesis producing two spermatozoan types, called eupyrene and apyrene, which differ in morphology and function. The eupyrene are typical sperm, with nucleus and acrosome, and are responsible for the egg fertilization. The apyrene are devoid of nucleus and acrosome and are related with the sperm competition. Both sperm types develop in a testicular cyst with 256 cells each. The coffee miner leaf (Leucoptera coffeella) is the principal coffee plague in Brazil. Here, we analyzed the number of spermatozoa per cyst and their arrangement in the testis and seminal vesicle. Therefore, adult males of L. coffeella, collected in the Instituto Agronômico de Campinas, were dissected, removing their testis and seminal vesicle for transmission electron microscopy. The apyrene and eupyrene spermatozoa are organized in separate testicular cysts with a different arrangement from that observed in other Lepidoptera. Besides, each cyst possesses 128 (2^7) spermatozoa, and the pattern is 256 (2^8). In the seminal vesicle, both spermatozoa are dispersed in the lumen, different from that observed in all other Lepidoptera species, where the eupyrene are organized in bundles. These preliminary observations were never been reported in other Lepidoptera species. Detailed analyses will be necessary to describe the ultrastructure of these spermatozoa and verify if these data are related with sperm morphology.

B-064 MORPHOLOGY AND ARRANGEMENT OF THE SPERMATOZOA OF ARMY ANT NEIVAMYRMEX SP (HYMENOPTERA)
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Seminal vesicles of Neivamyrmex sp were processed for transmission electron microscopy and suspensions of spermatozoa from seminal vesicle were processed for light microscopy. The spermatozoa are filiform, measuring 165 µm and are organized in spiraled bundles. The anterior region is composed of the acrosome and nucleus. The acrosome presents an acrosomal vesicle and perforatorium. The dense nucleus measures 13 µm and presents several clear areas. The flagellum is composed of the axoneme, centriolar adjunct, two mitochondrial derivatives and two accessory bodies. The axoneme presents a 9+9+2 pattern. In the posterior region, the central microtubules disappear first, followed by the doublets and finally by the accessory tubules. The centriolar adjunct assembles at the nuclear base and extends parallel to the axoneme and mitochondrial derivatives. Both mitochondrial derivatives, in transverse section, have a triangular shape with an electron-dense central area, two amorphous areas and a cristae region. The accessory bodies are located laterally between the axoneme and mitochondrial derivatives. For all the Aculeata studied, this is the first time that spermatozoa from seminal vesicle were observed in bundles in sexually mature adults. This characteristic was observed only in Symphyla the basal Hymenoptera group, but their bundles are not spirally twisted Probably, in Neivamyrmex sp, this is a derived characteristic, that distinguishes this species from all Aculeata and all known ants. FAPESP-03/08366-2.

B-065 ULTRASTRUCTURE OF THE UTERINE TUBE EPITHELIUM OF UCHA AND UCHB RATS LINEAGE: ETHANOL VOLUNTARY CONSUMERS
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Alcoholic women frequently present menstruation irregularities, infertility and problems in the fetus development during pregnancy. The UCHA (ethanol low consume) and UCHB (ethanol high consume) variety rats are important for the study of unique characteristics to human alcoholism. The aim of the present study was to analyse the structural alterations of the uterine tube epithelial cells from the UCHA and UCHB rat lineages. The uterine tubes of seven animals from each group (UCHA, UCHB and control), killed in estrus phase, were processed for TEM routine. The intramural region of UCH rats presented vacuoles and dilated cisterns in the cytoplasm. Nucleus with irregular shape, scarce secretory granules and microvilli, and lipidic drops. The ishthmus region of UCH rats showed modified microvilli electron-density, cilia with irregular distribution and the characteristics cited in intramural region. The ampulla region of UCH rats showed an increase of lamellar bodies and lipidic drops in the cytoplasm. The infundibulum and fimbiae regions of UCH rats showed modified mitochondria and an increase in the number of lipidic drops. It has been concluded that, due to the morphological alterations, the reproductive process is compromised in the UCH rats.
Two major populations of endometrial stromal cells are present during early implantation in the mouse: the antimesometrial and mesometrial decidual cells (MDC), and the mesometrial fibroblasts. Remodeling of mesometrial compartment takes place before establishment of the hemochorial placenta. We studied the morphology of primary cultures of MDC plated on glass and on type I collagen gel. Decidual reaction was induced before establishment of the hemochorial placenta. We collected on the 7th day of pseudopregnancy. The tissue was injected with almond oil, and the mesometrial endometrium was glass and on type I collagen gel. Decidual reaction was induced by the expression of desmin and α-smooth muscle actin.

In fish, the germinal epithelium/GE is composed of Sertoli/SC and germ cells/GC that are organized in spermatocysts. During the annual reproductive cycle, the GE changes, being continuous or discontinuous. These changes and the developmental stages of GC were used to determine five annual reproductive classes: Regressed/RGR; Early/E; Mid/M; Late Maturation/L and Regression/R. Little information exists on the biology of testol Leydig cells/LC associated with the changes in the GE. To document the ultrastructural alterations in LC during the changes in the GE, testes of S. spilopleura, in different reproductive classes, were fixed and processed using standard methods for transmission electron microscopy. During RGR-mitosis-dominated-period, the GE is continuous and composed of only spermatogonia and SC. LC are abundant and have typical features of steroidogenic cells. Some interactions between LC-macrophages were also observed during this class. During maturation, E/L-meiosis-dominated-classes, the GE becomes discontinuous due to spermatozoa formation followed by spermiogenesis, without replacement of cysts. During these classes, steroidogenic features of LC increased: 1-numerous/large dense mitochondria with tubular cristae; 2-abundant/extensive smooth endoplasmic reticulum. In the R-mitosis-dominated-period, the GE is discontinuous and formed by scattered cysts of spermatogonia/SC. LC had some features indicating degeneration: 1-degenerating mitochondria; 2-mytelin figures; 3-electron-dense chromatin; 4-extensive perinuclear spaces. The results indicate that probably the activity of LC is higher during the meiosis-dominated-period and lower in the mitosis-dominated-period.

The precise site of fertilization and the mechanism by which male gametes encounter the oocytes is still largely unknown in ticks. To document the ultrastructural alterations in LC during the changes in the GE, testes of S. spilopleura, in different reproductive classes, were fixed and processed using standard methods for transmission electron microscopy. During RGR-mitosis-dominated-period, the GE is continuous and composed of only spermatogonia and SC. LC are abundant and have typical features of steroidogenic cells. Some interactions between LC-macrophages were also observed during this class. During maturation, E/L-meiosis-dominated-classes, the GE becomes discontinuous due to spermatozoa formation followed by spermiogenesis, without replacement of cysts. During these classes, steroidogenic features of LC increased: 1-numerous/large dense mitochondria with tubular cristae; 2-abundant/extensive smooth endoplasmic reticulum. In the R-mitosis-dominated-period, the GE is discontinuous and formed by scattered cysts of spermatogonia/SC. LC had some features indicating degeneration: 1-degenerating mitochondria; 2-myelin figures; 3-electron-dense chromatin; 4-extensive perinuclear spaces. The results indicate that probably the activity of LC is higher during the meiosis-dominated-period and lower in the mitosis-dominated-period.
B-071 MIGRATION OF MOUSE TROPHOBLAST CELLS IN THE PRESENCE OF EXTRACELLULAR MATRIX COMPONENTS TGF BETA
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Mouse trophoblast cells display an invasive behavior when they penetrate into the endometrium during embryo implantation and the early stages of placentation, coming into contact with stromal cells and extracellular matrix (ECM). The interaction with the ECM is thought to participate in the regulatory mechanisms of trophoblast differentiation, proliferation and migration. In the present work, we have tested how different substrata induce changes in the behavior of implanting trophoblast cells (TC). For this, embryos were collected on the 3.5th day of pregnancy and plated onto laminin (LN), acid hialuronic (AH), matrigel (M) in Eagles’ medium containing 10% FN-free fetal calf serum and incubated for 72 h. Blastocysts were also poured in traswells containing collagen with LN, AH and M in Eagle’s medium, overnight to watch for invasiveness. Cells growth was determined by counting the cells number and the amount of DNA every 72h. The migration of TC cells was estimated by measuring the total area occupied by the growth blastocysts and the invasiveness was observed by the number of blastocysts collected from the traswells after 24 h. We have found significant changes in the pattern of migration and invasiveness of TC in the presence of LN. This may indicate that LN can play an important role in TC spatial arrangement for implantation during pregnancy.

B-072 MORPHOLOGICAL COMPARISON BETWEEN DECIDUA AND DECIDUOMA IN MICE.
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During the implantation stage, endometrial fibroblasts transdifferentiate into decidual cells forming a new structure in the uterus, called decidua. The decidua has a short period of life (transient structure) and forms an interface between the embryo and the mother. Decidual reaction can be artificially induced during the uterine receptivity period, through the introduction of many substances in the uterine lumen. The artificially induced structure is called deciduoma and represents a good model to study the process of decidualization without the influence of the embryo. The goal of this work was to compare the antimesometrial region of the uterus of mice on days 8, 9 and 10 of pseudopregnancy (dopp) and pregnancy (dop). This period comprises the end of the growth and part of the involution period of both structures. The artificially decidual reaction was obtained by the injection of 30 µl of almond oil in each uterine horn on the 4th dopp. Samples were fixed, embedded in paraplast, sectioned and analyzed under light microscopy. By morphometrical analysis we found that the maximal thickness of the decidualized area was reached on the 8th dopp for deciduoma and on day 9th for the decidua, showing that the deciduoma develops faster than the decidua. We also observed that contrary to the decidua, involution area of the deciduoma was displaced from the health area of the uterus, as small abortions.

B-073 THE ESTABLISHEMENT OF A NEW UTERINE LUMEN IN PSEUDOPREGNANT MOUSE. A RADIOAUTOGRAPHIC STUDY.
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The mouse blastocyst implants within an antimesometrial uterine chamber and the embryo later attaches to the mesometrial uterine wall where the placenta is formed. This event causes the obliteration of the uterine lumen at the mesometrial region of the uterus. Later on, the uterine lumen is reformed in the antimesometrial region. Although no embryo is present in pseudopregnancy, the uterine lumen becomes occluded by the development of the mesometrial decidua. This study describes the development of a new uterine lumen in mouse bearing deciduoma. The deciduoma was induced by injecting 50µl of almond oil into the uterine lumen on day 4 of pseudopregnancy. Animals on days 9, 10 and 11 of pseudopregnancy received an ip injection of 3H-thymidine diluted in PBS. Control animals received PBS. Samples were collected after 1 hour, processed by embedding in paraffin. Sections were covered by nuclear emulsion, and photographically developed. A qualitative radioautographic analysis showed that epithelial gland cells placed at the antimesometrial region were able to incorporate 3H-thymidine. The distribution of silver grains suggests that the new lumen was rebuilt by the proliferation of glandular epithelial cells and later fusion of these glands. Moreover, epithelial cells that lined the interimplantation sites migrated to these sites.

B-074 DIFFERENTIAL lysosomal ENZYME expression in the cat placenta
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The endothelio-chorial placenta exhibits two distinct areas: the endothelio-chorial labyrinth (main placenta) and the marginal hematoma (paraplacenta), where extravasated maternal blood is in close contact with trophoblast cells. At these sites, trophoblast cells phagocytose mainly erythrocytes, as a mechanism for iron and nutrients uptake. We investigated the expression of enzymes in the cat placenta aiming to correlate them to functions of the trophoblast. Term cat placentas were processed to detect acid phosphatase (AP), peroxidase, NAD(P)H-oxidase activities, iron deposits, cathepsin D (Cat-D) and biochemical evaluation of AP and Cat-D. Except for the presence of iron, found exclusively in paraplacenta, the remaining enzymes were present in all placental areas. Peroxidase and Cat-D were more intense in paraplacenta, in contrast to AP, predominant in the main placenta. Biochemical studies confirmed these data, suggesting that the predominance of the Cat-D over AP in the marginal hematoma may be related to the erythropagocytosis and, the AP activity in the main placenta to molecular metabolic exchanges. NAD(P)H-oxidase activity was seen in both placenta regions, predominantly in endosomes vesicles of trophoblast cells, indicating that oxygen reactive species take part of the phagocytic process. Our findings demonstrate differential enzymatic activities in trophoblast cells coherent with the specific roles played by the different cell populations in the term placenta. Supported by CAPES.
B-075 MOUSE IMPLANTING TROPHObLAST CELLS CAN INDUCE CELL DEATH
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At the implantation phase the trophoblast exhibits intense phagocytic activity, whereas the adjacent maternal endometrial cells show cell death morphology. Therefore, the aim of this study was to determine, whether trophoblast may induce cell death in neighboring cells, before phagocytosing them. A fibroblast cell line, A31, confluent culture was used as target cells. Each culture received 2-3 explants of ectoplacental cones. After six hours, trophoblast cells were attached on the fibroblast cell layer. At each 12 h, the co-cultures were fixed in glutaraldehyde 2.5 % in PBS and, processed for light and electron microscopy observation. Trophoblast on the co-culture system rapidly displaced A31 cells, which become pilled at the periphery of the explant. At the interface between both cell types, evident signals of degeneration and cell death were seen in A31 cells, which were also seen being phagocytosed by the trophoblast. Ultrastructural analysis and TUNEL reaction confirmed that a part of A31 cells was dying either by necrosis or by apoptosis. NADH-oxidase activity was found on the trophoblast surface, in the cells in close contact with degenerating A31 cells. Our findings indicate that the trophoblast, probably through the secretion of ROS, can interfere with the fate of neighbor cells, before phagocytosing them. On the framework of the rodent implantation process it may explain the short time spent by the trophoblast for successfully reaching the maternal blood supply. Supported by FAPESP, CNPq and CAPES.

B-076 GENERATION OF AN ANTI-MOUSE ECTOPLACENTAL CONE ANTIBODY LIBRARY BY PHAGE DISPLAY TECHNOLOGY
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This work aims to generate anti-mouse ectoplacental cones (mEC) antibodies using an antibody phage display library from immunized chicken. To that, three female White Leghorn chicken (14 weeks old) were immunized 3 times, over 15 days intervals, with 100 mEC suspended in Freud’s incomplete adjuvant. The animals were sacrificed 15 days after the last immunization, the spleen was collected under sterile conditions and the RNA isolated by Trizol reagent protocol. The first cDNA strand was synthesized from total RNA by RT. Subsequently, light and heavy-chain variable regions of immunoglobulins were amplified by PCR and linked through an overlap PCR step to provide final products (scFv) to be used for cloning. After agarose gel analysis, the PCR product was inserted into pComb3HSS vector phagemid and, XL-1 Blue (E. coli) cells were electroporated to incorporate the vector. The bacteria were selected by antibiotic selection and a large-scale cell transformation and expansion of the new library, called as MBR2 was performed. The phage library was produced with cooperation of Helper Phage. Phage selection consisted of binding phages to 1) immobilization of the substrate, 2) histological mEC sections. Selected Phages infected new XL1-Blue E. coli cells for isolation of single clones by immunohistochemical in situ localization. Preliminary results show that the protein expression of this selected phage library is ectoplacental cone cell-specific.

B-077 EFFECT OF IFN-γ ADMINISTERED IN THE POST IMPLANTATION PERIOD ON MOUSE ECTOPLACENTAL CONE CELLS IN VIVO AND IN VITRO
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Interferon (IFN)-γ is considered an abortion-inducing factor, however its direct effect on ectoplacental cones cells also point to a function in stimulating proliferation and phagocytosis on trophoblast cells. Here, on the other hand, we show that IFN-γ administered in CD1 pregnant mice on gestation day (gd) 5.5, 6.5 and 7.5 does not alter reproductive parameters neither the morphology of ectoplacental cones and outcome placenta, although the serum, spleen, uterus and placental concentrations of IFN-γ become higher in comparison to pregnant animals that do not received IFN-γ during the pregnancy. Cultured 7.5gd-ectoplacental cones, in presence of IFN-γ also did not show modifications in the morphology and cell death indexes. These findings may provide new information to explain the pregnancy success in the presence of significant levels of IFN-γ in uterus during gestation.

B-078 MORPHOLOGY OF THE REPRODUCTIVE ADULT FEMALE SYSTEM OF Drosophila mulleri (DIPTEROUS: DROSOPHILLIDAE) IN LABORATORY CONDITIONS
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Drosophilas are small dipterous usually founded over fermented fruits, really important in Genetics, once it is know their genes control their development and genes which controls vertebrates development, including human being, are very similar in structure and frequently controls analogues parts in both embryos. The aim of this study is describes the morphology of the female reproductive system in adults Drosophila mulleri on different development stages. The flies were maintained in banana cultivation environment and agar-agar, in camera with constant temperature set on 25 ± 1°C, relative humidity of 70 ± 10% and photo phase of 12 hours. The gap of emergency oviposition in adult of this specie is about 15 days, in this way the flies birth can be followed in defined intervals. These ones were anesthetized in ether etlic vapor, ventrally dissected by the posterior abdominal side in physiologic solution, and, after that, the reproductive system was fixed in acid acetol solution and assembled in slides with permount solution. The slides were analyzed and it was verified that the reproductive system of adult females until 24 hours of emergency are low developed and younger flies have a higher filaments amounts in ovary than the older ones. The more evident morphological differences the older are the flies.
the embryos which were considered viable were cultured in uterine horns with PBS with the addition of 20% of FBS. All later the female mice were euthanased by cervical hours later, another 10 IU of hCG was injected IP. Four days peritoneal (IP) administration of 10 IU of eCG and 46 to 48 two groups. Superovulation was carried out with one intraages 6 and 10 weeks were distributed randomly in one of the

The objective of this work was to compare the eclosion rate when compared to the cultivated embryos from the control and super ovulation group. Financial support: FAPESP, CNPq.

The endocrine disruptors, including DES, a synthetic estrogen, are a class of chemicals that modify the hormonal balance of the organism, affecting its reproductive capacity. Previous work from our laboratory showed that adult rats exposed to DES presented histological alterations in reproductive organs. The objective of the present work was to perform an analysis of the epididymis and testis of DES-exposed rats using transmission electron microscopy. Adult male rats were used, control (n=3) and treated (n=4) with DES sc, 10μg, diluted in 0.2 mL corn oil, for 12 days. After this period the rats were killed and testis and epididymis samples were collected, immersed in Karnowsky solution and prepared for study by electron microscopy. In the treated group, degeneration of primary spermatocytes was found in the testis. In the caput epididymidis an increase in the epithelial ductular height was evident, while in the cauda region the number of clear cells apparently diminished. DES exposure provoked an increase in the number of spermatozoa with the cytoplasmic droplet in the lumen of the epididymal duct. These observations corroborate previous morphological and functional data, and confirm the alteration of the normal function of the reproductive system of rats exposed to the DES.

The development and organization of the trophoblast cells in the placenta remain obscure. In this study, the stages of the junctional placental zone formation were followed from day 8.5 to 13.5 of gestation by morphological and immunohistochemical studies. Pregnant mice were sacrificed by cervical dislocation, the placentas were dissected and fixed in 10 % formaldehyde. Four μm paraffin serial sections were submitted to HE staining, PAS reaction and to immunolocalization of cytokeratin and vimentin. Our findings showed that the junctional zone is formed by differentiation of the outer layer of ektoplacental cone trophoblast cell population, which on day 8.5 contained giant polyploid cells and few clusters of small basophilic cells. On the subsequent days, this region increased, apparently due to: i) increase in cell number; ii) continuous differentiation of giant; iii) differentiation of clusters of basophilic and glycogen cells. During the zone junctional completion, progressive maternal capillary invasion by trophoblast giant cells was also observed. Closer the embryo, the endothelium was completely replaced by giant cells, while in distant regions, only part of this tunic was substituted. Cytokeratin filaments confirmed the trophoblastic origin of the invasive cells. There was no temporal correlation between vascular trophoblast invasion and the maternal arterial vessels alterations in the mesometrial gland region. The strategic location of the invasive trophoblast cells into the maternal vessels, however, suggests specific functions to be played by these cells.

The EDS is a cytotoxic drug that selectively destroys Leydig cells in rats leading to marked diminution of testosterone levels and, consequently, gradual disruption of spermatogenesis, mainly at the spermatocytary and spermiogenic phases. To our knowledge, there is no comprehensive study about the effects of this drug in spermatogonial proliferation. In this regard, adult rats that received intraperitoneal injection of EDS (7.5mg/100g BW) were sacrificed 10days after injection, and had their blood samples taken to measure testosterone and LH levels. The testis fragments, fixed in glutaraldehyde and embedded in plastic, were routinely prepared for histological and morphometric evaluation under light microscopy. As expected, testosterone levels were barely detected in treated rats, showing the effectiveness of the treatment. Due to the almost complete absence of testosterone and alteration in its negative feedback mechanism, LH levels were dramatically increased in EDS animals. A clear trend for the diminution of the numbers of the different spermatogonial types investigated and for pre-leptotene was observed 10d after treatment. However, only the values found for type A and intermediate spermatogonia were statistically significant (P<0.05). The results found indicate that 10d after treatment the absence of testosterone affected the proliferation of type A and intermediate spermatogonia but not the proliferation of type B spermatogonia. Financial Support: Fapemig, CNPq.
**B-084 APOTOPSIS IN THE INVOLUTION OF OVARIAN SPERMATOZOA OF Leporinus taeniatus LÜTKEN, 1874 (PISCES: ANOSTOMIDAE) SUBMITTED TO INDUCED SPAWNING.**

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The role of apoptosis during the involution of ovaries of piap-jejo, Leporinus taeniatus, submitted to induced spawning was analyzed. In this study, adult females in advanced gonadal maturation were submitted to hormonal induction using crude carp pituitary extract. Ovarian fragments, immediately after spawning, and at various times postspawning were fixed in 4% buffered paraformaldehyde, and embedded in paraffin for the method of TUNEL. Some specimens were also fixed in solution of Karnovsky, embedded in Epon-Araldite plastic resin and examined using transmission electron microscope. The involvement of postovulatory follicles showed hypertrophy and death of follicular cells, altered basement membrane and thickening of connective theca. The atretic follicles showed shrinkage and reabsorption of yolk, alteration and reabsorption of zona pellucida, hypertrophy and death of follicular cells. The TUNEL method allowed the cell death identification in follicular layer of postovulatory follicles and atretic follicles. The area of postovulatory follicles decreased and apoptotic index of follicular cells increased in a time-dependent manner. These results showed the involvement of apoptosis on the involution of ovaries in L. taeniatus after spawn. Support: Capes, FAPEMIG, CNPq and CODEVASF.

**B-085 SPERMIOMEGENESIS AND SPERMATOZOA ULTRASTRUCTURE OF Apterodonotus albifrons (GYMNOTINOTIDAE).**


**Technical support: CAUNESP.**

Spermiogenesis and sperm morphology have been recognized as useful data to phylogenetic analysis. However in the classic axoneme structure (9+2), and has two short lateral fins. Curiously the Gymnotiformes spermatozoa are more similar to some Characiformes species than to Siluriformes. Gymnotiformes and Siluriformes (the catfishes) are considered sister-groups.

Technical support: CAUNESP.

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**B-086 HISTOLOGY OF UTERUS OF ADULTS FEMALE RATS TREATED WITH ANABOLIC STEROID DECA-DURABOLIN AND SUBMITTED TO THE PHYSICAL EFFORT.**

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The Nandrolone Decanoate (Deca-Durabolin- DD) is an injectable synthetic anabolic used indiscriminately by a large parcel of the population, athletes or not. The goal of this study was to analyze the uterine histology of adult female rats treated with DD and submitted to the physical effort (swimming). Adult female rats Wistar (n=20) were distributed in two control groups (submitted or not to the physical effort) and two treated groups (submitted or not to the physical effort). The drug (6mg/kg) and physiologic solution were administered by a single intraperitoneal injection once a week, during 4 consecutive weeks. Swimming activity was used as model of physical effort (20 minutes/day, during 5 days/week, in 4 weeks of treatment). After this period, the females were sacrificed and the uterus was collected and prepared for histological analysis. The female rats of control groups presented uterine morphology typical of estrous phase, with columnar high epithelium and fibrocellular stroma. The oestral aciclicity of treated female rats resulted in cubic or low columnar uterine epithelium and predominantly fibrous stroma. The use of DD, associated or not in the physical effort, similarly affected the morphology of the endometrium of adult female rats.

**B-087 ULTRASTRUCTURE OF TESTIS SPERMATOZOA OF Microstigmus sp (HYMENOPTERA: APOIDEA).**

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Microstigmus sp is a Sphecid wasp. This wasp group, together with the bees, constitutes the Apoidea superfamily. These spermatozoa consist in two regions: the head, formed by acrosome and nucleus; and the flagellum, formed by two mitochondrial derivatives, a centriolar adjunct, an axoneme (9+2) and two accessory bodies.The singular morphological aspect of this sperm is the head arrangement. The acrosome is very long and basically formed by a paracrystalline structure. In anterior transverse sections, it is oval with two lateral projections at its greater axis. Transverse sections of the central head region show a circular acrosome, inserted into nucleus that is observed laterally at the paracrystalline structure. In this region, the acrosome represents about one third of the total area of the sperm section. Posteriorly, again in transverse sections, the nucleus becomes circular up to the flagellar insertion. The acrosome continues to be circular and paracrystalline; however it is narrower and dislocated toward the nucleus periphery. The nucleus forms a cone shaped projection at its posterior end. These morphological characteristics of the acrosome and nucleus of the Microstigmus wasp have not been previously described in Apoidea and could be used for a phylogenetic evaluation of this superfamily.
The aim of this work was the chemical induction of prostatic tumors in the gerbil Meriones unguiculatus, focusing the importance of the establishment of new animal model that can refine the study of the carcinogenic prostate process. The treated group received a single intraperitoneal dose of the carcinogen N-methyl-N-nitrosourea (30mg/Kg of weight of the animal), besides weekly intraderm doses of the testosterone propionate (2mg/Kg of weight of the animal) diluted in wheat germ oil. The control group received a single dose of the same carcinogen. After 03 and 06 months of experiment, the treated and control prostates were removed and processed for light microscopy. Intraepithelial neoplasia (PIN) was observed in the acini of ventral prostate with significant frequency, as well as hypertrophy and increase in the amount of smooth muscle cells. After 06 months of experiment, the treated group showed in the ventral prostatic lobe the occurrence of adenomatous invasive tumors. All the murine models of prostatic carcinoma already established, except transgenic mice, need long experimental periods, from 10 to 12 months, for the occurrence of the prostate cancer. Thus, the gerbil seems to be an important rodent model, once that the establishment of the prostatic carcinoma happened in shorter experimental periods. Financial support: FAPESP, CAPES and CNPq

Several cells of the maternal-fetal interface produce isoforms of the enzyme oxide nitric sintase (NOS), whose unbalanced expression can led to embryonic/fetal losses and embryonic abnormalities in different species. The product of this enzymatic activity is the nitric oxide (NO), able to perform a great number of biological activities such as prevention of leukocytes and platelet adhesion to the trophoblast surface and control of the uterine blood flow. In macrophages (Mφs), to the NO generated from inducible (i)NOS has also been attributed cytotoxicity and defense functions. Here, we evaluate in vitro the effect of Interferon (IFN)-γ on the iNOS expression, on the IFN-signaling pathway components Jak1, Jak2, Stat1 and Stat2 expression and, the effect of the PKC inhibitor, staurosporine, on these expressions in mice trophoblast cells. Ectoplacental cones dissected from embryos on day 7.5 of gestation were the source of trophoblast cell for 24-96 h-cultures. The cultures received IFN-γ (100 U/mL) and/or staurosporine (100nM) for additional 6 h. The protein expression for iNOS, Jak1, Jak2, Stat1 and Stat2 was analyzed by immunohistochemistry and Western-Blotting and the message for these proteins, by RT-PCR. Cultures of mice peritoneal Mφs were also prepared as positive control. Mφs and trophoblast cells revealed the presence all proteins. A maximum reactivity has been found in the presence of IFN-γ alone, whereas IFN-γ + staurosporine led to a very low immunoreactivity. Similar results were obtained by Western-Blotting. mRNA for these proteins were also found in trophoblast and Mφs under IFN-γ stimuli, but not so intensely in the samples IFN-γ + staurosporine-treated. These results indicate that the signaling pathway via PKC is fundamental for iNOS expression-dependent on IFN-γ in trophoblast cells, in a very similar mechanisms to those reported for Mφs. GRANTS: FAPESP
B-092 THE PRECISE FIBROBLAST ARRANGEMENT CONTRIBUTES TO THE STROMAL COMPARTMENTALIZATION AND EPITHELIUM REGIONAL VARIATION IN THE RAT VENTRAL PROSTATE DUCTAL SYSTEM.

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A perfect knowledge of the normal organization and composition of stroma is extremely important for better understand its pathological modification in a process called “stromal response”. Here, we examined in details the fibroblasts arrangement in the different regions of the rat ventral prostate ducts. Normal and castrated Wiistar rats were sacrificed at 0, 3, 7 and 21 days after castration. Ventral prostate (VP) was examined by histochemistry, immunohistochemistry and Transmission Electron Microscopy. In normal and castrated VP, fibroblasts formed an almost continuous unicellular sheath between epithelial basement membrane and smooth muscle cells sheath, and also made a last outermost continuous unicellular layer around the ducts. In both locations, these cells presented a flattened and longed cytoplasmatic expansions. In all the fields examined, the interductal stroma, with macrophages, mast cells and others fibroblasts, was separated by the periductal stroma, by fibroblasts cytoplasm. Castration promotes a progressive accumulation of folded fibroblasts cytoplasm. The results showed fibroblasts arrangement contributes to a complex prostate stromal architecture. Furthermore, because folding and branching, distal and proximal regions of the ducts are sometimes side-by-side, in these case, fibroblasts probably compose a barrier that guarantees the distinct influences of stromal variations in the epithelium morphology and physiology along prostatic ductal system.

B-093 EFFECT OF TESTOSTERONE REPLACEMENT IN GERBIL (Meriones unguiculatus) PROSTATE LONG TIME AFTER SURGICAL CASTRATION

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Testosterone plays an essential role in the prostatic development and differentiation. Therefore, the aim of this investigation was to evaluate by histological/morphometric methods the effects of testosterone supplementation after a long lifetime hormonal deprivation by bilateral orchiectomy. In the experimental group (G.Ex.01, n=25), the animals were surgical castrated and received for 21 days 0,25mL of testosterone cypionate, at intervals of 48 hours. The control groups were formed by 5 not castrated adult animals (G.C.01) and 5 animals surgically castrated that received 0,5mL of the vehicle (G.C.02). The animals were sacrificed at 0, 7, 14 e 21 days after the beginning of the treatment. The prostes were then removed and processed by histological methods to be employed histochemical and stereologic-morphometric evaluation. After 30 days of castration, the prostate showed a drastic decrease in the height of the epithelium, in the relative luminal volume (reduction of the glandular compartment) and reduction of the muscular layer. After 7 days of testosterone administration, there was a gradual increase in the height of the epithelium and muscular layer, which was stabilized at day 21 after treatment. These results indicate a prostatic high capacity to reorganize and retake its secretory functions after hormonal replacement with testosterone, even after the involution of the tissue components after long time castration. Support: FAPESP, CNPq.

C-001 CYTOPROTECTIVE EFFECT OF AMIFOSTINE (WR-2721) ON THE SEMINIFEROUS EPITHELIUM OF DOXORUBICIN-TREATED PREPUBERTAL RATS

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The cytotoxic effect of doxorubicin on the seminiferous epithelium results in depletion of germinative cells and can provoke irreversible damage to male fertility. Amifostine, an organic thiophosphate, is an efficient cytoprotector to normal cells against the toxicity of ionizing radiation and cisplatin. The target of this study is to investigate the amifostine cytoprotection against the doxorubicin toxicity on the testis. Eighty 30-day old male rats were distributed into four groups: Doxorubicin (D – 5mg/kg), Amifostine (A – 400mg/kg), Amifostine/Doxorubicin (A/D; amifostine was administered before doxorubicin) and “Sham” Control (SC – Physiological Saline). All treatments were intraperitoneal. The animals of each group were sacrificed at 3 different ages (45, 60 and 90 days). Testes were fixed in Bouin’s Liquid and Paraplast-embedded and 3µm-thick cross sections were stained with HE for histomorphometric analysis. Ninety-day old rats were submitted to sperm analysis. Sixty and 90-day old doxorubicin-treated rats showed a significant diminution of tubular diameters and seminiferous epithelium height in comparison to amifostine/doxorubicin-treated rats of the same age, in doxorubicin-treated rats the sperm/ml concentration was also lower than those observed in the Amifostine/Doxorubicin and Control groups. The results suggest that amifostine partially protect the seminiferous epithelium against the deleterious effect caused by doxorubicin; this protection was more conspicuous in 90-day old adult rats.

Financial Support: CNPq

C-002 IMMUNOHISTOCHEMICAL AND ULTRASTRUCTURAL EVALUATION OF SERTOLI CELLS OF ALBINO RATS TREATED WITH ETOPOSIDE DURING PREPUBERAL PHASE

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Sertoli cells are essential for normal spermatogenesis and secrete substances which are indispensable for maintenance of germ cell lineage. Among them is transferrin, a Fe3+ carrier. Under adverse situations, such as chemotherapeutic treatment, Sertoli cell role is poorly understood. Etoposide is a potent chemotherapeutic drug which provokes serious damage to the testis. Thus, we decided to investigate if Sertoli cells are harmed by etoposide. Prepuberal albino rats received 5mg/Kg of etoposide for 8 consecutive days and were sacrificed 12 hours and 13, 32, 95 and 148 days after the treatment, when they were 32, 45, 64, 127 and 180 days old, respectively. The control groups received 0,9% saline solution. The left testis was fixed in Bouin’s liquid for transferrin immunolabeling. The right testis was perfusion-fixed for Sertoli cell analysis under Transmission Electron Microscope. In all control and etoposide-treated groups, labeling was observed in Sertoli cell cytoplasm. In 64, 127 and 180 day-old control rats labeling was also observed in elongate spermatids. All etoposide-treated groups showed reduction of transferrin labeling in the seminiferous epithelium. Both control and experimental groups showed accentuated labeling of the interstitial tissue, and no differences were noted among the groups. Sertoli cells showed abnormal nuclear morphology, cytoplasm vacuolation and increase of lipidic inclusions. Some Sertoli cells showed degenerative features. These data suggest that etoposide treatment can cause Sertoli cell damage.
C-003 EFFECTIVE PROTECTIVE EFFECT OF AMIFOSTINE AGAINST GERM CELL APOPTOSIS IN THE CISPLATIN TREATED PREPUBERAL RATS

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Cisplatin, an alkylating antineoplastic agent, causes cytotoxic effects on the testes, including germ cell apoptosis. Since amifostine (WR2721) is a cytoprotective drug utilized against toxicity caused by ionizing radiation and chemotherapeutic drugs, we proposed to evaluate the cytoprotection of amifostine against germ cell apoptosis induced by cisplatin in prepuberal rat germ cells. Fifty-two 30-day old male rats were distributed into four groups: Sham Control (SC), Amifostine Experimental (AE), Cisplatin Experimental (CS) and Amifostine/Cisplatin Experimental (ACE). The AE and EC groups received cisplatin (5 mg/Kg) by intraperitoneal route. The AE and ACE groups received intraperitoneally 400 mg/Kg of amifostine which was injected before the cisplatin. The animals of SC group received intraperitoneal injection of 0,9% physiological solution. The animals were anesthetized with thiopental and their testes were fixed in Bouin’s liquid. Late apoptotic cell number and seminiferous epithelium height were obtained by morphometric analysis in fifty seminiferous tubule cross-sections stained with H.E.; an apoptotic index was also obtained by the relation: late apoptotic cell number/seminiferous epithelium height. The transmission electron microscopy was performed with the aim to confirm apoptosis occurrence in the testis. According to the data, the amifostine/cisplatin treated rats showed lower apoptotic index in comparison with the cisplatin treated rats.

C-004 DEVELOPMENT OF THE Calomys callosus TESTIS UNTIL THE PUBERTY

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The main objective of this study was morphologically to determine the developmental changes in the testis of Calomys callosus until the puberty. The animals were housed under a 12 h light, 12 h dark light period and received water; food and sunflower seeds “ad libitum”. Experimental groups contained two animals each, in the following ages: 1, 2, 5, 7, 10-20, 30, 32 and 35 days. Fragments of testis were fixed with 4 % formaldehyde in 0.1M phosphate buffer, at pH 7.4, and were embedding in methacrylate resin. Sections of 2 µm thickness were stained with H.E.; an apoptotic index was also determined in interphase nuclei of nauplii for light microscopic analyses. During the period of sexual maturation the testis exhibited several changes. It initially showed a thick albuginea rich in vessels, an exuberant interstitial area and three layers of myoid cells around the seminiferous tubules. During the testis maturation, there was an increase in the tubular dimensions and decrease of the interstitial area. At the developmental day 13, a lumen started to form inside the tubules and at the day 30, spermatides were commons nearby the luminal area of the tubules. At the day 35, the seminiferous epithelium showed an adult organization and the tubular lumen already exhibited high proportions of spermatozoids, characterizing the sexual maturity of these rodents.

C-005 HETEROCHROMATIN VARIATION IN FOUR ARGENTINEAN POPULATIONS OF Artemia (CRUSTACEA, BRANCHIPODA, ANOSTRACA)

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The genus Artemia is distributed worldwide except Antarctica and inhabits hypersaline environments. A.franciscana is the dominant species in the Americas, while A.persimilis is restricted to Argentina and few Chilean localities. It has been suggested that the number of chromocenters is a reliable chromosome marker of intra and interspecific genetic differentiation in the American species. In the present contribution the amount of C positive heterochromatin was determined in different Argentinean Artemia populations. Great Salt Lake, GSL(USA) and Salinas Grandes de Hidalgo, SGH(Argentina) populations were used as reference strains for A.franciscana and A. persimilis, respectively. The number of chromocenters and the percentage of C positive heterochromatin, determined in interphase nuclei of nauplii were: GSL: 2-26chromocenters, 8.97±4.74%; Salinas Grandes: 3-22chromocenters, 6.79±3.99%; Pampa de las Salinas: 1-14chromocenters, 4.06±2.71% and Mar Chiquita: 3-13chromocenters, 4.16±2.38%, all of them for A.franciscana, and SGH: 1-6chromocenters, 1.33±1.92% for A.persimilis. The statistical analysis revealed significant differences in the percentage of heterochromatin among populations. Furthermore, no correlation was found between the number of chromocenters and the amount of heterochromatin. It is suggested that the percentage of heterochromatin could be a better cytogenetic marker than the number of chromocenters. Besides, the amount of heterochromatin could be related to environmental conditions rather than to taxonomic categories.

C-006 ENVIRONMENTAL CUES INFLUENCE THE ACCESS OF VAGAL NEURAL CREST CELLS TO THE GUT.

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Vagal neural crest cells of neuronal lineage populate the gut, where they originate the Enteric Nervous System (ENS). There are several molecules involved in directing neural crest cells migration, among them PNA-positive glycoconjugates and ephrin ligands. PNA-positive glycoconjugates are related with the inhibition of migration of the thoracic neural crest cells, while ephrin ligands play a dual role, promoting melanoblasts migration and inhibiting neuronal lineage migration. We aimed to observe if these molecules have the same influence on vagal neural crest cells, reported to be more invasive than thoracic neural crest cells. With the use of PNA-Fitc, HNK-1 (antibody that recognizes neural crest cells) and Eph-B2-human Fc chimaeras, stage 11 to 22 (HH) chicken embryos were labeled. PNA-positive glycoconjugates and ephrin ligands were never observed at locations occupied by neuronal lineage neural crest cells at vagal level. These results suggest that PNA-positive glycoconjugates and ephrin ligands might play the same role on vagal and thoracic neural crest cells migration.
C-007 MORPHOLOGICAL, HISTOLOGICAL AND ULTRASTRUCTURAL CHARACTERIZATION OF THE OVARY OF THE TICK *Rhipicephalus sanguineus* (Latreille, 1806).

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Ticks are important vectors of pathogenic agents that cause various diseases in domestic and wild animals, birds and man. The present study had the object of discerning the morphology, histology and ultra-structure of the ovary and to understand the dynamics of vitellogenesis of the eggs of females of the tick *Rhipicephalus sanguineus* through the techniques of SEM, histology, histochemistry, and TEM. The ovary of *Rhipicephalus sanguineus* is a single tubular structure resembling a horseshoe and it is of the panoistic type. The ovarian wall is constituted by a simple epithelium of small cells that delimit a lumen and by larger cells the oocytes at different developmental stages. The oocytes are attached to the external margin of the ovary by means of a multicellular structure known as pedicel. The oocytes were classified into developmental stages, varying from I to V, based on the visualization of the germ vesicle and on the characteristics of the cytoplasm and the yolk. The results allowed us to suggest that there are three probable sources of vitellogenic substances for the growth of the oocytes: one is endogenous and it is represented by the oocyte itself, and two are exogenous: the haemolymph and the cells of the pedicel.

Supported by Fapesp - proc. 02/01180-8.

C-008 SALIVARY GLANDS IN FEMALES OF THE TICK *Amblyomma cajennense* (ACARI: IXODIDAE), HISTOLOGICAL AND STRUCTURAL ASPECTS OF TYPE I ACINI.

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The cayenne tick *Amblyomma cajennense* is considered one of the most important groups of arthropods. Their economical and sanitary importance is widely acknowledged due to the fact of being vectors and agents of diseases that affect animals and men. The present work had the object of studying the histology and ultra-structure of cells of type I acini of the salivary glands of *A. cajennense* through the use of techniques of resin inclusion and transmission electron microscopy (TEM). The salivary glands were fixed in 4% paraformaldehyde, calcium formaldehyde, Bouin’s fixative, and 2.5% glutaraldehyde and processed according to routine histological and TEM techniques. The results showed that this type of acini is composed by cells of pyramidal morphology with rounded nuclei and non-granular cytoplasm. The histochemical tests resulted in a weak positive reaction for the presence of lipids and polysaccharides, but a strong positive reaction was obtained for the presence of proteins. Ultrastructurally, the cells of this type of acini possess numerous mitochondria, a modest Golgi complex, few ribosomes and lack rough endoplasmic reticulum. The function performed by this type of acini would be related to autophagical activities and hydration of the individual during the feeding stage.

Supported by FAPESP nº 01/08866-0

C-009 THE THYROID HORMONE RECEPTOR BETASPECIFIC AGONIST GC-1 SELECTIVELY AFFECTS THE BONE DEVELOPMENT OF HYPOTHYROID RATS INDEPENDENTLY OF THE GH/IGF-1 AXIS.

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We studied the effects of the TRβ-selective thyromimetic GC-1 on bone development. 21 day-old hypothyroid rats (Hypo) were treated for five weeks with 0.3mg/100g BW/day of T3 (1xT3), 5xT3, or equimolar doses of GC-1. Hypothyroidism impaired growth and BMD gain, delayed ossification, decreased bone metabolism, reduced the number of hypertrophic chondrocytes [H; 72% vs. euthyroid, p<0.001] and resulted in disorganized growth plate (GP) chondrocytes. Serum IGF-I was 67% reduced vs. euthyroid (p=0.001) and the expression of IGF-I protein and collagen II and X mRNAs were undetectable in the GP of Hypo. T3 completely or partially normalized these parameters. GC-1 did not influence serum concentrations or GP expression of IGF-I, failed to organize the GP, and barely affected growth. Nevertheless, GC-1 induced ossification, HC differentiation and collagen II and X mRNA expression and increased GP thickness to euthyroid values. GC-1 group had higher BMD gain than Hypo (p<0.05). These changes were associated with increased trabecular volume (48%, p<0.01), mineralization rate (2.3-fold, p<0.05), mineralizing surface (4.3-fold, p<0.01) and bone formation rate (10-fold, p<0.01). These findings suggest that TRβ mediates some essential actions of T3 on bone development independently of GH/IGF-1 signaling.

C-010 EFFECTS OF in vitro PIG OOCYTES MATURATION IN CULTURE SYSTEM WITH PIRUVATE.

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The present study was conducted to identify an in vitro culture system capable of supporting pig oocytes maturation. Ovaries were obtained from a slaughterhouse and transported to the laboratory within 3 h at 25–28°C, and rinsed three times in 0.9% NaCl supplemented with 50 µg/ml streptomycin. Cumulus-oocyte complexes (COCs) were harvested from ovaries and in vitro maturation was evaluated in a SM chemically defined culture system with sodium pyruvate (22.3mM). COCs were selected and separated into three groups: SM, SM supplemented with follicular fluid and SM supplemented with follicular fluid and eCG, hCG. After 44 hours of culture at 37 °C, oocytes completed meiotic maturation to the metaphase II stage and increased cumulus expansion. The maturation rate of oocytes cultured in SM medium was 24/60 (40.0 %); in SM + follicular fluid 26/60 (43.3%) and in SM + follicular fluid + eCG + hCG. 34/60 (56.0%). These results shows that sodium pyruvate in nonserum maturation medium supports nuclear and cytoplasmic maturation of pig COCs. The use of this chemically defined culture system to investigate nuclear maturation, functions of cumulus cells or the effects of several factors during maturation will be useful for understanding of their mechanisms involved in the process of pig oocyte maturation.
C-011 IS THE DEVELOPMENTAL MUTATION Muddled of Drosophila A NOVEL REGULATORY ALLELE OF ROUGHEST?  
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Muddled is a dominant mutation of Drosophila melanogaster, associated with an inversion involving polytene chromosome bands 3C3-4 and 5A6-B1, phenotypically characterized by rough eyes with brownish color, frequently fused facets, irregularly distributed mechanosensory bristles and abnormal pigment cell differentiation. Its phenotype is strikingly similar to rst, a regulatory mutant allele of the pleiotropic locus roughest, involved in several embryonic and post embryonic developmental processes. Here we report a genetic and molecular characterization of Mud and also explore possible genetic relationships between Mud, rst and echinus, another gene affecting the final steps of ommatidial formation. We show that females double heterozygotes for Mud and either rst or the partial loss of function allele rst display a dramatic increase in eye phenotype severity while double heterozygotes Mud/echinus show the opposite effect. The Rst protein in a Inv(1)Mud background shows an abnormal redistribution dynamics along the interommatidial/pigment cell border as well as a gapped appearance. We also demonstrate that the distal breakpoint of the Mud inversion maps within rst second intron, just proximal to the start of the coding region. Finally Mud mutants also interfere with embryonic myogenesis. We conclude that the phenotype associated with the Mud inversion is due an alteration of rst gene expression and Mud is in all probability an allele of the roughest locus.

C-012 SPECIFICATION OF CARDIAC CHAMBERS BY RALDH2: EXPRESSION PATTERNS OR CELL MIGRATION?  
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Establishment of cardiac antero-posterior polarity is an early decision in heart development. Previously, we showed that cardiac cells commit to ventricular or atrial fates when a caudal-rostral wave of RALDH2 divides the cardiac field into anterior and posterior sections, respectively. To determine how the caudal-rostral wave is formed we performed fate maps, explant culture and whole mount in situ hybridization of the chicken cardiac field. Explant studies indicated that the cardiac field requires the apposition of posterior mesoderm to express RALDH2, as it is not competent to express RALDH2 when cultured in isolation. To define whether the caudal-rostral wave of RALDH2 is dependent on migration of RALDH2-expressing cells or induction by posterior signals we performed fate maps. Fate map of the wave indicated that its anterior two thirds are formed by cells that did not express the RALDH2 gene before wave initiation. However, the posterior third of the wave receives a significant contribution from cells in the posterior mesoderm that express RALDH2 before wave initiation. We conclude that the wave of RALDH2 results from limited cell migration and from signals from the posterior mesoderm that instructs the cardiac field to express RALDH2.

C-013 AUTOPHAGIC CELL DEATH IN Drosophila melanogaster SALIVARY GLANDS: A NEW FUNCTION FOR THE ROUGHEST LOCUS?  
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The Drosophila roughest (rst) locus encodes a type Ia transmembrane glycoprotein with 5 immunoglobulin-like domains in its extracellular portion and has been implicated in a wide variety of developmental processes, including myogenesis, blastoderm cellularization, patterning of sensory organs in the antenna and programmed cell death and differentiation during ommatidial assembly. Here we describe a hitherto unknown role of rst in the autophagic elimination of larval salivary glands during early pupal stages. Ubiquitous overexpression of the Rst protein ectodomain in early pupa leads to persistence of salivary glands up to at least 36 hours after head eversion. The same phenotype can be observed in individuals carrying the dominant regulatory allele rst, but is absent in loss-of-function alleles. Analysis of these persistent glands at the ultrastructural level shows characteristic morphological changes compared to wild type. Finally we show that Rst is dynamically expressed in pupal salivary glands and its expression pattern appears to be delayed in rst. Since a similar rst expression delay during the development of the pupal retina has been previously described for these mutants, leading also to a late onset of the programmed cell death in that tissue, we speculate that common molecular mechanisms might underlie rst function both in eye and salivary gland.

C-014 DPP AND MSH UNBALANCED DOSAGES AFFECT DISTINCT Drosophila DEVELOPMENTAL STAGES  
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In embryonic dorsal ectoderm, dpp gene is expressed in a gradual manner, from dorsal to ventral regions and, together with Dorsal signaling, specifies embryonic subdivisions in DV axis. msh gene is expressed in neurogenesis in the dorsal column of neuroectoderm, repressed dorsally by dpp signaling and ventrally by ind. In mesoderm, msh is expressed in myoblasts that will give rise to specific muscle fibers in larvae. In order to verify if dpp affects msh regulation in different stages during Drosophila development, we verified dpp and msh expression in mutant embryos for each of these genes. We could detect defects in dpp expression in E9 stage msh-embryos and in msh expression in E12 stage dpp-embryos, but no phenotype in adult flies were observed. Later on development, imaginal discs analysis also presented alterations in dpp and msh expression in the corresponding mutant discs, suggesting that msh and dpp expression depend on each other to the correct formation of embryonic and larval structures. We also present evidence that dpp and msh genes dosages unbalance disrupt signaling pathways important for nervous system and mesoderm formation, causing a high lethality frequency and severe defects in CNS, PNS and visceral mesoderm cells. Besides that, we tried to elucidate whether the relationships between these genes in embryogenesis and larval stage are similar or if it varies in Drosophila different developmental stages.
C-015 MORPHOLOGICAL AND MORPHOMETRIC STUDIES OF THE BUFFALO COW UTERUS (Bubalus bubalis).
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This study was directed towards the morphological and morphometric aspects of the buffalo cow uterus during the estrous cycle. Fragments of uterus and blood from 20 buffalo cows from a commercial slaughterhouse were utilized. The fragments were processed by light microscopy. The animals that showed vascularization and glandular mitoses in the endometrium from moderate to intensive degree, and stromal edema from moderate to intensive degree, were classified in the follicular (38%) phase, and those that presented glandular secretion and supranuclear vacuolation of the glandular epithelial cells were classified in the luteal phase (62%). The average diameter of the basal and superficial glands was, respectively, 36.53 µm ± 8.86, and 62.31 µm ± 10.40. For the basal and superficial glands, and basal epithelial, variables there were differences among the regions of the uterus, whereas for the variable basal and superficial glands, basal and superficial lumen, basal and superficial epithelium, and lumen epithelium, differences were observed among the animals within one or both the phases. Significant correlation was also observed between variable basal gland and the variable basal lumen and basal epithelium, between the superficial gland with the superficial lumen and superficial epithelium, between basal lumen and basal epithelium, and between superficial lumen and superficial epithelium.

C-016 GENETIC AND MOLECULAR CHARACTERIZATION OF THE echinus LOCUS OF DROSOPHILA AND ITS RELATIONSHIP WITH roughest
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The Drosophila eye comprises about 800 ommatidia and has a precise repeating structure. roughest is involved in the final stages of the development leading to the highly regular ommatidial pattern in the retina. It encodes a transmembrane glycoprotein involved in cell adhesion, with five Ig-like domains in its ectodomain, and an intracellular tail unusualy rich in serine and threonine. Double heterozygotes for rst and the molecularly uncharacterized locus echinus (ec) show an unexpected eye rougheness (both are recessive). To identify the ec genetic function we analysed the expression of the predicted transcription units in the ec cyogenetic region (3F1-F2) in three echinus stocks and in wild type control, using RT-PCR. For one of them, BcDNA:GH07910 (a putative serine threonine kinase), two mutant stocks show a strong 1.3 kb amplification product in addition to the 0.9 kb fragment expected. The same 1.3 fragment is also amplified, albeit weakly, in the third mutant, but barely detectable, in wild type controls. Preliminary sequence analysis of the ec-specific amplification product suggests that it could be structurally related to the BcDNA:GH07910 serine threonine kinase, but most likely non-functional. Interestingly, the embryonic expression pattern of the two echinus stocks is indistinguishable from wild type. Detailed transcriptional and functional analysis of BcDNA:GH07910 in echinus and wild type flies are currently underway.

Supported by FAPESP, FAEPA and CAPES

C-017 MELANOCYTES IN DERMIS OF JAPANESE SILKY CHICKEN EMBRYOS: WHAT FOR?
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The Japanese Silky (SK) chickens can be easily distinguished from others because of their extensive pigmentation in the dermis and connective tissue of internal organs. For thousands of years they have been credited with famous medicinal and health-promoting values and some researchers believe that melanin present in melanocytes is the key on the medicinal function of Silkie fowl. However, a modern scientific approach is missing. This work analyses the distribution and morphological aspects of melanocytes in the dermis of SK embryos at various days of incubation and their relationship with other types of cells. Dorsal skin was examined by light and electron microscopy. The dermis is populated by widely distributed melanocytes that in the deeper layers are round and have many mature spherical melanosomes, while the ones closer to the epidermis are elongated, protruding thin cytoplasmic processes. Their cytoplasm contains elongated melanosomes in all stages of maturation. These results point to a local environmental control of melanogenesis allowing completion of the process in the dermis. The exocytosis of melanosomes and their phagocytosis by macrophages is observed. Besides macrophages, mast cells and adipocytes are seen in close proximity to melanocytes. A role for the interaction between those cells might point to a function for dermal melanocytes besides pigmentation.

C-018 DISTRIBUTION OF STI1 (STRESS INDUCIBLE PROTEIN 1) IN MOUSE EMBRYOS
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This work was carried out to determine the distribution of STI1 in 13-day mouse embryos. STI1 is a very conserved heat shock protein that binds cellular prion protein (PrPc). The exact function of STI1 is unknown, although the protein has been proposed to play an important role in gene regulation, pseudopodia extension, formation of macromolecular complexes with Hsp70 and 90, and in the internalization of PrPc, which is related to neurodegenerative diseases. The embryos were collected at 13 dpc, fixed and Paraplast embedded. Sagittal, coronal and transverse sections were processed for immunohistochemical analysis, using a rabbit polyclonal STI1 antibody. The secondary antibody was a peroxidase-labeled donkey anti-rabbit IgG. We observed a wide distribution of STI1. Immunopositive cells were found in cartilage, mesenchyme, respiratory and intestinal epithelia, mesonephric tubules, heart, peripheral nerves, mantle and marginal zone in neural tube and cortical plate in cerebrum. Once STI1 belongs to the group of heat shock proteins its wide distribution was expected, reinforcing the suggestion that these are housekeeping proteins. These data reflect an expression of STI1 in differentiated cells, since undifferentiated cells like germinal neuroepithelium and perichondrium were immunonegative. Co-localization of STI1 with the reported expression of PrPc was observed, corroborating the hypothesis of interaction between these proteins.
C-019 MISEXPRESSSION OF THE ROUGHEST PROTEIN CAUSES ABNORMAL VENTRAL CORD FORMATION IN Drosophila melanogaster.
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Roughest (Rst) is a Type I transmembrane glycoprotein of the immunoglobulin superfamily required for several post-embryonic developmental processes in Drosophila, including axonal pathfinding in the optic lobe, pattern formation in the foalaty epithelium and programmed cell death and differentiation during the last steps of ommatidial assembly. Null rst mutants develop to adulthood but ubiquitous overexpression of a transgene encoding the Rst ectodomain under the control of an inducible heat shock promoter, has previously been shown to either block blastoderm cellularization or disrupt somatic myogenesis by preventing myoblast fusion, depending whether it is induced in the first 2 hours or after 10hs of embryonic development (ED) (Moda et al, An Acad Bras Sci 72, [2000]; Strünkelnberg et al, Development,128 4229, [2001]). Here we extend these findings by additionally showing that transgene induction for 2 hours around 3 hs ED, followed immunostaining 9 hours later with a neuronal specific marker antibody, often exhibit a longitudinal defect is highly reproducible and neuronal specific marker antibody, often exhibit a longitudinal separation, around the midline, of the normally fused two halves of the ventral cord. The defect is highly reproducible and generally affects only the two or three most proximal abdominal parasegments. In the most extreme cases the ventral cord is also ruptured along the dorsoventral axis in that same region. The detailed genetic and molecular characterization of this phenotype is underway.

C-020 SEARCH FOR REGULATORY ELEMENTS IN THE RALDH2 GENE
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Retinoic acid (RA) is critical for heart development. In the presence of RA, cardiac precursors develop as atrium and sinus venosa, while in its absence, they form ventricles. We showed that simatrial/ventricular divisions are established by RALDH2, a RA synthetic enzyme, through a two-step mechanism reflected in changes in its expression pattern. We used an evolutionary approach to identify conserved, regulatory elements in the RALDH2 genes of vertebrates such as human, chimpanzee, mouse, rat, chicken, zebrafish, puffer fish and the urochordate Ciona intestinalis. We identified several conserved blocks with more than 75% of homology in the 5’ untranslated region and in intron 1 of vertebrates. Conservation in intron 1 was especially high and transgenic mice established that an intrinsic enhancer drives expression in the posterior back region. Two-vertebrate conserved blocks (3 and 5) showed clusters of putative binding sites for cardiac transcription factors such as Nkx2.5, Gata, COUPTF-II, MEF-2 and Tbx-5, while others (1 and 4) were active in renal cells. Only one block of homology was found between fishes and other vertebrates and there were no conserved blocks between vertebrates and urochordates. In summary our analysis identified several conserved blocks of regulatory sequences among vertebrates, suggesting that the RALDH2 gene is controlled in a highly modular fashion.

C-021 NORMAL AND ABNORMAL EMBRYOS OF Macrobrachium olfersi (Crustacea,Palaeonidae) SUBMITTED AT DOMESTIC DETERGENT.
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Ovigerous females of M. olfersi incubate centrolecithal eggs in a brood pouch until hatching. The embryonic development time of this palaemonid is 14 days and the observation of morphological features on developing embryos allowed the characterization of ten stages. This study evaluates the effects of domestic detergents on the egg volume and embryonic cephalic structures. Prawns were maintained in two tanks (21.5 liters deionized water), an experimental (tank I) and a control (tank II). Three doses of 0.25% of detergent were added to the tank I on the 1st day, 2nd day and 3rd day. The prawns were monitored on 140th exposure day. Embryos in C-shape stage (E6) and Pigmented-eye stage (E12) of the both tanks were fixed in Bouin (24h), embedded in parafin, sectioned (7µm) and stained with HE. The egg volumes of E6 and E12 (tank I) were 0.012 and 0.013 mm³, respectively and the volume of the control group were, in the same embryonic stages, 0.009 and 0.010 mm³, respectively. Our results show that the optical lobes of experimental group are larger than the control group while the eye size of the experimental group had a reduction when compared to the control group. These results suggest that domestic detergent had a differential action on the embryonic structures.

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The fat body of insects is a special kind of tissue composed by cells arranged in laminae dispersed in the hemocoel, filling all cavities. It is an energetic source, stores of nutrients, detoxifies the insect from noxious substances, and produces and accumulates lipids, carbohydrates, and proteins. It is divided into parietal and visceral layers. Adipocytes are the main cell type in the fat body and accumulate reserves (lipids, proteins, and glycogen). The oenocyte is the other cell type, which acts in the intermediary metabolism and produces some of the constituents of the cuticular wax. Our aim was to investigate the ultrastructure of the fat body of wasps. The parietal and visceral layers were fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer and prepared for TEM studies. We observed that the fat body presents two different cellular types, the oenocyte and the adipocyte, which are intimately associated. The oenocyte is highly distributed, mainly in the visceral layer. They have a rounded nucleus and a dense and homogeneous cytoplasm. The adipocyte presents an irregular nucleus and a cytoplasm with large lipidic vacuoles and dense granulations. The ultrastructural aspects are similar to those found in other insects, but in the wasp adhesion structures were observed between the cells.
In the present work, we identified the mouse NC cell. However, the pluripotentiality of NC is not elucidated in mice. CCB - UFSC. *PIBIC/UFSC brunoc@grad.ufsc.br

Objective: The neural crest (NC) is a transient structure of the vertebrate embryo formed by the lateral borders of the neural primordium. Its constitutive cells lose their epithelial characteristics and migrate throughout the embryo to establish the dorsoventral axis of the embryo. CCB - UFSC. meline@grad.ufsc.br

Objective: Quail cephalic neural crest (CNC) cells are heterogeneous in proliferation and differentiation capacities, comprising highly pluripotent stem-like cells able to give rise to neurons, glial cells, melanocytes and cartilage as well as diverse developmentally restricted oligopotent precursors and committed cells. However, the pluripotentiality of NC is not well elucidated in avian. Materials: CNC cells were isolated from 24 hs explanted neural tubes of 6-8 somite quail embryos at mesencephalon–rhombencephalon level. Secondary cultures were grown on collagen or 3T3 mouse fibroblast feeder-layers. Cell phenotypes were identified by immunocitochemistry using lineage-specific markers to melanocytes (MelEM), glial cells (SMP), Neurons (TH and NF) and Myofibroblasts (SMA). cartilage cell aggregates were observed by phase contrast microscopy. Results and Conclusions: CNC grown on collagen differentiated in Schwann cells, myofibroblast and melanocytes in large proportion. We did not observe neurons and cartilage on this substratum. On the other hand, 3T3 fibroblast feeder-layers provided favorable conditions to CNC differentiation in neurons, and cartilage in addition to the previous ones. These findings suggest the existence of neural-mesectodermal precursors that can be considered as NC stem cells. Such highly pluripotent cells can, during migration and proliferation, generate precursors of more restricted developmental options and finally yield unipotent committed precursors in the sites of differentiation.

Supported by: CNPq, CAPES/PROCAD, MCT/INFRA, PRONEX, FUNCITEC.

C-024 CHARACTERIZATION OF MICE CEPHALIC NEURAL CREST STEM CELLS
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Objective: The neural crest (NC) is a transient structure of the vertebrate embryo formed by the lateral borders of the neural primordium. Its constitutive cells lose their epithelial arrangement and migrate through embryonic tissues to stop at selected sites where they differentiate into various cell types. However, the pluripotentiality of NC is not elucidated in mice. In the present work, we identified the mouse NC cell phenotypes at the cephalic level in vitro. Materials: Cephalic NC (CNC) cells were isolated from 8.5 day mouse embryos by 48 hs explant cultures of neural tube at mesencephalon–rhombencephalon level. The cell phenotypes were identified by immunocitochemistry and RT-PCR using lineage-specific markers to melanocytes (MelEM), glial cells (SMP), Neurons (β-Tubulin III), Myofibroblasts (SMA). Results and Conclusions: The NC cells can differentiate in various cell types, both neuronal and non-neuronal. Immunocitochemistry and RT-PCR using lineage-specific markers identified neuronal, glial, myofibroblasts, and melanocytes. The identification of these phenotypes will permit studies about the CNC developmental mechanism in mammals, specially the identification of common precursors to mesectodermal and neuronal cell lineages and the proposition of a model about the balance control between the differentiation and maintenance of the NC Stem cells. Besides, it can bring the comprehension of the numerous cranial-facial anomalies mechanisms occurred during the vertebrates and human embryogenesis.

Supported by: PIBIC/UFSC, CNPq, CAPES/PROCAD, MCT/INFRA, PRONEX, FUNCITEC

C-025 IN VITRO STUDIES OF PLURIPOTENCY OF CEPHALIC NEURAL CREST STEM CELLS
Costa, M. C. Calloni, G.W. Trentin, A. G. Laboratório de Neurobiologia e Hematologia Celular e Molecular – BEG – CCB - UFSC. meline@grad.ufsc.br

Objective: Quail cephalic neural crest (CNC) cells are heterogeneous in proliferation and differentiation capacities, comprising highly pluripotent stem-like cells able to give rise to neurons, glial cells, melanocytes and cartilage as well as diverse developmentally restricted oligopotent precursors and committed cells. However, the pluripotentiality of NC is not well elucidated in avian. Materials: CNC cells were isolated from 24 hs explanted neural tubes of 6-8 somite quail embryos at mesencephalon–rhombencephalon level. Secondary cultures were grown on collagen or 3T3 mouse fibroblast feeder-layers. Cell phenotypes were identified by immunocitochemistry using lineage-specific markers to melanocytes (MelEM), glial cells (SMP), Neurons (TH and NF) and Myofibroblasts (SMA). cartilage cell aggregates were observed by phase contrast microscopy. Results and Conclusions: CNC grown on collagen differentiated in Schwann cells, myofibroblast and melanocytes in large proportion. We did not observe neurons and cartilage on this substratum. On the other hand, 3T3 fibroblast feeder-layers provided favorable conditions to CNC differentiation in neurons, and cartilage in addition to the previous ones. These findings suggest the existence of neural-mesectodermal precursors that can be considered as NC stem cells. Such highly pluripotent cells can, during migration and proliferation, generate precursors of more restricted developmental options and finally yield unipotent committed precursors in the sites of differentiation.

Supported by: CNPq, CAPES/PROCAD, MCT/INFRA, PRONEX, FUNCITEC.

C-026 DIFFERENTIAL ACTIVITIES OF SOG FRAGMENTS EXPRESSED DURING OOGENESIS MAY ESTABLISH THE DORSOVENTRAL AXIS OF THE DROSOPHILA EMBRYO
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The formation of the DI gradient in the embryo depends on a series of events that take place during oogenesis: dorsal activation of the Gurken-Egfr pathway and ventral activation of a proteolytic cascade that generates the signal for activation of Toll-Dorsal pathway. In addition of these pathways, the maternal sug and dpp genes play a role, during mid-oogenesis, to influence the formation of the nuclear Dorsal gradient, thus helping to define the dorsoventral axis. In this work, we show that driving misexpression of intact Sog protein, truncated constructs of Sog, Tolloid and Twisted Gastrulation in follicle cells, during mid-oogenesis, disrupts the position of dorsoventral domains of the embryo. Superexpression of the Supersog-like fragment has dorsalizing activity. The embryos derived from mothers that superexpress Tld and TSG in the follicle cells show simultaneously dorsalized and ventralized phenotypes. These results suggest that Tld and TSG generate at least two Sog fragments with different activities: a SogCR1-like fragment with dorsalizing activity and a other fragment with ventralizing activity. In addition, superexpression of TLD and TSG disrupts the formation of the dorsal appendages of the chorion. These results show that different forms of Sog protein have distinct activities to modulate the maternal Dpp pathway during oogenesis and to pattern the dorsoventral axis. This work was supported by FAPERJ and FIRCA/NIH.
C-027 RETINOIC ACID AND THE ATRIAL-SPECIFIC EXPRESSION OF THE TRANSGENE SMyHC3-HAP IN MICE EMBRYOS.
Michelle Vasconcelos e José Xavier-Neto. Laboratório de Genética e Cardiologia Molecular, Instituto do Coração – InCor – HC-FMUSP. Av. Dr. Enéas de Carvalho Aguiar, 44. São Paulo. SP. Vasconcelos777@hotmail.com

The SMyHC3 promoter is currently the only regulatory sequence able to restrict gene expression to sino-atrial tissues from the earliest stages of mammalian cardiogenesis up to adulthood. Since retinoic acid (RA) has been established as a critical player in atrial chamber morphogenesis, it is important to define how RA signaling regulates expression of SMyHC3-HAP, an atrial-specific transgene. In this work, we standardized post-implantation mouse embryo cultures and employed this technique to study the role of RA signaling in SMyHC3-HAP expression. RA signaling was manipulated in SMyHC3-HAP through treatments with exogenous all-trans RA and BMS 493, a RA-receptor pan antagonist. Inhibition of RA signaling by BMS 493, reduced SMyHC3-HAP expression and, when performed at the critical period, prevented atrial morphogenesis turning the heart into a single ventricular chamber. On the other hand, exogenous RA increased transgene expression and, when performed at the critical period, prevented ventricular morphogenesis, turning the heart into a single atrial chamber. Our results are consistent with a model where RA stimulates SMyHC3-HAP activity in both indirect and direct ways. In the former, RA initiates SMyHC3-HAP expression through the induction of an atrial genetic program. In the latter, RA modulates SMyHC3-HAP expression acting through RA receptors bound to response elements in the SMyHC3 promoter.

C-028 DENTINOGENESIS IMPERFECTA TYPE II. HISTOLOGICAL AND ULTRASTRUCTURAL ASPECTS.
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The Dentinogenesis Imperfecta (DI) is an autosomal, dominant, mendelian inheritance disease classified as: Dentinogenesis Imperfecta type I, being the one linked to Osteogenesis Imperfecta; Dentinogenesis Imperfecta type II, without Osteogenesis Imperfecta; and Dentinogenesis Imperfecta type III or Brandywine, a rare form of the disease reported in a triracial isolate from Brandywine (USA). DI is mapped to a 7q22. It is apparently not sex-linked, and appears equally in males and females. The aim of this study was to show the histological, ultra structural and radiographic characteristics of patient’s teeth affected by DI. This research was performed using teeth of one normal patient, and teeth of one family with DI (mother and daughter). The teeth with DI observed through MEV and polarization microscopy demonstrated few and disorganized or inexistent dentinary canaliculi. These teeth showed high degree of amelobrite translucency. Through the radiographic features of roots were short and slender and complete obliteration of pulp chamber was seen. Because of the social importance of this disease, physiological and stetic consequences, a professional, as dentist would know as to recognize it and to proceed an appropriate treatment.

C-029 MOLECULAR BASES OF ATRIAL-SPECIFIC TRANSCRIPTION: BINDING SITES FOR NUCLEAR RECEPTORS IN THE SMYHC3 PROMOTER
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To elucidate genetic pathways controlling cardiac chamber formation we analysed the regulation of an atrial-specific promoter from the quail slow myosin heavy chain 3 gene (SMyHC3). Atrial-specific expression of the 840-bp SMyHC3-HAP in transgenic mice is controlled by a distal 72-bp fragment. Using computerised simulations of the interaction between nuclear receptors and their response elements we identified a 26 bp motif containing a complex nuclear receptor response element (CNRRE). The CNRRE binds hetero and homodimers ofRAR, RXR, VDR, PPAR and homodimers of ER. Using energy minimisation followed by molecular dynamics we identified the lowest energy positions when scanning the CNRRE sequence with DNA-receptor structures. This analysis predicted 3 binding sites for nuclear receptors monomers (A and C) in the CNRRE. Electrophoretic mobility shift assays (EMSA) with purified RAR/RXR and VDR/RXR demonstrated that these 3 binding sites are functional and bind nuclear receptors dimers in three mutually exclusive configurations: distal (A+B); proximal (B+C) and “spaced” (A+C). Both simulation and competition assays indicated that the distal and spaced configurations bind RAR/RXR and VDR/RXR heterodimers more efficiently than the proximal configuration. In summary, atrial-specific expression of the SMyHC3-HAP in mice is controlled by a CNRRE, rather than by a dual RAR/VDR response element as reported in the quail context.

C-030 RETINOIC ACID AND THE EVOLUTIONARY ORIGIN OF CARDIAC CHAMBERS
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Combining studies on cardiac anterior-posterior (AP) patterning, comparative cardiac anatomy and expression patterns of retinoic acid (RA) synthetic enzymes we suggest a new paradigm for vertebrate cardiac development and evolution. Our paradigm pictures hearts as pumps projected to perform the dual functions of inflow and outflow, functions dispensed by units, the cardiac chambers. We propose that the inflow/outflow project is expressed in the dorsal/ventral chamber organization typical of the S-shaped vertebrate heart. The inflow/outflow pattern is laid down early in development as an AP code established by differential RA signaling, dividing the cardiac field into precursors of sinus venosus plus atrium (inflow) and ventricle plus conus arteriosus (outflow). We propose that cardiac AP patterning by RA is a common vertebrate theme, but that the basic elements required for partition of the heart in this axis are already present in basal chordates. Furthermore, we speculate that the ancestral chordate had a main urochordate-like pump into vasculature similar to that of amphioxus. This pump was retained in urochordates, most likely regressed in cephalochordates and developed further in vertebrates to give rise to their typical four-chambered hearts.
Tooth development is promoted by a sequence of signaling events that controls interactions between epithelium and mesenchyme cells. In the initiation stage, tooth appears as a thickening of the oral epithelium, and it acts as the first inductor center of the mesenchyme. Then, the epithelium invaginates into a bud and the underneath mesenchyme becomes the inductor. In the cap stage the inductor appears as the enamel knot in the epithelium, which leads the cells to proliferate and promote the formation of tooth shape. Many factors have been implicated in cell proliferation and differentiation during the first stages of tooth development. CTGF (Connective Tissue Growth Factor) is a protein implicated in differentiation, migration, and proliferation, and it interacts with TGF-β (Transforming Growth Factor-β) promoting an increasing of its signaling. In this work, we show that CTGF and members of TGF-β signaling, such as TGFRII, Smads2/3, Smad4 and TGFβ1 itself are mostly expressed in the epithelium of the dental lamina at embryonic day 11.5 (E11.5), in the mesenchyme of bud stage (E13.5) and in the enamel knot in the cap stage (E14.5). Our data suggest that CTGF could cooperate with TGF-β signaling components to modulate development of tooth.

C-032 ESTABLISHMENT OF calpain MUTANTS TO STUDY CALCIUM-DEPENDENT DEGRADATION OF CACTUS IN THE Drosophila EMBRYO
Rodrigo Agrellos Costa; Adriana de Oliveira e Silva; Helena Araujo. Depto de Histologia e Embriologia, ICB, UFRJ. rodrigoagrellos@hotmail.com

Dorso-ventral (DV) patterning of the Drosophila embryo depends on maternal genes acting during oogenesis and embryogenesis. Among this genes, dpp and sog act antagonistically to regulate the degradation of the IkB-related Cactus protein, releasing the NF-κB-related Dorsal to the nucleus. The Dpp maternal pathway acts in parallel to the well-described Toll pathway to form a nuclear gradient of Dorsal by mechanisms still not clear. Several evidences indicate the involvement of Calpain-dependent proteolysis of Cactus, regulated by maternal Dpp. In this work, we are trying to establish mutant lines for calpainA, since there aren’t mutants available for this gene. For this purpose, we have devised a genetic scheme for imprecise excision of a transposon near the calpA locus. A P element inserted 100 pb from the initiation codon for calpainA was used. We selected the flies that lost the P element through the use of phenotypic markers. Imprecise excision was valued by PCR with primers designed for the P element and for calpainA. Moreover, we are also developing transgenic flies transformed with a UAS-calpainA construct. These two lines will be used for analyzing the effect of calpain on DV patterning, as well as interaction between the calcium-dependent degradation of Cactus and the Dpp maternal pathway.

This work was supported by FAPERJ, CAPES and NIH/FIRCA.

C-031 CTGF IS CO-EXPRESSED WITH MEMBERS OF TGF-β SIGNALING IN THE INDUCTORS CENTERS OF THE TOOTH DEVELOPMENT
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Connective tissue growth factor (CTGF) is a 38 KDa secreted protein with a great capacity of promoting fibroblast proliferation, migration and extracellular remodeling. CTGF has been also implicated in a variety of process during organogenesis like angiogenesis and cartilage differentiation. CTGF binds directly to TGF-β1 enhancing its signal and has a opposite effect antagonizing BMP4 activity by blocking its binding to the receptor. In Xenopus laevis, CTGF is a maternal gene, expressed in early stages until pre-gastrula. At gastrula, CTGF expression is silenced and at post-gastrula stages, CTGF is expressed in notochord, floor plate and in somites. Since CTGF is expressed by mesoderm derived structures, we tested the ability of TGFβ superfamily members to activate CTGF expression. To do this, we co-transfected pCS2-Xnr1 or pCS2 empty (Control) with a construct containing the entire CTGF promoter, controlling a luciferase reporter gene (pCTGF-lux) in MV1LU cells. Xnr1 transfected cells induced four-fold more luciferase expression than the control, indicating that Xnr1 modulate CTGF transcription. In addition, CTGF protein secretion was increased in the supernatant of the Xnr1 transfected but not in control cells. These results suggest a possible role for CTGF during Xnr-1 expression during early embryogenesis.

Supported by: CNPq, MCT-PRONEX, CAPES, FAPERJ, TWAS and FUJB

C-033 XENOPUS NODAL RELATED-1 INDUCES CONNECTIVE TISSUE GROWTH FACTOR GENE EXPRESSION AND PROTEIN SECRETION
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The human glioblastoma (Gbm) is the major malignant glial tumor cells and represents 40% of all neoplasias of the central nervous system. Interactions between tumor cells and surrounding normal cells are not well known. Transforming growth factor-β1 (TGF-β1) is a peptide, which belongs to the multifunctional cytokines family that regulates some cellular functions, like proliferation and differentiation in several tissues, controlling its development and homeostasis. TGF-β1 induces Connective Tissue Growth Factor (CTGF), which is a protein secreted by different cell types like neurons and glial cells and is involved in many cellular events including angiogenesis and wound healing. Recently, it has been demonstrated that CTGF acts sinergically with TGF-β1 increasing signaling pathway and gene transcription. In this work the expression of TGF-β1 and CTGF was analyzed in tumor cells co-cultured with embryonic and neo-natal rat neurons and conditioned medium of rat neurons from the same ages and were maintained for 24 hours. Expression of CTGF, TGF-β1, GAPDH and β-actin transcripts were observed by semi-quantitative RT-PCR. In Gbm cells co-cultured with neo-natal neurons, CTGF was downregulated and TGF-β1 remains unaffected. However, preliminary experiments showed that CTGF and TGF-β1 transcripts increased in Gbm cultured with embryonic and neo-natal neuron conditioned medium. These results suggest that CTGF expression can be modulated during tumor and neurons interaction.

Supported by: CNPq, MCT-PRONEX, CAPES, FAPERJ, TWAS and FUJB

C-034 NEO-INALTAL NEURONS MODULATES CTGF EXPRESSION IN GIOBLASTOMA CELLS
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The human glioblastoma (Gbm) is the major malignant glial tumor cells and represents 40% of all neoplasias of the central nervous system. Interactions between tumor cells and surrounding normal cells are not well known. Transforming growth factor-β1 (TGF-β1) is a peptide, which belongs to the multifunctional cytokines family that regulates some cellular functions, like proliferation and differentiation in several tissues, controlling its development and homeostasis. TGF-β1 induces Connective Tissue Growth Factor (CTGF), which is a protein secreted by different cell types like neurons and glial cells and is involved in many cellular events including angiogenesis and wound healing. Recently, it has been demonstrated that CTGF acts sinergically with TGF-β1 increasing signaling pathway and gene transcription. In this work the expression of TGF-β1 and CTGF was analyzed in tumor cells co-cultured with embryonic and neo-natal rat neurons and conditioned medium of rat neurons from the same ages and were maintained for 24 hours. Expression of CTGF, TGF-β1, GAPDH and β-actin transcripts were observed by semi-quantitative RT-PCR. In Gbm cells co-cultured with neo-natal neurons, CTGF was downregulated and TGF-β1 remains unaffected. However, preliminary experiments showed that CTGF and TGF-β1 transcripts increased in Gbm cultured with embryonic and neo-natal neuron conditioned medium. These results suggest that CTGF expression can be modulated during tumor and neurons interaction.

Supported by: CNPq, MCT-PRONEX, CAPES, FAPERJ, TWAS and FUJB
Intriguing and might suggest some local role. Heterochromatic, attaching rDNA from the chromosome X is specific for the chromosome regions, frequently mitotic chromosomes. In this sense, the early idea of a ramified physiology and biophysics São Paulo University, Biomedical Sciences Institute, São Paulo, Brazil. The phagocytosis process in mice trophoblastic cells is induced by IFN-γ and involves the NADPH-oxidase system that provides reactive oxygen species (ROS). In this context, the aim of this study was to analyze the antioxidant enzyme balance and the production and releasing of ROS by mouse placental cells treated and untreated IFN-γ. For this purpose, mouse placentas were collected on the 14th day of pregnancy, submitted to trypsin digestion, cultured for 48 h and then were incubated with IFN-γ (100 U/mL) for 12 h. The enzymes, CuZn and Mn superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (CAT) were analyzed through the gene expression (RT-PCR) and enzyme kinetics. The release of free radicals was measured by TBARs technique. The IFN-γ stimulus induced an increase of ROS synthesis and in concomitant to an increase in the activity (SOD CuZn and GHS-Px) and expression (SOD Mn, SOD CuZn and GHS-Px) of antioxidants enzymes. These data show an IFN-γ direct induction of different mechanisms for ROS production and antioxidants enzymes activation, probably related to the maintenance of the integrity of placental cells. However, the high expression of SOD Mn indicates that IFN-γ may regulate the respiratory burst in mice placental cells. The high of ROS production and antioxidant enzymatic levels (SOD CuZn and GHS-Px) in this model, also suggest that an oxidative stress situation may be involved in pathological conditions.

Grants: FAPESP

C-036 THE LOCALIZATION OF RIBOSOMAL DNA IN SCIARIDAE REVISITED
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In polytene chromosomes of Sciaridae (Diptera: Nematocera), the nucleolus lacks the well-formed aspect that is observed in other dipterans such as Drosophila and Chironomus. The nucleolar material in sciarids is frequently associated with the chromosome X, being also organized as micronucleoli. In Rhynchosciara americana, ribosomal DNA (rDNA) is mainly localized in the chromosome sections X-12 and C-11. rDNA probes also hybridize regularly at the regions B-15, B-13, X-1 and A-11. In the later sections, the intensity of the hybridization signals can vary significantly. Such variations could be due to the underreplication of ribosomal cistrons in the salivary gland when compared with diploid tissues of R. americana. The putative effect of the rDNA underreplication on the in situ hybridization results might be thus avoided if the localization of the rDNA was studied in mitotic chromosomes. In situ hybridization was then carried out with chromosomes from ovaries. Surprisingly, the rDNA probe was only localized in the chromosome X. The results show that rDNA localization in polytene chromosomes of R. americana is not reflected in mitotic chromosomes. In this sense, the early idea of a ramified NOR from X-12 cannot be ruled out. On the other hand, the specificity of the chromosome regions, frequently heterochromatic, attaching rDNA from the chromosome X is intriguing and might suggest some local role.

C-037 MORPHOLOGICAL ASPECTS OF COLLAGEN FIBER OF Rana catesbiana DERM
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Collagens belong to a family of proteins highly characterized and found in all multicellular animals. They are highly distributed in the extracellular matrix, being the main product of fibroblasts. Using the Sirius-Red methodology collagen fibers disposition and arrangement can be observed and both thick and thin fibers show different colour patterns in the polarization microscopy. Rana catesbiana specimen were collected in a frog creation from the ONG, OICA-BRASIL (JacaréI/SP) and taken to the laboratory to be manipulated. Skin fragments in the dorsal and ventral regions were taken and fixed in buffered formaldehyde solution and submitted to histochemical reaction of picrosirius-hematoxilyn. The thin fibers show different colour patterns in the polarization microscopy showing a great variation in colour. The results showed that collagen type I was thick and strongly birefringent. In this way the collagen type I form thick fibers (collagen fibers), composed by thick fibrils closely connected and with intense birefringence in redish.

C-038 A LIM-HOMEOBOX GENE UPREGULATED DURING Mesocestoides corti STROBLAR DEVELOPMENT

In order to understand the molecular processes regulating morphological changes during parasitic platyhelminthes life histories we focused on homeodomain (HD) proteins, a family of transcription factors essential for pattern formation during development. Mesocestoides corti is a parasitic platyhelminthes cestodo that present remarkable developmental capacities, as sexual and asexual reproductive stages, and dual developmental ability of the larval forms, tetrahyridia. In this work we report the isolation of the partial sequence of MvLim, a LIM-HD gene of Mesocestoides corti. Other members of this gene family, characterized in Drosophila melanogaster, Caenorhabditis elegans and vertebrates contribute to cell fate determination of various neuronal subtypes. Phylogenetic analyses showed that MvLim clusters with members of the LIN-11 group and that platyhelminthes have at least two different LIM-HD genes. By real time PCR we determined that MvLim expression is 20-fold greater in segmented worms than in tetrahyridia. The enhancement of MvLim expression during strobilation could be associated to changes in the innervation pattern occurring in proglostids development.
Poisoning by Bothrops snakes is frequent and though the morality rate is low, the coagulating action, proteolytic and vascular toxicity may cause systemic complications. The purpose of this study was to identify the alterations promoted in the spleen of mice injected with the poison from Bothrops moojeni poison at 0.4mg/ml (GE, n=18). Control animals were injected with 0.9% saline solution (CG, n=18). Samples were collected after 3, 12, 24 hours and 3,7 and 21 days. The rough portion was received from the Zoology Laboratory of UNIDERP and kept at -20°C. The most common lesions in the different periods from the experimental group (3, 12 e 24 hours e 3, 7 e 21 days) were the following: hiruditic degeneration, follicular atrophic areas, pholiculus hyperplesia (after 24 hours) and vascular congestion after 21 days. The macroscopic alterations observed in the spleen after 24 hours were not very important as the organ showed itself a little darkened in relation to the natural color and lightly rigid. In what concerns to the microscopic alterations it showed with a thick capsule and with invaginations inside the tissue. In conclusion it was noticed that the poison was harmful to the organ as in 21 days after the exposure there were still congested blood vessels.

D-001 DISTRIBUTION OF TGF-β DURING CHICK VERTEBRAL BODY DEVELOPMENT
Renato M Salgado; Luiz Carlos Nogueira & Terezinha J. Sirotheau-Corrêa. Depto. de Morfologia, Instituto Biomédico, UFF, 24240-130. biouff@uol.com.br, sirotheau_correa@uol.com.br

Endochondral bone develops through a complex process whereby a cartilage model, originated from mesenchymal cells, is replaced with bone. Members of the TGF-β superfamily are secreted growth factors that regulate many aspects of development, including growth and differentiation. Although it is highly distributed during vertebral body development, its effects on terminal differentiation and endochondral ossification are less clear. The objective of this study is to analyze the sequential distribution of TGF-β in the phases of endochondral ossification. Chick embryos, stages 17 through 45, were fixed in methacarn, processed and embedded in paraplast. Immunohistochemistry was performed using monoclonal primary antibody against TGF-β. The results achieved showed that during mesenchymal cells migration and condensation (stages 17-27), strong positive reaction was observed in these cells and the surrounding matrix, in the notochord sheath and notochordal cells. In the mature cartilage (stage 35), positivity was absent in the extracellular matrix and very intense in the perichondrium matrix. Finally, during osteogenesis (stages 40-45), strong positive reaction was present in the hypertrophic chondrocytes and their extracellular matrix, also in the osteocytes and osteoblasts from bone trabeculae. Many recent studies revealed that TGF-β plays an important role in sclerotome differentiation, but inhibits chondrocytes maturation in vitro. It is also relevant for proper bone development. Our results suggest that this growth factor is essential for normal endochondral ossification during vertebrae development.

In many tissues, carefully regulated cell-matrix interactions are responsible for maintaining tissue homeostasis. Fibronectin binds to β1 integrin, which activates actin polymerization. These specific interactions in cartilage might be expected to regulate processes such as cell survival, growth and differentiation. Nevertheless, these events are not yet clearly understood in chick vertebral body development. The aim of this study is to analyze the distribution of macromolecules involved in cell-matrix interactions during endochondral ossification. Chick embryos, stages 17 through 45 were fixed in methacarn, processed and embedded in paraplast. Immunohistochemical localization was performed using anti-fibronectin, anti-β1-integrin and anti-actin antibodies. Our results showed similar distribution among the molecules studied. During mesenchymal cells migration and condensation (stages 17-27), strong positive reaction for fibronectin was observed in the notochordal sheath and in the mesenchymal extracellular matrix and for β1 integrin and actin, in the mesenchymal cells. During cartilage maturation (stages 32-35), fibronectin was absent in the cartilaginous extracellular matrix, but abundant in the perichondrium matrix, while β1 integrin and actin had a mild expression in chondrocytes and stronger in chondroblasts. In osteogenesis (stages 40-45), the positivity was again present in the hypertrophic cartilage and bone extracellular matrices and in the cells observed. As shown in previous studies, inhibition of cell-matrix contacts through integrins enhances chondrogenesis. Our results suggest these regulated expression patterns might be essential for proper vertebral development.

D-002 CELL-MATRIX INTERACTIONS DURING VERTEBRAL BODY DEVELOPMENT: AN IMMUNOHISTOCHEMICAL APPROACH
Renato M. Salgado & Terezinha J. Sirotheau-Corrêa. Dept. de Morfologia, Instituto Biomédico, UFF, 24240-130. biouff@uol.com.br, sirotheau_correa@uol.com.br

In chick embryos, primordial germ cells (PGCs) migrate in three distinct phases. First of all, in the separation phase, the PGCs separate from hypoblast. Second, during the migration phase, embryonic blood vessels are formed around these cells at the germinal crescent, which begin to circulate through the bloodstream, leaving the capillaries and migrating interstitially. Finally, in the colonization phase, PGCs reach and colonize the genital ridges. As the expression of glycoproteins in the migratory pathway of chick primordial germ cells in all its phases has not yet been described, the present study aimed to analyze the expression of these matrix components during these phases of migration. Using periodic acid Schiff (PAS) histochemical technique, the present study identified, during separation phase (stage 7), positive reaction in the hypoblast basement membrane and surrounding matrix. In stages 9, 15 and 17, which characterize the migration phase when the cells reach the splanchnic mesoderm, positivity was observed in the extracellular matrix. In stage 21, intense reaction was present in the dorsal mesentery, coelomic angle and genital ridges extracellular matrix, regions well known as part of the migratory pathway and colonization areas. Our results suggest that the successive steps of the PGCs migration require the expression of glycoproteins, so that these cells can achieve their goal properly.

C-039 MOUSE SPLEEN ALTERATIONS CAUSED BY Bothrops moojeni POISONING

Bothrops moojeni, a common envenoming snake in Brazil and other South American countries, is responsible for many deaths and serious medical conditions. The study aimed to analyze the sequential distribution of TGF-β (growth factor) in the spleen of mice injected with the poison from Bothrops moojeni. The main findings were that TGF-β was distributed in the extracellular matrix, indicating its role in splenic tissue proliferation. Further studies are necessary to understand the mechanism of action of TGF-β in this specific tissue.
D-004 DISTRIBUTION OF MAST CELLS IN RAT FEMORAL BONE MARROW
Devandir A.S. Junior; Michel F. Guiraldelli; Constance Oliver; Maria C. Juniar. Department of Cellular and Molecular Biology and Pathogenic Bioagents, FMRP USP, 14049-900, Ribeirão Preto, SP, Brazil. dsjunior@rbp.fmrp.usp.br

In addition to mast cells precursors, bone marrow contains mast cells in all stages of maturation, from immature to mature. The mature mast cells can be readily identified by the presence of metachromatic cytoplasmic granules, but specific markers are needed to identify immature and very immature mast cells, that do not contain metachromatic granules. In this study we investigated the distribution of metachromatic mast cells in situ in the rat femur. The mature and immature mast cells had a similar distribution in the bone marrow, but the number of metachromatic immature mast cells was higher. The mast cells are nonrandomly distributed in the bone marrow and are preferentially located at the distal diaphysis. Metachromatic mast cells were also present in association with the endosteum, bone spicules as well as near the epiphyseal cartilage. The quantification of bone marrow mast cells was done using the rodent mast cell specific monoclonal antibody AA4 that recognizes mast cells in all stages of maturation, mature, immature and very immature. The femur was removed, cut in half and the marrow was detached from the bone, dissociated and immunolabeled with mAb AA4 directly conjugated to FITC. The cells were analyzed by FACS. The number of mast cells was significantly higher in the distal diaphysis of the femur, confirming the results obtaining with toluidine blue staining. Thus, the distribution of mast cells in the femoral bone marrow is heterogeneous.
Financial Support: FAEPA

D-005 PORCINE PERICARDIAL MATRIX: TOPOCHEMICAL AND TOPOPHYSICAL STUDY
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Pericardial tissue has been used in the bioprosthesis fabrication, which are used in the repairing of several injuries, especially cardiac ones. However, shortcomings such as calcification and mechanical failure are the major causes of limited durability of cardiac bioprosthesis. Optical anisotropy and basophilia were used to find morphological and ordering aspects of pericardium that was the purpose of this work. Porcine pericardia were collected and fixed in paraformaldehyde. Part of sample was subjected to routine processing for paraffin embedding and microtomy and part was destined to in toto preparation. Some samples were stained with Toluidine Blue pH 4.0, while others remained without staining. Microscopy of pericardium revealed high cellularity and these cells are mainly fibroblasts and some mast cells. A very ramified vascular tree constituted of capillaries, small arteries and veins was observed. Collagen fibers did not stained in Toluidine Blue pH 4.0. Polarized light analysis allowed detection of collagen fibers by means of its birefringence, as well as its distribution, orientation and aggregation. Observation at different focal planes has been revealed collagen fibers in multidirectional layers with preferred collagen fibers directions. The left anterior side showed collagen fibers predominantly oriented in oblique direction. In the right anterior side preferred directions were parallel to base-to-apex of the heart. The lack of metachromasia may be related to absence or unavailable poly-anionic radicals.

D-006 AGE INFLUENCE IN THE ORDERED AGGREGATIONAL STATE IN TENDONS SUJECTED TO COMPRESSION FORCES
Flávia de Paoli; Benedicto C. Vidal. Departamento de Biologia Celular, Instituto de Biologia, UNICAMP, 13083-863. depaoli@unicamp.br

Birefringence is the best way to determine the direction of the electronic transitions between peptide bonds in collagen and consequently the molecular orientation and biopolymer crystallinity. Changes on diameter and packing of fibers in response to biomechanics or physiological changes may influence birefringence. Tendons that are localized on articular regions undergo compressive forces that give differential optic anisotropies. The aim of this work is to compare the same tendon at two ages, by verifying alterations in the molecular order and crystallinity caused by biomechanical and physiological changes with the ageing. For this calcaneus communis tendons were utilized from chickens with 21 and 41 days. So the tendons were fixed in paraformaldehyde and paraffin embedded. Slices with 7 µm thick were dewaxed and embedded in media with increasing refraction index. Optical retards values were measured using Sernamont’s λ/4 and Bräce-Köhler’s 1/100 compensators. The data were analyzed by ANOVA and birefringence curves have been made. 21 days tendons showed highest ROs values, consequence of the high ordered aggregation state of its fibers along the main axis of the tendon. However, alterations in the intrinsic birefringence, revealed by the electronic transactions in collagen, were not detected. These data are evidence of an adaptation of 41 days tendons to compressive forces, by alterations in the concentration and arrangement of its components influencing form birefringence.

D-007 MOLECULAR ORDER AND CRYSTALLINITY IN COLLAGEN BUNDLES OF NON-OBSENE DIABETIC (NOD) MICE
1Marcela Aldrovani Rodrigues; 2Ana M.A. Gualandro; 1María L.S. Mello; 1Benedicto de Campos Vidal. 1Dept. Cell Biology, IB/Unicamp, 13083-863; 2CEMIB, Unicamp, 13084-971. marcela_aldrovani@yahoo.com.br

The non-enzymatic glycosilation of collagen is one of the factors potentially responsible for many clinical alterations observed in diabetes. Advanced glycosilation gives rise to end-products that form intermolecular cross-linkings in collagen fibers and modify some of its physical properties, such as optical anisotropies. In the present study we examined birefringence in collagen bundles from calcaneal and tail tendons of NOD/Uni mice in the 4th-week of diabetic expression and BALB/C/Uni (control) mice, in order to investigate the collagen molecular order/crystallinity during the diabetic expression. Some BALB mouse tendons were glycosilated in vitro before being fixed in paraformaldehyde. After fixation, all the tendons were rinsed in water, dehydrated, embedded in paraffin and cut into 7 µm sections. The sections were immersed in solutions with various refractive indices. Optical retardations (OR) were measured in a polarizing microscope with Sénarmont λ/4 and Bräce-Köhler λ/10 compensators. The results indicated that diabetes and glycosilation in vitro increase the OR values of the tendons in all the imbibing solutions used, except in tail tendons immersed in pure glycercel (intrinsic birefringence/crystallinity). The non-enzymatic glycosilation turned the collagen fibrils/bundles with a molecular packing tighter than that occurring in control. In addition it promoted variable changes in the collagen crystallinity, suggesting the existence of different physio-pathogenics meanings as a function of the structure. (CAPES, CNPq)
The therapeutic ultrasound has been used, for more than 50 years, to reduce pain and to accelerate the healing process of bone after injury. However, there are a few scientific reports about biochemical effects of pulsed therapeutic ultrasound on bone repair. Sixty male albino rats were divided into 4 experimental groups: reference; control; 16Hz and 100Hz ultrasound-treated. The rats (45) undergone bone fracture were treated with ultrasound 24h after the surgery, once a day, for 3min, with an intensity of 0.5 w/cm² pulsed mode 1/5, pulse modulated frequency of 16Hz and 100Hz. The animals were euthanised 7d, 14d and 21d after surgery, the blood was sampled by cardiac puncture for alkaline phosphatase and calcium analysis and the parameters were compared by ANOVA. The blood alkaline phosphatase and calcium levels were significantly (p<10⁻⁷) different among experimental groups:

<table>
<thead>
<tr>
<th>Groups 7d</th>
<th>Alkaline phosphatase (U/L)</th>
<th>Calcium (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference</td>
<td>66.78±7.97</td>
<td>9.84±4.04</td>
</tr>
<tr>
<td>Control</td>
<td>58.78±14.2</td>
<td>9.72±1.72</td>
</tr>
<tr>
<td>UST 100Hz</td>
<td>37.68±7.04</td>
<td>5.72±1.44</td>
</tr>
<tr>
<td>UST 16Hz</td>
<td>47.84±11.93</td>
<td>5.97±1.23</td>
</tr>
</tbody>
</table>

The pulsed ultrasound treatment accelerated the fibula repair and the most effective treatment was achieved using the 100 Hz pulse modulated, and it modulates the blood level of alkaline phosphatase and calcium.
Several methods have been used to stimulate bone repair. The nature of the formed tissues seems to be determined by the quantity of the local deformation (mechanical signal) in the bone-healing region. The purpose of this work was to investigate the effect of 16 and 100 Hz pulse modulated 1 MHz therapeutic ultrasound in fibula repair after experimental fracture. Six male albino rats were divided into 4 experimental groups: reference, control, 16 Hz and 100 Hz ultrasound-treated. The rats with bone fracture were treated with ultrasound, once a day, for 3 min, with an intensity of 0.5 W/cm² pulsed mode 1/5, pulse modulated frequency of 16 Hz and 100 Hz. The animals were euthanized 7 d, 14 d, 21 d and 28 d after surgery and the fibula removed and analyzed. The density of bone matrix, chondrocytes and fibroblasts were determined and the parameters compared by ANOVA. Seven days after surgery, changes in the studied parameters could be found among experimental groups: 100 Hz (40.75 ±2.81) and 16 Hz-pulse (23.37 ±1.18) relevantly increase the bone matrix density (in relation to the control, 18.62 ±1.62, p<10⁻⁶). The number of chondrocytes did not vary significantly and, the number of fibroblast relevantly decreased in both ultrasound treatments (p<10⁻⁶) as compared to the control (100 Hz, 12.37 ±2.18; 16 Hz, 10.87 ±1.19; control, 26.75 ±1.19). These results indicate that the pulsed ultrasound treatment is able to accelerate the ossification process and that the most effective treatment was achieved using the 100 Hz pulse modulated.

D-011 CALCIFIED DERMAL LAYER OF THE Bufo ictericus INTEGUMENT
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Amphibian integument is a high-specialized organ, and its structure reflects its participation in several functions such as respiration, osmoregulation and hydric control. Besides the classic structural organization in epidermis and dermis, anuran integument presents a no cellular layer, where organics constituents coexist with inorganic calcium. This layer is usually between the stratum spongiosum and stratum compactum of the dermis, which is named the Eberth-Katschenko (EK) layer. The knowledge about the interaction between calcium and extracellular matrix components is essential; since this phenomenon has been regarded as one of the ossification start factors. This study characterized the EK layer, using histochemical techniques as Alcian Blue stain in different critic electrolytic concentrations with and without enzymatic treatment, and von Kossa method with and without acid treatment, for light microscope analysis. The results showed the coexistence between calcium and glicosaminoglycans, mostly hyaluronic acid, in the EK layer; and that calcium is probably linked to the GAG. Chondroitin sulfate, which was previously pointed out in Bufonidae family frogs, was not detected. The EK layer of Bufo ictericus may be directly associated to hydric balance and integument structural maintenance, and represent a good model of organic and inorganic elements interaction in extracellular matrix.

D-012 THE ROLE OF METALLOPROTEINASES DURING PREGNANCY OF THE MOUSE.
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Studies of pubic symphysis in mice showed, that during pregnancy it is gradually replaced by a fibrous connective tissue, forming a flexible and elastic interpubic ligament. Following labor, the ligament undergoes rapid involution and around the second week it got back together almost completely. During pregnancy uterine and cervix tissue undergoes to radical modifications induced by relaxin/metalloproteinases. Even though there are almost no reports that recognize the specific role of metalloproteinases (MMPs) involved in the remodeling of the symphysis or in interpubic ligament development. In order to evaluate the main characteristics of the mice interpubic ligament in vivo and production of MMP during 24 h of incubation were used light and electron microscopy to identify cell types and zymography for MMPs invitro secretion. Ligament cells were observed to migrate in a limited window in the explant. The late pregnant ligaments showed accelerate cells migration. Electron micrographs demonstrated that these ligament cells were fibroblasts, sometimes associated with extracellular fibrils. Morphological analyses confirmed that the processes of cell migration from ligament explants to plate were different for all days of pregnancy. This study in vitro demonstrates that the interpubic ligament contains active fibroblasts capable of migration and MMPs synthesis. These findings support the hypothesis that metalloproteinases can facilitate the growth and remodeling of interpubic ligament by intrinsic processes. Supported by FAPESP.
D-013 EXTRACELULAR MATRIX ORGANIZATION IN DIFFERENT TENDON REGIONS OF CHICKEN
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Some tendons experiment compressive in addition to tensional forces, as passes under a joint. Taking into account the presence of biomechanical forces, the purpose of this work was to investigate the organization of the extracellular matrix of the superficial flexor tendon of chicken. So the tendon was divided in proximal (p) and intermediate (i) regions, both receiving compression, and distal (d) region, withstanding only tension. Some sections were stained with toluidine blue and observed under common and polarized light microscopies. In p and i regions, chondrocyte like cells and basophilia in the matrix was observed, indicating presence of proteoglycans. Analysis under polarized light microscopy, showed that the collagen bundles run in different directions, and are associated to proteoglycans. In the region under tension, fibroblasts appear disposed in a row, close to the collagen bundles, which were arranged in the same direction of the longest axis of the tendon. No basophilia was found, indicating a low concentration of proteoglycans in this region. Measurements of optical retardation, showed higher values in the region under compression than in the region under tension, indicating that in the former, there is a larger compactness of the collagen fibres. It must be considered that in the compressive region, there is a significant presence of proteoglycans contributing to the supramolecular organization of the tendon. Fapesp * 01/04820-5; ** 00/09080-7.

D-014 HISTOCHEMICAL AND MORPHOLOGICAL EVALUATION OF THE REMODELING IN THE MANDIBULAR CONDYLE OF THE TEMPOROMANDIBULAR JOINTS AFTER PLACEMENT OF UNILATERAL OCCLUSAL INTERFERENCE IN RABBITS.
1Karen Chaves; 2Milene Sanches Galhardo; 2Oswaldo Alves Mora; 3Cláudia Naves Battlehner; 3Elia Garcia Caldini; 20gla M. S. Toledo. 1Fisiopatologia Experimental, FMUSP ; 2Depto. Morfologia, UNIFESP; 3Depto. Patologia FMUSP. olmatoledo@uol.com.br

The effect of occlusal imbalance on the structure and function of the temporomandibular joint is a controversial issue. Structural adaptation of the articular surface of the mandibular condyle is necessary for normal development and for the demands imposed by changing forces. Remodeling involves morphological changes in the articular components. In a rabbit model of unilateral occlusal interference insertion, the articular cartilage as well as the osseous portion of the mandibular condyle were studied both in the ipsilateral and in the contralateral sides, 7, 30 and 60 days after the placing of the device. Histological sections were stained by H&E and by the Picrosirius-polarization method. Our results demonstrated that at both sides the condyles are affected by the unilateral bite rise. The changes include cell proliferation and growth of a cartilaginous model for the posterior deposition of osseous tissue. Meanwhile, the original bone goes through a remodeling process that includes resorption. Although the same general kind of changes can be observed at both sides, they do not seem to be identical. The fact that each side of the face is subjected to distinct biomechanical forces in this case, may account for the differences observed between the joints.

D-015 MORPHOLOGICAL ANALYSIS OF THE TRACHEAL CARTILAGE OF CHICKENS WITH DIFFERENT AGES
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The extracellular matrix of trachea is composed by collagen, proteoglycans and non-collagenous proteins, which are organized to provide flexibility to the tissue. Considering that the trachea undergoes alterations with the age, in this study were analysed the morphological aspects of the tracheal cartilage of chickens with 1, 30, 60 and 90 days. The tracheas were fixed in 4% paraformaldehyde and sections stained by toluidine blue and weigert’s for optical microscopy. For electron microscopy the fixation was in 3% glutaraldehyde and staining with ruthenium red and cuprolinie black. A larger amount of cells in animal with 1 day. The rough endoplasmatic reticulum and secretion vesicles were more relevant in animals with 1, 30 and 60 days than in animals with 90 days. Basophilia indicating the presence of proteoglycans was observed in the territorial and interterritorial matrix in cartilage of animals with 1, and 30 days, but in animals with 60 and 90 days it was restricted to the territorial region. Elastic fibres detected in the perichondrium exhibited ortogonal distribution in animals with 1 and 30 days, while in animals with 60 and 90 days they are circumferencialy disposed along the ring. Our results demonstrated that the distribution of proteoglycans and the arrangement of elastic fibers are deeply altered with the age. Fapesp 01/04821-1.

D-016 BONE MARROW STROMA OF ADULT MICE
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Bone marrow (BM) is the major hematopoietic organ in adult mice. Its stroma assembly is scarcely studied. Here, BM stromal cells and extracellular matrix (ECM) of 20 days mice were studied. Ribs and spine were fixed in Carson’s Millonig formalin and embedded in paraffin. Sections (5 μm) were stained with HE, Lennert’s Giemsa, PAS-AB pH=1.0/ 2.5 and analyzed by brightfield microscopy. PMA-Picrosirius stain (PMA-PRS) and immunofluorescence to a-smooth muscle actin (aSMA), tropomyosin (TP), fibronectin (FN), laminin (LM) were analyzed by confocal microscopy. The results were: 1) two cellular populations (high/low) were distinguished by a-SMA, 2) TP was presented in endosteum, hypertrophic chondrocytes, in some hematopoietic/stromal cells and megakaryocytes. 3) FN showed sparse distribution through ECM, and was detected in the endosteum, cytoplasm of megakaryocytes and hypertrophic chondrocytes. 4) Proteoglycans (PG) lined the endosteum and some venus sinususes (pH=2.5>1.0) and were often seen around hematopoietic cells (pH=2.5<1.0). 5) Collagens I and III (PMA-PRS) exhibited a mild expression in BM, specially near endosteum and medullary sinususes. 6) LM showed a discontinuous pattern along the basal membrane of medullary sinususes. In conclusion, a)PGs, specially those with low sulphation, were the main component of bone marrow ECM; b) stromal population is heterogenic, with some cells expressing a-SMA and/or TP; c) the endosteum microenvironment is very peculiar, showing cells positive to TP and ECM composed by FN, collagens I and III and PGs like hyaluronic acid.
Embryonic development is a complex process that constitutes an excellent model for the study of extracellular matrix (ECM) molecules. Considering the biological characteristics of glycosaminoglycans and proteoglycans we may expect that they play a role during embryonic organogenesis. Hyaluronan (HA) is important as space filler during embryonic development. HA also participate in chemical signaling between cells. Versican (VER) regulates the cell survival, proliferation and migration and participates in the structural reorganization of ECM molecules, necessary for the development and maintenance of the tissues during embryonic development. This work shows the distribution of AH and VER in embryonic tissue of mice from E9 to E15 stages. Embryos on E9 to E15 stages of the development were fixed in Methacarn. HA and VER was localized by immunocytochemistry using specific antibodies. Both HA and VER were present in embryonic tissues in all developmental stages. HA was distributed in the extracellular spaces of connective tissues whereas VER was present around connective tissue cells and epithelial cells. Both molecules were present in the epithelial/mesenchymal interface. Both HA and VER are expressed in the connective and epithelial interface suggesting that these molecules play a role in the establishment of epithelial/mesenchymal interactions. The presence of these molecules in the early stages of the embryonic development indicates that both participate in the morphogenesis and organogenesis of mice.

The physical, chemical and mechanical properties of collagen bundles change during aging. The collagen fibers become more resistant to enzymes and consequently, its solubility is reduced. Modifications in glycosaminoglycan sulfatation degree are also resistant to enzymes and consequently, its solubility is reduced. An inhibitory effect over sulfated glycosaminoglycan synthesis. Hyaluronic acid predominated over other glycosaminoglycans; dermatan sulfate and condroitin sulfate were detected while condroitin destroyed, due to proteolytic activity of the matrix metalloproteinases. The volume fraction of the extracellular matrix in the alveolar septa (normalized by septal length) was measured with a computerized image analysis system. Both collagen and elastic fibers proportions were increased in HIV/PCP patients when compared with controls (collagen: 0.44±0.10 and 0.24±0.06, respectively, p<0.05; elastic: 0.42±0.05 and 0.24±0.06, respectively, p<0.05). Our findings demonstrated that in HIV positive patients that died of respiratory failure there is also an increase in the amount of collagen-containing and elastic system fibers in the alveolar interstitium. This observation may have therapeutic implications.
D-021 COLLAGENS TYPE I, III AND V CONSTITUTE THE THICK COLLAGEN FIBRILLS OF THE MOUSE DECIDUA

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Deciduization in the mouse involves cell growth and a severe reduction of the extracellular spaces. In mice, one of the distinguishing events is a remarkable increasing of the collagen fibril diameter occurring exclusively in the decidualized regions of the endometrium. Previous biochemical studies show that in mice, an unusual homotrimer form of collagen type V (α1(V)3) is present in the decidualized endometrium. The goal of this study is to investigate the molecular composition of the thick collagen fibrils in the decidua of mice. For this propose we analyzed by immunoelectron-cytochemistry the presence of type I, III and V collagen in thick collagen fibrils of the decidua, using the double-labeled method. Uteri from animals on the day seven of pregnancy were treated with urea to disrupt the hydrogen bonds of collagen and to expose the collagen epitopes. Following, samples were double immunolabeled, with anti-collagen type I-III; I-V; or V- III specific antibodies. After, samples were incubated with rabbit IgG anti-goat or with goat IgG anti-rabbit conjugated to 5nm and 10 nm colloidal gold respectively. Our results show that the thick collagen fibrils present in the mouse decidua are heterotypic fibrils formed by collagen types I, III and V.

D-022 PROLIFERATIVE ASPECT OF TESTOSTERONE-INDUCED PROSTATIC REGROWTH IN CASTRATED RATS

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Androgen ablation in adulthood promotes an extensive involution of prostate gland with elimination of epithelial cells by apoptosis. In the involuting rat ventral prostate, apoptosis occurs mainly in the distal region of the ducts and the proximal region have minor effects. In this work, we investigated the epithelial cell proliferation in ventral prostate during androgen reposition after 21 days of involution. Wistar rats were castrated and treated with testosterone (4mg/kg body weight). Prostates were removed at 0, 3, 5, 7 and 10 days after testosterone replacement and processed for histochemistry and immunocytochemistry for PCNA. Sham operated animals were used as control. The proliferative index (PI) was determined counting PCNA-positive nuclei between unlabeled nuclei and expressed in percentage. Control prostate presented a PI about 1% and 0% in the distal region and proximal region, respectively. Castration abolished any proliferation in the epithelial cells. Testosterone replacement induced a remarkable proliferation in the epithelial cells and the PI at 3, 5, 7 and 10 days was respectively, 35%, 20%, 20% and 10% in the distal region and about a half of these values were obtained in the proximal region. Considering that the PI at 10 days after regrowth was 10 times higher than in control group, we can say that the gland was probably recovering.

D-023 COMPARATIVE METRIC AND STRUCTURAL PARAMETERS OF THE TESTOSTERONE-INDUCED REGROWTH IN PROSTATE AND SEMINAL VESICLE OF CASTRATED RATS.

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Hormone-dependent secretory organs are targets of the many human diseases, including hyperplasia and cancer. Testosterone plays a fundamental role on maintenance of secretory activity of prostate and seminal vesicle. Androgens withdraw and replacement has been used as model for understanding the physiology of these glands. Here, we examined the prostate and seminal vesicle regrowth with androgen replacement after 21 days of castrated-induced involution. Wistar rats were divided into 3 groups: control (CT), castrate (CS) and treated-castrate (TR). Prostate and seminal vesicle were removed at 3, 5, 7 and 10 days after testosterone replacement, weighted and processed for histochemistry. Statistical data of wet weight showed significant prostate and seminal vesicle reduction in CS groups. On prostate, 10 days of testosterone treatment resulted in an incomplete glandular wet weight recovery, while the seminal vesicle weight overcome significantly the CT values. Morphological analyses showed prostate and seminal vesicle from TR recovered the glandular structure when compared with CT. The results showed a distinct ratio response for androgen replacement on each gland. Probably, the structural complexity of prostate with branched ducts may require more time to recover. Furthermore, differences in the velocity of secretion production and accumulation between the glands may also contribute for this distinct response.

D-024 HUMAN PROSTATIC SMOOTH MUSCLE CELLS EXPRESS DECORIN, BIGLYCAN AND TYPE VI COLLAGEN

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The prostatic stroma is composed by different cell types and a complex and regionalized extracellular matrix. We have previously shown that type VI collagen is a component of the prostatic extracellular matrix and that it forms part of the matrix microfibrils observed by transmission electron microscopy (Carvalho et al. Tissue Cell, 29: 163, 1997). In this work we have investigated whether sub confluent smooth muscle cells isolated from the human prostate can produce type VI collagen. We have also investigated the expression of the small proteoglycans decorin and biglycan. Immunocytochemistry and immunoblotting procedures using polyclonal antibodies were employed. We have observed that cultured human smooth muscle cells produces and secrete type VI collagen, decorin and biglycan, which were detected by immunoblotting of extracts of the cell layer. Immunocytochemistry have identified either of these extracellular matrix components around the cells. However, it was clear that these cells are not able to assemble an extracellular matrix and probably secrete these components to the culture medium. We are now engaged in investigating the ability of these cells to establish an extracellular matrix layer after confluence. The results suggests that the smooth muscle cells contribute to the formation of the prostatic extracellular matrix, at least with the production of type VI collagen and the small proteoglycans decorin and biglycan.
Wound healing is a complex process that involves several molecules. To investigate the effects of nitric oxide synthesis inhibition on cutaneous wound healing, L-NAME was administrated to rats, daily, for 21 days. In the first day a full-thickness excision wound was done. Control group had free access water. The lesion surface was measured in the day of lesion, 7, 14 and 21 days later. Blood pressure was measured in the beginning and at the end of experiment. Twenty-one days after lesion, the scar, with adjacent normal skin, was formalin-fixed and paraffin-embedded for histological analysis. At the end of experiment the blood pressure of L-NAME group was higher than that of control group. Lesion contraction of L-NAME group was slower than that of control group 7, 14 and 21 days after lesion. In control group granulation tissue a higher amount of "fibroblast-like" cells was observed in superficial and deep dermis. In control animals thick red-yellow collagen fibers were observed, resembling normal skin arrangement. In superficial dermis of L-NAME group greenish collagen fibers were observed parallel to surface and in deep dermis thick yellow collagen fibers were present. In deep dermis of L-NAME group a high amount of vessels was observed. Our results showed that the blockade of nitric oxide synthesis affects wound closure and granulation tissue organization on rats cutaneous wound repair.
D-029 MATRIX METALLOPROTEINASE-9 EXPRESSION IN RAT SKELETAL MUSCLE WITH MONOCROTALINE-INDUCED HEART FAILURE
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Heart failure (HF) is characterized by a reduced tolerance to exercise due to early fatigue and dyspnea; this may be, in part, due to skeletal muscle myopathy, with atrophy and shift from type 1 “slow” to type II “fast” fibers. The skeletal muscle atrophy suggests the participation of matrix metalloproteinases (MMPs), which cooperatively degrade all components of the extracellular matrix. The purpose of this investigation was to determine MMPs expression in skeletal muscle from rats with heart failure consequent to pulmonary arterial hypertension induced by monocrotaline administration (30 mg/Kg, i.p.). Wistar rats, male (30 days-old), were divided into two groups: control (C) and heart failure (HF), sacrificed 21 days after drug administration, when overt HF had developed. MMPs expression was study by gelatin zymography and Western Blot in three muscles with distinct contractile activities: soleus, longus extensor digital (EDL) and diaphragm. MMP-2 was expressed in the skeletal muscles from both groups, but MMP-9 was only expressed in HF animals and with higher levels in the diaphragm. Our data demonstrated that MMP-9 expression is related with skeletal muscle myopathy in rats with HF and its levels are different among muscles with distinct contractile activities.

D-030 ACTIN GENE EXPRESSION IN NILE TILAPIA (Oreochromis niloticus) TISSUES
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Actin is a highly conserved cytoskeletal protein of eukaryotes organisms and represents a key component of cells. Two actin classes can be found in higher eukaryotes: muscle actin (a) and cytoplasmic actin (b and g). Although, there is already a great amount of data on the evolution of actins in mammals, few studies have been conducted in lower vertebrates. At least six different isoforms of actins exist in mammals: two cytoplasmic actins and four muscle actins. In fishes, however, only a few representatives of the actin multigene family have been cloned and sequenced, and little is known about their expression and evolution. To address a better understanding of the expression of actin multigene family among fishes, we performed RT-PCR to detect a-actin gene expression in gill, liver, heart, ovarian, myotomal muscle and brain of Nile tilapia. The set of primers aActF 5’-ATG AGA CTA CCG CCC TTG TG, and aActR 5’- AAT CCA CAT CTG CTG GAA GG, designed from a-actin gene sequence of Fugu rubripes (GenBank accession: U38850), were used to amplify the products of the transcription reverse reaction of Nile tilapia total RNA. Expression products of a-actin genes were used to amplify the products of the transcription reverse reaction of Nile tilapia total RNA. Expression products of a-actin genes were amplified in cDNA from different stages of development and from different tissues. These results indicate a tissue-specific expression of a-actin genes in Nile tilapia. Support: FAPESP

D-031 HYALURONAN IN THE PUBIC SYMPHYSIS OF VIRGIN AND PREGNANT MOUSE
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Distribution of HA in the interpubic tissue of virgin and pregnant mice was studied using: cuprolinic blue associated with treatment with Streptomyces hyaluronidase enzyme and a binding-specific probe for HA. The HA distribution could be demonstrated ultrastructurally on the pericellular matrix and between the collagen fibrils. Digestion by hyaluronidase results in a pericellular halo and spaces between collagen fibrils. The thickness of the halo is much larger at late pregnancy, as well as the spaces between the collagen fibril when compared with virgin interpubic pericellular halo and spaces. Quantitative analyses showed a significant increase in HA at late pregnant period compared with virgin symphysis. These results suggest that HA may contribute to the increased length and pliability of ligament observed during mouse pregnancy.
Supported by CAPES

D-032 MMPs AND RECK EXPRESSION IN THE RAT VENTRAL PROSTATE
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Androgens regulate development and function of the prostate gland. Growth is dependent on pubertal increase in androgen and androgen deprivation leads to marked changes in the prostate. Matrix metalloproteinases are involved in these alterations. The prostatic regression after castration involves extensive changes in the stroma. In this work we have examined the variations in MMPs and RECK (reversion-inducing-cysteine-rich protein with Kazal motifs), an inhibitor of MMP-activity, by using zimography and semi-quantitative RT-PCR during post-natal growth and after castration. Protein and RNA extracts were obtained from the ventral prostate of control, castrated and testosterone-treated castrated rats. Gelatin zymography has shown a diffuse band of EDTA-sensitive proteinases in young individuals that resolved to MMP2 in 42d-old rats. MMP-7 appeared after 21 days, following epithelial maturation. Besides an increment in MMP-2 activity, castration also induced the expression of MMP-1 and MMP-9; this increase was modulated negatively with time after castration and by testosterone treatment. On the other hand, MMP7 activity was diminished by castration. RECK mRNA, on the other hand, was diminished after castration and re-induced with testosterone treatment. The results demonstrated that RECK is expressed in the rat ventral prostate and that the inverse correlation between the expression of MMP-1, -2 and -9 and RECK described in other systems also applies to the rat ventral prostate. Opposite correlation was seen with MMP-7.
D-033 POSTNATAL DEVELOPMENT OF THE CHICKEN TRACHEA
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The tracheal segment of the respiratory system initiates at the cricoid cartilage extending until the dichotomy of the pulmonary bronchus. In adult chickens it is mainly constituted of hyaline cartilage forming concentric and interlaced half-rings with different diameter, whereas in newborn chickens it is formed by several individual cartilage plates. This work analyzed the formation of the tracheal half-rings in chickens from the birth until the 30th developmental day (dd). Fragments of the tracheas were fixed in 4% paraformaldehyde, processed for paraffin embedding and the sections stained with hematoxylin-eosin for light microscopy observations. The tracheal half-rings were completely formed on dd 30, by the fusion of adjacent cartilaginous, small plates. From dd 0 to 30, rounded condrocytes formed isogenic groups inside the extracellular cartilaginous matrix, whereas in the periphery of each plate, the cartilaginous precursors cells, with a peculiar elliptical shape, were more common. During the 30 dd, the perichondrium around each and every plate was prominent and included cells in varied degrees of the differentiation process. Fellowship SAE-UNICAMP.

D-034 THE EFFECT OF REPETITIVE MUSCULAR ELECTRICAL STIMULATION ON THE JOINT
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The tracheal segment of the respiratory system initiates at the cricoid cartilage extending until the dichotomy of the pulmonary bronchus. In adult chickens it is mainly constituted of hyaline cartilage forming concentric and interlaced half-rings with different diameter, whereas in newborn chickens it is formed by several individual cartilage plates. This work analyzed the formation of the tracheal half-rings in chickens from the birth until the 30th developmental day (dd). Fragments of the tracheas were fixed in 4% paraformaldehyde, processed for paraffin embedding and the sections stained with hematoxylin-eosin for light microscopy observations. The tracheal half-rings were completely formed on dd 30, by the fusion of adjacent cartilaginous, small plates. From dd 0 to 30, rounded condrocytes formed isogenic groups inside the extracellular cartilaginous matrix, whereas in the periphery of each plate, the cartilaginous precursors cells, with a peculiar elliptical shape, were more common. During the 30 dd, the perichondrium around each and every plate was prominent and included cells in varied degrees of the differentiation process. Fellowship SAE-UNICAMP.

D-035 COLLAGEN BUNDLES AND PROTEOGLYCANs IN PIG MITRAL VALVE
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The mitral valve is bicuspid and each cusp is bound to the ventricle wall and papillary muscles by the tendineal cords. The objective of this work was to identify the sulfated glycosaminoglycans (GAGs) present in the leaflets and tendinea cords, and to know how is the organization of collagen and proteoglycans. The extracts of leaflets and tendinea cords were applied on a DEAE-sephacel column and fractions analyzed in SDS-PAGE. For morphological analysis whole valves were mounted on slides, stained with toluidine blue and observed in polarized light microscopy. The results of determinations of proteins and GAGs were respectively 14.3 ± 1.2 and 1.81 ± 0.30 mg/g of leaflet, and 32.8 ± 0.89 and 4.83 ± 0.58 mg/g of tendinea cord. Analysis of GAGs in agarose gel showed that chondroitin sulfate (CS) is prominent in the leaflet, and dermatan sulfate in the tendinea cord. A striking metachromasy observed in some regions of the whole mounting of the leaflet corroborates our biochemical findings for CS and indicates the existence of domains of CS-proteoglycan in the leaflet. Birefringence analysis showed that in the leaflet the collagen fibers exhibit multidirectional orientation, but in the cords they are highly orientated following the longest axis of the tendinea cord. This differentiated orientation of the collagen bundles is related to the different biomechanical properties of those two structures.

D-036 BIOCHEMICAL ANALYSIS OF ARTICULAR CARTILAGE FROM OSTRICH
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The articular cartilage has extracellular matrix in abundance, that is composed of collagen, non-collagenous glycoproteins, and proteoglycans. In this study the extracellular matrix composition of articular cartilage from ostrich, that in captivity conditions may undergo arthrosis at the tarsometatarso joints, was described. Articular cartilage from lateral (PL) and medial (PM) portions of proximal surfaces and the central (DC) portion from distal surface of the tarsometatarsus were used. The components of the several regions were extracted utilizing 4M guanidine chloride, fractionated in DEAE-sephacel and analyzed in SDS-PAGE. The glycosaminoglycans were analyzed in agarose-propilenediamine gel. The protein and sulfated glycosaminoglycans amount were respectively 6,63 ± 0,98 and 4,25 ± 0,87 (PL), 4,86 ± 0,82 and 5,47 ± 1,35 (PM), 9,1 ± 0,76 and 19,76 ± 0,80 (DC) in mg/g tissue. SDS-PAGE analysis showed the presence of the same proteins at the 3 regions, with Mr of 17, 21, 36, 47, 61, 121 kDa. Analysis of GAGs in agarose gel showed that chondroitin sulfate (CS) is prominent in the leaflet, and dermatan sulfate in the tendinea cord. A striking metachromasy observed in some regions of the whole mounting of the leaflet corroborates our biochemical findings for CS and indicates the existence of domains of CS-proteoglycan in the leaflet. Birefringence analysis showed that in the leaflet the collagen fibers exhibit multidirectional orientation, but in the cords they are highly orientated following the longest axis of the tendinea cord. This differentiated orientation of the collagen bundles is related to the different biomechanical properties of those two structures.

*Fellowship CAPES.
Control of blood vessel growth is an essential feature of tissue physiology. A reduction in blood supply and endothelial cell death is part of the early prostate response to androgen ablation. In this work we have attempted to isolated prostatic endothelial cells aiming to establish a proper model for the studies of cell-cell communication in the prostate gland. We have used immunomagnetic, immunoblotting and immunocytochemistry to isolate and characterize the prostatic endothelial cells. After collagenase and trypsin treatments, prostatic cells were isolated using immunomagnetic beads (Dynabeads) coupled with anti-CD31 antibody. The isolated cells were cultured in HAM-F12 containing L-glutamine. They grown as a monolayer and showed contact inhibition. To characterize these cells, we have checked their reactivity to anti-von Willebrand factor antibody and to Ulex europaeus agglutinin (UEA). More than 95% of the cells were reactive to von Willebrand factor, which appeared as granules in the cell cytoplasm. The UEA reaction was also positive in more than 95% of the cells, appearing as a uniform reaction at the cell surface. Furthermore, we also checked the expression of two VEGF receptors, flt-1 and flk-1, in immunoblotting. Both receptors were expressed by the isolated cells. The present protocol resulted in a highly homogeneous culture of prostatic endothelial cells.

The leaflets of the aortic valve are formed mainly by collagen and proteoglycans, which organize to maintain the valve functionality. In this work, using birefringence and images of basophilia, we present another view about the extracellular matrix organization in the porcine aortic valve. Whole mount and histological sections of the leaflets were fixed in paraformaldehyde, stained in toluidine blue pH 4.0 and observed in polarization microscopy. Analysis of the whole mounted leaflets showed the presence of fibers that emerged from the regions of insertion of the valve at the aorta wall, following the main axis of the leaflet. The birefringence bright of the collagen fibers and fibrils evidenced an highly ordered distribution, forming crimps. Intense metachromasy was observed along the collagen fibers and in many regions of the leaflet forming real domains. This indicates the presence of proteoglycans at these regions, possibly as a consequence of compression at these regions. Transversal sections analysis showed that two fibrous and one spongy layer form the valvar leaflet, and that at these layers the degree of aggregation of the collagen fibers changes from highly compacted, at the insertion at the aorta wall, to ramified fibers that intercrosses at many directions. Thus, the aspect of a basket was observed, allowing an adequate distribution of forces, compatible with the viscoelasticity and biomechanics of the leaflet.

Wound healing process increases metabolic and nutritional demands on patients. Undernourished patients not heal adequately, especially those protein depleted. The objective of this study was to verify how protein restriction affects wound contraction in male and female rats submitted to an excisional cutaneous lesion. Wistar rats (male and female) were divided into experimental groups. The groups 1 (male) and 2 (female) were fed with 23% protein level diet, and groups 3 (male) and 4 (female) with 0% protein level diet, during the experiment. After 8 weeks of diet, the animals were submitted to an excisional wound, and its surface was measured on the day of lesion and 14 days latter. The body mass was measured weekly during the experiment. After 8 weeks of diet, animals from groups 3 and 4 presented body mass loss (p<0,0001) that was higher in female group. The wound contraction in animals from groups 3 and 4 animals was slower than that of animals from groups 1 and 2 (p<0,0001), 14 days after lesion. Our results show that protein restriction affects wound healing process delaying wound contraction. Further studies are required to determine exactly how protein depletion affects this process and allow the proposition of adequate therapies to decrease the wound related complications caused by protein restriction.

Multidrug resistance (MDR) in tumor cells involves overexpression of ABC transport proteins, P-glycoprotein (Pgp) or MDR related protein (MRP). There is growing evidence that increased MRP expression may be a factor involved in brain tumors MDR phenotype. However, the expression of MDR proteins is rarely studied in brain metastasis. Lung tumors present high incidence of brain metastasis. We are studying the influence of neural microenvironment in MDR proteins expression. We developed an experimental model in vitro that mimetize brain metastasis using a human small cell lung cancer cell line (GLC4/ADR), chemotherapy resistant, which overexpresses MRP. We cultured GLC4 ADR cells during 4 days in vitro (DIV) at three distinct conditions: (1) control culture with GLC4/ADR cells in suspension, (2) culture with GLC4/ADR cells upon extracellular matrix (ECM) of rat cortical astrocytes and (3) GLC4/ADR cells cultured in astrocytes conditioned medium. After incubation, we analyzed MRP expression by flow cytometry. Our data show that control resistant GLC4 ADR cell line exhibited 2.0 media fluorescent intensity (MFI). In parallel, in the second condition, GLC4/ADR cells exhibited 2.3MFI and third situation the cells exhibited 3.2MFI. In conclusion our data suggest that ECM and soluble factors secreted by astrocytes may influence MDR expression and play a role in the chemotherapy resistance of brain metastasis.
There are important differences between healing and chronic wounds. In chronic wounds quiescent fibroblasts are observed and in healing wounds fibroblasts are extremely active. One of the latest important findings is the different expression of metalloproteinases (MMPs), capable to degrade the extra cellular matrix (ECM). During normal wound healing there is a balance between “construction” and “destruction” of ECM. In chronic wounds an imbalance is observed, with high levels of MMPs, specially MMP-2. Dermax® is a new device in the market that claims to decrease the production of MMP-2. Chronic wounds were biopsied in day 0, 2 and 6 weeks. We analyzed the morphological changes in comparison with fibrocytes MMP-2 expression during the healing process. Biopsies were performed in 4 chronic wounds during treatment with Dermax®. The samples were evaluated morphologically for light microscopy. The MMP-2 expression by fibroblasts was evaluated by immunocytochemistry using monoclonal MMP-2 antibodies. During the treatment the most striking immunohistochemical changes were the loss of the fibro-necrotic cap covering the wound bed, the re-activation of the fibroblasts in the granulation tissue and the sharp decline in the expression of fibroblast MMP-2. In this study the application of Dermax® induced an improvement of wound healing both clinically and histologically. Somehow Dermax® changed the environment of the wound and MMP-2 fibrocytes producers were substituted by fibroblasts ECM producers, leading to wound healing.

To approach the question of the functional role of stromal cells in the thyroid epithelial cells activity, we established and characterized a homogeneous stromal cell population of rat thyroid gland (TS7 cells). These fibroblastoid cells presented many short processes and expressed the cytoskeleton proteins α-smooth muscle actin and vimentin, and the extracellular matrix components laminin, fibronectin, type IV collagen, decorin, chondroitin sulfate and heparin sulfate. Co-culture assays were performed with FRTL5 thyroid epithelial cells and TS7 stromal cells substrate. In these conditions, these ECM components were disposed in a more organized way, concentrated around epithelial cells. These preliminary results showed that interactions between human gastric epithelial and stromal cells modify cell morphology and ECM distribution, and raise a model to study the effect of Hp or its toxin on gastric mucosa cells.

Support: Millennium Institute for Tissue Bioengineering, CNPq, FUJB, FAPERJ.
D-045 ISOLATION AND CHARACTERIZATION OF HUMAN PROSTATIC STROMAL CELLS

Introduction: The aim of this study was to establish a homogeneous prostate stromal cell population from primary culture of benign prostatic hyperplasic tissue samples, obtained from transurethral resection of the human prostate. After mechanic and enzymatic treatment, the cells, exhibiting different shapes, elongated or rounded, were cultivated in DMEM with 10% fetal bovine serum (FBS). Immunocytochemical methods using antibodies against cytoskeleton proteins: vimentin(VM) and α-smooth muscle actin (α-SMA); extracellular matrix (ECM) components: fibronectin(FN), laminin (LN), collagen IV (CIV), chondroitin sulfate (CS); the metalloproteinases 1 (MMP 1), 3 (MMP 3) and 7 (MMP 7) and their tissue inhibitors 1 (TIMP-1) and 2 (TIMP-2) were used to characterize and observe the morphology of the cells. These prostate stromal cells were defined as elongated fibroblastoid-like cells and muscle cells that expressed VM. α-SMA was observed in the majority of the cells. A strong cytoplasmatic immunoreactivity for FN, LN and CS was also observed, as compared to a weak and diffuse CIV distribution. MMP 1 and MMP 3 were not detected, while MMP 7, TIMP-1 and TIMP-2 were present in all cells. Our preliminary results, show that prostate stromal cells shares common characteristics with other glandular stromae and raise questions about the study of the effect of these cells on the epithelial cells behaviour.

Support: Millennium Institute for Tissue Bioengineering, CNPq, FUJB, FAPERJ.

D-046 EFFECTS OF LERCANIDIPINE ON PLASMA METALLOPROTEINASE (MMP)-2 AND MMP-9 ACTIVITY IN HYPERTENSIVE (H) AND HYPERTENSIVE DIABETIC (HD) PATIENTS

Introduction: Increased activity of MMPs has been described in many cardiovascular disorders and is of major significance in vascular remodelling. Inhibition of MMPs may be a pharmacologic target. We examined the effects of Lercanidipine on plasma MMP-2 and MMP-9 activities in H and HD patients. Methods: Lercanidipine 20 mg (or placebo) was given to H (n=7) and HD (n=7) patients for 30 days. Venous blood samples were drawn after 1ER (or placebo) treatment and gelatin zymography of MMP-2 and MMP-9 from plasma was performed. Samples were subjected to electrophoresis on 12% SDS-PAGE co-polymerized with gelatin (0.1%) as the substrate. Then gels were washed Triton X-100 and incubated (37°C, 16 h,) in Tris–CaCl2 buffer, and stained with Coomassie Brilliant Blue. Enzyme activity was assayed by densitometry. Results: Lercanidipine decreases MMP-9 and MMP-2 activity in plasma from HD patients by 536%. Lower increases (77%) in lung MMP-2 were observed when L-ARG 0.5 mM was added to the lung perfusate solution 5 min before induction of APE. Gelatin zymography of MMP-2 and MMP-9 from lung and plasma samples were performed. Samples were subjected to electrophoresis on 12% SDS-PAGE co-polymerized with gelatin (0.1%) as the substrate. Then gels were washed Triton X-100 and incubated at 37°C for 16 h in Tris–CaCl2 buffer, and stained with Coomassie Brilliant Blue. Enzyme activity was assayed by densitometry. Results: APE did not affect MMP-2 and MMP-9 plasma activities, and MMP-9 lung activity. However, APE increased lung MMP-2 activity by 536%. Lower increases (77%) in lung MMP-2 were observed when L-ARG 0.5 mM was added to the lung perfusate. Discussion: Our results suggest that L-ARG attenuates the increase in MMP-2 activity caused by APE and may help to explain the beneficial effects of L-ARG.

Financial support: FAPESP-CAPES-CNpq

D-047 EFFECTS OF L-ARGININE (L-ARG) ON ACUTE PULMONARY EMBOLISM (APE)-INDUCED INCREASES IN LUNG METALLOPROTEINASE-2 ACTIVITY

Introduction: Matrix metalloproteinases (MMPs) are proteinases involved in the degradation of extracellular matrix. MMP-2 regulates the vascular reactivity by cleaving big endothelin-1, a pulmonary vasoconstrictor released during APE. We examined whether MMP-2 and MMP-9 are activated during APE and whether L-ARG, a precursor of nitric oxide, affects MMPs activation. Methods: APE was induced in isolated Wistar rat lung perfusions by injecting 6.6 mg/kg of Sephadex microspheres into the pulmonary artery. L-ARG 0.5 mM (or saline) was added to the lung perfusate solution 5 min before induction of APE. Gelatin zymography of MMP-2 and MMP-9 from lung and plasma samples were performed. Samples were subjected to electrophoresis on 12% SDS-PAGE co-polymerized with gelatin (0.1%) as the substrate. Then gels were washed Triton X-100 and incubated at 37°C for 16 h in Tris–CaCl2 buffer, and stained with Coomassie Brilliant Blue. Enzyme activity was assayed by densitometry. Results: APE did not affect MMP-2 and MMP-9 plasma activities, and MMP-9 lung activity. However, APE increased lung MMP-2 activity by 536%. Lower increases (77%) in lung MMP-2 were observed when L-ARG 0.5 mM was added to the lung perfusate solution 5 min before induction of APE. Gelatin zymography of MMP-2 and MMP-9 from lung and plasma samples were performed. Samples were subjected to electrophoresis on 12% SDS-PAGE co-polymerized with gelatin (0.1%) as the substrate. Then gels were washed Triton X-100 and incubated at 37°C for 16 h in Tris–CaCl2 buffer, and stained with Coomassie Brilliant Blue. Enzyme activity was assayed by densitometry. Results: APE increased lung MMP-2 activity by 536%. Lower increases (77%) in lung MMP-2 were observed when L-ARG 0.5 mM was added to the lung perfusate solution 5 min before induction of APE. Gelatin zymography of MMP-2 and MMP-9 from lung and plasma samples were performed. Samples were subjected to electrophoresis on 12% SDS-PAGE co-polymerized with gelatin (0.1%) as the substrate. Then gels were washed Triton X-100 and incubated at 37°C for 16 h in Tris–CaCl2 buffer, and stained with Coomassie Brilliant Blue. Enzyme activity was assayed by densitometry. Results: APE decreased active MMP-2 and MMP-9 from lung perfusate. Discussion: Our results show that the non-specific MMPs inhibitor doxycycline attenuates APE-induced hypotension in rats. This finding suggests that MMPs have a role in the hemodynamic changes caused by APE.

Financial support: FAPESP-CAPES-CNpq

D-048 INHIBITION OF METALLOPROTEINASES (MMPs) WITH DOXYCYCLINE ATTENUATES ACUTE PULMONARY EMBOLISM (APE)-INDUCED SYSTEMIC HYPOTENSION

Introduction: MMPs may affect vascular reactivity. In this study we examined whether MMPs inhibition with doxycycline attenuates MMP activation and the hemodynamic responses to APE. Methods: Anesthetized Wistar rats had their carotid artery and femoral vein cannulated for the measurement of mean arterial blood pressure (MAP) and drug administration, respectively. The experiments were initiated after 20 min of stabilization. Doxycycline (40 mg/kg) or saline was injected intravenously 10 minutes before APE was induced by injecting 9 mg/kg of Sephadex microspheres (or saline) intravenously. Gelatin zymography of MMP-2 and MMP-9 from lung and plasma samples were performed. Samples were subjected to electrophoresis on 12% SDS-PAGE co-polymerized with gelatin (0.1%) as the substrate. Gels were washed Triton X-100 and incubated (37°C, 16 h,) in Tris–CaCl2 buffer, and stained with Coomassie Brilliant Blue. Enzyme activity was assayed by densitometry. Results: MAP decreased by 34 ± 3 mmHg 10 minutes after APE induction in rats pre-treated with saline. With doxycycline, significantly lower decreases in MAP were observed (8 ± 3 mmHg; P<0.05). MMPs were not activated in plasma. Lung samples are now being studied. Discussion: Our results show that the non-specific MMPs inhibitor doxycycline attenuates APE-induced hypotension in rats. This finding suggests that MMPs have a role in the hemodynamic changes caused by APE.

Financial support: FAPESP-CAPES-CNpq
D-049 ATORVASTATIN ATTENUATES ACUTE PULMONARY EMBOLISM (APE)-INDUCED INCREASES IN LUNG METALLOPROTEINASE-9 ACTIVITY

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Introduction: Matrix metalloproteinases (MMPs) may be activated during APE. Atorvastatin has pleiotropic effects that may lead to downregulation of MMPs. We examined whether MMP-2 and MMP-9 are activated during APE and whether atorvastatin affects MMPs activation. Methods: Wistar rats received water or atorvastatin 30mg/kg/day p.o. for 14 days. APE was induced by injecting 9 mg/kg of microspheres (or saline) into the caudal vein. One day after APE, rats were killed by decapitation and gelatin zymography of MMP-2 and MMP-9 from lung and plasma were performed. Samples were subjected to electrophoresis on 12% SDS-PAGE co-polymerized with gelatin (0.1%). Gels were washed Triton X-100 and incubated to electrophoresis on 12% SDS-PAGE co-polymerized with gelatin (0.1%). Gels were washed Triton X-100 and incubated at 37°C for 16 h in Tris-CaCl₂ buffer, and stained with Coomassie Brilliant Blue. Enzyme activity was assayed by densitometry. Results: APE increased active MMP-9 in plasma by about 100%. Moreover, APE increased lung active MMP-9 by 200% and pro-MMP-9 by 480%. Pre-treatment with atorvastatin attenuated (P<0.05) the increases of MMP-9 in the lungs (active MMP-9 increased by only 100% and pro-MMP-9 by 320%). No significant changes were observed in plasma and lungs (active MMP-9 increased by only 100% and pro-MMP-9 by 480%). Pre-treatment with atorvastatin increased the 24-hour survival rate from 35% to 52% (P<0.05).

Discussion: Our results show that atorvastatin attenuated the increases in lung active MMP-9 and significantly reduce APE-mortality rate.

Support: FAPESP-CAPES-CNPq

D-051 NANTOTEXURING OF TITANIUM SURFACES INDUCES CHANGES IN MMP-2 LEVELS IN A RAT CALVARIA OSTEOGENIC CELL CULTURE MODEL

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Matrix metalloproteinases, MMPs, are proteases from extracellular matrix. These enzymes were shown in osteogenic cell cultures. The aim of this study was to verify the presence and possible variations in the MMP-2 profile in calvaria-derived osteogenic cell cultures, grown on polystyrene (PS), titanium (control cpTi), and nanotextured cpTi (nano-cpTi). At 2, 5, 7, 9, 12, and 14 days, 1 mL conditioned media was collected from each well (n=2). Gelatin zymography results show considerable variations in the MMP-2 band pattern. The highest level of MMP-2 was observed at day 12, with the onset of matrix mineralization. Another important finding was the marked difference seen when samples from control cpTi were compared to samples from nano-cpTi and PS. Samples from these 2 groups demonstrated a similar pattern of MMP-2, which differed from the one exhibited by control cpTi samples. Particularly after day 7, the quantity of active MMP-2 was much higher in control cpTi samples. Polystyrene and nano-cpTi surfaces appear to elicit similar MMP-2 expression, as can be observed from band profile and quantities of active and inactive MMP-2 forms. Strikingly, both surfaces support increased amounts of bone-like nodule formation after 14 days.

Thus, a possible link might exist between titanium nanotopography, MMP-2 quantity, and /in vitro/ osteogenesis.

D-050 COMPARISON OF THREE DIFFERENT METHODS FOR ENAMEL PROTEINS EXTRACTION IN DIFFERENT DEVELOPMENT STAGES.

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Dental enamel has one characteristic: the more mature it is, the smaller is its protein content, making protein analysis in different development stages a difficult task. The aim of this study was to analyze the lipid deposits in spores and hyphae of Lepista sordida. Fresh mushrooms were collected during January, 2004. Its gills and spore prints were removed for the experiments and the mycelium developed over Potato-Dextrose-Agar culture medium. All these biological materials were stained by Red Nile for lipid detection and analyzed through Radiance 2001 Confocal Microscope. The results showed basidiospores from the gills organized in groups of four, a common feature among Basidiomycetes. These spores were fulfilled with a big lipid body, which occupies most of its cellular compartment. Spores derived from spore prints, nearly twenty days old, showed mostly this same characteristic. However there were also spores with less lipid content. Supposedly this energetic reserve is being used during aging, a phenomenon already observed in other fungi species. Smaller lipid bodies were observed inside young hypha, whereas less quantity was observed in older ones, suggesting lipid relocation along the mycelium. These results indicate that lipid bodies play important roles during the different steps of L. sordida life cycle.

Supported by CNPq and Paraná Tecnologia.

E-001 CYTOCHEMICAL IDENTIFICATION OF LIPID RESERVES IN Lepista sordida BASIDIOSPORES AND HYPHA THROUGH CONFOCAL MICROSCOPY

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Lipid bodies are stored inside spores of many fungi, being dynamic structures that play an important role in the cellular metabolism. One of its functions is to supply the energy required during aging, when external nutrition is deficient. The aim of this study was to analyze the lipid deposits in spores and hypha of Lepista sordida. Fresh mushrooms were collected during January, 2004. Its gills and spore prints were removed for the experiments and the mycelium developed over Potato-Dextrose-Agar culture medium. All these biological materials were stained by Red Nile for lipid detection and analyzed through Radiance 2001 Confocal Microscope. The results showed basidiospores from the gills organized in groups of four, a common feature among Basidiomycetes. These spores were fulfilled with a big lipid body, which occupies most of its cellular compartment. Spores derived from spore prints, nearly twenty days old, showed mostly this same characteristic. However there were also spores with less lipid content. Supposedly this energetic reserve is being used during aging, a phenomenon already observed in other fungi species. Smaller lipid bodies were observed inside young hypha, whereas less quantity was observed in older ones, suggesting lipid relocation along the mycelium. These results indicate that lipid bodies play important roles during the different steps of L. sordida life cycle.

Supported by CNPq and Paraná Tecnologia.
E-003 ULTRA STRUCTURE OF Croton macrobothrys Baill. (EUPHORBIACEAE) LEAVES
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Croton L. (Euphorbiaceae) species, known in folk medicine as dragon’s-blood has been used in human health because of its antibacterial and anti-inflammatory properties. It has been shown that diterpenes, alkaloids and phenolics compounds are the main secondary metabolites biosynthesized by Croton species. In this work the ultrastructure and the localization of phenolic compounds at tissue, cell and subcellular compartments of Croton macrobothrys leaves was carried out. The leaves were hypostomatic and covered by stellate, fasciculate dentritic and lepidote trichomes, which developed from epidermic and hypodermic cells. The leaf blades were dorsiventral. In the young leaves, the cytoplasm of palisade cells was dense, with enlarged nucleus, numerous mitochondria and little plastids evidencing the thylakoids and granum slightly developed and starch grains, which may occupy a large portion of the plastids. The vacuole was reduced, presenting granular deposits corresponding to the early stage of phenolic accumulation. In mature leaves the mesophyll showed 2-3 layers of palisade tissue and 3-6 layers of spongy parenchyma. In this region, it was observed sclereids connecting the trichomes localized on both leaf surfaces. The palisade cells showed dense cytoplasm and chloroplasts with thylakoids, granum, starch grains which occupied a large portion and numerous plastoglobules. The vacuole of these cells accumulated electrondense substances that correspond to phenolic compounds.

E-004 CYTOCHEMICAL STUDY OF CARPOSPORE GERMINATION IN NEMALION HELMINTHOIDES (NEMALIALES, RHODOPHYTA).
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The spores of the red alga are released without a cell wall and embedded in a mucilage sheath. Following attachment a thin cell wall is produced after which the germination can be observed. The formation of the cell wall, mucilage composition and subcellular structure were studied with calcofluor, toluidine blue (AT-O), alcian blue (AB), periodic acid Schiff (PAS) and Coomassie brilliant blue (CBB). The mucilage sheath present in non-germinated carpospores exhibited a positive reaction to PAS and AB, and was metachromatic to AT-O, indicating the mixture of acidic and neutral polysaccharides that forms this region, which was negative to CBB. In this stage, the cytoplasm was positive to PAS showing large quantities of starch grains, but was negative to AB. The cytoplasm reacted positively to CBB, mainly in the region of the chloroplast. Germination proceeds with the production of a germ tube to which most of the cytoplasmic content of the spore migrates. During the early stage of germination the spore content of the germ tube was orthochromatic to AT-O and reacted slightly to PAS. The acidic polysaccharides were present only on the germling surface as shown by a positive reaction with AB and a metachromatic reaction with AT-O. Cellulose deposition was demonstrated with calcofluor by the fluorescence around the spore body and germ tube.

E-005 EFFECTS OF CYTOSKELETON CONTROLLING DRUGS AND CALCIUM ON SPORE GERMINATION IN THE RED ALGA GELIDIUM FLORIDANUM (RHODOPHYTA).
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Spore germination is a crucial step in the process of seaweed development. The germination events in Gelidiales, beginning with spore attachment and culminating in germ tube formation and rhizoid differentiation, are prerequisites for substrate colonization. Here we discuss the role of Ca 2+ and cytoskeleton on the polarization and germination of recently released tetraspores of G. floridanum. Cytochalasin-B was dissolved in DMSO to 5 mM. This stock solution was diluted in seawater to get 2, 10 and 40 µM concentrations. Colchicine was dissolved in seawater in concentrations from 1 mM to 100 mM. EGTA in distilled water at 50 mM was diluted in seawater to 0.1 mM to 5mM. The spores treated with cytochalasin-B showed a low percent of germination at 2 µM, but although remaining alive did not germinate on concentrations of 10 µM or higher. Spores treated with 50 to 100 mM of colchicine became vacuolated and did not germinate, while at lower concentrations spores exhibited a germination rate slower than the controls. The differentiation of the germ tube was inhibited due to non-polymerization of microtubules and microfilaments. Calcium chelated by EGTA at concentrations of 3 mM seriously impaired germination indicating that Ca 2+ has a key role in spore germination in red algae.
The essential amino acids lysine, threonine, isoleucine and methionine are synthesized by the aspartate pathway. Lysine and threonine are present in very low concentration in cereal seeds. Aspartate kinase (AK) and homoserine dehydrogenase (HSDH) control the regulation of lysine and threonine biosynthesis, respectively, whereas lysine 2-oxoglutarate reductase (LOR) and succinoglycine dehydrogenase (SDH), have been shown to play a key role in the lysine catabolism. We have studied the biochemical characteristics of AK, HSDH, LOR and SDH from several cereal crops, such as maize, coix, rice and sorghum. Chromatographic techniques, such as anion exchange and gel filtration have been shown to be very efficient to isolate the isoforms for these enzymes. The activity of these enzymes have also been studied in maize endosperm mutants and shown to vary considerably. Although the opaque-2 maize mutant and other high-lysine mutants exhibited a drastic reduction in LOR and SDH activities, however in other mutants the reduction in lysine catabolism could not explain the high concentration of lysine.

We are grateful for the financial support from FAPESP and The British Council.

Seed storage proteins serve as the major food reserve for germinating seedlings and their polypeptides determine the nutritional quality of the grain for human and livestock consumption. Cereal proteins have poor nutritional value due to the reduced content of lysine and threonine. The seed storage protein major fractions include albumin, globulin, prolamin and glutelin and the concentration of these have been compared by SDS-PAGE profiles for different genotypes of maize, rice and sorghum. A higher amount of albumin was observed in maize o2 (11.93%) and fl2 (12.88%) when compared with Oh43+ (6.24%). A similar behavior was observed in sorghum for IS11758 (31.42%) and IS16199 (35.30%) when compared with MASSA03+ (15.61%). In rice, glutelin was the principal fraction representing about 70%, but no differences in quantity between of genotypes were observed. SDS-PAGE revealed protein bands between the molecular weights of 10 to 100 kDa. Difference in the profile and intensity of the bands were observed between genotypes, suggesting that protein fractions could be responsible for higher lysine amount in cereal seeds. Through these analysis, it may be possible to obtain a better understanding of the expression of storage proteins in cereals. Financial FAPESP/CNPq/British Council.

Cadmium (Cd) is a toxic element that in areas that have been subjected to application of sewage sludge the concentration can be high. Cd can increase the production of reactive oxygen species (ROS). Cd may be detoxified in plants by phytochelatins and the effects of ROS minimized by antioxidant enzymes. In this work we report the effects of these antioxidant enzymes in radish, sugarcane, Crotalaria and soybean plants; sugarcane callus and in vitro cell cultures of coffee and tobacco. All plant species exhibited a growth inhibition due to Cd treatments. CAT and SOD activities varied among plant species and tissues, however, GR activity increased in all plants and tissue tested. The results suggest that GR is stimulated by Cd in roots, to produce reduced glutathione, which can be used in the synthesis of phytochelatins or possibly in response to the generation of ROS induced by Cd. In addition, GR, GST, CAT and GPX activities in tobacco cells suggest a differential and temporal response for ROS scavenging.

Financial support FAPESP, CNPq and British Council.

Financial support by FAPESP.
E-010 LIPID BODIES, NUCLEI AND VACUOLES CORRELATION IN Lepista sordida BASIDIOSPORES THROUGH CONFOCAL MICROSCOPY

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The majority of Basidiomycetes produce four haploid basidiospores, which are released from a single cell called basidium. Inside these spores, as well as in other fungi structures, the vacuoles play an essential role for the cellular metabolism. They maintain different functions, such as lisosomal, storage and homeostasis control. The aim of this study was to analyze the correlation among lipid bodies (stained by Red Nile), nuclei and vacuoles (stained by Acridine Orange), from basidiospores of Lepista sordida. The spore prints obtained from fresh mushrooms and after twenty days were analyzed through Radiance 2001 Confocal Microscope. The results show, in most of the spores, a big lipid body occupying a large volume of the spore cytoplasm. Basidiospores nuclei showed irregular morphology. They were flattened against the cellular wall when the lipid body presented its maximum size, and were more spherical in spores with less lipid body content. Our evidences indicate that the lipid body determines the nuclei morphology and its cellular localization by mechanical compression. The results showed an inverse correlation between vacuoles and lipid bodies volumes. Cells with less lipid body content presented more acid vacuoles, as well as bigger ones. Altogether, these results suggest that the cellular metabolism and internal organization are modified in a dependent manner with lipid consumption. Supported by CNPq and Paraná Tecnologia.

E-011 MORPHOQUANTITATIVE STUDY ON THE KIDNEYS OF MICE (Mus musculus) UNDER TREATMENT WITH AQUEOUS EXTRACT OF Dioclea grandiflora

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Medicinal plants have been used extensively by brazilian people to treat several diseases; however the toxicological effects on tissular and cell structure do not have been investigated. The aim of this study was to analyze and to quantify the morphological alterations in mouse kidney. 16 Mus musculus mice were used (8 each group). In the control (C) group saline was injected intraperitoneally (IP) and in the experimental (EX) group 62mg/ml of stem peel aqueous extract of Dioclea grandiflora was IP administrated. Under deep anesthesia by ether inhalation, both groups animals were killed, then the kidney were dissected out and immersed in Bouin’s fixative solution by 24h. After this period the kidneys were sliced in several pieces and randomly processed for histological routine. 4µm thick sections were stained by HE and PAS+ Hematoxylin. To morphometrical analysis a computadorized semiautomatic system Image-Lab (Softium) was used. In the (EX) group microscopic alterations were observed: glomerular congestion; decrease of the sub-capsular space; epithelial tubular damage and lymphocytes infiltration. These results show that the histological and morphoquantitative alterations are current of changes in the magnitude of the toxic action of the plant on the renal structure, demonstrating the importance of studies involving medicinal plants and their possible toxic effects.

E-012 CELLULAR CHANGES INDUCED BY CADMIUM ON RADISH AND MAIZE SEEDLINGS.

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The objective of this study was to examine cadmium (Cd) effects on growth of radish, turnip and maize seedlings, showing structural and ultrastructural modifications. Raphanus sativus L. (cv. redondo vermelho and comprido branco), Brassica napus L. (cv. marteau and nancy) and Zea mays L. (IAC8333) seedlings were grown with different Cd concentrations during 3 and 6 days in controled conditions. The materials were fixed in Karnovsky solution, embedded in JB-4 resin and thick sections (1 to 5 m) were stained for light microscopy. For electron microscopy samples were embedded in Spurr resin and cut with 50 nm thickness. The results showed a strong deleterious effect on growth in the higher concentrations of Cd, mainly for R. sativus cv redondo vermelho. Reserve mobilization was inhibited, the leaf epidermal cells presented several modifications and the mesophyll cells showed palisade damages. Nuclei and nucleoli structures changed in size, with a more irregular shape in treated cells and the nuclei became more compacted in relation to the control cells. Ultrastructure revealed chloroplast alterations, with thylacoid system disorganization. The major effects were observed for concentrations higher than 300 M in R. sativus and B. napus, and higher than 5 mM in Z. mays, showing that these plants reacted in different ways to absorb cadmium. (CNPq/SAE-Unicamp)

E-013 INFLUENCE OF PH AND TEMPERATURE ON THE PRODUCTION OF EXTRACELLULAR AMYLASE BY Pleurotus pulmonarius AND Colletotrichum lindeimuthianum

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In the extraction process of cassava starch for each ton of processed root, 250Kg of the starch is produced and about 140 Kg of the starch is wasted in the cassava fibrous residue. The purpose of this study was to investigate the production of amylases (1,4-α-glucanohydrolase) by two fungi (Pleurotus pulmonarius and Colletotrichum lindeimuthianum) using this residue. The enzymatic production was carried out in liquid media with 1% of moist cassava fibrous residue as carbon source and inducer of amylase synthesis, and the fungi were grown on Klausen liquid culture medium at 50ºC, showing structural and ultrastructural modifications. The major effects were observed for concentrations higher than 300 M in Pleurotus pulmonarius and Colletotrichum lindeimuthianum) using this residue. The enzymatic production was carried out in liquid media with 1% of moist cassava fibrous residue as carbon source and inducer of amylase synthesis, and the fungi were incubated at 25ºC, 35ºC and 50ºC for 7 days under static conditions. The amylolytic activity were determined by hydrolysis of starch and the reducing sugar were then quantified by 3,5 dinitrosalicylic acid. Czapeck, Khouvine and Klausen media were tested. The highest production of amylase from Pleurotus pulmonarius (1.57 U/ml) was obtained when this fungi were growth on Klausen liquid culture medium at 50ºC, and Colletotrichum lindeimuthianum showed high amylolytic activity (2.07 U/ml) on Khouvine liquid culture medium at 50ºC. The properties of crude amylases were studied, and displayed the optimum pH was at 6,5 and the optima temperature ranges of 45 to 50ºC. Supported by: UNIAMÉRICA and UNIOESTE.
E-014 PROTOPLASTS: ISOLATION, CULTURE AND PLANT REGENERATION IN SPECIES OF THE GENUS Passiflora.
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The isolation, culture and plant regeneration has been studied in five species of passion fruit: Passiflora edulis, P. alata, P. cincinnata, P. nidita and P. setacea. Cotyledons were excised from seedlings germinated in vitro. The cell wall was removed with a mixture of enzymes 0.4% Macerozyme and 2% Cellulase. Protoplasts were cultured in K&P medium and K8 cells medium. The osmoticum of the culture medium was progressively lowered, by replacing the medium each 3 or 4 days. After 28 days of culture, the microcalli formed, were transferred to MD medium, and then to MS medium with 6-BA, under light, aiming plants regeneration. The average result observed in protoplast number isolated by gram in fresh material, were: 1.1 x 10^7 ± 0.71 x 10^7, 1.5 x 10^7 ± 0.52 x 10^7 edulis; 1.5 x 10^8 ± 0.52 x 10^8 alata; 1.5 x 10^8 cincinnata; 3.6 x 10^8 nidita and 9.2 x 10^9 ± 1.6 x 10^9 setacea. The plating efficiency or the division frequency observed were: edulis 45.8%, alata 36. 8 % and nidita 24.5 %. Until now cincinnata was the only specie that showed regeneration, in MS medium supplemented with 0.5 or 1.0 mg. L^-1 6 -BA.

EE-001 NARCOSIS EFFECT ON THE GERM CELLS OF THE QUEEN OVARIOLES IN Apis mellifera LINNÉ, 1758. (HYMENOPTERA, APIIDAE).
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Honeybee queens treated with CO2 start ovoposition earlier than non-treated queens, besides present vitellogenin titles in the hemolymph increased. Nevertheless, the development and possible morphological changes of the queens ovaries on narcosis have been not investigated. To understand the mechanisms that influence the queen reproduction behavior and physiology on narcosis, ovarioles of 3, 10, 15 and 18 days old narcotized virgin queens of A. mellifera were analyzed under routine light and fluorescence microscopy. The results showed that the CO2 treatment enhanced and even stimulated the virgin queen ovary development, taking the germ cells to the differentiation and follicle formation in the ovarioles, a situation only seen in mated queens. The ovary viability of the virgin queens was also maintained even after the mating period, when ovaries of non-treated virgin queens present signs of tissue degeneration. The morfometrical analyses showed that the number of picnotic nuclei has not relationship with the oocyte damage, once the number of oocyte per ovariole is not significantly different among 4 to 18 days old queens. Probably the deing cells of the ovarioles are those that will be differentiate in nurse cells, which feed the oocytes and modulate their development. The present results corroborate the behavior and physiological data of the queens on narcosis and generate new questions for investigation.

Financial support: FAPESP

EE-003 FUNCTIONAL AND ULTRASTRUCTURAL CHARACTERIZATION OF BLOOD AND PERITONEAL EXUDATE GRANULOCYTES FROM THE LIZARD AMEIVA AMEIVA
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The fine structure of blood and peritoneal exudate leukocytes is presented aiming the granulocytes identification of Ameiva ameiva, a lizard largely distributed in the Americas. Blood leukocytes were separated through a Percoll cushion and peritoneal exudate cells were obtained after 24 h stimulation with thioglycollate injection into the peritoneal cavity of the lizards. The blood presented erythrocytes, monocytes, thrombocytes, lymphocytes, plasma cells and 4 types of granulocytes. Type I and III granulocytes had round granules and the same basic morphology. However, type III had a bilobulated nucleus and condensed chromatin suggesting an advance maturation stage. Type II granulocyte showed fusiform granules and type IV granulocyte was classified as the mammalian basophil. Macrophages and granulocytes were found in the normal peritoneal cavity. Nevertheless, the inflamed peritoneum presented high number of granulocytes. The peritoneal granulocytes were related to type III blood granulocyte, based on the morphology, cytochemical localization of alkaline phosphatase and basic proteins. Taken together, these results indicate that type I and III granulocytes correspond to the mammalian neutrophils and type II to the eosinophil granulocytes. Supported by CNPq, CAPES, FAPERJ, PROCAD.

EE-004 HISTOLOGICAL FEATURES OF Mytella sp. GILL FILAMENTS (BIVALVIA: MYTILIDAE).
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Bivalvia have been extensively used to monitor aquatic habitats. Due to their sedimentary filter feeding habit they are able to accumulate elements from the environment. Mytella genus is of special interest because it gives informations about the conditions of the substrate since it is found buried in estuary system. Here we present the histological features of Mytella sp gill filaments which can be used in further studies of pathomorphology. Gill of five individuals were dissected, fixed in 4% paraformaldehyde, dehydrated in ethanol and included in JB4 resin; slices of 4μm were made and stained with haematoxilin-eosin. The gills are formed by two V-shaped demibranchs, each one containing the outer and the inner lamella of the gill filament. The gill filament present a branchial vessel covered outside by squamous lateral cells and inside by endothelial cell with flattened nucleus. The abfrontal region present cubic cells with round nucleus and no ciliation. The frontal region presents three different types of ciliation: frontal cilia, short and inserted in columnar cells forming a row; cu-latero-frontal cilia, longer also inserted in columnar cell, forming tow rows in each side of Doral cilia; lateral cilia, the longest cilia are located lateraly inserted in columnar cells. Cells with brush border and no ciliation are found between eu-latero-frontal cilia and lateral cilia. Ciliary disks formed by columnar cells are found conecting the filaments.

Honeybee queens treated with CO2 start ovoposition earlier than non-treated queens, besides present vitellogenin titles in the hemolymph increased. Nevertheless, the development and possible morphological changes of the queens ovaries on narcosis have been not investigated. To understand the mechanisms that influence the queen reproduction behavior and physiology on narcosis, ovarioles of 3, 10, 15 and 18 days old narcotized virgin queens of A. mellifera were analyzed under routine light and fluorescence microscopy. The results showed that the CO2 treatment enhanced and even stimulated the virgin queen ovary development, taking the germ cells to the differentiation and follicle formation in the ovarioles, a situation only seen in mated queens. The ovary viability of the virgin queens was also maintained even after the mating period, when ovaries of non-treated virgin queens present signs of tissue degeneration. The morfometrical analyses showed that the number of picnotic nuclei has not relationship with the oocyte damage, once the number of oocyte per ovariole is not significantly different among 4 to 18 days old queens. Probably the deing cells of the ovarioles are those that will be differentiate in nurse cells, which feed the oocytes and modulate their development. The present results corroborate the behavior and physiological data of the queens on narcosis and generate new questions for investigation.

Financial support: FAPESP
EE-005 HISTOCHEMISTRY AND ULTRASTRUCTURE OF THE MUSCLE TISSUE IN PACU (PIARACTUS MESOPOTAMICUS) DURING THE INITIAL GROWTH PHASES AFTER DIFFERENT INCUBATION TEMPERATURE.

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Histological and ultrastructural methods were used to evaluate muscle tissue in pacu after different incubation temperature, during the initial growth phase. Eggs were incubated at 25°C, 27°C and 29°C until hatching and then they were transferred to 500 L tanks (25°C - 28°C). Specimens were sacrificed from hatching at 60 days by MS-222 anesthesia. Muscles were frozen in liquid nitrogen and transverse sections were submitted to NADH-TR and mATPase. Fragments were fixed in 2.5 % glutaraldehyde, for ultrastructural analysis. At hatching, we observed a superficial monolayer of undifferentiated columnar muscle fibers, with few myofilibrils and many ribosomes, surrounding several round muscle fibers. From 5 days, we observed two distinct compartments: red, in a superficial region with fibers presenting oxidative metabolism and slow contraction and white fibers with glycolytic metabolism and fast contraction. Small fibers, with few myofilibrils, close to well differentiated fibers were observed. Undifferentiated cells with little cytoplasm were frequent in the connective tissue. In all temperatures, there was increase in muscle mass with intermediate fibers and proliferating zone. This increase occurred by hypertrophy and hyperplasia in red, intermediate and white muscle layer, mainly in this proliferating zone.

EE-006 HEK293T CELL LINE AS A MODEL TO EVALUATE CELLULAR ALTERATIONS CAUSED BY HUMAN PRION PROTEIN POLYMORPHISMS

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Cellular prion (PrP) is a cell surface protein encoded by a single-copy gene, PRNP, for which some genetic variants have been described. Some polymorphic sites at the human PrP molecule since the presence of 4 or 5 octarepeats and a rare polymorphism at codon 171 were related to protection against oxidative stress or associated to epileptic syndromes, respectively. Therefore, it is important to generate tools that will allow comparison between PrP polymorphisms and protein activity. We have previously generated expression vectors containing PrP fused to the Green fluorescent Protein (GFP) in order to transfect cell lines and evaluate different aspects of the PrP biology. HEK-293T is a human embryonic kidney cell line which is easily transfected by inexpensive methods and will be an important tool for our study. Herein, we evaluate HEK-293T in order to uncover the polymorphisms presented in the endogenous PRPN. We extracted DNA from these cells, performed a PCR and evaluated PRNP open reading frame by Denaturing High Performance Liquid Chromatography (DHPLC), restriction endonucleases and direct sequencing. We found no mutations or polymorphisms in HEK-293T endogenous PRNP what indicates that this cell line is an adequate tool to transfect PrP molecules with different polymorphisms and establish how they can alter the cellular physiology.

EE-007 FOOD RESTRICTION INDUCES MORPHOLOGICAL CHANGES IN THE CARDIAC MUSCLE IN MIDDLE-AGED RATS

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The effects of protein-calorie undernutrition (PCU) on the cardiac muscle morphology are incompletely defined. Recent research has shown that PCU induced focal alterations in the left ventricular morphology in young rats. The purpose of this experiment was to analyze the influence of chronic PCU on the cardiac muscle morphology in middle-aged rats. Male Wistar-Kyoto rats (12 months-aged) were divided in two groups: control (C, n=3) and food restriction (FR, n=3). The FR received 50% of amount of food consumed by the C. FR was maintained on this dietary regimen for 90 days and then all animals were killed for the ultra-structural analysis. The papillary muscle of C showed normal morphological aspects. Food restriction caused alterations in the ultrastructure of the papillary muscle. These alterations were present in the majority of the muscle fibers and included deep infolding of the sarcolema; reduction of sarcoplasm content due to myofilaments and Z line disorganization; hyper-contracted myofilibrils; presence of polymorphic and swelling mitochondria with disorganized cristae. The increased space between muscle fibers due to the reduction of sarcoplasm content was filled by a great quantity of collagen fibrils. Our results show that PCU induces alterations in cardiac muscle in middle-aged rats.

EE-008 IN SITU HYBRIDIZATION FOR HUMAN PAPILLOMAVIRUS INFECTION IN CERVICAL, VULVA AND VAGINA REGIONS.

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Cervical cancer is currently the most common sexually transmissible disease occurring among young women reaching up to 20% to 46% in some countries. HPV is the major etiological factor in human uterine cervical cancer. HPVs which induce important lesions and invasive cancers are called high risk oncogenic viruses (types 16 and 18), moderated risk oncogenic viruses (31,33) and low-risk viruses (6,11) which usually are associated with condylomatous lesions. For HPV DNA detection, 332 specimens from 269 patients were studied. By the ISH technique, 113 (34,04%) were positive for HPV infection, at which 24,39% (40/164) of the uterine cervix, 38,02% (46/121) of the vulva and 57,45% (27/47) of the vagina. By the ISH technique, 113 (34,04%) were positive for HPV infection, at which 24,39% (40/164) of the uterine cervix, 38,02% (46/121) of the vulva and 57,45% (27/47) of the vagina. The present study showed positive statistically significant results were in specimens from the uterine cervix and vulva. Significant numbers of positive results were observed using A/E probe in the three regions. The prevalence of HPV infection in Cervical Intraepithelial Neoplasia was 88,88% (8/9) and 75% (3/4) in VDNa cases. Different techniques used for prevention of cervical, vulvar and vaginal neoplasms are important, and the ISH is a technique that can correlate morphology with HPV infection with high specificity and allows retrospective analyses of filed specimens. The results demonstrated that morphological evidence of viral infections observed in histopathological preparations is confirmed in most cases by ISH in these anatomical sites.
Baccaro RBF

USING INFUSION IN SITU SYSTEM.

p53 and HPV 16/18 infection. The results showed association between immunoreactivity of p53 expression and immunohistochemistry staining to detection of HPV 16/18 in wax embedded cervical and vulva tissues were examined. In situ Hybridization was used to detection of HPV 16/18 ORFs are transcriptionally active, producing E6 proliferative protein. Expression of E6 protein inhibits the apoptotic integration leads to disruption of the viral genome, the E6 often integrated into the host chromosome and , although leads to growth regulation in ZF. In conclusion ACTH and FGF2 on regulation of these protein families in the zones of adrenal cortex (glomerulosa-zG, fasciculata-zF and reticularis-zR) has not been analyzed following alterations in the adrenal tropic state of an animal. Male Sprague Dawley rats were HP according to standard procedures and kept under normal diet. The operation was confirmed by physical inspection of sella turcica. HP received an i.p. injection of either saline, 10-3M ACTH or/and 20ng/mlFGF2, 2h prior to harvesting the glands. Animals were killed and the gland harvested, fixed, embedded in paraffin, cut at 5µm. Morphological alterations was measured using HE stain and the protein expression was determined by immunohistochemistry. The atrophic effect of HP was in the inner adrenal cortical zones (zF and zR) and had less effect in zG and was seen within 3 days following HP. HP rats treated with ACTH showed cFos protein expression in zF. Supported by FAPESP.

F-002 EFFECTS OF HYPOPHYSECTOMY AND ACTH ADMINISTRATION ON TROPHIC RESPONSE OF RAT ADRENAL GLAND.

F-003 STIMULATION OF EARLY RESPONSE GENES IN RAT ADRENAL PRIMARY CULTURE CELLS TREATED WITH ACTH AND FGF2.

Introduction and Goals: Mammalian cell cycle control is exerted at the G0-G1-S transition by hormones and growth factors inducing early response genes, fos and jun proto-oncogene families. FGF2 (fibroblast growth factor) mimic the mitogenic actions of growth factors in Y1 cell line while ACTH (adrenocorticotropin) does not present signaling potential sufficient to this response. We analyzed the kinetic of regulation of expression of Fos and Jun proteins in rat primary culture cells, treated by ACTH or/and FGF2. Methods: Adrenal primary culture cells, glomerulosa (G) and fasciculata/reticularis (F/R) cells were obtained from Wistar male rats through and seeded in DMEM medium+10%FCS at 37°C and 5%CO2. After serum-starved the cells were treated with 10-3M ACTH(A), 1ng/ml FGF2(F) or A+F for different times and analyzed by immunoperoxidase or Western Blotting. Results and Conclusion: cFos proteins are not detected in the nuclei serum starved cells but is induced by ACTH and FGF in G and F/R cells with the same kinetic, with peaking at 2h, but with different intensity. FGF2 induction reaches 50% of the level attained upon ACTH induction. A+F presented maximal cFos activation in labeled nuclei but present an additive effect on protein expression. The FGF2 is poor inductor of cFos protein however A+F combination results suggest that FGF2 may have an important role in the growth response of ACTH in adrenal cells. Supported by FAPESP.
We have investigated the effects of fractions of a new type of Brazilian propolis containing phenolic acid derivatives (FRRP) on cell growth and proliferation in a human multiple myeloma cell line (RPMI 8226). The effects on cell cycle progression in the RPMI 8226 cell line were assayed with propidium iodide using DNA histograms and multiparametric flow cytometry. The inhibitory concentrations 50 (IC_{50}) were estimated by cytotastic assay of tetrazolium (MTT) reduction. The IC_{50} (± S.E.M., n =3) of FRRP at 48 h exposure, was 28 ± 8 µg/ml. FRRP at a concentration of 20 µg/ml inhibited the G1/S transition of the cell cycle of the human multiple myeloma cell line, an action consistent with its cytotastic effect. Our results also revealed that mRNA expression of cyclin-dependent kinase inhibitors, p16, as assayed by RT-PCR, did not change in multiple myeloma. All procedures were performed in triplicate with appropriate controls. The chemical composition of FRRP was evaluated by High performance liquid chromatography coupled to mass detector. Our observations suggest that the FRRP from Brazilian propolis may be a powerful therapeutic drug in myeloma pathogenesis.

F-004 INHIBITORY EFFECT OF PHENOLIC ACID DERIVATIVES FROM BRAZILIAN PROPOLIS ON CELL GROWTH IN A HUMAN MULTIPLE MYELOMA CELL LINE

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We have investigated the effects of fractions of a new type of Brazilian propolis containing phenolic acid derivatives (FRRP) on cell growth and proliferation in a human multiple myeloma cell line (RPMI 8226). The effects on cell cycle progression in the RPMI 8226 cell line were assayed with propidium iodide using DNA histograms and multiparametric flow cytometry. The inhibitory concentrations 50 (IC_{50}) were estimated by cytotastic assay of tetrazolium (MTT) reduction. The IC_{50} (± S.E.M., n =3) of FRRP at 48 h exposure, was 28 ± 8 µg/ml. FRRP at a concentration of 20 µg/ml inhibited the G1/S transition of the cell cycle of the human multiple myeloma cell line, an action consistent with its cytotastic effect. Our results also revealed that mRNA expression of cyclin-dependent kinase inhibitors, p16, as assayed by RT-PCR, did not change in multiple myeloma. All procedures were performed in triplicate with appropriate controls. The chemical composition of FRRP was evaluated by High performance liquid chromatography coupled to mass detector. Our observations suggest that the FRRP from Brazilian propolis may be a powerful therapeutic drug in myeloma pathogenesis.

F-005 SILK PRODUCTION BY THE SALIVARY GLAND OF THE WEAVER ANT Camponotus sexen Sarcodes

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The weaver ant Camponotus sexen uses the larval silk for nest construction and cocoon spinning. We present the ultrastructure and the macromolecular array of silk formation. The salivary glands were submitted to routine for TEM. The histophotographic array was studied under polarized light microscopy using whole gland mounts. The salivary glands were composed by four secretory tubes, two reservoirs, and two lateral ducts that join to form the anterior duct. The secretory tubes show cubic cells characterized by few lipid droplets, rounded nucleus, abundant rough endoplasmatic reticulum, rounded Golgi Bodies, and a great amount of fibrous secretory vesicles, probably fibroin. The luminal tactoids are centrally located. Under polarized light, they are birefringent and show a net pattern of optical directions when the first order red filter was used. The reservoir presents flattened cells that are ultrastructurally similar to the secretory tubes. The nucleus is flattened and there are large lipid droplets in the basal portion. These lipids are probably used in the sericin layers to cover the fibroin. The lumen is full of tactoids and the birefringence in a net pattern is higher than in the secretory tubes. In the lateral duct, the silk fiber is structured showing two optical directions (zigzag pattern), similar to what was observed for larvae of Polistes versicolor wasp and Pachycondyla villosa ant.

F-006 EFFECT OF ACLF, A RECOMBINANT SNAKE VENOM METALLOPROTEASE ON THE PROLIFERATION OF HUMAN FIBROBLASTS

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Snake venom metalloproteases (SVPs) comprise a family of Zn^2+ dependent enzymes, which contain many different biological activities. They can cause bleeding by interfering with the blood coagulation and/or by degrading extracellular matrix components. ACLF is a fibrinolytic non-hemorrhagic metalloprotease from the venom of the snake Agkistrodon contortrix laticinctus. This enzyme hydrolyses natural substrates such as thrombospondin, laminin, fibronectin and proinsulin. ACLF can be produced as a recombinant prometalloprotease and activated in vitro. The aim of this work was to study the activity of ACLF on human fibroblasts proliferation and morphology. Human fibroblasts were grown to confluence in DMEM and 10% FBS. After detachment with trypsin, cells were suspended in serum-free DMEM supplemented with 2mg/ml bovine serum albumin. Cells were plated (2 x 10^3 cells/well) in 96-well plates and incubated with ACLF (0.015 to 0.5 µM) for 24 and 48 h at 37º C (5% CO2). To determine the number of cells after treatments, the MTT method was used and blue formazan product was detected by measuring of absorbance in MRX Dynex plate reader. ACLF increases the number of cells in all concentrations tested when compared with cells grown without the enzyme. 1,10-phenantroline treated enzyme (ACLFi) induced the same effect. After 48 h incubation, 0.5 µM ACLFi induced rounded cell shape and detachment.

Support: FAPESP

F-007 JUVENILE HORMONE EFFECT ON THE VENOM GLAND ULTRASTRUCTURE IN WORKERS OF Apis mellifera (HYMENOPTERA, APIDAE)

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The present study analyzed, the influence of the treatment with juvenile hormone on the ultrastructure of Apis mellifera workers' venom glands. Newly emerged workers received topical application of 1µl of juvenile hormone diluted in hexane, in the concentration of 2µg/µl. Two controls were used; one control received no treatment (group C1) and other received topical application of 1µl of hexane (group C2). The aspect of the glandular cells, in not treated newly emerged workers, showed that they are not yet secreting actively. Cellular modifications happened according to the worker age and to the glandular area considered. The most active phase of the gland happened from the emergence to 14 days, and at 25 days the cells already lost its secretory characteristic, being the distal area the first to suffer degeneration. The treatment with juvenile hormone and hexane altered the temporal sequence of the glandular cycle, forwarding the secretory cycle and degeneration of the venom gland.
F-008 PROGNOSTIC SIGNIFICANCE OF PROLIFERATIVE ACTIVITY MEASURED BY Ki-67 (clone MIB-1) IN GASTRIC AND COLORECTAL CANCER

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The growth of tumours is highly variable and this probably reflects in its clinical course. The monoclonal antibody Ki-67 recognises an antigen present in the nuclei of cells in all phases of the cell cycle except G0. We analysed by immunohistochemistry the proliferative activity, based on Ki-67 labelling index, in formalin-fixed and paraffin-embedded sections of 152 tumours, being 70 gastric and 89 colorectal cancer. The results were correlated with the clinicopathologic factors. The carcinomas showed a wide range of Ki-67LI, reflecting a variation in proliferative activity. The tumour labelling index ranged from 10 to 85 per cent positivity, being the mean level on gastric cancer tissue 0.52 and in colorectal cancer 0.44. There was also heterogeneity of labelling within many of the tumours. No significant correlation was found between Ki-67LI and sex, age, clinical stage in these cancers. In colorectal cancer, but not in gastric cancer, high levels of Ki67LI have been correlated with poor survival. Ki-67 staining is a simple and useful method for estimating proliferative activity. In colorectal cancer this index may be used as a marker of prognosis.

F-009 ALTERATIONS IN ALUMINUM UPTAKE CAPACITY IN TOBACCO BY-2 CELLS DURING THE CELL CYCLE

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Sensitivity of plant cells to aluminum depends on their growth and developmental status. Root cells of the distal transition zone are the most sensitive to Al. In culture, cells that are undergoing intense growth and division are the most Al sensitive. It is possible that changes in Al sensitivity may occur during the cell cycle. The objective of this work was to synchronize a culture and to verify if differences in Al uptake capacity occurred during the cell cycle. Stationary-phase cells of a tobacco BY-2 culture were incubated for 24h in a fresh medium containing aphidicolin, then washed and immediately incubated for another 2h in propyzamide, washed again and then released in a fresh medium. Aliquots of the synchronized cells were collected every 2h and were exposed to 50μM Al (in 2mM CaCl2, 10mM KCl, pH 4.2) for 2h. Aluminum uptake was determined fluorometrically with morin (2',3',4',5',7'-pentahydroxyflavone). Cells were capable of Al uptake in all phases of the cell cycle but this capability doubled when cells proceeded through the M/G1 transition. Thus, higher Al uptake capacity appears to coincide with a phase in which membrane fusion and cell wall construction are occurring. This is the first work to report changes in sensitivity to Al during the cell cycle. Support: FAPESP

F-010 THE CYTOCHEMICAL LOCALIZATION OF ACID PHOSPHATASE IN THE DEGENERATION OF THE SILK GLAND OF Apis mellifera UNDER THE INFLUENCE OF THE JUVENILE HORMONE.

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The present study aimed to define, at the ultra-structural level, the activity of the acid phosphatase during the degeneration process of the silk gland of Apis mellifera under the influence of the Juvenile Hormone (JH) using paranitrophenilphosphato (PNPP) as substrate. The silk glands of control larvae (at the beginning and end of the 5th instar) were analyzed and compared to the glands of larvae treated with Juvenile Hormone (JH-III) from the same stage. The cytochemical analyses demonstrated a slight increase of the activity of the acid phosphatase from the beginning to the end of the 5th instar. At the beginning of the 5th instar, the activity of the acid phosphatase at the Golgi regions and vesicles was related to the origin of lysosomes and to the elimination of the secretion. At the end of this instar, the incidence of a positive activity of this enzyme at the glandular lumen and in the microvilli was related to the elimination and degradation of the secretion and of the cells. The activity of the acid phosphatase was similar in the glandular cells of larvae treated with JH and in control larvae at the beginning of the 5th instar, which indicates that the JH delays the process of cellular degeneration.

F-011 RETINOIC ACID INHIBITS TUMOR AND NORMAL LIVER CELLS PROLIFERATION IN CULTURE

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In the last decades alternative therapies have been searched for the treatment of the liver cancer to improve the probability of cure for the disease. Several studies investigated the biological activity of the retinoids in different tumor cell lines, since they act on the regulation of the cell proliferation and differentiation processes. Retinoic acid, a biologically active metabolite of vitamin A play a critical role during normal development and regulates growth and/or differentiation in a variety of tumor cell lines. The present work investigated the effect of the retinoic acid on the proliferation in normal and tumoral liver cell lines. The results obtained by the “kit CellTiter 96R AQueousNon-Radioative Cell Proliferation Assay” (MTS) after the treatment with retinoic acid (10-7 M) for 48h plus 24h of recovery in normal medium showed 38% proliferation inhibition in the normal cells and 45% in the tumoral ones. Flow cytometry (FACS) analysis indicated that G0/G1 cell population decreases drastically with 48h of recovery as well as polyploid cell population increased. The immunofluoresce assay showed morphologic alterations in normal cells after the treatment. The cell organization in culture resembled that of hepatic lobules presenting an increased number of binucleated cells. Present data agree with the suggestion of the retinoic acid used as an alternative therapy in the treatment of the cancer, including hepatic tumors. (FAPESP, CAPES, CNPq)
The corn leafhopper, *Dalbulus maidis* (DeLong and Wolcott), is a significant pest because it is a vector for three stunting pathogens: the corn stunt spiroplasma, the maize bushy stunt phytoplasma and the maize rayado fino marafivirus. The diseases triggered by these pathogens occur sporadically in many regions, but can also cause widespread and serious diseases in newly introduced non-adapted corn varieties. In addition, leafhoppers are also of genetic interest due to the fact that their chromosomes present diffuse kinetochores, distributed along the chromosome – holocentric chromosome. In this study, special emphasis was given to the spermatogenesis analyzes and to the number of the chromosomes of this leafhopper. Testes of adult males were fixed in acetic acid and stained with lacto-acetic orcein. The analysis showed polyploid nuclei in the nutritive cells of the tube wall and spermatogonial metaphases with 18 chromosomes. The nuclei displayed a single corpuscle during mitosis and, at metaphase II, an autosomal ring and the sex chromosomes, were located in the center. The study of gametogenesis in these insects allowed an analysis of meiotic behavior in the order Homoptera as to the type of kinetochore organization of the group.

**G-002 EFFECTS OF THE ASCORBIC ACID SUPPLEMENTATION ON THE CELLULAR PROLIFERATION OF THE ILEUM MUCOSA IN DIABETIC RATS**

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The aim of this study was to determine the metaphasic index in the cryptal cells of the ileum mucosa in diabetic rats supplemented with ascorbic acid (AA). Fifteen 90-day old rats (Rattus norvegicus) were divided in the following three groups: control (C), diabetic (D) and diabetic treated with AA (DA). Diabetes mellitus (DM) was induced through streptozotocin administration (35 mg/Kg), whereas the group DA was obtained by supplementing the water during 120 days with AA (1g/L/day). After this period Vincristin was administered to the animals (1mg/Kg), 2 hours before the sacrifice. The segments were collected and processed for paraffin embedding. Semi-seriated sections were stained with H&E. The mucosa metaphasic index was calculated by obtaining chromosome morphology, C-banding and NOR interchanges were responsible by evolutionary chromosomal differentiation of these two species. Financial support: CAPES

The species *Tradescantia pallicia* Hunt. cv. purpurea Boom, that is adapted to tropical weather, was used for the *Tradescantia* micronuclei (Trad-MCN) assay in order to analyze air quality in two urban areas in Sorocaba: one in a residential block (Campolim), and the other at Dom Aguirre highway, compared to “Floresta Nacional de Ipanema” in a neighbor town, Ipiré. The plants at Dom Aguirre highway presented the highest and more significant increases in the frequency of micronuclei tetrads (2.23%) in comparison with 1.53% found in plants from the residential block and a similar frequency to the samples from “Floresta Nacional” (2.35%). These results suggest that the concentration of pollutants influences the frequency of micronuclei tetrads and indicates the need of continuous biomonitoring of “Floresta Nacional”, since this area is potentially not affected by high concentrations of pollutant in the air and micronuclei tetrads frequency was close to what was found in the highway area.

**G-003 AIR QUALITY EVALUATION IN TWO URBAN AREAS FROM SOROCABA CITY THROUGH THE USAGE OF *Tradescantia pallicia* BIOINDICATOR PLANT**

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The species *Tradescantia pallicia* Hunt. cv. purpurea Boom, that is adapted to tropical weather, was used for the *Tradescantia* micronuclei (Trad-MCN) assay in order to analyze air quality in two urban areas in Sorocaba: one in a residential block (Campolim), and the other at Dom Aguirre highway, compared to “Floresta Nacional de Ipanema” in a neighbor town, Ipiré. The plants at Dom Aguirre highway presented the highest and more significant increases in the frequency of micronuclei tetrads (2.23%) in comparison with 1.53% found in plants from the residential block and a similar frequency to the samples from “Floresta Nacional” (2.35%). These results suggest that the concentration of pollutants influences the frequency of micronuclei tetrads and indicates the need of continuous biomonitoring of “Floresta Nacional”, since this area is potentially not affected by high concentrations of pollutant in the air and micronuclei tetrads frequency was close to what was found in the highway area.

**G-004 CHROMOSOMAL CHANGES BETWEEN *Conoderus dimidiatus* AND *Conoderus ternarius* (COLEOPTERA, ELATERIDAE) OCCURRED DURING EVOLUTIONARY PROCESSES.**

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The family Elateridae possesses a great karyotype heterogeneity. The purpose of this study is the cytogenetic characterization of *Conoderus dimidiatus* and *Conoderus ternarius* in relation to the karyotype, the C-banding, and the chromosomes carriers of the NORs. The chromosomal preparations were obtained from gonads of adult individuals. Mitotic cells of *C. dimidiatus* and *C. ternarius* showed 2n=17 for males and 2n=18 for females, and X0/XX type sex determination system. In both species, the chromosomal morphology was acrocentric for all chromosomes, with the exception of 6th and 8th pairs of *C. ternarius*, which are metacentrics. Metaphase cells of these two species exhibited constitutive heterochromatin block in the centromeric region of almost all chromosomes. However, differences involving the presence of telomeric C-band on three autosomal pairs and X chromosome were noted between *C. dimidiatus* and *C. ternarius*. Silver impregnated mitotic chromosomes evidenced 4 NORs in *C. dimidiatus*, occupying pair 2 pericentromeric region and pair 4 long arm telomeric region, and 2 NORs in *C. ternarius*, occurring on pair 1 long arm distal region. The obtained chromosome morphology, C-banding and NOR pattern indicate that pericentric inversion, addition and/or deletion of constitutive heterochromatin, and NORs interchanges were responsible by evolutionary chromosomal differentiation of these two species. Financial support: CAPES
Approximately 1% of the Lampyridae species has been analyzed on the cytogenetic point of view and the majority showed similar cytogenetic features, 2n=19=18+X0=9II+X0 in males. The aim of this work is to determine the karyotype characteristics and meiotic behaviour of Apisoma lineatum from Rio Claro, São Paulo State. The chromosomal preparations were obtained from gonads of three male adults and stained with 3% Giemsa. The analysis of A. lineatum preparations evidenced 2n=19=18+X0 in mitotic metaphases, mitotic cycle 9II+X0 in spermatocytes I, and n=9 and n=10=9+X in spermatocytes II. The A. lineatum karyotype exhibited pair 1, 6, 7, 8 and 9 metacentric, pair 3, 4 and 5 submetacentric, pair 2 and X chromosome acrocentric. During diplotene and diakinesis, the majority of the bivalents possessed one interstitial or terminal chiasma, with the exception of one bivalent, which revealed two chiasmata, one interstitial and other terminal, assuming ring configuration. The analysis of metaphases II confirmed the X chromosome regular segregation during preceding anaphase I. The A. lineatum obtained data were similar to those described for Apisoma aegrotatum, Apisoma ignitum, and Apisoma laterale in relation to the diploid number and type of sex determination system. However, the A. lineatum X chromosome disclosed pre-reductional behaviour, contrasting with that post-reductional found in the previously analyzed Apisoma species. Supported by FAPESP (Process number 03/10633-9).

The aim of this work was to evaluate the effects of therapeutic ultrasound on muscle regeneration after lesion caused by Bothrops poison. Groups of five male, white Wistar rats were used weighing about 270± 20 g. They were divided into two groups: control and treated ultrasound and analyzed seven days after the lesion. The front tibial muscle of back limb was injured (0.5 mg/kg) with Bothrop poison by intramuscular injection. And treated for five days with pulsive ultrasound, at 1 MHz frequency with intensity of 0.5 W/cm², for five minutes, once a day. The animal tissue of all groups was extracted for histological preparation on a regular basis. The densities of macrophages, lymphocytes, fibroblasts, blood veins and fresh muscle fibers, were determined. The Bothrops poison was the most miotoxic, due to the largest number of injured fibers (p< 0.01). The morphometric analysis showed that the animals treated with ultrasound presented higher densities of macrophage (p< 0.01), blood veins (p< 0.05) and of fresh muscle fibers (p< 0.001) and lower density of lymphocytes (p< 0.01) and fibroblasts (p< 0.001). The results of this work indicated that ultrasound was efficient in regenerating the injured muscle by different Bothrops poison.

The genus Epidendrum Linnaeus, with about 900 species is distributed largely in tropical and subtropical areas of the Americas, being represented in Brazil by many endemic species. In the present study chromosome number, ploidy level, interphase nuclear types and effect of polyploidy in the foliar epidermis to species E. cinnabarinum Salzm were investigated. For cytogenetical analyses root tips were pretreated with 0,002M 8-hydroxyquinoline for 4h at room temperature, fixed in Carnoy for 18-24h, transferred to 70% alcold, stored at 4°C and then hydrolyzed in 1N HCl for 8 min at 60°C. The stain follows the Feulgen method. For meiotic analyses young flower buds were placed directly in Carnoy for 24h and stored to 70% alcold at 4°C. Squash preparations were made in a 2% propionic carmine. Polyploid effect on lower epidermis cells of E. nocturnum (diploidy 2n=40) and E. cinnabarinum was compared. The chromosome number 2n=ca.240 for E. cinnabarinum was determined, sugesting the polyploidy in the evolution of the genus. Meiotic alterations with presence of laggard chromosome, early segregation chromosome and anaphase bridges were observed. The interphase nuclei of the species study were found to be categorized into simple chromocenter type. As the ploidy level increase in plants, a greater surface area and smaller stomata number were observed.
G-009 CYTOGENETICAL ANALYSES IN SPECIES OF ORCHIDACEAE OCCURRING IN “DUNAS OFABAETÉ – SALVADOR/BA”.
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The environment protection area of “Lagoas e Dunas de Abarité” represents one of the few coastal ecosystems of dunes still found in the city of Salvador, presenting a high taxonomical diversity and endemic species. Floristic survey indicate the occurrence of 17 species of 11 orchid genera, many of which without chromosome recording. In the present study, chromosome number and ploidy level were investigated to the species Cyrtopodium parviflorum, Vanilla bahiana and Galeandra sp. and meiotic analyses in V. bahiana. Mitotic metaphases of the root tips pretreated with 0,002M 8-hidroxyquinoline and stained following the Feulgen method were analysed. For meiotic analyses, young flower buds were fixed in Carnoy for 24h and stored in 70% ethanol at 4ºC and squash preparations were made in a 2% propionic carmine solution. C. parviflorum showed the somatic number 2n = 46, confirming the basic number x = 23 for genus. For V. bahiana, the number chromosome 2n = 32 was determined, bimodal confirming the basic number x = 28 for genus was not confirmed, suggesting the occurrence of dysploidy, so that the correct identification of this species is still needed.

G-010 ANALYSIS OF THE EFFECT OF THE THERAPEUTIC ULTRASOUND IN THE TREATMENT OF MUSCLE LESION INDUCED BY Bothrops newviedi POISON.
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This work aimed to quantify the morphological alterations induced by the treatment with ultrasound in the recuperation of previous tibial muscle of rat injured by Bothrops poison. Groups of five white, male, Wistar rats were used weighing 270±20 g. They were divided in three groups: control, treated with ultrasound and placebo. The treatment had beginning 24 h after injury, three or five days by week, once a day, for five min, with ultrasound of reduced headstock, pulsatile way, 1 MHz and 0,5 W/cm². In placebo was used the off device. The muscles were analyzed 3, 7, 14 and 21 days after injury through histological preparations. The macrophage densities, lymphocytes, fibroblast, injured and new muscle fibers were determined. The quantitative analysis of the group treated with ultrasound was significantly (p<0.05) different from the groups control and placebo. The density of new muscular fibers increased with the time of treatment: 4,50±2,38 (3 days), 12,33±1,52 (7 days), 15,00±0,05 (14 days) and 22,1±7,07 (21 days). The fibroblast rate decrease: 10,43±4,83 (3 days), 12,33±1,52 (7 days), 4,44±0,88 (7 days), 4,00±7,77 (14 days) 5,00±1,73 (21 days). The data indicated that ultrasound accelerate the inflammatory reaction and the proliferation of muscle fibers showing regeneration with smaller tissue fibrosis.

H-001 HEALING EFFECT OF AN OINTMENT PHYTOTHERAPY
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A phytopathic salve, popularly called miraculous ointment, has been for long used as healing. Thus, the goal of this study was to evaluate the healing effect of the salve produced with the following vegetal species: Sambucus australis Cham. Et. Schecht; Arctium lappa Linn; Malva sylvestris L.Ck.; Plantago australis L.; Achillea millefolium Lino and other components, such as: bee wax, Gum Rosin and swine lard. To that, 36 male Wistar rats were divided in three groups, anesthetized and had a wound done at their dorsal area. The first group received an ointment treatment, the second a sugar treatment and, the third, the control, received nothing. At the 7th, 14th and 21st days postoperative, four animals of each group were euthanized and samples collected for paraffin embedding and histological analysis. In all groups, 21 days after the lesion, an epithelial layer completely covered the wounds but, at deeper derme layers, signals of an inflammatory process were still present. Only in the ointment-treated wounds, a superficial dense keratinization and, sebaceous gland and hair follicles were observed. These results indicate a relevant potential for ointment phytotherapy as it improves the cicatrisation process.

H-002 HEALING EFFECT OF COMMERCIAL PRODUCTS (SKILLFUL AND DERSANI®) CONTAINING MEDIUM CHAIN-TRIGLYCERIDES AND ESSENTIAL FATTY ACIDS: A MORPHOLOGICAL AND COMPARATIVE STUDY
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In view of the recent applications of products containing medium triglycerides and essential fatty acids as healing, it becomes of great help to histologically compare the efficiency and effectiveness of the commercialized product known as Skillful and Dersani. This experimental design included 24 male Wistar young rats, whose dorsal regions were surgically performed bilateral wounds of 1,5 cm of diameter. In twelve rats Skillful and Dersani were applied daily, respectively at the right and left wound. The remaining 12 rats remained as controls and, they did receive any product on the wounds. The healing evolution was evaluated at the days 3, 7, 14 and 21 postoperative in histological sections. Morphological differences were found in the development of the treated and non-treated wounds until day 14. At day 21, however, no microscopic and macroscopic differences between the treated and control groups could be found. These results indicate that although, some differences may occur during the evolution of the wounds, using any of the commercial products, they did not influence the time of the healing process.
H-003 ACUTE ANTI-INFLAMMATORY EFFECT OF Averrhoa carambola L. EXTRACSTS
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Averrhoa carambola is a plant of the family Oxalidaceae, popularly used as anti-inflammatory. To add new information about this popular treatment, in this study, it was determined the morphology of the inflammatory response in the presence or absence of this phytotherapeutic drug and the results compared to glyocorticoid treatment in rats. Wounds were surgically performed at the dorsal skin region of the animals. In the experimental group, the animals received A. carambola extracts i.p. at the dose of 500 mg/Kg. In the control group it was administered glyocorticoid (dexametasona), at doses compatible to the animal weight. The wounds were evaluated at 6, 12 and 24 h postoperative by histological observations. The comparative analyses of the wound showed that both treatments were able to adequately control the acute inflammatory response, in equivalent period of time. In both groups there was a prominent decrease of the inflammatory response and stimulation of tissue repair processes. These findings indicate pharmacological functions to A. carambola, which certainly deserves more detailed studies to be used as a phytotherapeutic for inflammatory process treatment.

H-004 ANTI-INFLAMMATORY ACTION OF AVERRHOACARAMBOLA AT MORPHOLOGIC LEVEL IN DIFFERENT FORMULAS OF POMADES
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The development of new pharmaceutical forms of topical use with medicinal plants, in order to help in the process of tissue reparation, has been increasingly studied. In this work, it was aimed to evaluate the anti-inflammatory effects of the pomade of Averrhoa carambola extract in hydrophilic and lipophilic bases in different concentrations (0%, 5%, 2%, 5%, 10%) on a skin injured by rats. Pomade was prescribed twice a day, for 3 days. For the injury, the animals were anesthetized and a clean wound was induced in the cranial back thoracic region, using a 0,8mm diameter metallic mould. The groups treated with the pomade in lipophilic base at 2% and 5% presented a higher-quality cicatrizing pattern in relation to the groups of hydrophilic pomade in different concentrations, represented by neo-formation of blood vessels, reduction of inflammatory cells and intense reepithelization. However, regarding the formation of collagen fibers, it was observed intensively in concentration of 10% of lipophilic base and 2%, 5% and 10% in hydrophilic base, suggesting that cicatrization was more accelerated, though not organized, as occurred in the lipophilic base at 2% and 5%, where cicatrizing phases were followed continuously with dermic structure in a gradual and organized way.

H-005 GROWTH CHARACTERISTICS OF THE MUSCLE TISSUE IN NILE TILAPIA LARVAE (Oreochromis niloticus) FED WITH A DIET CONTAINING LYSINE SUPPLEMENTATION.
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The present study evaluated the effect of the aminoacid lysine supplementation in the growth of the skeletal muscle tissue in Nile tilapia larvae (O.niloticus). Two groups were studied: the lysine group that received the supplementation in increasing levels and another that received a regular diet, during 30 days. Muscle samples (7µm thick) were submitted to HE stain for morphological analysis and for the count of the number of muscle fibers. Other sections were submitted to PCNA for analysis of cellular proliferation. The morphology analysis did not show differences between the groups, both with muscle mass distributed in red superficial and white deep muscle layers. The group that received regular diet had superior diameter and number of fibers, and the PCNA expression was lower. This means that muscle growth occurred by the association of hypertrophy and hyperplasia in this group and in the lysine group, the recruitment of the fibers was predominant. The lack and lysine supplementation in the diet do not contribute to the hypertrophy growth mechanism in this developmental period of tilapia.

Apoio Financeiro: FAPESP, Proc. 02/10550-3 FUNDUNESP, Proc. 00101/03-DFP

H-006 MAPPING THE TRANSFORMATION PATHWAY OF MGAL CELLS, A NOVEL MURINE MELANOMA CELL LINE.
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Tumorigenesis is a complex process characterized by accumulation of genetic changes, leading to the deregulation of essential genes to cellular homeostasis. Embryonic cells have been useful models to investigate the molecular basis of malignant transformation. Here we report on the characterization of a novel cell line (Mgal3) isolated from an embryonic cell culture. Mgal3 cells were isolated from the 10th passage of a finite culture of C57bl-6 embryonic cells. Mgal3 cells were isolated from the 10th passage of a finite culture of C57bl-6 embryonic cells.

Mgal3 cells were characterized by the production of a dark brown pigment. Ultrastructural analysis allowed for their identification as melanocyte-derived cells, since melanosomes at different stages of maturation were observed. Mgal3 cells were also tumorigenic in syngeneic mice, giving rise to metastatic melanomas. Mgal3 cells produce a large number of intraciserial viral particles. The expression of retroviral genes was confirmed by Northern blots and flow cytometry. Transcription reactivation of integrated ecotropic viruses are markers for chromatin hypomethylation. The tumor suppressor INK4a and galectin-3 genes were not expressed in Mgal3 cells, but their expression could be induced by azadecocytidine, suggesting that their promoters were hypermethylated. Thus, an imbalance of epigenetic mechanisms was associated with the generation of the Mgal3 cell line.

Support: FAPESP.
Cisplatin is an antineoplastic agent used to treat some malignancies, like ovarian, testicular and bladder tumours. However, both in vitro and in vivo, cisplatin has been shown to be mutagenic and tumorigenic. We investigated the possible changes in the adhesion pattern and cytoskeleton and extracellular matrix components, induced by cisplatin treatment, to obtain adhesion curves of treated and control Vero cells, and evaluated for cytoskeleton and extracellular matrix components by immunocytochemistry. Vero cells were treated with cisplatin (50µg/ml) during 24h. After 10 successive subcultures this treated population grew in multiple layers, forming cellular aggregates and was named VT (transformed Vero cells). VC (control Vero cells) presented a characteristic adhesion pattern with monolayer growth. The adhesion pattern, actin and fibronectin distribution were altered in VT. After confluence, VC presented fibronectin accumulation in lateral cell-cell contact regions, while VT cells presented reduced accumulation of fibronectin between cells, with a diffuse distribution. This may signify a reduced fibronectin synthesis or deficient fibronectin accumulation in VT, which may lead at the alterations and decreased of adhesion index with multilayered growth observed in these cells transformed by cisplatin treatment.

H-009 ALTERATIONS IN THE ADHESION PATTERN AND CYTOSKELETON OFvero cells INDUCED BY Cisplatin

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Cisplatin is an antineoplastic agent used to treat some malignancies, like ovarian, testicular and bladder tumours. In vivo and in vitro, cisplatin has been shown to be mutagenic and tumorigenic. We investigated the possible changes in the adhesion pattern and cytoskeleton and extracellular matrix components, induced by cisplatin treatment, to obtain adhesion curves of treated and control Vero cells, and evaluated for cytoskeleton and extracellular matrix components by immunocytochemistry. Vero cells were treated with cisplatin (50µg/ml) during 24h. After 10 successive subcultures this treated population grew in multiple layers, forming cellular aggregates and was named VT (transformed Vero cells). VC (control Vero cells) presented a characteristic adhesion pattern with monolayer growth. The adhesion pattern, actin and fibronectin distribution were altered in VT. After confluence, VC presented fibronectin accumulation in lateral cell-cell contact regions, while VT cells presented reduced accumulation of fibronectin between cells, with a diffuse distribution. This may signify a reduced fibronectin synthesis or deficient fibronectin accumulation in VT, which may lead at the alterations and decreased of adhesion index with multilayered growth observed in these cells transformed by cisplatin treatment.

H-008 CYTOGENETICAL ALTERATIONS IN V-79 CELLS INDUCED BY CISPLATIN TREATMENT.

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Cisplatin is an antineoplastic agent used to treat lymphomas and some malignancies, like ovarian, testicular and bladder tumours. In vivo and in vitro, cisplatin has been shown to be mutagenic, genotoxic and tumorigenic. We investigated the effect of cisplatin on cytogetenical characteristics of V-79 cells in culture. V-79 cells were arrested in metaphase by the addiction of colchicine and chromosomes were prepared according routine techniques. Trypsin-Giemsa (GTG) banding was performed, the modal chromosome number was determined and the mitotic and polyploidy index were obtained. Treated population presented altered modal chromosome number. Control population presented 59.5% of cells with 21 chromosomes (range of 20-22). Treated population presented 42.5% of cells with 21 chromosomes and 38% of cells with 20 chromosomes (range of 19-24), indicated genetical instability. Polyploidy and mitotic index were enhanced in treated cells. Control and treated populations presented a polyplody index of 1.58% and 2.15% respectively. Mitotic index was 18.12% in control cells and 22.82% in treated cells. We can conclude that the cisplatin treatment are responsible for the cytogetenical alterations and genetical instability observed in treated V-79 cells.

H-007 CADMIUM INDUCED OXIDATIVE STRESS IN SKELETAL MUSCLE CELLS IN VITRO.

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The effects of cadmium (Cd) exposure were verified on the oxidative stress in skeletal muscle cells (C2C12). Myoblasts cells were differentiated into myotubes during 4 day with DMEM horse serum supplemented. Myotubes cells were treated in different cadmium concentration (1, 3, 5, 7.5, 10 and 12.5µM CdCl2) to observe the oxidative stress analysing the glutathione S-transferase activity (GST; nmol.min⁻¹.g protein⁻¹), malondialdehyde content (MDA; µM.µg protein⁻¹), and morphological alterations using light (ML) and electronic scanning microscopy (MEV), after 24h. GST activity was increased in 1µM and 3µM Cd (Cd1= 36.9 ± 5.6; Cd3= 32.1 ± 6.0, respectively) although in high cadmium concentration, the GST activity was decreased compared to control group (C=21.8 ± 1.5 vs Cd5= 21.7 ± 2.7; Cd7.5= 15.9 ± 3.3; Cd10= 15.9 ± 4.6; Cd12.5= 10.5 ± 2.8). Elevated lipid peroxidation occurred in high cadmium concentration (C=7.3 ± 0.5; Cd1= 5.7 ± 0.4; Cd3= 6.9 ± 1.5; Cd4= 7.8 ± 1.3; Cd7.5= 11.2 ± 3.1; Cd10= 14.6 ± 3.8; Cd12.5= 20.5 ± 6.5). Proportional to cadmium treatment, ML and MEV analyses showed enhanced in adhesion cells lost and several bladders, evidencing possible cells death. These results suggested that Cd induced high oxidative damage end products, compromising the cells control mechanisms and cell adhesion. Financial support: Fapesp, Capes, FAEP-UNICAMP

H-010 ROLE OF LIPID BODIES IN THE DEVELOPMENT OF COLON CANCER

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accumulating evidence suggest that the inflammatory process and COX-2 play a pivotal role in colon carcinogenesis. Moreover, nonsteroidal anti-inflammatory drugs (NSAIDs) are used as chemopreventive agents for colon cancer. It has been demonstrated that lipid bodies are sites involved in the production of eicosanoids during the inflammatory process. Thus, the objective of this study was to evaluate the involvement of lipid bodies in the development of colon carcinoma. Our results demonstrated a drastic increase in the number of lipid bodies in human colon adenocarcinoma cell lines (Caco-2 and HT-29) when compared to a non-transformed intestinal epithelial cell line (IEC). Furthermore, Caco-2 and HT-29 cells present an increased expression of COX-2 when compared to IEC cells. Interestingly, most COX-2 immunoreactivity was localized in the lipid bodies. Treatment with aspirin significantly reduced the number of lipid bodies in CACO-2 cells. Analysis of adenocarcinoma of colon from patients demonstrated a large number of lipid bodies in the tumor tissues. Our results demonstrated that adenocarcinoma of colon present an increased number of lipid bodies that is correlated with the overexpression of COX-2. Furthermore, lipid bodies could be the major source of eicosanoids related to the pathogenesis of colon cancer.

Financial Support: INCA/FAF, CNPq and FURNAS.
This work presents the analysis of a mammary carcinoma metastasis (cm) in placenta using Digital Image Processing techniques. To classify the several structures present in the immunohistochemistry images of placenta, a classifier based on fuzzy logic was used. The training of the classifier consists in selecting regions of interest and calculating statistical parameters, such as sample mean, sample pattern deviation, maximum and minimum values and the sample median of the pixels within such regions. These parameters are employed to mount fuzzy membership functions (fp) centred at the sample mean (symmetric trapezoid method) or at the sample median (asymmetric trapezoid method). Each fp corresponds to a class in an specific band and the product of the several fp forms a discriminant function (fd). Therefore, the class called "image background", e.g., will be represented by an fd obtained from the fp's which express the class "image background" in the bands red, green and blue. The decision criteria is the winner class, that is, a pixel in a class if its fd gives the greater value among the other fd's for a given pixel. Using the fuzzy classification methods mentioned above, we can get very expressive images showing clearly the interface between the metastatic cm and the placental barrier, which avoids the placenta invasion by the maternal cm.

H-013 RHOB, RAC1 AND CDC42 RHO-GTPASES DURING CARDIAC MORPHOGENESIS
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Organogenesis of the heart involves the morphogenesis of the cardiac valves and septa, which are derived from the endocardial cushions – localized thickenings within the atrioventricular canal and outflow tract regions of the tubular embryonic heart. The endothelium in these regions undergoes a process known as an epithelial-mesenchymal transformation (EMT), in which the endothelial cells acquire a mesenchymal phenotype and differentiate into fibroblasts. Rho-GTPases are involved with cytoskeleton control and gene regulation. The aim of this work was to determine if there is any correlation between Rhob, Rac1 and Cdc42 and the EMT that occurs in the endocardial cushions. By immunohistochemistry, Rhob, Rac1 and Cdc42 were detected in the endocardium, mesenchymal cells and myocardium of chick embryo atrioventricular endocardial cushions. These Rho-GTPases were detected within the analyzed regions during most of the stages studied, but were most evident during stages 15 to 18. In addition, Rhob was detected in cells within a three-dimensional culture of endocardial cushions. Based on its known actions on the actin cytoskeleton and gene regulation, we propose that Rhob acts on the EMT program and cardiomyocyte development in the embryonic heart. In addition, Rac1 and Cdc42, which also act on the cytoskeleton and gene regulation, may act on endothelial and cardiomyocyte shape changes and on mesenchymal cell migration. These results suggest important roles for Rhob, Rac1 and Cdc42 during cardiac morphogenesis.

H-014 ULTRASTRUCTURE OF MAST CELLS IN INFLAMMATORY/REPARATORY PROCESS IN RATS
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The mast cells are globous, large and have their cytoplasm replete of basophils granules. They trigger inflammatory process by releasing the chemical mediators stored, promoting the recruitment of leukocytes and relaxation of blood vessels. This study compares the mast cells with and without influence of moderated physical activity. Twenty male Wistar rats distributed in 2 groups, sedentary (S) and trained (T), with 10 animals each one. All of them received implants of PVC sponges in the dorsal region. The T animals were submitted to moderated physical activity by daily swimming. After 5, 10, 15, 20 and 30 days animals were killed and their implants were removed. The analysis was carried out through light and transmission electron microscope. By comparing the S and T groups, numerical and morphological differences were observed. The number of mast cells was approximately 13% higher in T animals. In the S group, we observed the fusion of granular membranes during the degranulation, forming canals that fuse with the plasma membrane and communicate with the extracellular medium. On the other hand, in the T group, the plasma membrane undergoes major alterations, exhibiting a torn aspect. These results are probably not caused by the physical effort, since it is seen in peripheral and central regions of the implant, and the cells around it are intact.
In insects, the midgut is the organ where food digestion and absorption take place. The midgut epithelium has, at least, two cell types: digestive and regenerative cells. In Hymenoptera, the regenerative cells are in nests scattered among the digestive cells and are the responsible for the renewal of the midgut epithelium. Cell renewal occurs by regenerative cell differentiation without cell proliferation. Therefore, the longevity of the insect may be associated with the number of regenerative cell nests and cells per nest. In the bee *Melipona quadrifasciata anthidioides* queens live about two years against 50 days of adult lifespan of workers. So this study investigated the quantities of regenerative cell nests and cells per nest. Sections 3 µm thick from midguts of nurse and forager workers and virgin and physogastric queens were analyzed and the numbers of regenerative cell nests and cells per nest were determined with aid of the software Image Pro-Plus 4.1. Preliminary results have shown differences in the number of regenerative cell nests between castes and a higher number of cells per nest in both virgin and physogastric queens than workers.

### H-015 THE REGENERATIVE CELL IN THE MIDGUT OF BEES: A QUANTITATIVE STUDY.
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Collagen has been described as a beneficial material in bone tissue engineering. Selective hydrolysis of carboxyamides of asparagine and glutamine residues of collagen was employed to improve mechanical strength and piezoelectric properties. Anionic collagen scaffolds were prepared by hydrolysis for 24 hours (BP 24) or 48 hours (BP 48). Primary bovine fetal osteoblasts were cultured in regular and mineralization medium. The samples were processed for osteocalcin detection and SEM with energy dispersive spectrometric analysis (EDS). In regular medium, we observed multilayered cells with irregular morphology and a small quantity of vesicles and mineral deposits on their surface, confirmed by EDS. All samples did not present significant staining for osteocalcin, except for BP48. In mineralization medium, we verified multilayered cells with richer amount of vesicles and spots of mineral deposits at native and BP 48. Osteocalcin was expressed in all matrices and was stronger at BP48 and native, what corroborate the greater amount of calcium deposits found on them, once osteocalcin is related to mineralization. Our results showed that anionic collagen is capable of inducing osteoblastic differentiation, mainly when conditions are supplied. BP 24 did not present any significant gain in the induction of osteoblasts differentiation, while BP 48 present satisfactory results, comparable to the native ones.

### H-016 DIFFERENTIATION OF OSTEOBLASTS ON ANIONIC COLLAGEN SCAFFOLDS FOR BONE REPAIR
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Bone repair after mechanical injury is a complex process involving proliferation of osteoblastic cells, synthesis of bone matrix and reabsorption of local bone matrix portions. Osteoblastic-like cells have been used as a cell culture tool to study bone metabolism, bone diseases, tissue responses to implant biomaterials and morphological differentiation on distinct substrates. Morphological interaction between hFOB 1.19 cells (obtained from American Type Culture Collection) and fragments of demineralized bone matrix (DBM), extracted from rowett mice skulls was studied. On DBM, 1x10^5 cells/mL were plated for 120h. The samples were fixed, processed, and observed on light and electron microscopy and exposed to energy dispersive spectrometry analysis (EDS). The hFOB 1.19 cells on DBM revealed filopodia and cytoplasmatic prolongations emitted in direction of the DBM inner, growth in molayer, deposition of reticulated material on them and a DBM, encounter of cytoplasmatic prolongations from two distinct cells and an increase on calcium deposition. This work demonstrated DBM as a biomaterial with capacity of stimulating human osteoblastic cells spreading and differentiation in vitro.

### H-017 GROWTH PATTERN OF HUMAN FETAL OSTEOBLASTIC (hFOB 1.19) CELLS CULTURED ON DEMINERALIZED BONE MATRIX (DBM)
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Fibroblastic cells were cultured on and in collagen I gels and on glass coverslip under collagen with medium without fetal calf serum (FCS) and with 10% or 20% of FCS. On samples culture with 10% FCS, or without it, we observe that cells were capable of migrating in collagen matrix. Inside collagen, around of the migrating cells, we found deposition of extracellular granulations. Cytochemical data indicate that this material is proteoglycans and/or glycosaminoglycans. Moreover, the cells formed a structure similar to a connective tissue. However, cells cultivated with 20% FCS did not migrate into the collagen matrix, remaining on its surface, forming a structure similar to an epithelial tissue. When cultured on coverslip, under collagen, the cells migrated into the collagenic matrix. Around migrating cells in the collagen, we found the deposition of fibronecin in the substrate. On samples where we found a mono or multi layer, we could observe a deposition of a basement membrane formed by collagen IV and laminin on cells. We found two distinct differentiation patterns in the cells studied. Thus, we concluded that environment could induce phenotypic alterations on fibroblastic cells.

### H-018 MIGRATORY AND DIFFERENTIATION PATTERN OF FIBROBLASTIC CELLS CULTURED ON AND IN TYPE I COLLAGEN GELS,
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In recent years, there is a great interest in the development of biomaterials that could be utilized in the reposition of bone defects. Collagen matrix shows the advantage of being able to suffer alterations by chemical modifications, which can result in the improvement of its mechanical properties. In the present work, we aim the evaluation of the threedimensional matrix of native or anionic (submitted to alkaline treatment for 48h or 96h) collagen matrix, in the consolidation of osteoporosis bone fractures resulting from the gonadal hormonal alterations caused by oophorectomy, submitted or not to hormonal replacement. Macroscopic results showed the absence of pathological alterations on impled areas, suggesting that the materials were biocompatible. Microscopic analysis verified that areas that received native membrane had apparently a minor quantity of neoformed bone, compared to those areas bone fracture that received the anionic membranes. In oophorectomized animals that received anions membranes, we could observe a delay in bone regeneration, mainly in animals that did not receive hormonal replacement. Thus, we conclude that the anions membranes, especially with 96h of alkaline treatment, presented better results regarding to neoformation of bone tissue.

H-021 NADPH OXIDASE SYSTEM REGULATION IN HUMAN COLOSTRUM MACROPHAGES
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Phagocytes have a NADPH oxidase system, responsible for production of oxygen reactive intermediates. The aim of this work was to compare the NADPH oxidase activity and gp91-phox expression in colostrum macrophages, blood monocytes, and THP-1 cells. These cells were cultured with IFN-γ and TNF-α during 48 hours. The assays included the spontaneous and PMA-induced superoxide release and the relative expression of gp91-phox by RT-PCR. Results: 1) Spontaneous superoxide release: Colostrum macrophages cultured in basal conditions release more superoxide than blood monocytes. IFN-γ and TNF-α increased superoxide release of blood monocytes but not of colostrum macrophages. 2) PMA-induced superoxide release: IFN-γ and TNF-α increased superoxide release by colostrum macrophages, blood monocytes, and THP-1 cells. Colostrum macrophages and blood monocytes released more superoxide than THP-1 cells in all conditions. 3) gp91-phox expression: IFN-γ and TNF-α did not interfere in gp91-phox relative expression in colostrum macrophages and in blood monocytes, but caused a dramatic increase in THP-1 cells. After IFN-γ and TNF-α incubation, all studied cells presented the same relative level of gp91-phox expression. We conclude that other factors in macrophages up-regulate the NADPH oxidase activity, providing strong evidence that the NADPH oxidase activity in the myelomonocytic lineage is regulated only in part by gp91-phox gene expression.
Suported by: FAPESP
H-022 ISOLATION AND CHARACTERIZATION OF MESENCHYMAL CELLS FROM MURINE BONE MARROW
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Mesenchymal Stem Cells (MSCs) have the capability for differentiation into various lineages of mesenchymal tissues. When these cells are cultured, they adhere to culture plates, resemble fibroblasts and form colonies. In this study we isolated MSCs from murine bone marrow, cultivated using two different culture medium and characterized them with immunostaining with anti-vimentin antibody. We used six Balb-c mice with 2 week-old. To isolate murine MSCs, the bone marrow was flushed out of tibias and femurs. The cells were ressuspended in DMEM high glucose and DMEM Knockout supplemented with 10% FBS, to a final concentration of 6x10^6/ml, and kept in a humidified 5% CO₂ incubator at 37°C for 72 hours, when non-adherent cells were removed. The analysis of the cell growth curve and the presence of adherent fibroblast-like colonies was different with the two medium used: 72 hours of cell adherence in almost all the plate with DMEM-Knockout versus 10-20 days to get the same cellular concentration when the medium was DMEM. The cells gotten in culture of both protocols were positive for anti-vimentin, characterizing them as MSCs. The use of the two medium allowed to find out the superiority of the DMEM-Knockout that determined a lesser apoptotic index in the cells when compared with values obtained when they had grown in DMEM.
H-023 QUANTITY EVALUATION OF STEM CELLS CULTURED ON DIFFERENT TITANIUM SURFACES
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The implantology initiated a new technologie that solved cases complex, mainly those involving bone lost. The objective of this work was to elaborate a protocol of attainment of osteoblasts to evaluate the total behavior of the cells in presence of different surfaces of commercially pure titanium implantations and functional aspects through the determination of alkaline fosfatase and total proteins. The stem cells (SCs) had been collected of the peripheral blood and cultured in the medium: α-MEM, DMEM-Knockout and DMEM supplemented with hormones for differentiation in osteoblasts. They had been analyzed still: curve of cellular growth, cellular differentiation and test of adhesion. Before the culture an aliquot one it was analyzed by flow cytometry having used the following antibodies: anti-HLA-ClasseI, anti-HLA-ClasseII, CD34 and CD19. The titanium discs with smooth and rough surface had been placed in each plate of the SCs culture. The culture was observed during 28 days. The implantations of rough surface had better had performance in the adhesion of osteoblasts cells, pointing with respect to a positive effect in the formation of bone tissue in vitro. Differences in the levels of alkaline fosfatase and total proteins had not been observed. In this work it was possible to differentiate the SCs in osteoblasts and to better demonstrate to its cellular adhesion in titanium discs of rough surface.

H-024 MODULATION OF FIBER TYPES IN SOLEUS MUSCLE FROM RATS TREATED CHRONICALLY WITH N -NITRO-L-ARGININE METHYL ESTER (L-NAME).
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Nitrice oxide (NO) regulates glucose transport, contractile force and oxidative metabolism in skeletal muscle. In this work, we examined the changes in soleus muscle fiber types in rats treated chronically with L-NAME. Male Wistar rats (150 g at the start of the experiment) received L-NAME (20 mg/rat/day) in the drinking water for 2, 4 and 8 weeks (n=5 each). Control rats received tap water. The soleus muscle was excised, oriented in traganth gum and immediately frozen in isopentane cooled to −159°C in liquid N2 and stored at −74°C until fiber typing. Sections 12 μm thick were pre-incubated at pH 4.55, 4.25 and 10.5 to determine the number and percentage of each fiber type based on m-ATPase activity. Two weeks after the onset of treatment, there was a significant reduction (13.2%) in the number of type I fibers and a concomitant rise in the number of type IIa (75.7%) and IIb (40%) fibers. After 8 weeks of treatment with L-NAME, type I fibers remained unchanged, whereas type IIa and IIb fibers increased markedly by 202.4% and 39.6%, respectively. These results indicate that NO modulates the expression of fiber types in rat soleus muscle.

Financial support: CNPq, FAEP, FAPESP.

H-025 CALCULUS SIGNALING OF ENDOTHELIN, ACETYLCHOLINE AND GLUTAMATE RECEPTORS DURING NEURONAL DIFFERENTIATION OF P19 CARCINOMA CELLS
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We are using P19 teratocarcinoma embryonal cells as an in vitro model for studying neuronal differentiation. Upon addition of retinoic acid and plating in non-adherent tissue culture plates, P19 cells form embryonic bodies and following replating undergo neuronal differentiation. During this process, neuropeptide and neurotransmitter receptors are expressed and direct calcium signaling events that induce the expression of neuronal proteins and trigger neurite outgrowth and synapse formation. Differential expression of endothelin-B, acetylcholine, and NMDA glutamate receptors during neuronal differentiation has been reported previously. We show that stimulation of these receptors by their respective ligands endothelin-2, carbamoylcholine and NMDA results in transient increase in intracellular calcium concentration [Ca2+], in neuronal differentiation, whereas undifferentiated, embryonic P19 cells do not respond to application of these ligands.

Acknowledgments: H.U. is grateful for a financial support from FAPESP and CNPq. P.M.’s doctoral thesis are supported by FAPESP.

HH-001 RAFTS AND CHOLESTEROL ARE INVOLVED IN SKELETAL MUSCLE DIFFERENTIATION
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The formation of a skeletal muscle fiber begins with the withdraw of committed mononucleated precursors from the cell cycle. These myoblasts elongates while aligning to each other, guided by the recognition between their membranes. This step is followed by cell fusion and the formation of long and striated multinucleated myotubes, with a highly organized contractile apparatus. Since fusion involves the sarcomella, and particular membrane micro-domains have been described in muscle, we used the drug methyl-β-cyclodextrin (MCD, 2 mM) in primary cultured chick skeletal muscle cells, to deplete membrane cholesterol and investigate its the role during myogenesis. MCD enhanced myoblast fusion and induced the formation of large multinucleated myotubes with nuclei centrally clustered and not aligned in the cell periphery. MCD-myotubes were perfectly striated, as indicated by sarcomeric β-actinin immunofluorescence staining, and microtubules and desmin filaments distribution was not altered. Pre-fusion MCD-treated myoblasts form large aggregates, with cadherin and β-catenin accumulated in cell-cell adhesion contacts. We also showed that the membrane microdomain marker GM1 was not visualized as clusters in the membrane of MCD-treated myoblasts. Our data demonstrates that cholesterol is involved in the early steps of skeletal muscle differentiation.
HH-002 INFLUENCE OF LONG TERM GLICEMIC TREATMENT ON SALIVARY GLANDS OF AUTOIMMUNE NOD MICE
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Diabetes mellitus is one of the major world health problems. It’s considered the 4th causa mortis in Brazil. The diabetes mellitus affects salivary glands changing their morphology and salivary production, which are important to oral health. The aim of this study was to analyze the effects of long-term glicemic control on organelles involved in secretory process in autoimmune non-obese-diabetic mice. Moreover, this work will attempt to associate the cellular structure to proliferative and degenerative processes. Fifty-Four female mice were divided in three groups: Diabetic Nod (I), Insulin Treated Diabetic (II) and Nondiabetic Balb/c (III). The group II daily received 0.2 ml/100g of insulin the groups III and I received the similar doses of saline solution following the same experimental procedure. Samples from salivary glands were analyzed by histochemistry and scanning and transmission microscopies. Group I showed atypical and atrophic cells, disorganization of the membrane system with dilatation of organelles involved in secretory process, extra-cellular matrix compounds increasing and occurrence of inflammatory process. These alterations were minimized in insulin treated diabetic group. It was concluded that not only in diabetic mice but also in insulin treated diabetic mice there was damage of cellular integrity changing secretory process and leading to glandular pathologies.

HH-003 ASYMMETRICAL DIFFERENTIATION OF THE GILL EPITHELIUM IN THE RED FRESHWATER CRAB, Dilocarcinus pagei.
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Freshwater crustaceans maintain a strong ionic gradient between the surrounding medium and their hemolymph due to active ion pumping by the gills, and by impeding osmotic water entry. We examined the structural basis for ion transport in the gills of D. pagei by routine microscopy. The eight gill pairs are subdivided into anterior and posterior types. The latter exhibit a central, osmophilic region that corresponds to a thick epithelium, absent from the anterior gills, which display a typical, thin, respiratory-like epithelium with little amplification in membrane area. The posterior gills exhibit asymmetrical epithelia in the osmophilic area: the proximal epithelium consists of a thick cell layer, which exhibits basal mitochondrial pumps and membrane invaginations, differentiated apical membrane junctions, and an apical membrane amplified by evaginations. The thin, distal epithelium, like that of the anterior gills, exhibit few mitochondrial pumps, and few apical cell membrane evaginations. The apical evaginations measure 0.78 ± 0.08 µm [N=4] in height with 6.3 ± 0.8 [N=4] evaginations/µm² on the proximal epithelium, and 0.44 ± 0.08 µm [N=4] in height, with 1.2 ± 0.3 [N=4] evaginations/µm² on the distal side. This epithelial differentiation in the posterior gills may reflect a structural adaptation to the freshwater habitat. Apparently, the invasion of freshwater by the Brachyura stems from a gradual shift in respiratory to transport function by the posterior gill epithelium.

FAPESP #01/08730-0

I-001 APOPTOTIC INDEX IN THE EPITHELIAL RESTS OF MALASEZ OF YOUNG AND ADULT RAT MOLARS
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Epithelial rests of Malassez are clusters of cells derived from Hertwig’s root sheath that remain in the periodontal ligament throughout life. It has been shown, however, that the number of Malassez’s rests decreases with aging. We felt, therefore, that it was necessary to determine whether reduction in the number cells of Malassez’s rests could be due to apoptosis. Wistar rats aged 29, 45 and 120 days were sacrificed and fragments containing maxillary molars were removed and fixed in formaldehyde, decalcified in EDTA, and embedded in paraﬃn. Sections were stained with hematoxylin/eosin and the TUNEL method, for detection of apoptosis. Specimens were also processed for transmission electron microscopy. Epithelial rests of Malassez containing round/ovoid dense structures strongly stained by hematoxylin were found in all animals examined. The TUNEL method revealed positive structures, mainly in the nuclei of the cells of Malassez’s rests. Some tonofilaments-rich epithelial cells contained nuclei with condensed peripheral chromatin and a shrunken cytoplasm with intact organelles, typical of apoptosis. Moreover, round/ovoid electron-opaque structures, presumably apoptotic bodies, were also observed; some of them appeared to be in the process of being engulfed by neighbouring epithelial cells. Thus, our results may be interpreted as indicating that apoptosis may play a role in the decrease and/or turnover of the epithelial cells of Malassez’s rests in the developing and in the adult periodontium.

Supported by FUNDUNESP (00429/04)

I-002 APOPTOTIC INDEX IN SEASONAL SPERMATOGENESIS OF BULLFROG
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In bullfrogs, the seminiferous lobules have maximal diameters during winter and decrease significantly during summer when germ cells in all phases of spermatogenesis and spermiogenesis are often observed. Among several factors controlling normal spermatogenesis, programmed cell death (apoptosis) has been related to the control of spermatogenic cycle. Thus, we proposed to compare the apoptotic index between breeding (summer) and non-breeding (winter) periods in bullfrogs. For this purpose, twenty adult male frogs were divided into two groups: winter (WG) and summer (SG), containing ten animals each. The animals were anaesthetized and the testes were perfusion-fixed with Bouin’s liquid and embedded in paraffin. For the ultrastructural analysis of apoptotic germ cells, the frogs were perfusion-fixed with glutaraldehyde/formaldehyde and Araldite-embedded. The paraffin sections were submitted to the TUNEL assay and the numerical density of TUNEL-positive germ cells (apoptotic index) was obtained by an Image Analysis System. TUNEL-positive germ cells were observed in both groups and the ultrastructural aspects of degenerating germ cells were compatible with characteristics of apoptosis. The apoptotic index increased significantly in the frogs of SG in comparison to WG. Thus, we suggest that apoptosis is one of the control mechanisms during period of active spermatogenesis, when additional survival factors are intensely required.

Supported by FUNDUNESP (00435/04)
I-004 ANTIPROLIFERATIVE EFFECTS OF A 7-KETOCHOLESTEROL- CONTAINING LIPID EMULSION (LDE/7KC)

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Oxysterols, oxygenated derivatives of cholesterol formed either by autooxidation or enzymatically, play a role in several pathophysiological processes, including atherosclerosis. Among oxysterols, 7-ketocholesterol (7KC) induces cell death. We propose that 7KC cytotoxicity could be used therapeutically. To approach that we have delivered 7KC to B16F10 melanoma cells, using the LDE lipid emulsion (LDE/7KC). While 7KC alone was highly cytotoxic to melanoma cells, its emulsification with LDE was shown to be cytostatic, leading to a polyploid population. Our results indicated that besides serving as a delivery particle, LDE attenuated the cytotoxicity of 7KC.

Supported by FAPESP and CAPES.

I-005 SERRATAMOLIDE (AT514), A NEW ANTICANCER CYCLIC DEPSIPEPTIDE. APOPTOSIS CHARACTERIZATION


Serratamolide (AT514) is a new molecule leader from bacteria origin with proved anticancer properties. Cytotoxic studies performed in 25 human cancer cell lines from hematopoietic, skin, breast, lung, gastrointestinal, prostate and neural origin, have established AT514 as an interesting cytotoxic agent which showed a great-major sensitivity for leukemia and lymphoma cells (IC50: 5 µM and 7.5 µM respectively), skin carcinoma (IC50: 7.5 µM) and breast cancer (IC50: 8 µM). In studies performed in non-cancer cell lines, AT514 showed non-significant toxicity. AT514 induces cell death by apoptosis, as confirmed by morphological and biochemical analysis. Apoptotic bodies were observed by Hoechst 33342 staining. We have characterized that this process induces caspase activation (2, 3, 8 and 9) and PARP cleavage. DNA laddering was also observed in agarose gel electrophoresis. In addition, we have demonstrated that the effect of AT514 is p53 independent, and induces a decrease in the Bcl-2/Bax ratio. We are currently analyzing the effect of AT514 in breast cancer xenografted tumors. In addition, pharmacogenomic experiments using macroarrays and other singular experiments have also being performed to identify the AT514 molecular target. An international PCT application (PCT/ES 03/00489) protects the use of AT514 for cancer treatment.

Acknowledgements: Support: CIDEM (Generalitat de Catalunya), Fundació Bosch I Gimpera (ref.301888).

I-006 ULTRASTRUCTURAL FEATURES OF DEGENERATING SALIVARY GLANDS IN THE TICK Boophilus microplus (CANESTRINI, 1887) (ACARI: IXODIDAE)

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Boophilus microplus, known as the southern cattle tick, belongs to the family Ixodidae and is considered important vector of microorganisms. The female salivary glands are composed by approximately 400 acini, classified into three types. Immediately after attachment, there is an enormous increase in size of these organs following by degeneration after engorgement. This work studied the ultrastructural characteristics of the salivary glands of semi-engorged females of Boophilus microplus, in order to obtain a better understanding of the biology of this tick aiming at an alternative control strategy. TEM techniques evidenced details of the degenerative process: amorphous and/or fragmented nuclei with intense vacuolization; masses of condensed chromatin next to the internal surface of the nuclear envelope; nuclear membrane appeared retracted and folded or even broken with structures that resembled bubbles; few organelles such as rough endoplasmic reticulum, degenerating mitochondria, lysosomes, and lipid inclusions; cytoplasmic vacuoles with contents of weak electron-density or even degenerating organelles; retraction of plasma membrane and infoldings towards the interior of the cell; rounded shape nucleoli strongly electron-dense containing a more lucid region with a granular appearance. The study showed that the degenerative process of the salivary glands of Boophilus microplus females began before completing the engorgement, opposite to observations done in other tick species.

Supported by Fapesp – Process number 02/08393-7.
The apoptosis is a genetically mediated suicide mechanism, associated with many distinctive morphological changes. In insect ovary the apoptosis process has been mostly studied in the Drosophila vitellarium, where the nurse cell death has been reported. However, Diatraea saccharalis is considered the most destructive pest attacking sugarcane in several Latin American countries. This investigation aims to describe the morphological features of nurse cells death in the vitellarium of the sugarcane borer. Ovarioles of adult insects were prepared for light microscopy, submitted to HE and Feulgen staining methods. Also the ovarioles were conventionally prepared for transmission electron microscopy. Many fragments of degenerated cells, visualized as masses with nuclear and cytoplasmic condensation were clearly revealed by the HE and Feulgen staining, and interpreted as apoptotic bodies. The electron microscopic analysis showed that the nurse cell initially shrinks and loses contact with the neighboring cells. The cells gradually condense and the chromatin forms dense clumps. Finally, the cells start to dismantle themselves, by pinching off portions of cytoplasm and nucleus, as apoptotic bodies. According to the literature, cells that are no longer necessary to the development of the organism are selectively deleted by apoptosis process. In this way, we believe that nurse cells are cleared from Diatraea saccharalis follicle by apoptosis.

Supported by FAPESP(01/10849-6).

**I-008 DIFFERENTIAL EFFECT OF RIBOFLAVIN ON MITOGEN-ACTIVATED PROTEIN KINASES AND PHOSPHATASES FROM HL60 CELLS**

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Flavins are known as versatile compounds which function as electrophiles and nucleophiles, forming covalent intermediates and substrates often involved in catalysis. Flavins are thought to contribute to oxidative stress through their ability to produce superoxide. Nevertheless, they are also frequently involved in hydroperoxides reduction and recent reports have been linking this class of compounds to programmed cell death. In this work we demonstrated that irradiated riboflavin caused expressive HL60 cells death as well as differential effect on mitogen-activated protein kinases and phosphatases activities. Riboflavin was irradiated with UV light for 30 minutes and subsequently used to treat HL60 cells for 24h. Western blotting analysis showed activation of JNK and protein tyrosine phosphatases (PTPs) by 50µM riboflavin. On the other hand, p38 and p42/44 activities remained unchanged. Interestingly, protein serine/threonine phosphatases were inhibited by this flavin. Apoptosis was assessed by caspase 3 activation. Our results demonstrated that apoptosis induction of HL60 cells by riboflavin is dependent on JNK and PTPs activities. Moreover, our findings suggest that riboflavin could be a candidate to coadjuvant in photodynamic therapy of tumors. In addition, the mechanism by which riboflavin acts on HL60 cells showed to be independent on oxidative stress induction, since PTPs were activated.

**I-009 EVALUATION OF TUMORAL PROGRESSION IN ANIMALS TREATED WITH THE LECTIN KM+**

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Lectins are proteins possessing at least one domain that recognizes and binds specific sugars and play a fundamental role in innate immunity. KM+, a D-mannose-binding plant lectin, is known to be neutrophil migration inducer and to activate IL-12 released by macrophage. The present study was undertaken to evaluate the progression of Sarcoma-180 cells in mice treated with the lectin KM+. Tests were performed by subcutaneous inoculation of ascite cells (1x10^5/cells) into the right groin of animals. KM+ (50µg/ml) was injected intraperitoneally 24h after tumor implantation. Control animals were inoculated with saline solution. All mice were sacrificed after 7, 14 and 21 days of treatment. The tumors were processed by histopathology analysis. Areas with cells in mitosis were observed in the tumors obtained from the control animals after 7, 14 and 21 days. On the other hand, the tumors of the animals treated with KM+ revealed areas of cells in necrosis. The tumors of the control animals obtained 21 days after the inoculation shown to be bigger when compared to the tumors obtained from the animals treated with KM+. Tumors of the animals treated with KM+ showed mostly necrosis areas while the tumors of the control animals showed smaller areas of necrosis surrounded by cells in mitosis. These results suggest that KM+ play a role in the antitumoral activity.

**I-010 ANTITUMORAL ACTIVITY OF CRUDE EXTRACTS AND FRACTIONS OF Didymopanax vinosum.**

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Most of the chemotherapeutic agents present an “antiproliferative” activity rather than an “anticancer” one. For this reason, there is a growing need for less toxicity and more efficacy and selectivity in antineoplastic drugs. For the last decades, several plant-derived compounds have been successfully used in the treatment of cancer. Due to the great diversity of Brazilian ecosystems, the Cerrado is a rich source of new drugs for many diseases including cancer. In this study, the species Didymopanax vinosum was selected through the in vitro antiproliferative assay using nine human tumor cell lines: leukemia (K.562), prostate (PC0.3), kidney (C786.0), ovary (OVCAR), melanoma (UACC-62), colon (HT.29), lung (NCI.460), breast (MCF.7) and multi-drug resistant breast cells (NCI/ADR). The crude hydroalcoholic extract (EBH) obtained from the leaves of D. vinosum was achieved by mechanic extraction with ethanol 70%, this extract was partitioned using H_2O / ethil acetate giving an aqeous (F1) and an organic (F2) fractions. F1 presented the most significant antiproliferative activity which seems to be related to the presence of glycosylated flavonoids, revealed by its chemical hydrolysis.
I-011 ANTI-TUMORAL ACTIVITY OF GOMESIN, AN ANTI-MICROBIAL PEPTIDE ISOLATED FROM THE SPIDER Acanthoscurria gomesiana
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Melanoma treatment is a great challenge in Medicine. A low percentage of patients respond to usual chemotherapy, several of them develop drug resistance and a low survival rate is expected. Alternative melanoma treatments are actively being pursued. Recently, it has been described anti-tumoral activity for some cationic antimicrobial peptides, components of innate defense of many vertebrates and invertebrates. Gomesin, isolated from the tarantula spider Acanthoscurria gomesiana, has 18 aminoacids and four cysteine residues forming two disulfide bridges, adopting a -hairpin structure. This peptide has 18 aminoacids and four cysteine residues forming two disulfide bridges, adopting a -hairpin structure. This peptide shows anti-bacterial, -fungicidal and -protozoan activity. Tumoral cells may be sensitive to antimicrobial peptides in vitro, including multi-drug resistant cell lines. Promising results were also obtained after in vivo treatment of tumor-bearing mice. Murine and human melanoma cell lines were treated in vitro with recombinant gomesin, and our results showed that this molecule abolished completely cell proliferation at the concentration of 10 M. In order to establish the relationship between structure and function of gomesin, many isoforms of gomesin were synthesized and tested in vitro: D-gomesin (D aminoacids); Linear-Ser (Cys-Ser substitution); Linear-Thr (insertion of Thr, to mimic the secondary structure of the peptide); Monocyte (one disulfide bridge). Our data showed that disulfide bridges are important for anti-tumoral activity of gomesin.

I-012 B- CYCLODEXTRIN COMPLEXED WITH DEHYDROCROTONIN INDUCE APOTOSIS IN HL60 CELLS AS SEEN BY FLOW CYTOMETRY.
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Compounds derived from plants are currently being investigated for their ability to regulate apoptosis due to its importance in cancer therapy. Recently, our group demonstrated that dehydrocrotonin (DHC), extracted from Croton cajucara, shows cytotoxic effects on human leukemia cells (HL60). To improve the solubility and biological activity, DHC/B-cyclodextrin complex (DHC/B-CD) was prepared. In this work we observed apoptosis and terminal differentiation induction on HL60 cells by flow cytometry and NBT reduction, respectively. We also analysed the occurrence of the membrane mitochondrial permeability transition. HL60 cells were incubated with DHC 250µM and B-CD/DHC 500µM, concentrations around IC50 values and different treatment periods. DHC and B/CD/DHC induced after 72 h of treatment 78% and 92% of apoptosis, respectively. The NBT reduction was increased in almost 100% after cell treatment with 500µM of the complexed form. We also verified an increase of mitochondrial swelling of 15% and 35% after 72 h of treatment with B/CD/DHC and DHC (both to 250 µM), respectively. In conclusion, our results showed that DHC and its B-CD complex present efficient activity against leukemic cells, inducing apoptosis and terminal differentiation.
Supported by FAPESP, CAPES and CNPq.

I-014 CYTOTOXIC ACTION OF Bothrops alternatus SNAKE VENOM IN CULTURED MDCK CELLS
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Bothrops snake venoms are cytotoxic to a variety of cell types in vitro and in vivo. Most of these effects are mediated by enzymes present in the venom, particularly L-amino acid oxidase, metalloproteinases and phospholipase A2. In this work, we investigated the cytotoxicity of Bothrops alternatus venom in cultured Madin-Darby canine kidney (MDCK) cells. Incubation with venom (10 and 100 µg/ml) significantly (p<0.05) decreased the cellular uptake of neutral red dye by 25.6±7% (n=12; mean±SD) and 43.4±12.2% (n=12), respectively, after 1 h, and by 65.3±7.2% (n=12) and 85.3±10% (n=12) after 3 h. The transepithelial electrical resistance (R T) was measured to assess whether the venom altered the epithelial barrier function. Venom (100 g/ml) decreased the R T across MDCK monolayers from 319.1±52.3 to 52.6±14.5 /cm² (n=3) after 2 h, and after 4 h the R T were zero. Staining with rhodamine-conjugated phalloidin revealed disarray of the cytoskeleton that involved the stress fibers at the basal cell surface and the focal adhesion-associated F-actin in cell-to-matrix contact region. These effects were particularly marked at a venom concentration of 100 µg/ml. These results indicate that B. alternatus venom is cytotoxic to cultured MDCK cells and could partly explain the nephrotoxicity seen after envenoming by this species.
Financial support: FAPESP
The hepatopancreas is the digestive gland in crustaceans. It is sensitive to toxic agents in the diet, and can be used as an aquatic indicator of contamination. This study attempted to analyze the effects of domestic detergents in this digestive gland of adults of freshwater prawns Macrobrachium olfersi. Prawns were maintained in two tanks (21.5 liters deionized water), a experimental (tank I) and a control (tank II). Three doses of 0.25%, 1.25%, 1.5% and 5% ethanol were added to the tank I on the 14th, 12th and 39th days, and the prawns were monitored on 140th exposure day. Females from the two tanks were cooled and the hepatopancreas were dissected and fixed in Bouin (24h), embedded in parafin, sectioned (10µm) and stained with HE and Hoescht. Analyses of secretory region of hepatopancreas (middle region) submitted to the tank II showed tubules constituted by B-cells with a well evident vacuolar apparatus. The animals exposed to the tank I showed a decrease in the number of B-cells and significant reduction of vacuolar apparatus. There were no apparent alterations in apoptotic pattern in tubule epithelial cells in both experimental conditions. Our data indicate that studies of the distal and proximal regions of the hepatopancreas are necessary to better understand the effect of domestic detergents in this species.

When an endodontic treatment fails and, a new intervention is needed, and solvents, such as, orange oil, eucalyptol, chloroform are used to remove the original filling (gutta-percha) from the dental roots. Our objective was the analysis of the potential cytotoxic effects of these solvents to macrophages. Initially 5% ethanol was added to the solvents to improve their effects in macrophages. Peritoneal macrophages from Swiss mice were incubated at 37°C/30 min with the solvents. Cell counting, after incubation with Trypan blue showed that at 45.5%, the solvents led to macrophage death. Transmission electron microscopy of the cells treated with 23.8% of the three solvents showed dilatation of both the nuclear envelope and endoplasmic reticulum, mitochondrial alterations, i.e. loss of the matrix’s electron density and disappearance of the inner cristae, and extensive cytoplasmic vacuolization. We conclude that these solvents are toxic, and their leakage could lead to damage of the oral microenvironment and to envisage the study of new and efficient solvents.

This Work Was Financed By FAPERJ – Number Of Process: E-26171593-02

I-015 HEPATOPANCREAS CELL MORPHOLOGY of Macrobrachium olfersi (Crustacea, Palaemonidae) TREATED WITH DOMESTIC DETERGENT.
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Cryopreservation of embryos is important for application of reproductive biotechnology, but causes cell injuries. Haematoxylin – Eosin (HE) stain may be useful to biotechnologies by allowing the differentiation of cell death types under optic and fluorescence microscopies. Our goal was to compare different trials for apoptosis detection and characterize and quantify the embryonic cell death, caused by cryopreservation. After thawing, embryos were evaluated regarding their quality, fixed and stained for Hoechst and Propidium Iodide (H/PI) to assess integrity of cell membrane; HE for morphologic analyses and cytoplasm fluorescence and electron microscopy (MET) for ultrastructural characteristics of apoptosis. Vitrification group showed more dead cells (69,7%) detected by H/PI trial, than slow-freezing (48,4%) and fresh (13,8%) groups (p<0,05 Wilcoxon’s test). Morphologic evaluation by HE revealed that vitrification and slow-freezing induced picnosis, nuclear retraction and chromatin condensation. Mitotic pattern was observed in the fresh and slow freezing group, but not at the vitrification group. Cytoplasm scarce and degenerated cells at vitrification indicated oncosis, while in the slow freezing group, cytoplasm condensation and eosinophilic structures, indicated apoptosis. The ultrastructure morphology visualized at MET, confirmed HE observations. Staining with HE showed being a efficient trial in detecting oncosis and apoptosis in cryopreserved embryos. By these data, vitrification caused more cellular injuries, with oncosis predomination and consequently low survival rate.
I-019 DLC2, BMF AND MYOSIN-Va PROTEINS ON MELANOMA APOPTOSIS

Myosin-Va sequesters the proapoptotic Bmf factor to the actin cytoskeleton through its interaction with dynein light chain, DLC2, preventing its binding to the prosurvival Bcl2 and consequently triggering apoptosis in lymphoblastic cells. To study this pathway in melanoma cell lines, we transiently transfected B16-F10 cells with a small segment (iMVa) from myosin-Va that contains part of the characterized region known to be responsible for interaction with DLC2. The number of cells expressing iMVa GFP decreased 90% 72h post transfection to be responsible for interaction with DLC2. The number of cells expressing iMVa GFP increased 12% within the same period, demonstrating that Bcl2 GFP inhibits the iMVa GFP-induced death. The number of cells expressing Bcl2 GFP increased 2% confirming its known role in the inhibition of cellular proliferation. Apaf-1 detection, reduction of the mitochondrial membrane potential, and apoptotic morphological characteristics in iMVa GFP transfected B16-F10 cells, as well as the demonstration that bacterially expressed iMVa GST is sufficient for in vitro interaction with DLC2, suggest that iMVa GST affects cellular viability via its role in competing for DLC2/Bmf, releasing Bmf from its anchorage sites. DLC2 GFP transfected cells showed exponential growth during the 72h interval and also inhibited the iMVa GFP-induced death. Preliminary results demonstrate that DLC2 and Apaf-1 are differentially expressed among different melanoma cell lines. Expression profile and functional assays for other proteins herein studied are being pursued.

Financial Support: FAPESP, CNPq, CAPES, FAPEA

I-020 APOPTOSIS AND REDUCTION IN THE NUMBER OF OSTEOCLASTS IN ESTROGEN-TREATED RATS

Although it is generally accepted that estrogen inhibits bone resorption, the mechanism by which estrogen acts upon skeletal tissues remains unclear. We have therefore investigated the possibility that estrogen may promote reduction of osteoclasts by apoptosis and thereby prevent bone destruction. For this purpose, ten 22-day-old female rats were divided in control and experimental groups. In the experimental group, the rats received daily 0.125 mg/100g of estrogen during 6 days. After 24h, the rats were sacrificed and fragments containing the maxillary molars were removed, fixed in formaldehyde, decalcified and paraffin embedded. The sections were stained with hematoxylin/eosin for morphological observation. The TRAP method, a marker of osteoclasts, and TUNEL method for detection of apoptosis were also used. The bone surface was measured using an imaging analysis system (Leica Qwin) and the number of osteoclasts/mm of bone surface was obtained. Numerous osteoclasts showing conspicuous TRAP activity were observed in the control group. In the experimental group, some osteoclasts showed condensed nuclear chromatin and a shrunken cytoplasm exhibiting, occasionally, weak positivity to the TRAP method. In addition, the TUNEL method revealed osteoclasts containing TUNEL-positive nuclei. The number of osteoclasts/mm of bone surface decreases significantly in the experimental group. Thus, our results appear to suggest that estrogen reduces alveolar bone resorption/remodeling possibly by promoting apoptosis of osteoclasts.

Financial Support: FAPESP, CNPq, CAPES, FAPEA

I-021 EXPRESSION AND FUNCTIONS OF MYOSIN-Va IN MALIGNANT MELANOMA

Establishment of a malignant melanoma involves progression through radial growth phase (RGP), vertical growth phase (VGP) and metastasis. Anoikis, which is considered one of the barriers to metastasis, is a specific type of apoptosis induced by loss of cell attachment. In healthy cells myosin-Va, through its association with dynein light chain 2, sequesters a pro-apoptotic protein, Bmf. Under loss of cell attachment, Bmf is released allowing it to bind to the pro-survival Bcl-2 proteins, leading to anoikis. In the present study we showed an increase of myosin-Va mRNA and protein levels in two metastatic cell lines when compared with their parental VGP cell lines and also in RGP and VGP cell lines. Searching for an association of high expression of myosin-Va and possible cellular characteristics involved in tumor progression, we found a positive correlation with the proliferation rate, resistance to anoikis, and the ability of facilitated attach/detachment. Constructs of the full-length myosin-Va and its globular tail domain fused with EGFP were also obtained. To confirm the relevance of the correlation between myosin-Va expression levels and the functional aspects herein studied, we are presently overexpressing different structural domains of myosin-Va to determine their ability of interfering with cellular phenotypes, such as apoptosis, cell proliferation and adhesion.

Financial Support: FAPESP, CNPq, CAPES, FAPEA

I-022 EFFECTS OF EUTERPÉ OLERACEA (ASSAI) EXTRACT ON THE CULTURED HUMAN PROSTATIC SMOOTH MUSCLE CELLS

Benign prostatic hyperplasia (BPH) is a common affection of the elderly man. It is promoted by hypertrophy and hiperplasia of the prostatic smooth muscle cells (SMC), causing urethral obstruction. α-adrenergic antagonist treatments are currently in use to relief BPH symptoms. Phytosteroids are possible alternatives, and the saw palmetto oil is commercially available. The oil from E. oleracea pulp has been suggested as a regional substitute for saw palmetto. In this work we have tested different concentrations of an oil extract containing 0.3mg/mL campesterol, 0.95 mg/mL stigmasterol and 2.9 mg/mL sitosterol on cultured human prostatic cells. We have observed that cells treated with the oil accumulated lipid droplets in the cytoplasm and die in a time and dose dependent manner. The morphological aspects revealed nuclear picnosis and fragmentation, and apoptotic bodies, before detachment. We have also observed that some cells have larger nuclei, which also showed aspects of fragmentation. Lower dosages showed no effect on cell survival. Further studies are necessary to identify the death pathway induced by the oil and to verify the effects of lower (close to physiological dose reached after oil intake). These responses differ between the cultures obtained from two different patients, one of them being much more resistant to the treatment. Given the differences between the two cell cultures, we may anticipate differential response to assai oil usage.

Financial Support: FAPESP(02/10171-2)/FUNDUNESP(293/03)
**I-023 MORPHOLOGICAL ALTERATIONS IN CHO-K1 CELLS AFTER TREATMENT WITH TESTOSTERONE PROPIONATE**

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The Anabolic-Androgenic Steroids (AAS) include testosterone in its natural form along with its innumerable synthetic derivatives, such as the testosterone propionate. Steroid hormones are capable of producing rapid effects (within 2 minutes) in various cell types. These rapid responses are not compatible with the classic mechanism proposed for these hormones, which involve the binding to intracellular receptors, processes of transcription and protein synthesis. This work had the objective of studying the morphological alterations in nucleus and endoplasmic reticulum through fluorescence microscopy. The cell line CHO-K1 was incubated with the hormone at different concentrations and periods. Photomicrographs reveal that cells incubated with 50 mM of hormone for periods of 6 and 24 hours showed vesicles in endoplasmic reticulum and loss of network arrangement. The cells incubated with 100 mM for 6 hours showed cell stress, nuclear fragmentation and complete disappearance of the reticulum. After 24 hours, there was also loss of cell adhesion and the membrane integrity was compromised. Through these results we can conclude that 100 mM of the testosterone propionate is lethal for the cell line used in this experiment.

**I-024 PHOTODYNAMIC THERAPY AND APOPTOSIS**

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The Photodynamic Therapy (PDT) is a new therapy in cancer treatment that involves administration of a photosensitizer agent which binds to tumor cells, followed by an activation by a specific length of light. Studies suggest the involvement of multiple ways during cellular death mediated by PDT. A complete knowledge of the mechanisms involved in PDT can lead to a better therapeutic effectiveness. Both in vitro and in vivo studies have shown the involvement of an apoptotic process during the cellular death mediated by PDT. Hep-2 cells were incubated with the photosensitizers Chloroaluminum Phthalocyanine Tetrasulfonate (AlPcS4) 10 µM/mL and Chloroaluminum Phthalocyanine Tetrasulfonate (AlPcS4) 10 µM/mL, for 1 hour with each photosensitizer separately and submitted to irradiation with a diode laser (λ = 670 nm, 4.5 J/cm², 45 mW/cm²). After irradiation, the cells were incubated for 30 minutes, 24 and 48 hours. The following aspects had been evaluated: the cytotoxic effect by MTT test, presence of caspases 3 and 6 and ultrastructure. The results demonstrate that the submitted cells the PDT, suffer to severe damages in relation its internal structures and the type of cellular death. The AlPcS4 presented a lethal dose of 90% and liposomal CIAIPc presents a low power of photosensitization, however when encapsulated in liposomes to one low concentration it has this optimized potential, front to other phthalocyanines.

**I-025 VIOLACEIN CYTOTOXICITY PROCEEDS VIA APOPTOSIS AND IS MEDIATED BY INDUCTION OF CASPASE-3 AND MITOCHONDRIAL PERMEABILITY TRANSITION IN A MURINE RENAL CANCER CELL LINE (RENA)

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Drugs that can selectively sensitize cancer cells to apoptosis induction are crucial and we have previously determined the ability of violacein to induce apoptosis in several cell lines. The murine renal tumor model (RENA) mimics the tumor progression characteristically observed for human renal cell carcinoma. This study finds the effects involved in this type of cell death in RENCA tumor model. Since apoptosis is a complex phenomenon during which several events occur, the role of mitochondria was studied in cultures of RENCA cells treated with violacein (0.2 - 0.6 μmol/L) for 4 - 72 h. Changes in mitochondrial potential (ΔΨm) were analysed by flow cytometry in samples stained with JC-1, a ΔΨm sensitive probe. Caspase-3 intracellular activity was determined by flow cytometry using the PhiPhiLux® TM1D2 kit (Calbiochem). A dose-dependent decrease in ΔΨm was observed within 4 - 12 h of treatment, indicating mitochondria depolarization. At these same conditions, apoptosis induction was confirmed by the increasing percentage of cells presenting caspase-3 activation. Our data suggest that a mitochondrial-dependent apoptotic pathway is involved in violacein induction of apoptosis in RENCA cells. Supported by FAPESP.

**I-026 MORPHOLOGICAL ALTERATIONS PRODUCED BY TRITERPENE ACTIVE COMPOUND (β, 3β dihydroxy-urs-12-ene-28-oic), ISOLATED FROM Gaylussacia brasiliensis (Spreng.) Meissn. IN OVCAR-03 CELL LINE**

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Gaylussacia brasiliensis (Spreng.) Meissn. (Ericaceae) is used in Brazilian popular medicine. The triterpene active compound (β, 3β dihydroxy-urs-12-ene-28-oic) was evaluated on sulforhodamine B antiproliferative assay in four concentrations (0.25; 2.5; 25 and 250 μg/mL), for 48 h, using eight human cancer cell lines: MCF-7 (breast), NCI-ADR-RES (breast expressing multi-drug resistance phenotype), NCI-460 (lung), UACC-62 (melanoma), 786-0 (renal), OVCAR-03 (ovarian), PC-03 (prostate) and HT-29 (colon). This compound showed concentration-dependent activity and selectivity for 786-0 and MCF-7 cell lines. The aim of this work was to assess the in vitro morphological alterations produced by this compound in OVCAR-03 cell line, after 48 hours exposition by optical and scanning electron microscopy, using non treated cells as negative control. The morphological analysis revealed mitotic aberration, such as multi-nucleation, giant cells and micromucleus, in addition to nuclear fragmentation. Moreover, by scanning electron microscopy, the cells treated by triterpene showed membrane blebbing and pits on their surface, which are associated with the mechanisms of cell death by apoptosis. These results suggest that the triterpene cytotoxicity may induce apoptosis in OVCAR-03 cells.
I-027 STUDY OF THE IMMUNOCYTOCHEMICAL AND MORPHOLOGICAL ALTERATIONS INDUCED BY Talsia esculenta LECTIN (TEL) IN CULTURED MAMMALIAN CELL
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In this study, the immunocytochemical and morphological examination was performed in Vero cell culture after 2.5 and 5 h TEL treatment (300 µg/ml). Confluent monolayers of untreated and TEL treated Vero cells were subjected to scanning electron microscopy and provided comparisons of size, shape and surface characteristics. The fibronectin distribution pattern was observed by using the indirect immunofluorescence method. DNA fragmentation was investigated in situ by TUNEL assay using an In situ Apoptosis Detection Kit. The results showed that TEL treated Vero cells exhibited marked cellular condensation in comparison to control and loosening of the cellular junctions. Additionally, formation of apoptotic bodies was observed. Immunodetection demonstrated fibronectin accumulation in treated cells while control cells presented diffused fibronectin distribution. The cleavage of nuclear DNA occurred in TEL treated Vero cells which showed intense and non-uniform labeling; some untreated cells showed moderate labeling. These results showed that TEL induces morphological and intracellular changes in Vero cells which are compatible with programmed cell death.

Supported by: CNPq, FUNDECT and FAPESP

I-028 APOPTOTIC-LIKE PROCESSES IN Trichomonas vaginalis (PROTOZOAN THAT LACKS MITOCHONDRIA): AN ULTRASTRUCTURAL AND SEM-EDAX MICROANALYSIS
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We have investigated morphological changes and elemental composition in Trichomonas vaginalis, an anaerobe unicellular parasite devoid of mitochondria. T. vaginalis is characterized by the presence of hydrogenosomes. At TEM, we have detected morphological features that are typical of cell-death, such as a visible decrease in Trichomonas volume, cell blebbing, cytoplasm vacuolation, and nuclear fragmentation. An important change was the empty hydrogenosomes. The spatial resolution of electron probe X-ray microanalysis (SEM-EDAX) is an analytical technique that combines simultaneous compositional and morphological observation. At SEM-EDAX, energy dispersive spectra show a high peak for oxygen, the relative semi-quantitative weight % (k) for this element was 61; for Na+ 15 % and it was larger than K+, 6 %. Chloride anion was present in T. vaginalis, with 11 %. Ca2+ was not significant with 1.9 %; but we have demonstrated a plasma membrane calcium pump (PMCA), in this parasite, by indirect immunofluorescence. In conclusion, the massive intracellular accumulation of oxygen and specially the influx of Na+ lead to cell death. These data are discussed in relation to apoptotic-like processes in Trichomonas vaginalis an anaerobe flagellate devoid of mitochondria.

I-029 IONS, ELECTROCYTE VOLUME AND OXIDATIVE STRESS: AN ELECTRON PROBE X-RAY MICROANALYSIS

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We have investigated the elemental composition and morphological changes in electrocytes of Psammobatis extensa (Rajidae) by using SEM-EDAX. P. extensa belongs to the Rajidae, one of the three groups of weakly electric fish. The electrocytes, electric organ cells, are semi-circular in shape and have their concave face receiving innervation from electromotor neurons of the spinal cord. The convex face, is non-innervated and shows a system of caveolae. Adult fish were from two different places from Argentina: A-) Golfo San Matías, Río Negro Province, and B-) Estuario de Bahía Blanca, Buenos Aires Province. Electric organ segments treated for SEM were oriented for observation by a JEOL 35 SEM-EDAX (Si (Li) energy dispersive X-ray detector. Significant differences between A and B were found in the morphology and elemental composition of the electrocytes, namely: i-) an increase in electrolyte volume was shown in B; ii-) energy dispersive spectra show a high peak for oxygen, the relative semi-quantitative weight % (k) was 70, compared to A, the peak was 2.5-fold larger. The peak for Na+ was larger than K+ in contrast with A; iii-) a new peak corresponding to Al3+ ion was observed. In conclusion, the massive intracellular accumulation of oxygen and the influx of Na+ and Ca2+ and the presence of Al3+ lead to electrolyte swelling. These data are discussed in relation to electrolyte oxidative stress and death.

I-030 MORPHOLOGICAL CHANGES AND IONIC MIGRATION PRODUCED BY THE INTRACELLULAR LOCALIZATION OF TETRAPHENYL-PORPHYRIN DERIVATIVE PHOTOSENSITIZER IN RAJIDAE ELECTROCYTES

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In this work we used Scanning Electron Microscopy (SEM) coupled with Energy Dispersive X-ray Analyzer (EDAX) system to obtain information about the morphological changes observed after the intracellular localization of the 5,10,15,20-tetrakis(4-n-dodecylphenyl)-porphyrin (TPP) in Psammobatis extensa electrocytes. Electrocytes, electric organ cells, are large and highly polarized. They are semi-circular in shape and have their concave face receiving innervation from electromotor neurons of the spinal cord. The other face, convex is non-innervated and shows a system of caveolae. Cryostat sections were treated with TPP derivative in three different systems and observed with an epifluorescence microscope. In contrast to what is usually the case, this TPP derivative was localized in the electromotor nerves and in the nuclear chromatin of the electrocytes. All structures exhibited intense fluorescence, whereas, the mitochondria were only slightly fluorescent. Immediately after the penetration of the photosensitizer, electrocytes swell and the convex face loses all its invaginations. According to the electron-probe X-ray microanalysis, this effect is due to the flux of chloride and sodium ions into the electrocytes, which might be implicated in chloride and cationic channels activation. These data are discussed in relation to electrolyte death in a weakly electric fish. This research reports the first instance in which the electric organ was used to study photosensitizers.
I-031 MOLECULAR MECHANICS OF CYTOTOXICITY INDUCED BY PALLADACYCLE COMPLEX IN HL60 AND JURKAT CELL LINES.

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Evidences are growing that Angiotensin-I-converting enzyme (ACE) inhibitors play important roles in antagonizing angiogenesis, growth and invasion of the tumoral tissue. Recently, we have synthesized and investigated the cytotoxicity and apoptosis induction of a new organometallic compound with ACE inhibitory properties in Jurkat and HL60 leukaemic cells. Our results demonstrated that BPC induces apoptosis in both cell lines, even in the presence of normal Bcl-2 expression.

By using the MTT tetrazolium reduction test, we obtained an IC_{50} of 5.9 µM and 5.7 µM for HL60 and Jurkat cell lines, respectively, after 72 h incubation. The trypan blue assay showed an IC_{50} less than 3.0 µM for both cell lines. Moreover, the Feulgen reaction and genomic DNA fragmentation suggested that BPC triggers apoptosis in these cells. Interestingly, preliminary studies showed that bcl-2 and bax expression, as assessed by RT-PCR, were similar to untreated HL60 and Jurkat cells. The Bcl-2 expression were also confirmed to be similar to controls by immunocytochemistry. Our results points to BPC as a promising drug with ACE inhibitory properties and cytotoxic activity in leukaemia cells. Hence, it seems important to further investigate the mechanisms involved in the induction of cell death.

Financial support: FAPESP, CNPq and FAEP/UMC.

I-032 CYTOTOXICITY AND APOPTOSIS INDUCTION BY BIPHOSPHINIC PALLADACYCLE COMPLEX IN V79 CELL LINE.

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All of the anticancer drugs in the current pharmacopoeia can induce the morphological features of a programmed cell death, or apoptosis. Biphosphinic Palladacycle Complex (BPC) is reported to be a potential drug for the treatment of cancer. Previous studies performed by our group showed that BPC is effective against leukemia and lymphoma cell lines in culture. Moreover, BPC induced apoptosis in these cell lines. In this work, using the 3-(4,5-dimethylthiazole-2-yl)-2,5-biphenyl tetrazolium bromide reduction, DNA fragmentation, and trypan blue dye exclusion assays, we analyzed the cytotoxicity of the BPC in V79 fibroblasts cell line, which is commonly used in mutagenicity and toxicity studies. Our results demonstrated that BPC exhibited an IC_{50} of 3,28 µM and 3,37µM, using MTT and the trypan blue dye exclusion assays, respectively. The DNA fragmentation, typically observed in programmed cell death, suggest that apoptosis is also present in this cell line after incubation with BPC. These results suggest that a new organometallic complex with potential to induce apoptosis was identified. Thus, elucidation of the molecular mechanisms involved in this result is in progress in our laboratory.

Financial support: FAPESP, CNPq and FAEP/UMC.

I-033 FLUORESCENCE MICROSCOPICAL AND IMMUNOHISTOCHEMICAL DETECTION USING THE MONOCLONAL ANTIBODY M30 OF APOPTOTIC CELLS IN THE BUFALLO PLACENTA

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Apoptosis plays a key role in the development, remodeling and aging of the placenta. Several methods are widely used to identify apoptotic cells and a novel technique that determines and quantifies apoptotic bodies (ABs) is the fluorescence microscopy, based on strong eosin fluorescence of ABs in H&E-stained tissue sections. Immunohistochemical methods are even more specific. The aim of the present study was to evaluate the use of both methods to assess apoptotic cells in the buffalo placenta throughout gestation. Sixteen placentomes of buffaloes on the different phases of pregnancy were sectioned, fixed in paraffin, and submitted to a fluorescence microscopy study. The fluorescent microscopic method has identified ABs in the placenta, confirmed by transmitted light microscopy by switching between fluorescent and transmitted light and also by the M30 CytoDeath method. We identified CK18 in villous trophoblasts cells, resembling human data. Apoptosis was present in all the phases of pregnancy, mainly in the final and a progressive increase in the number of apoptotic cells was observed throughout gestation, analogous to human. Many giant cells showed inten-labeling.


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The information conveyed in the above title represents a novel contribution to the study of apoptosis. The MPT is a state of the organelle in which in addition to the changes cited in the title there is collapse of the mitochondrial transmembrane potential (ΔΨm). It occurs in apoptotic and possibly also in necrotic cells. We observed, with the TEM, MPT in 5 different types of rat tissue cells in apoptosis and in the apoptotic cells of six different lines of cultured cells. From the following cell lines HL-60, HeLa, WEHI-164 and a special batch of PC-12 cells, eight samples subjected to various apoptotic agents were studied morphometrically to evaluate the mitochondrial volumes in µm^3 and the volume fraction of the cytoplasm occupied by mitochondria with ruptured outer membrane in cells at early stages of apoptosis and also in mitochondria with no ruptured outer membrane from companion non apoptotic cells. A considerable fraction, some 65% (47-86%), of the total mitochondrial volume of the apoptotic cells is occupied by mitochondria expressing permeability transition.
I-035 IN THE EARLY STAGES OF THE MITOCHONDRIAL PERMEABILITY TRANSITION (MPT) THE RUPTURE OF OUTER MEMBRANE IS SMALL SIZED; CORRELATION BETWEEN APOPTOTIC INDICES AND MPT.

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The initial breach of the outer mitochondrial membrane may appear literally punctual and openings of this membrane in the range of ~30 nm to ~100 nm are observable. To cover the expanding swollen matrix the inner membrane utilizes membrane organized in the cristae. The initial small sized aperture of the outer mitochondrial membrane may appear literally punctual and openings of this membrane in the range of ~30, ~50 and ~100 nm are observable. To cover the expanding swollen matrix the inner membrane utilizes membrane organized in the cristae. The initial small sized aperture of the outer membrane is preceded by a localized intermembranous swelling. The initial small sized aperture of the outer mitochondrial membrane may appear literally punctual and openings of this membrane in the range of ~30, ~50 and ~100 nm are observable. To cover the expanding swollen matrix the inner membrane utilizes membrane organized in the cristae. The initial small sized aperture of the outer mitochondrial membrane may appear literally punctual and openings of this membrane in the range of ~30, ~50 and ~100 nm are observable. To cover the expanding swollen matrix the inner membrane utilizes membrane organized in the cristae. The initial breach of the outer mitochondrial membrane is preceded by a localized intermembranous swelling. The initial small sized aperture of the outer mitochondrial membrane is preceded by a localized intermembranous swelling. The initial small sized aperture of the outer mitochondrial membrane is preceded by a localized intermembranous swelling. The initial small sized aperture of the outer mitochondrial membrane is preceded by a localized intermembranous swelling. The initial small sized aperture of the outer mitochondrial membrane is preceded by a localized intermembranous swelling. The initial small sized aperture of the outer mitochondrial membrane is preceded by a localized intermembranous swelling. The initial small sized aperture of the outer mitochondrial membrane is preceded by a localized intermembranous swelling. The initial small sized aperture of the outer mitochondrial membrane is preceded by a localized intermembranous swelling.

I-036 FLUORESCENCE MICROSCOPY OF THE CYTOSQUELETAL ELEMENTS, ACTIN, MICROTUBULES, CYTOKERATIN AND VIMENTIN IN APOPTOTIC CELLS

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During the orchestrated cytolysis occurring in apoptotic cells we investigated the chronology of disappearance of the following cytoskeletal elements: microfilaments of F-actin (Alexa Fluor 488 or Alexa Fluor 594 phallolidin, for green and red fluorescences, respectively), microtubules (monoclonal anti α or β tubulin and goat anti mouse Alexa Fluor 488 or 568 and 594), intermediate filaments of cytokeratin (monoclonal anti cytokeratin and goat anti mouse Alexa Fluor 488 or 594) and vimentin (monoclonal anti vimentin and Alexa Fluor 488 or 594). PC-12 (rat pheochromocytoma) LLC-WRC-256 (rat carcinoma), BHK (“baby hamster kidney cells”), HeLa (carcinoma, human), HT 29 (adenocarcinoma, human) cells and also in cells rendered permeable by fixation. We have labeled mitochondria from BHK, WRC-256 and PC-12 cells transfected with plasmids carrying the sub unit 8 of cytochrome oxidase tagged with a red fluorophore, the DsRed. Upon transfection the cells start to produce mitochondria that fluoresce in red. Apoptotic cells were identified after Hoechst staining. The majority of apoptotic cells exhibited no fluorescence by the mitochondrial probe. Surprisingly, part of these dead cells possessed a variable accumulation of the probe in the cytoplasm and in many cases individual mitochondria were distinctly fluorescent. In some apoptotic and more rarely in non apoptotic cells the fluorescent mitochondria have lost their filamentous configuration becoming spheroid. This change in form also occurred in some cells, apoptotic and non apoptotic, transfected with DsRed. Electron microscopy confirmed these shape variations.

I-037 IN SITU AND IN VIVO EVALUATION OF THE MITOCHONDRIAL PERMEABILITY TRANSITION (MPT)

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The decrease or abolition of the mitochondrial transmembranous potential (ΔΨm), revealing the onset of MPT, has been evaluated mainly by cyto fluorimetric analysis with the flux cytometer. The active mitochondria are labeled with special mitochondrial potential sensitive probes. Some of these probes must be used only in vivo. They are lost from cells whose mitochondrial membrane potential is lost, like in dead cells and also in cells rendered permeable by fixation. We have labeled various types of cultured cells under the action diverse apoptogenic agents to evaluate in vivo the mitochondrial uptake of the followings probes, in apoptotic and in non apoptotic cells: a) JC-1, b) -tetramethylrhodamine (TMRM), c) 3,3’-dihexyloxocarboxyanine iodide (DIOC-6), c) – Mitotracker green FM. In addition we studied the mitochondria from BHK, WRC-256 and PC-12 cells transfected with plasmids carrying the sub unit 8 of cytochrome oxidase tagged with a red fluorophore, the DsRed. Upon transfection the cells start to produce mitochondria that fluoresce in red. Apoptotic cells were identified after Hoechst staining. The majority of apoptotic cells exhibited no fluorescence by the mitochondrial probe. Surprisingly, part of these dead cells possessed a variable accumulation of the probe in the cytoplasm and in many cases individual mitochondria were distinctly fluorescent. In some apoptotic and more rarely in non apoptotic cells the fluorescent mitochondria have lost their filamentous configuration becoming spheroid. This change in form also occurred in some cells, apoptotic and non apoptotic, transfected with DsRed. Electron microscopy confirmed these shape variations.

I-038 CYCLOPALLADATE-DPPE COMPLEXES REVEALS ANTI-TUMOR ACTIVITY AGAINST A MURINE MELANOMA CELL LINE

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In 1969 Barnett Rosenberg and Loretta Van Camp discovered the antitumoral activity of the cisplatin, represented by cis-[PtCl2(NH3)2]. This outcome has generated considerable interest in the metallic complexes pharmacology. Since then, new chemotherapeutic agents based on the transition metals have been investigated constantly, especially those belonging to the Platinum group. In this context, regarding palladium compounds, several researches indicate the class of cyclopalladacycle complexes as a promising chemotherapeutic agents. We describe the screening of the several cyclopalladated compounds in the form of biphosphinic complexes, for in vitro and in vivo anti-tumor activity in a B16F10 murine melanoma model. Depending on the cyclopalladated fine structure, different anti-tumor properties were observed involving the respiratory activity inhibition and proton output in the tumor cells. Complex [Pd2(µ-Cl)(C2,N-dmpa)2(µ-dppe)Cl2] affects actively the respiratory metabolism of the cell causing a collapse in the mitochondrial proton gradient as reflected by an abrupt decrease in the extracellular acidification rate measured by an microphysiometer. Complex [Pd2(R+)C2,N-dmpa)(µ-dppe)Cl2] also causes decrease of acidification but with a different kinetics preceded by respiratory stimulation. The results of the present work introduce the cyclopalladated dppe complexes as a promising anti-tumor drugs in which no apparent toxicity in experimental animals is found. Further this drugs can be elaborated for maximal activity. Grateful to FAPESP by financial support.
I-040 INDUCTION OF THE MURINE MELANOMA CELL LINE APOPTOSIS BY A NEW CYCLOPALLADATED DPPE COMPLEXES ON GANGLIOSIDE GD3 PATHWAY

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Cancer chemotherapy has achieved major progress during the last 4 decades. The successful use of anticancer agents can lead to complete tumor regression followed by prolonged disease-free periods, and increased survival in some patients. Alternative drugs of lesser toxicity other Cisplatin and with wider spectrum of activity based on transition metals, especially those of the platinum group are being investigated. Cyclopalladated complexes showed the most stable and most importantly, less toxic, suggesting they could have a more specific anti-tumor activity in vivo. Active complexes in vivo, [Pd2(S-H)C2,N-dmpa)2(dppe)Cl2] cause DNA degradation in vitro but do not increase the levels of caspase 1 and 3, suggesting the induction of a caspase-independent apoptosis. The drug, however, in analogy to cisplatin, could enter the nucleus and complex with DNA contributing to its aggregation and/or degradation. Mechanistic studies on Infrared spectroscopy show the interaction of the drugs with ganglioside GD3, through a residue of the acid group of the sialic acid present in this molecule, being this probably the mechanism of action of the Cyclopalladated dppe complexes. Grateful to FAPESP by financial support.

I-041 ATP-SENSITIVE K' CHANNEL (mitoK_ATP) OPENING PROTECTS AGAINST CELLULAR DAMAGE PREVENTING MITOCHONDRIAL PERMEABILITY TRANSITION

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Mitochondrial permeability transition (MPT) is a crucial event in the ischemic injury cell resulting in swelling and impairment of mitochondrial functionality and integrity in a manner inhibited by cyclosporin A. On the other hand, pharmacological opening of MitoK_ATP by diazoxide, a selective opener of the mitoK_ATP, has been proposed to mediate ischemic protection in cardiac muscle. This study tested the viability and ROS generation of HL-1 cells submitted to ischemia/reperfusion and treated with diazoxide (30 μM) and cyclosporin A (1 μM). MitoK_ATP opening by diazoxide (10 - 30 μM) in isolated heart mitochondria was tested to verify its effect on Ca2+-induced MPT under physiological and non-respiratory conditions. We found that the treatment with cyclosporin A (1 μmol/L) or diazoxide (30 μM) significantly protected HL-1 cells from damage by ischemia and reperfusion and decreased ROS generation during reperfusion, without additive effects. In this context, mitochondrial swelling secondary to MPT induced by Ca2+, measured by a decrease in light scattering (520 nm), was prevented by diazoxide only under non-respiratory conditions. On the other hand, in medium without K+ diazoxide does not alter mitochondrial swelling under physiological or isoelectric conditions. Swelling was inhibited by cyclosporin A (1 μmol/L). These results suggest that the opening of mitoK_ATP prevents cellular death under conditions of ischemia/reperfusion by decreasing ROS damage during reperfusion and inhibiting MPT. Supported by FAPESP and CNPq.

I-042 THE INVOLVEMENT OF 1,4,5-TRISPHOSPHATE INOSITOL RECEPTORS ON APOPTOSIS.

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Increase of intracellular calcium (Ca2+) is involved in a high range of cellular functions. The rise of Ca2+ is mediated mainly by the release of that ion from the 1,4,5-trisphosphate inositol receptors (InsP3R) and/or the ryanodine receptors. In this work, we investigated the role of InsP3R on apoptosis. We used the interfering RNA technique (siRNA) to silence the expression of the three isoforms of InsP3R in CHO cells. The oligos used in the in vitro transcription of the siRNA were designed to silence specifically each InsP3R. The efficiency and specificity of the silencing of each isoform were shown through western blot and immunofluorescence. The densitometry of the western blot results showed a reduction of about 90%, 40% and 50% in InsP3R-I, II and III, respectively. To study cell death, CHO cells were induced to enter apoptosis through transfection with a plasmidial vector that express the biliar acid transporter protein (pNTCP-GFP) followed by incubation with a apoptotic inducer Sodium Glycochenodeoxycholate-Sigma. A large number of apoptotic cells were observed by its condensed cromatin visualized with DAPI. Silencing InsP3R, but not the other two InsP3R isoforms, prevented the apoptosis. In conclusion, our results suggest a positive participation of InsP3R-III in apoptosis.

I-043 EXPRESSION OF APOPTOTIC CELLS IN ACTIVE CUTANEOUS LESIONS OF PATIENTS WITH AMERICAN TEGUMENTAR LEISHMANIASIS

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American tegumentar leishmaniasis (ATL) is a human cutaneous (CL) caused by Leishmania (Viannia) braziliensis and endemic in Brazil. The disease is characterized by one or more cutaneous ulcer, frequently associated with satellite lymph-node enlargement. Programmed cell death (PCD) or apoptosis is a genetically encoded program that results in the cell death and represents a fundamental biologic concept, with large relevance in the cutaneous diseases. The implication of PCD in the pathogenesis of L. (V.) braziliensis is still very discussed. In the present work we have analyzed the expression of apoptotic cells in active lesions of patients with ATL, with different infection times. Cutaneous biopsies were obtained from inhabitants from endemic areas of Rio de Janeiro State. The studies were accomplished by immunofluorescence TUNEL. Our results demonstrated the presence of a high number of apoptotic cells in the active cutaneous lesions of patients with ATL. The number of apoptotic cells was variable according to the time of infection. Supported by CNPq and FIOCRUZ.
I-044 CARVEDIOL PROTECTS ISCHEMIC CARDIAC MITOCHONDRIA BY PREVENTING OXIDATIVE STRESS
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Ischemia negatively affects mitochondrial function by inducing mitochondrial permeability transition (MPT). The MPT is triggered by oxidative stress, which occurs in the mitochondria during ischemia as a result of diminished antioxidant defenses and increased oxidants species. MPT causes mitochondrial dysfunction and can ultimately lead to cell death. A drug able to minimize mitochondrial damage induced by ischemia may prove to be clinically effective. We analyzed the effect of carvedilol, a β-blocker with antioxidant properties, in mitochondrial dysfunction. Carvedilol reduced mitochondrial swelling; however it did not alter calcium-uptake. It also decreased the levels of TBARS (thiobarbituric acid reactive substances), a product of lipid peroxidation which serves as an indicator for oxidative stress. Because of its antioxidant properties, carvedilol prevents the occurrence of oxidative stress. Therefore, we concluded that carvedilol protects ischemic mitochondria by preventing oxidative mitochondrial damage, and, by doing so, it may also inhibit the formation of the MPT pore. In what concerns cellular death by apoptosis, although ischemia did increase caspase-8-like activity, there were no changes in caspase-3-like activity, which is activated upstream of caspase-8. Therefore we conclude than the apoptotic cascade is not activated by 60 min of ischemia. Given that, we can not state whether carvedilol protects or not ischemic mitochondria against apoptosis.

I-002 MODULATION OF Rab5A DURING ENDOCYTOSIS INHIBITION BY OKADAIC ACID INVOLVES ERK 1/2 ACTIVATION IN HCT-116 CELLS.
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The functioning of the endocytic pathway is regulated by the activity of several kinases, phosphatases and GTPases. Among them, the small GTPase Rab5 plays key roles in this process. Rab5 was showed to be regulated by Erk 1/2 through GDI phosphorylation and to be phosphorylated in vitro by Erk 1/2. Okadaic acid (OA), a well known inhibitor of protein phosphatases 1 and 2A was previously observed to inhibit both endocytosis and activate Erk 1/2. Thus, the aim of this study was to evaluate OA effects on endocytosis, Erk 1/2 activation and the function of Rab5A. We observed that OA caused inhibition of vesicle fusion and HRP ingestion by HCT-116 cells using enzymatic cytochemistry and HRP enzymatic assay. This drug caused translocation of Rab5A from membrane to cytosolic fractions as observed by subcellular fractioning and immunoblotting and promoted activation of Erk 1/2 as revealed by pErk 1/2 immunoblots. Treatment with the Mek-1 inhibitor PD98059 prevented OA effects on Rab5A translocation. Furthermore, alterations at Rab5A phosphorylation state due to OA treatment were found. Finally, we propose a mechanism of signaling transduction in which okadaic acid performs its effects on endocytosis by Rab5A modulation, in an Erk 1/2 dependent manner.

J-003 HUMAN COLOSTRAL PHAGOCYTES ARE ABLE TO INGEST AND KILL GIARDIA LAMBLIA TROPHOZOITES
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Giardiasis is a worldwide distributed parasitosis and it is more prevalent in children up to four years of age, especially in collective environments. Breast-feeding, provide the newborn several immunological active components involved in anti-infectious protection. The objective of this study was to determine the phagocytical activity of polymorphonuclear (PMN) and mononuclear (MN) cells present in the human colostrum and verify the influence of opsonins in the adherence, ingestion and killing of trophozoites of Giardia lamblia. PMN and MN phagocytes were incubated with trophozoites of G. lambia, in the presence or absence of supernatant of human colostrum (opsonins source) for 120 minutes. The trophozoites/phagocytes rate was 1:1, and the percentage of trophozoites phagocytosed was previously observed to inhibit both the ingestion of acidine orange stained cells. The MN phagocytes presented greater functional activity more effective than PMN. The largest indexes of ingestion (68.90 ± 5.50) and kill (48.50 ± 4.85) were obtained with incubation of MN in the presence of colostrum supernatants. The phagocytes of the human colostrum are able to ingest trophozoites of G. lambia and presented microbicidal activity in vitro, suggesting that these phagocytes could act as an additional mechanism of protection against infant giardiasis through breast-feeding.

Phagocytosis, essential for host defense, depends on a complex process that requires coordination of a variety of signaling reactions. MT-III, a PLA2Asp49 with high enzymatic activity and MT-II, a PLA2Lys49, devoid of catalytic activity, isolated from B. asper, stimulate leukocytes for phagocytosis. The subject of this study is the signal transduction pathway mediating the sPLA2-induced phagocytosis. Macrophages were obtained from Swiss mice peritoneal cavity 96 hours after i.p. injection of thioglicolate 3%. Phagocytosis via mannose receptor was studied with non-opsonized zymosan (Zy) in the presence or absence of specific inhibitors. NO release and receptors, were assayed by Griess reaction and RT-PCR, respectively. Stauroporine and H7, PKC inhibitors, Herbyminic, a PTK inhibitor, Wortmanin, a P13 kinase inhibitor, L-NAME and L-NIL, cNOS and iNOS inhibitors, respectively, significantly reduced the sPLA2-induced Zy phagocytosis (p<0.05). MT-II and MT-III induced a significant release of NO (p<0.05) from macrophages, but did not interfere with iNOS mRNA expression. These results suggest that PKC, PTK, P13 kinase and NO, via iNOS, have a role in the signaling events required for myotyoxins-induced Zy phagocytosis. sPLA2-induced production of NO is not related to increase of iNOS mRNA expression.

Financial Support: FAPESP (02/01009-7)
K-001 WOUND HEALING IN CULTURED CORNEAL ENDOTHELIAL CELLS: ACTIN PURSE-STRING OR LAMELIPODIAL CRAWLING?
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Two mechanisms of epithelial repair have been described, the “purse-string” closure, typical of embryonic tissues, and lamellipodial crawling, frequently seen in adult tissues. However, some epithelia heal using both mechanisms, either sequentially or simultaneously. It has been suggested that the mechanism of closure depends on the size of the wound, with large circular wounds healing predominantly by lamellipodial crawling and small ones employing the purse-string mechanism. In this work, we investigated the dependence of the mechanism of closure on wound width of bovine corneal endothelial (BCE) cells in culture and whether this mechanism is influenced by the persistence of the extracellular matrix (ECM). Linear wounds of known widths were performed by scraping the monolayer with different instruments depending on whether the maintenance of the extracellular matrix was desired. Actin was localized by FITC-phalloidin and the ECM was put into evidence by Crystal Violet staining. The percentage of actin cable was assessed by image processing. We found that the mechanism of wound closure is independent of the width and affected by the presence of ECM. Two hours after injuring, the percentage of actin cable was around 50% irrespective of the cell substrate. When the ECM was removed, this percentage increased in time to up to 80% , whereas in the presence of ECM it gradually decreased reaching a value of 20% by six hours.

K-002 DYNEIN ATPASE, IS ASSOCIATED WITH A DISTINCT SUBSET OF PIGMENT GRANULES IN THE CHROMATOPHORES OF A FRESHWATER SHRIMP.
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Objectives: To separate the membrane-bound pigment granules from the amembranous granules found in the red, ovarian chromatophores of the freshwater shrimp, Macrobrachium olfersii, to allow independent probing for the presence of molecular motor proteins. Methods and Results: Immunocytochemical mounts were prepared in which formaldehyde-fixed chromatophores were labeled with monoclonal antibodies against the heavy chain of dynein ATPase. The preparations were stored in the refrigerator at 4 C for more than a week, during which time the two known pigment granule types separated. The red pigment, which corresponds to the membrane-bounded granules, appears to coalesce, forming areas in which it predominates, leaving regions of chromatophore cytoplasm in which a yellow pigment prevails. This pigment does not appear to coalesce. Immunocytochemical analysis revealed the complete co-localization of dynein ATPase labeling associated exclusively with the yellow-pigmented areas. Conclusion: The microtubule-dependent, molecular motor protein, dynein ATPase, appears to be associated with a distinct yellow pigment observable within the cytoplasm of fixed, red, ovarian chromatophores from the freshwater shrimp M. olfersii. This finding suggests that independent, subcellular regulatory mechanisms, which branch downstream from the initial hormone-receptor signal, may act during the process of unidirectional pigment granule translocation since the molecular motors, kinesin and myosin, are closely associated with the membrane-bounded pigment granules (Boyle and McNamara, 2004).
Financial support: FAPESP, CAPES

K-003 CYTOSKELETAL AND ADHESION PROTEINS IN THE ZEBRAFISH (DANIO RERIO) MYOGENESIS.
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The current myogenesis model is based on structural and biochemical analysis of in vitro cell cultures. Because embryos are more difficult to work with than cells, this differentiation process has scarcely been studied in avian and mammalian embryos in situ. Therefore, we used the optically clear and easy to handle embryos of the zebrafish Danio rerio. We studied the myofibrillar, cytoskeletal and cell-adhesion proteins using immunofluorescence microscopy and video-enhanced, differential interference contrast (DIC) of 24-48 hrs embryo. In the mature myotube, the monoclonal myoblasts displayed striations for all sarcomeric proteins tested (actin, myosin, alpha-actinin, troponin and titin). All desmin-positive cells were also myofibrillar proteins positive and striated, in contrast to what occurs in cell cultures. In the zebrafish the adhesion proteins integrin, dystrophin, paxillin and vinculin were distributed along the whole connective tissue septa between the somites, not confined to small patches as their in vitro counterpart. On the other hand, cell-cell contacts, important for muscle fusion and differentiation, were not observed in the zebrafish muscle cells using cadherin or catenin antibodies. The peculiarities of zebrafish myogenesis in situ could point to artifacts in the in vitro approach, or that muscle differentiation program is extremely adaptive, as these cells have to function while they are developing.
Support: FAPERJ, CNPq

K-004 PRESENCE OF ELEMENTS OF THE CYTOSKELETON DURING THE PROLIFERATION OF CYSTOCYTES IN THE OVARIES OF BEES
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During oogenesis the germ cells of bees remain connected by intercellular bridges. The bridges connecting the germ cells are the result of incomplete cytokinesis during the proliferating phase of the cystoblast. The cystocytes that result from such proliferation remain linked together and constitute a cyst. The bridges that interconnect these cells provide a way for the distribution of the substances that determine which one of the cells would become the oocyte and, latter, for the transport of the products synthesized by the remaining cells, known in meroistic ovaries as nurse cells, into the oocyte. Elements of the cytoskeleton sustain the bridges and orientate the flux of substances through them. In the present work, a study of the distribution of these elements in the ovary germarium of Scaptotrigona postica and Apis mellifera was conducted using the techniques of transmission electron microscopy and confocal microscopy. It was possible to observe that the cystocytes are linked by short and wide bridges, reinforced by actin rings and which present two bundles of thick filaments of unknown nature that extend throughout the width of the bridge. A large amount of tubulin with irregular distribution can be observed in the cytoplasm. Our results show not only the distribution but also the dynamics of the cytoskeleton during the proliferation of the cystocytes.
Support: FAPESP
K-005 INTERMEDIATE FILAMENT - LIKE IMMUNOREACTIVITY IN THE OPTIC STALK OF THE CRAB Ucides cordatus
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The literature is plenty of reports stating the absence of intermediate filaments in arthropods through observations using conventional electron microscopy and immunohistochemistry. Neither neurofilaments (NFs) nor glial fibrillary acidic protein (GFAP) have been reported in crustaceans. The present study was carried out to identify by immunohistochemistry, immunochemistry microscopy and Western blotting assays, NF and GFAP-like positive structures in the protocerebral tract (PCT) and optic ganglia of the crab Ucides cordatus obtained from Tubiacanga, Rio de Janeiro, Brazil. Our results indicated the presence of NF proteins using the Nin18 clone (antibody against the NF-medium subunit) and GFAP labeling by immunohistochemistry and immunoelectron microscopy. Besides that, using the Western blotting assay, we observed the bands of the same molecular weight of mammalian NF-medium subunit (160 kDa) and mammalian GFAP (48 kDa). In addition, we detected a band with approximately 90 kDa when used anti-GFAP antibody, raising the hypothesis of the presence of dimers or other different association with a different protein. Thus, we suggest that GFAP and NF proteins have not only been conserved in vertebrate evolution, but also that a homologous molecule may already have appeared in higher invertebrates, even though not in a filamentous form.

K-006 BIOAVAILABILITY OF THE SODIUM PERTECHNETATE AND MORPHOMETRY OF ORGANS ISOLATED FROM THE RATS: STUDY OF POSSIBLE INTERACTIONS OF A Ginkgo Biloba EXTRACT
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The nuclear medicine has the radiopharmaceutical as important compound used for the diagnosis and therapeutic treatment of human diseases. Many substances have been reported to affect the biodistribution of different radiopharmaceuticals. Ginkgo Biloba extract (EGb) is a phytoterpic that has several effects, specially, vasodilator, anti-inflammatory and anti-coagulant properties. We evaluated the influence of an EGb on the bioavailability of the sodium pertechnetate (99mTcO4-Na) and on the morphometry of the organs from rats. The animals were treated with EgB. After that, 99mTcO4-Na was injected. The organs were counted in a counter and the percentages of radioactivity per gram of each organ were calculated. The results showed that EgB altered the bioavailability of the 99mTcO4-Na in the kidneys, liver (but P>0.05) and duoden (P<0.05). Alterations on kidney, liver and duoden due to treatment were found and the morphometric analysis showed that they were significant (P<0.05). We suggest that this extract could generate metabolites capable to promote changes in the organs (kidney, duoden and liver) and to alter the biodistribution of the 99mTcO4-Na in the treated animals.

K-007 DISTRIBUTION OF RHO GTPASES DURING SUBMANDIBULAR GLAND DEVELOPMENT
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Objective: Rho GTPases are involved in morphogenesis and cytodifferentiation through their effects on actin cytoskeleton reorganization, cell proliferation, migration, differentiation and apoptosis. The aim of this study was to evaluate, using immunohistochemistry, the distribution of the GTPases Cdc42, Rac1, Rhox, Rhob and RhoC during the development of the rat submandibular gland (SMG). Methods and Results: Wistar rats were sacrificed at 17, 18, 19 and 20 days of gestation; newborns and male adults were also used. The SMG was fixed with Methacarn solution for 2 h at room temperature, processed and embedded in Paraplast®. The avidin-biotin-peroxidase technique was used for immunohistochemistry. All Rho GTPases studied were found in epithelial and mesenchymal tissues throughout the SMG development. During lumen formation and cellular polarization in the terminal tubule (17th-18th day of gestation), Rac1, Cdc42 and the phosphorylated form of Rac (P-Rac) were observed surrounding the lumen. During intense cytodifferentiation of secretory and ductal cells (19th day and newborn), RhoA and Rhob labelling was more evident. The expression of Rac1, P-Rac and Cdc42 in the adult gland was weak, while RhoA, Rhob and RhoC were observed in different glandular compartments. Conclusion: the expression pattern of Rho GTPases during the development of SMG strongly suggests that these proteins are involved in lumen formation and cytodifferentiation, probably being regulated by epithelial-mesenchymal interactions.

Financial support: FAPESP and CNPq.

K-008 RHO GTPASES ARE INVOLVED IN SALIVARY ACINAR FORMATION
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Objectives: Rho GTPases regulate processes such as proliferation, cytoskeletal organization, migration and apoptosis. The aim of this study was to evaluate the distribution and role of some Rho GTPases during acinar formation using as experimental model the Human Salivary Gland (HSG) cell line. Methods and Results: HSG cells were cultured within basement membrane extract (Matrigel®) or agarose gels under controlled conditions for 48 hours, and then fixed and processed for immunohistochemistry, the distribution of the GTPases Cdc42, Rac1, Rhox, Rhob and RhoA during the development of the rat submandibular gland (SMG). Methods and Results: Wistar rats were sacrificed at 17, 18, 19 and 20 days of gestation; newborns and male adults were also used. The SMG was fixed with Methacarn solution for 2 h at room temperature, processed and embedded in Paraplast®. The avidin-biotin-peroxidase technique was used for immunohistochemistry. All Rho GTPases studied were found in epithelial and mesenchymal tissues throughout the SMG development. During lumen formation and cellular polarization in the terminal tubule (17th-18th day of gestation), Rac1, Cdc42 and the phosphorylated form of Rac (P-Rac) were observed surrounding the lumen. During intense cytodifferentiation of secretory and ductal cells (19th day and newborn), RhoA and Rhob labelling was more evident. The expression of Rac1, P-Rac and Cdc42 in the adult gland was weak, while RhoA, Rhob and RhoC were observed in different glandular compartments. Conclusion: the expression pattern of Rho GTPases during the development of SMG strongly suggests that these proteins are involved in lumen formation and cytodifferentiation, probably being regulated by epithelial-mesenchymal interactions.

Financial support: FAPESP and CNPq.
K-009 PRODUCTION AND CHARACTERIZATION OF MONOCLONAL ANTIBODIES AGAINST THE SERINE 1650 PHOSPHORYLATION SITE IN THE TAIL DOMAIN OF MYOSIN-VA
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Myosin-Va (MVa) is a molecular motor phosphorylated by calmodulin-dependent protein kinase II (CaMKII) on serine 1650 in its globular tail domain. We generated monoclonal antibodies against the synthetic peptide, CL164RKRTS5(P02)IADE1654, which corresponds to the phosphorylation site in order to probe its role in MVa function. These antibodies specifically recognize heavy chain of MVa purified from chick brains or in unpurified homogenates, indicating that they recognized the antigenic site within the native molecule. The globular tail domain, expressed and purified from bacteria, is not recognized by these antibodies; however, when phosphorylated by purified CaMKII in vitro, the globular tail domain is strongly labeled by the monoclonal antibodies. Thus, these monoclonal antibodies are specific probes for the serine 1650 phosphorylation site in MVa. We have characterized these antibodies by probing western blots and by immunocytofluorescence of the neuroblastoma cell line N2A, as well as cells from a primary culture from cerebellum. We observed specific and strong labeling at several stages of cell division in N2A cells, whereas in cells of the cerebellum, cultured for 8 days, we observed granular labeling within the nucleus. The data suggest that phosphorylation of MVa is increased during mitosis and may be involved in nuclear events.

Finanical support, FAPESP

K-010 IMMUNOFLOURESCENT DETECTION IN CULTURED CELLS OF MYOSIN Va PHOSPHORYLATED ON SERINE1650
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Monoclonal antibodies against the phosphorylation site at serine 1650 in the globular tail domain of myosin Va (MVa) have been produced in our laboratory. We have characterized these antibodies by western blot and immunocytofluorescence of mouse melanoma cell lines B16 (wild-type for MVa) and S91-6 (null mutant of MVa), with or without hormonal stimulation (MSH), as well as human melanoma cells at several stages of tumor progression. An immunoreactive band consistent with MVa heavy chain labeling was obtained on western blots from these cell extracts, except for S91-6, indicating that these antibodies specifically recognize phosphorylated, endogenous MVa. Upon immunocytofluorescence the staining pattern of phosphorylated MVa was clearly different than the staining by polyclonal antibodies against non-phosphorylated tail domain of MVa. In interphase cells, prominent perinuclear staining was observed for phosphorylated MVa whereas the polyclonals showed mostly cytoplasmic, punctuate staining. At several stages of cell division specific and strong labeling by the monoclonal antibodies was observed. The data suggest that phosphorylated MVa is involved in events during cell division in all the cell lines investigated.

Financial support from FAPESP

K-011 SIGNIFICANCE OF THE DOMAINS OF SPAG6, AN ARMADILLO REPEAT PROTEIN THAT BINDS TO MICROTUBULES.
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Armadillo (Arm) repeat proteins function in various processes, including intracellular signalling and cytoskeletal regulation. Tandem Arm repeats form a superhelix of helices, which creates a surface for protein–protein interactions. It has been proposed either that the armadillo repeats are additive in function or that the N-terminal and the C-terminal ends are essential to the function. Spag6 contains 8 armadillo repeats and is the mammalian homologous of Chlamydomonas PF16, a central apparatus protein required for algae flagellar movement. In order to test the Spag6 domains that could be involved in the interaction between Spag6 and other proteins, we cotransfected COS7 cells with Spag6-Red Fluorescent Protein (RFP) and Tubulin-Yellow Fluorescent-Protein (TYFP). We observed that Spag6-RFP binds avidly to polymerised microtubules. Cells cotransfected with Spag6-RFP and TYFP resists treatments that normally depolymerise microtubules like maintaining the cells 1 hour at 4 °C. We have also transfected the cells with modified constructs of Spag6 that lacks of the N and the C terminal ends of the protein. We analysed its interaction with tubulin by fluorescent microscopy. The tubulin-like pattern of Spag6 was lost after transfection of the cells with the transformed constructs, suggesting that these regions of the protein could interact direct or indirectly to tubulin. We will introduce smaller disruption to check which regions are responsible of these effects.

K-012 CYTOTOXIC EFFECTS OF CINNAMIC ACID ON MELANOMA CELLS IN CULTURE.
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Cinnamic acid (CA) is a naturally occurring aromatic fatty acid that has a long history of human exposure as a component of plant-derived scents and flavoring. Its effects include antimicrobial and anti-inflammatory activity. The present work propose to evaluate the CA cytotoxic potential on human melanoma cell line ,HT-144. Cell viability was determined by using MTT essay after 48h cell treatment with several drug concentrations. The IC50 was established as 2.40mM. Further, studies on the probable CA mechanisms of action were performed with two concentrations considered as mild and drastic, 2.20 and 3.24mM respectively. Alterations in the pattern of organization of microtubules were analyzed in cytological preparation stained by immunofluorescence with anti-α tubulin + anti-β tubulin monoclonal antibodies. Tubulin bundles seem to dissociate after 48h treatment with 3.24mM of CA. Microfilaments were also desorganized with both concentrations of the drug. This effect was visualized by immunofluorescence with phalloidin. The effects seem to increase the frequency of binucleated cells in HT-144 culture. The number of binucleated cells increased from 1.07% to 1.60% in cells treated with 0.20mM of CA and to 3% with 3.24mM. DNA quantification by image analysis did not show alteration in DNA indexes after CA treatment. Nevertheless, the M30 staining showed that the treatment caused an increase in the apoptotic cells frequency.
K-013 SHP-2 IS A POTENTIAL MODULATOR OF FAK ACTIVITY BY MECHANICAL STRESS IN CARDIAC MYOCYTES.  
Inoue, R.Y., Calixto, A.R., Theizen, T.H, Marins, T.M., Crosara-Alberto, D., Franchini, K.G. Department of Internal Medicine, FCM, UNICAMP, Campinas.

We have previously demonstrated that focal adhesion kinase (Fak) is rapidly activated and coordinate the initial activation of hypertrophic genetic program in cardiac myocytes in response to mechanical stress. Here, we examined whether the tyrosine-phosphatase SHP-2 interacts with FAK in the myocardium and in cultured neonatal rat ventricular myocytes (NRVM). Fak was highly expressed, but barely phosphorylated at Tyr-397 (activity) at baseline in myocardium or NRVM. Co-immunoprecipitation assay showed that FAK is highly associated with SHP-2 at baseline in both models. Mechanical stress, represented by aortic constriction or cyclic stretch of NRVM promptly activated Fak, as detected by phosphospecific antibody against Tyr397. This was paralleled by a reduction of FAK/SHP-2 association. Immunohistochemistry and confocal microscopy analysis showed that both FAK and SHP-2 were located most at sarcomeric A-band in both models. Mechanical stimuli led FAK to be re-located to sites such as costameres, Z discs and nuclei, while SHP-2 remained localized at the A-Band. Thus, the results of the present study indicate that in cardiac myocytes inactive FAK is associated with SHP-2 while FAK activation by mechanical stimuli is accompanied by a reduction in this association. These data indicate that inactivation of SHP-2 might be an important mechanism for FAK activation by mechanical stress in cardiac myocytes.

K-014 FAK PROTEIN INTERACTION IN CARDIAC HYPERTROPHY SIGNALING PATHWAY WITH TWO-HYBRID YEAST SYSTEM.  
Department of Internal Medicine, FCM, UNICAMP, Campinas and 8 Center of Structural Molecular Biology, National Synchrotron Light Laboratory, Campinas.

Fak has been implicated as a signaling molecule involved in the early response of cardiac myocytes to mechanical stress. Here, we report the load-induced Fak activation and its association with myosin heavy chain in cultured neonatal rat ventricular myocytes (NRVM). Cyclic stretch was shown to induce Fak phosphorylation at Tyr-397 as detected by phosphospecific antibodies. Yeast two-hybrid screening of an adult rat cDNA library revealed an interaction between Fak and C-terminal coiled-coil region of alpha myosin heavy chain. This was confirmed by co-immunoprecipitation and pull-down assay with GST-tagged myosin and native Fak from NRVM. Such interaction was detected in homogenates of non-stretched and stretched NRVM but it was markedly reduced at 10 and 30 min-stretched cells. Confocal microscopy of anti-Fak and anti-myosin stained cells showed that Fak is most located at sarcomeric A-bands in non-stretched cells, while after 10 min-stretching Fak was clustered at Z discs. Thus, our present data indicate that Fak interacts with myosin in non-stretched cardiac myocytes and suggest that this association might be involved in the mechanical stress-induced activation of Fak in these cells.

K-015 REDUCED ASSOCIATION WITH SHP-2 MAY EXPLAIN THE INCREASE TYROSINE PHOSPHORYLATION OF FAK IN MUSCLE DYSTROPHY IN MICE.  
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The absence of functionally competent dystrophin causes muscular dystrophy and dilated cardiomyopathy. Several abnormalities of cell signaling, including integrins, in human and mice (MDX) with muscle dystrophy have been reported and may play a role in the pathogenesis of skeletal muscle and myocardial degeneration. In this study, we examined whether focal adhesion kinase (FAK), a tyrosine kinase involved in mechanotransduction in cardiac myocytes, is altered in cardiac myocytes of MDX mice. Western blot of myocardial extracts, performed with phosphospecific antibody against FAK Tyr397, indicated that FAK is hyperphosphorylated and active in control MDX as compared to Swiss mice, but no difference was found between these models concerning the amount of FAK expressed in the myocardium. Mechanical stimuli induced by aortic constriction increased FAK phosphorylation at Tyr397 in MDX mice more than in Swiss. Co-immunoprecipitation assays performed with anti-FAK and anti-SHP-2 (tyrosine-phosphatase) antibodies indicated that there was a lower baseline association of FAK to SHP-2 in MDX as compared to Swiss mice. Moreover, aortic constriction induced no change in SHP-2 expression in Swiss but reduced its expression in MDX mice. Thus, the present data indicate that the lack of dystrophin is accompanied by a baseline activation of FAK in cardiac myocytes. This is probably related to the reduced baseline association of FAK with SHP-2 in MDX mice.

K-016 ANALYSIS OF ACTIN FILAMENTS ORGANIZATION IN TWO STRAINS OF ACANTHAMOEBA POLYPHAGA.  
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Acanthamoeba spp. is a group of free-living amoebas that are distributed in diverse environments. Occasionally, these microorganisms could be infectious agents of a variety of human diseases. It has been recognized that this protozoan presents a remarkable actin cytoskeleton. Actin polymerization has been involved in important biological phenomena in Acanthamoeba, including locomotion and phagocytosis. Since in Acanthamoeba, microfilament organization is directly related to the general behavior of Acanthamoeba, we looked for analyze actin filaments organization in two strains of Acanthamoeba polyphaga which in turn, possess different locomotion activity. The amebas were fixed previously or not to adhere onto glass surfaces, and then they were observed in a confocal microscope. Microorganisms were previously reacted with Alexa 488 – phaloidin. We clearly observed bundles of actin filaments at the ectoplasmic region underlying the plasma membrane, in pseudopodal areas and filling the microspikes which surrounds the cell body of the microorganism (termed acanthapodia). We also detected a high actin organization diversity in the strain that possesses high locomotory activity. Moreover, we also observe actin structures at the cellular basal region with a huge actin staining similar that described for adhesion plates in Entamoeba histolytica, indicating that A. polyphaga uses a remarkable adhesive actin structure to provide the protozoan attachment to glass surfaces.  
Financial Support: CNPq, FAPERJ, FUJB-UFRJ and MCT-PRONEX.
K-017 STAUFSN GRANULES ARE RECRUITED INTO STRESS GRANULES IN A CYTOSKELETON-DEPENDENT MANNER

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Cytoplasmic mRNA granules are functional units for a variety of cellular events such as mRNA transport, silencing, activation and degradation. We found that the double stranded RNA binding proteins Staufen 1 and Staufen 2 form granules that contain ribosomes and associate to the cytoskeleton. Upon induction of heat shock or oxidative stress, Staufen granules remodel into Stress Granules (SG), which are stress-induced organelles containing transiently silenced messengers. SGs are located in the nuclear periphery and remain attached to microtubules after a brief Triton extraction. We tested the effect of microtubule and microfilament-disrupting drugs on SG formation on primary cultures and cell lines and found that both treatments cause dramatic changes on SG assembly. Disruption of actin network induces the accumulation of a larger number of smaller SGs that remains dispersed throughout the cytoplasm. In contrast, disruption of microtubule network provokes the formation of normal-sized SGs which remain dispersed as well. Staufen is conserved among different organisms where it is required for microfilament and microtubule-dependent targeting of mRNAs granules. Our finding that Staufen molecules, are novel components of SGs and that the cytoskeleton is required for SG aggregation, suggest that Staufen may mediate motor recruitment for their clustering into SGs and likely, to allow relocation of mRNAs in response to different stimuli.

K-018 CYTOKERATIN AS A SUPPORT ELEMENT OF GERM FOLLICLES IN Bufo icterus ovary

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Due to specialization and complexity of cytoplasmic internal structures, eukaryotic cells have an internal cytoskeleton, which still needs a better understanding concerning its functions. Cytokeratin represents the intermediate filaments family that performs structural functions and those components show similar protein organization. Although answer for important questions like structure, complexity, origin, expression, and function of intermediate filaments is still little known, molecular and cellular biology studies have shown great progresses. The purpose of this study was to verify the cytokeratin presence as support element of Bufo icterus ovarian germ cells. Immunohistochemical technique was performed, using primary antibody (anti-human cytokeratin – clone AE1/AE3 – DAKO cat. no M3515) and secondary antibody (Envision, anti-mouse – DAKO). The immunohistochemical labeled slices revealed that cytokeratin was present in cytoplasm of ovarian follicular cells and also in mesothelial cells that recovered the ovarian sac surface. In cytoplasm of germ native cells was detected no positive reaction. The results in Bufo icterus suggest that the cytokeratin filaments play an important rule in structure integrity and shape maintenance of the germ cells of toad ovary.

L-001 ELECTRONMICROGRAPHS AS TOOLS FOR INTEGRATING THEORETICAL AND PRACTICAL CLASSES

Giovanna C. dos A. Almeida; Janile C. Fanhani; Anne Caroline Olivo, Sônia M. H. N. Mizoguchi. Cesumar – Centro Universitário de Maringá

The lack of supporting material that allows the students to establish logical connections between theoretical and practical subjects is commonly experienced in either elementary school or undergraduate levels. When related to Cell Biology, the theoretical-practical relationship is hampered by the students difficulty in establish an accurate microscopic idea of the cell. As most of the educational institutions use the light microscope, the acquisition of knowledge on cellular ultrastructure depends on the student’s ability to correlate theoretical information with the visual images seen in practical classes. Therefore, aiming at improving the dynamics of practical classes, we have adopted an album of electronmicrographs obtained at high magnifications, which became an important tool for the teaching of cellular morphophysiology. This album consists of 300 micrographs, each covered with a plastic film and entitled. A supporting text was to each micrograph to help the students to recognize and interpret them. The results of the use of this tool demonstrated that students from CESUMAR showed increased ability to correlate practical and theoretical subjects and significantly improved their knowledge on the cell.

L-002 STUDENTS OF MIDDLE, HIGH SCHOOL AND UNDERGRADUATE COURSE: THEIR KNOWLEDGE ABOUT CELLS.


The cell is a fundamental concept to understand the living world and the biological aspects of the organisms. In recent years, research on the teaching and learning of cell biology has increased, which has yielded many positive benefits for students. Thus, with the objective of knowing what students think about the cell, with the purpose of facilitating an educational intervention in teaching cell biology from the middle level until undergraduate we decided to make a survey . At the beginning, a questionnaire was given to a pilot group formed by 35 students from high school and after reviewing the questions it was enlarged and given to a group of 390 students from the middle level, high level and freshman students of Health Group Courses. The questions were about previous conceptions concerning structure, morphology and cell physiology . The initial results showed some mistakes suggesting inadequate methodologies, for, in many situations, the students were prompted to retain summarized information sometimes out of context, not taking into account their cognitive development .With this results we hope to establish new approaches about the cell concept and create alternative materials to give support to teachers in the future.
The production of scientific knowledge is renewed constantly, while schools elaborate their programs from didactic books that in many cases, do not follow the increasing number of information arising through the media and publications in specialized magazines. In spite of a trend of change in the approach concerning biological sciences studies in the last 30 years, the curriculum is based on descriptive content without practical or demonstrative activities. A major challenge in teaching and learning cell biology is the enormous and expanding information base in our discipline. The increasing wealth of information obliges science teachers to be capable to learn continuously and understand the scientific process as a tool of learning. Based on these data, the discipline “Update in Cellular Biology” (lato sensu course “Education in Biology and Health” Oswaldo Cruz Institute/FIOCRUZ) was organized. In the last three courses public school teachers of Rio de Janeiro state and health professionals took part. The purpose was to contribute in the search of scientific information and use of local resources as education strategies. The classes included experiments, demonstrations, visits to laboratories, discussions of articles and projects. In the presentation of final works teachers mentioned the importance of these practical approaches in the classroom. The experience with protocols and images led to the search for new tools of learning.

The Cell Biology program for students of the Medical School is a short term and intensive course. There is a general recommendation of not exceeding 1-hour lecture within a 4-hour period (morning and/or afternoon), followed by different teaching strategies to deal with additional concepts and theories. When different subjects are to be presented within these 4-hour period, one of them should be a non-formal lecture. In one such situation we simulated a “quiz-show” with students divided into two competing groups (55 students each). Rules were agreed before starting. Sections (six for each group) consisted of choosing the student to answer the question, introduction of the subject with presentation of concepts and informations, the question, and alternative responses. The student could answer the question (worthig 10 points) or ask the corresponding group for the correct answer (worthig 5 points). If the question was too difficult it could be asked to the opposing group to answer. The proposing group would get 10 points in case the challenged group did not score. Whatever the answer, the lecturer presented the correct explanation for the question. The advantages of this scheme was the use of a 2-hour period with segments of a formal lecture interspersed with segments of relaxation and joy. Careful explanation of the procedure is necessary for the students to be aware that the subject won’t be presented again, and prize for the winner must clearly not be prejudicial for the loosers.
L-007 PHAGOCYTOSIS AS A LEARNING SUBJECT TO TEACH BASIC CELL BIOLOGY

Cell biology has, since its beginning, generated a plethora of knowledge. A challenging effort has been to transfer this knowledge to schools, mainly due to lack of up-to-date methodology, teaching models and surpassed conceptions of teachers. As a result, what is now considered complex biological processes are taught in schools in such an inadequate way that it induces alternative conceptions. The phagocytosis paradigm involves different areas of cell biology, therefore, as a teaching subject, it may be used to integrate updated cell biology. The way in which phagocytosis is presented by textbooks, and the students’ concept (through questionnaires) was verified. Textbook analysis demonstrated that phagocytosis is presented in an inadequate manner; figures (all diagrams) did not show essential structures present in phagocytosis, as receptors and cytoskeleton. This incorrect approach leads students to construct alternative conceptions about cell biology. Questionnaires demonstrated that the majority of students had difficulties with elementary points concerning updated cell biology. Furthermore, textbook emphasis on inadequate biological concepts leads students to difficulties in relating organelle function to biological processes. Images of the phagocytic process, obtained through video microscopy, was generated to complement textbooks. Our intention is not to propose the teaching of all complexity of phagocytosis, but to use it, in a simplified updated way, as a tool to teach cell biology in a contextualized manner.

Supported: CNPq, CAPES, FAPERJ.

L-008 AN ALTERNATIVE EDUCATIONAL STRATEGY FOR TEACHING DEVELOPMENTAL BIOLOGY

We believe that training of Biology students must include the realization that it is necessary to include in their classes subjects related to current family, community and/or media themes. We also believe that these subjects need to be dealt with in accessible and clear ways, allowing further discussion and studies in the classroom. Our objective was the establishment of a procedure to improve the process of acquisition and transfer of knowledge, that students may use later with their own students. The strategy involves the use of softwares and other computer tools for the acquisition of knowledge and then the creation of three-dimensional models, casts and puppets exhibitions for the transfer of the acquired information. This dynamic was successfully applied. The students were deeply involved as it defies them for searching and discovering concepts and new way of teaching. It also represents an alternative to classical lectures on the approaching of relevant concepts and new way of teaching. It also represents an alternative to classical lectures on the approaching of relevant concepts and new way of teaching. It also represents an alternative to classical lectures on the approaching of relevant concepts and new way of teaching. It also represents an alternative to classical lectures on the approaching of relevant concepts and new way of teaching. It also represents an alternative to classical lectures on the approaching of relevant concepts and new way of teaching. It also represents an alternative to classical lectures on the approaching of relevant concepts and new way of teaching. It also represents an alternative to classical lectures on the approaching of relevant concepts and new way of teaching.
AGING: A LEADING THEME FOR CELL BIOLOGY TEACHING  
D. Cassâo Cecchini; M. Izabel Gallão. 1Depto. de Biologia Celular - IB - Unicamp, Campinas – SP; 2Universidade Federal do Ceará – UFC, Fortaleza - CE.

Aging is a typical phenomenon of most living forms and is manifested in different ways as one considers different groups or species. It is a dynamic and progressive process in which morphological, functional and biochemical events modify the organism, making it more prone to intrinsic and extrinsic aggressive factors. These modifications result in behavioral adaptations to decreased intellectual and physical capabilities to execute daily activities. At the cellular level, one observes nuclear, organelles and cytosolic changes. Aiming at making Cell Biology classes to the elementary school more productive, we adopted aging as the leading subject, enlisting cell modifications that take place during this life stage. This strategy also couples to the Cell Biology classes the humanistic view of aging, dealing with its nature and predictability, the massive growth of the elder population, and the omission by and prejudice from younger people. This methodology was applied to the third year elementary school students of a public school in Lages/SC. Classes had dynamic exercises using didactic situations experienced by the elders. It also involved contacts with a third-age group for the exchange of experiences. Students were actively involved with the proposed activities and showed themselves interested not only in the subject itself but also on the employed methodology.

PHOTOSYNTHESIS.  
L-012 DRAMA TO TEACH CELLULAR ORGANELLE PHYSIOLOGY: CHLOROPLASTS AND PHOTOSYNTHESIS.  
1Angelo Luiz Cortelazzo; 2Maria Izabel Gallão. 1Depto. de Biologia Celular - IB - Unicamp, Campinas – SP; 2Universidade Federal do Ceará – UFC, Fortaleza - CE. angelo@unicamp.br

Many different approaches have been used with the aim of finding new learning methods and a better understanding of organelle physiology, mainly of those in which the complexity of chemical reactions can be an obstacle transposed. Analysis of electron micrographs, study of clinical cases and dramatizations have been successfully applied together with cell biology lectures in the State University of Campinas. Specifically in the case of chloroplasts, a three act plot was developed: (1) photosynthetic electron-transfer reactions in thylakoid membranes, with electron energization of chlorophyll, water photolysis and NADP$^+$ reduction; (2) transmembrane electrochemical proton gradient and ATP synthase action; (3) carbon-fixation reactions with ATP and NADPH utilization, to drive the conversion of CO$_2$ to carbohydrate. Each of these acts consider the biochemical, morphological and physiological aspects associated to the organelle and is accompanied with classic or popular Brazilian music. In the professors’ evaluation, these activities have represented an enhancement of about 30% in relation to the average of global performance in periodic tests and, in some cases, 150% in relation to test results of other complex organelles as mitochondria, for example. In the students' evaluation, these activities are considered excellent, with a significantly larger frequency when compared to the theoretical and practical activities developed in Cell Biology classes.

BIOLOGY TEACHING  
L-013 “BIOMOLEULAS” COURSE IMPROVES TEACHERS KNOWLEDGE AND STUDENTS INTEREST IN BIOLOGICAL SCIENCES  
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The course “As Células, o Genoma e você, Professor” is part of the Cell-based Therapy Center Educational Project (CTC - CEPID/FAPESP). It aimed to improve didactic methods in Sciences Education and was destined to teachers from public and private schools at Ribeirão Preto region (SP), in Fundamental and Medium levels. Activities were held in a general module and into small groups led by researchers and post-graduate students. Our group, called “Biomoléculas”, dealt with basic concepts and applications of cellular molecules, biotechnology and media on Biology learning. Simple laboratorial techniques adaptable to the school were also developed. Each teacher elaborated an alternative lesson on one of the argued subjects defining goals and strategies, applied it to their students and discussed the results with the group. Moreover, games, enigmas and quizzes were created; questionnaires were developed and leaflets containing brief explanations about water treatment, transgenic food and gene expression, for instance, were written. These activities stimulated teachers’ creativity and writing skills, enhanced students’ interest on Sciences and enriched the communication between basic education and research.

Financial support: CTC (CEPID/FAPESP) and FUNDHERP

CELL OF DIATRAEA SACCHARALIS (LEPIDOPTERA) LARVAE.  
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The midgut epithelium of the sugarcane borer (Diatraea saccharalis) is composed by different cells: columnar, goblet, regenerative and endocrine. Spherical granules with concentric lamination and mineral content (spherite) have been described inside columnar and regenerative cells of several Lepidoptera species. The functional meaning of such structure is still controversial. This work describes spherites in the midgut epithelial cells of D. saccharalis larvae at different time of larval development (17-30 days old), maintained with two different artificial diets. Fragments of midgut (anterior, middle and posterior regions) were conventionally prepared for transmission electron microscopy observation. All the midgut epithelial cells from D. saccharalis larvae showed spherites independent of the their localization along the midgut. The spherites morphology was similar independent of either the diet or the cell type, without preferential localization into the cells. They were more abundant and bigger in older larvae, suggesting a function related with the degenerative process of the metamorphose (*FAPESP fellowship).
M-002 EFFECT OF PROTEIC DIET AND AGING IN THE HEPATOCYTES MORPHOLOGY OF RATS
Alessandra Lemes da Rosa; Thais Paz Alves; Emílio A. Jekel-Neto. Laboratório de Envelhecimento Celular, IBP-PUCRS. aleslemes@hotmail.com

To characterize the morphologic parameters of hepatocytes submitted to different percentages of protein on diet during aging, male Donryu rats were housed in a specific pathogen free (SPF) room and fed with 60% of the mean daily quantity of diet consumed by rats fed ad libitum. The animals were divided in three groups fed with 40% (P40), 18% (P18), and 10% (P10) of dietary protein. The animals were sacrificed at 6, 12, 18, 24 e 30 months of age. Their livers were embedded in paraffin, cut and stained by Mallory tetrachrome technique. Cellular size, mononuclear and binuclear cells by area were measured and counted using a microscope connected to a digital camera. The images obtained were analyzed with the Image-Pro Plus 4.0 software. The results showed that the size of P10 hepatocytes was significantly bigger than those of P18 group. There was a significant reduction in the number of hepatocytes on animals of P40 group. The animals of P10 group presented significant reduction on cell number, with aging, in relation to the P18 group. The quantity of binuclear cells showed a significant increase in the animals of groups P10 and P40. These results suggest that the amount of protein ingested during aging can provoke alterations on the liver parenchyma that compensate either metabolic changes or natural cell loss.

N-001 IN VITRO TRANSFECTION OF BOVINE MYOBLASTS FOR CELL-MEDIATED GENE TRANSFER
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Adult myoblasts are mononucleated cells located between the basal lamina and sarclemma of muscle and contribute to muscle repair and hypertrophy. Gene transfer to these cells is a potential tool for studying muscle growth and development. Moreover, muscle is an excellent tissue for delivery of recombinant proteins because of its ability to incorporate and express foreign DNA. However, efficient strategies to genetic manipulation of bovine myoblasts have not been developed. The aim of this study is to determine the most efficient method in vitro for incorporation and expression of reporter gene in bovine muscle cells. Myoblasts were isolated from semimembranosus and semitendinosus muscles of 6 months old bovine fetus. Primary culture cells was established in Dulbecco’s minimal essential medium (Sigma) containing 10% of fetal bovine serum (Sigma) and 1% of antibiotic. Myogenic cell lines were identified by myotubes fusion assay and presence of muscle specific marker, myostatin, by RT-PCR. Four different protocols for transfection (Calcium Phosphate, electroporation, and two commercial cationic liposomes Effectene®; Qiagen and FuGENE 6; Roche) were tested for their ability to transfect myoblasts with green fluorescent protein reporter gene under control of CMV promoter in semiconfluent cultures. Preliminary results show Effectene® as the most efficient methodology for bovine myoblast gene transfer becoming a powerful tool for muscle growth and development studies.

Acknowledgement: FAPESP 03/0156-9

N-002 ROLE OF THE LEISHMANIA (VIANNIA) BRAZILIENSIS GENES PGPA AND HTBF IN HEAVY METAL RESISTANCE
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Leishmaniasis is caused by species of the protozoan Leishmania. Its treatment consists of pentavalent antimonials administration, whose collateral effects are dose-cumulative. The study of the ability of Leishmania to evade chemotherapy is important to help the design of effective treatments. Leishmania major submitted to pentavalent antimonials, and other unrelated drugs, amplifies a locus of 48kb, the H region. One of the H-region genes, PGPA, confers resistance to antimonials and is associated to vesicular elements of the exocytic and endocytic pathways. HTBF mediates resistance to terbinafine and is related to vesicle docking and trafficking. The aim of this study was to investigate the activity of PGPA and HTBF in L. (Viannia) braziliensis. Using a stepwise selection protocol we isolated a L. braziliensis cell line resistant to 12mg/ml of glutatione. Southern analyzes revealed that PGPA was not amplified and PFGE analyses showed that the resistant line did not carried amplified episomal molecules. Therefore, different from other Leishmania spp., L. (V.) braziliensis does not seem to use gene amplification as a major mechanism underlying drug resistance. Our current efforts are focused in the identification of the molecular mechanism used by the glutacntime-resistant L. braziliensis.

Financial support: FAPESP and WHO

N-003 INFLUENCE OF RENIN ANGIOTENSIN SYSTEM (RAS) IN THE THYROID HORMONE -INDUCED CARDIAC HYPERTROPHY USING PRIMARY CULTURES OF NEONATAL CARDIOMYOCYTES.
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Objectives: We have previously demonstrated in vivo the participation of RAS in the cardiac hypertrophy induced by Thyroid Hormone. However, the contribution of local RAS for this effect have not been evidenced yet. The objective of present study was to investigate the role of local RAS to the development of cardiac hypertrophy induced by T3. Methods: Cultures were segregated from neonatal ventricles of Sprague Dawley rats by collagenase/pancreatin method. Myocytes were segregated using Percoll density gradient and cultured. Cells were treated with T3 10nM, Losartan 1.0 μM and/or Enalapril 1.0μM for 24h. Total RNA was isolated, and cDNA was obtained. The atrial natriuretic factor (ANF) and alpha-smooth muscle actin (alpha-sm actin) mRNA were employed like markers for hypertrophy. Results were expressed as mean ± SD and P<0.05 (n=2). Result: T3 promoted a significant cardiac hypertrophy, expressed by increased ANF and alpha-sm actin mRNA levels. The cardiac hypertrophy was prevented totally or partially by administration of losartan, but not by enalapril.

<table>
<thead>
<tr>
<th>ANF expression</th>
<th>alpha-sm actin</th>
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<tbody>
<tr>
<td>Control</td>
<td>2.69 ± 0.36</td>
</tr>
<tr>
<td>10nM</td>
<td>10.07 ± 0.78</td>
</tr>
<tr>
<td>10nM + Los</td>
<td>3.59 ± 0.52</td>
</tr>
<tr>
<td>10nM + Eta</td>
<td>7.63 ± 0.92</td>
</tr>
</tbody>
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* vs control p<0.01
** vs 10nM p<0.01
*** vs control p<0.05
N-004 EFFECT OF THYROID HORMONE ON TISSUE ANGIOTENSIN-CONVERTING ENZYME (ACE) ACTIVITY AND EXPRESSION.
Marcela Sorelli Carneiro-Ramos1; Vanessa Beatriz da Silva1 and Maria Luiza Barreto de Chaves1. 1Department of Anatomy, 2Department of Histology and Embryology, Institute of Biomedical Sciences - Universidade de São Paulo, SP, Brasil. msorelli@usp.br.

This study was designed to determine the role of thyroid hormone on local regulation of ACE activity and expression in different tissues. Methods: Hyperthyroidism was induced by intraperitoneal administration of L-Thyroxin: T4-2.5 and T4-10 (0.025mg or 0.1mg/Kg BW/day) for 14 days in adult male Wistar. On the 15th day, animals were decapitated; heart, lung, kidney were removed and weighed. Tissue samples were homogenized and ACE activity determined by a fluorescent assay. ACE expression was determined by Northern-Blotting assay. Results: There was no difference on ACE activity and expression in lung. Renal ACE activity and expression were significantly increased in both groups treated to thyroid hormone when compared to control group. However, cardiac ACE levels presented a significant decrease in groups that received thyroid hormone treatment. Conclusion: Thyroid Hormone regulates ACE activity and expression locally and differently in each tissue, suggesting that the Renin Angiotensin System presents a fine interaction with thyroid hormone levels, which can be very important in some circumstances as in cardiac and renal hypertrophy induced by thyroxin. Financial support: FAPESP (99/08184-4).

N-005 EFFECT OF THYROID HORMONE ON EXPRESSION OF RENIN ANGIOTENSIN SYSTEM COMPONENTS IN PRIMARY CULTURED CARDIAC MYOCYTES.
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Renin-Angiotensin System has been associated with cardiac hypertrophy induced by thyroid hormone. However, little is known regarding the regulation of its components in cardiac cells after hyperthyroidism. Methods: Newborn rat ventricular cardiomyocytes were obtained from 1 to 3 day old rats. Cardiomyocytes were isolated using a Percoll gradient and cells were treated with T3 (0.1 mM – 0.1 mM) during 24 hours. Angiotensinogen (AGT), Renin (REN), Angiotensin-Converting Enzyme (ACE) and Angiotensin II type I receptor (AT1) mRNA levels were analyzed using semiquantitative or Real-Time PCR. Results: Thyroid hormone did not promote alteration on AGT mRNA levels in cardiac myocytes. The REN and ACE gene expression presented increased with highest expression after treatment with 0.01 µM of T3 (80% and 150%, respectively). However, the treatment with T3 promoted a significant decrease on AT1 mRNA, characterizing a dose-response relation, which showed the lowest expression level with 0.1 µM of T3. Conclusion: Thyroid Hormone exerts influence on the various RAS components in cardiomyocytes. The effect of Thyroid hormone on decreasing AT1 mRNA levels in cardiac myocytes may imply an important regulatory mechanism considering that both hormones (angiotensin II and thyroid hormone) act inducing the cardiac hypertrophy. Financial support: FAPESP

N-006 SINGLE NUCLEOTIDE POLYMORPHISM IN THE GENE ENCODING THE PHAGOCYTE OXIDASE 67 KDA PROTEIN
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Chronic granulomatous disease (CGD) is an inherited immunodeficiency resulting from defects the multi-enzyme complex NADPH-oxidase (phagocyte oxidase, phox), which normally produces microbicidal reactive oxygen metabolites (ROM). This phagocyte oxidase is constituted by at least four subunits, one of which is the cytosolic component p67-phox and is located in chromosome 1q25 (called NCF2), mutations in this gene results in autosonomic chronic granulomatous disease (CGD) We report here one polymorphism of the NCF2 gene corresponding a A to G transition in the nucleotide –21 of the 3’ end of the intron 10 (IVS10-21A→G). To determine the frequency of this substitution a group of 114 healthy volunteers were analyzed by SSCP-PCR and DNA cycle sequencing. The allelic frequencies were 0.58 for A, 0.42 for G. The localization of this transition is involved in RNA lariat formation during pre-mRNA splicing, we observed that all of genotypes individuals presents multiple splicing patterns of this region, affecting partially the exon 11 splicing, and therefore may alter the expression of the p67-phox gene. Supported by: FAPESP and CAPES

N-007 THE REITERATED GENE BhSGAMP-1 PROBABLY CODES FOR AN ANTIMICROBIAL PEPTIDE AND ITS EXPRESSION, IN THE SALIVARY GLAND OF Bradysia hygida, IS DELOPMENTALLY REGULATED
IAP Gallina, IJAC da Silva, IGM Zanarotti, IJC de Almeida. 1Departamento de Biologia Celular e Molecular, FMRP, USP. jcdalmei@fmrp.usp.br.

We are interested in the control of the process of gene amplification, which takes place in the salivary glands of Bradysia hygida, and in the control of the activity of amplified genes. In our search for new amplified genes, we have selected a small cDNA that detects an abundant small transcript. It starts to be detected at the age E7, when the larva is actively spinning its individual cocoon and is more abundant at the age E7+24h. So, its expression is regulated in development. "Southern blot" and molecular “in situ” hybridization experiments showed that the gene is reiterated, with copies spread throughout the entire genome. The cDNA deduced amino acid sequence presents a small segment with homology to antimicrobial peptides of two plant species. Being the first gene, that possibly codes for an antimicrobial peptide, to be detected in the salivary gland of Bradysia hygida, it was named: BhSGAMP-1. Antimicrobial peptides are present in different kinds of organisms, including humans; they actively participate in the defense mechanisms against the attack of microbial pathogens. If here, as in all the other cases, the peptide is secreted and used in the cocoon construction; we propose that its production by the salivary gland is part of an external defense mechanism.
Periodontal disease (PD) is caused by interactions between host factors, specific microbial pathogens, and environmental factors. It is, therefore, of interest to investigate the nature of host factors as they may provide useful risk markers and reveal important information regarding the PD. The susceptibility of some immune and infections diseases have genetic association with Human Leukocite Antigen (HLA) - which are essential in the recognition of foreign antigens in humoral immune response. The aim of this study was to determine if PD is associated with a polymorphism in the HLA gene. DNA was extracted from buccal epithelial cells of 113 unrelated adult individuals: 44 health individuals (control group), 31 subjects with moderate periodontitis and 38 with severe periodontitis. A restriction fragment length polymorphism (RFLP) for HphI of the HLA-DRB1 gene was analyzed by PCR, followed by restriction digestion with HphI. The products were analyzed in 10% polyacrylamide gel electrophoresis and stained by silver staining. Significant differences in the allele (p=0.000) and genotype (p=0.000) frequencies of the polymorphism were found between control and groups with periodontal disease. We conclude that this RFLP of the HLA-DRB1 gene can serve as a useful marker of susceptibility to chronic PD in the population studied.

Cell proliferation in gastric epithelium may be inhibited during postnatal development under different conditions as dietary manipulation and glucocorticoid treatment. The transforming growth factor beta (TGFβ) can mediate these inhibitory responses, our aim was to evaluate the immunostaining for TGFβ1, 2, 3 and receptors TβRI and TβRII in the gastric mucosa of 18-day-old pups using the early weaning model for dietary induction. Rats were subjected to early weaning on the 15th day, to fasting on the 17th and sacrificed at 18 days. For hormonal treatment 18-day-old pups were injected i.p. with hydrocortisone (Sigma, 50 mg/kg) and sacrificed after 0, 1 and 3 hours. The stomachs were collected and processed for immunohistochemistry. Early weaning followed by fasting increased the number of TGFβ3 and TβRI immunolabelled cells (p<0.05). After 3 hs of hydrocortisone treatment there was an increase in TGFβ1 immunostaining (p<0.05) and a decrease of TGFβ2 labelled cells (p<0.05). We can suggest that the upregulation of specific TGFβ isoforms in the gastric epithelium after abrupt dietary change or hydrocortisone treatment, may be correlated to the inhibition of cell proliferation in the stomach during development. Supported by FAPESP.

O-002 EXPRESSION OF TRANSFORMING GROWTH FACTOR α IN GASTRIC EPITHELIUM OF SIALOADENECTOMIZED SUCKLING RATS SUBMITTED TO FASTING.
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Transforming growth factor α (TGF α), a member of the epidermal growth factor (EGF) family of peptides, induces a wide variety of biological responses, from mitotical stimulus to regulation of differentiation. The gastrointestinal tract of suckling rats receives EGF mainly from milk and TGF α from saliva. We demonstrated that fasting enhances cell proliferation in the gastric epithelium of suckling rats. Sialoadenectomy was performed in this model to determine the expression of growth factors in the absence of stimulus from saliva, as well as from milk (in fasting rats). 14-day-old suckling rats had their salivary glands ablated or were sham operated. Fed or fasted rats were sacrificed at 18 days. Immunohistochemical analysis of TGF α expression was made and positive cells in the gastric epithelium (surface mucous cells and glandular cells) were counted. Fasting caused significant increase in the TGF α expression in surface mucous cells and glandular cells (p<0.05). Sialoadenectomy caused a significant decrease in this expression in fasting animals. We suggest that TGF α from saliva may have a stimulatory function over the expression of TGF α in gastric epithelium more important than EGF or other factors in milk. Higher expression of TGF α was correlated with enhanced cell proliferation in gastric epithelium. Support: FAPESP, CNPq.
Transforming growth factor alpha (TGFα) is a 50-aminoacid peptide that stimulates epithelial cell proliferation. Fasting stimulates this process in the gastric epithelium of suckling rats and this effect is reversed when pups are submitted to early weaning. The aim of this study was to evaluate the expression of TGFα after nutritional manipulation, trying to correlate this growth factor with cell proliferation in the gastric epithelium. We collected the stomachs of 18 d-old Wistar rats submitted or not to early weaning, fasted or not. TGFα was identified by immunohistochemistry with a monoclonal antibody (Ab-2, GF10, Oncogene-Calbiochem). Reactions were analyzed under light microscopy and stained surface and gland cells were counted. Fasting increased the number of surface cells with apical reaction in early weaned rats (P<0.05). The number of TGFα immunostained gland cells increased after early weaning and it was even higher in fasted animals (p<0.05). Our results suggest that abrupt milk withdrawal and fasting alter the expression of TGFα, which may be related to the control of growth in the gastric epithelium. Supported by FAPESP and CNPq.
IN THE PERIODONTUM OF LOWER INCISOR OF HUMAN OSTEOBLASTS PROLIFERATION: AN IN VITRO ANALYSIS.
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The influence of different concentrations of platelet-rich-plasma (PRP) on proliferation of human osteoblasts hFOB1.19 was assessed. The PRP was obtained from a human source. Two experiments were conducted. In the first one, PRP was diluted to 50, 25, 12.5 and 6.125 (v/v) with culture medium (MEM:HAMD F12 and 1% antibiotics-antimicotic) supplemented with 10% of fetal bovine serum (FBS). In the second experiment, all conditions were identical except for the omission of FBS on the culture medium. Epyfluorescent microscopy was used to visualize the osteoblasts and the obtained results showed high nuclei activity and defined cell extensions. Results of the proliferation test were higher when stimulated by the 50% PRP dilution with or without FBS. A further study is suggested to determine if concentrations above 50% cause higher rates of osteoblast proliferation. In this study, we found that PRP promotes osteoblast proliferation and suggests its clinical application to bone graft procedures in implant dentistry.

ANALYSIS.

This study concluded that PRP promotes osteoblast proliferation and suggests its clinical application to bone graft procedures in implant dentistry.

O-007 INFLUENCE OF PLATELET-RICH-PLASMA IN HUMAN OSTEOBLASTS PROLIFERATION: AN IN VITRO ANALYSIS.

O-009 IN SITU EVALUATION OF HEMATOPOIETIC DYNAMICS IN MICE TREATED WITH G-CSF

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Granulocyte colony-stimulating factor (G-CSF) is largely used to mobilize hematopoietic progenitors from bone marrow to peripheral blood for stem cell transplantation. Despite this clinical importance, little is known about its influence on bone marrow hematopoesis and systemic hemostasis. To address this subject, Swiss Webster mice were daily subcutaneously injected with 6 µg (20 µL) of h-G-CSF or saline (“sham”) during four consecutive days. At the 5th day, they were killed and their bones (femurs, spine, ribs and tail), liver and spleen were collected, fixed in Carson’s Millonig formalin, decalcified in EDTA (bones) and paraffin embedded. Sections (5 µm) were stained with HE and Lennert’s Giemsa, and analyzed by brightfield microscopy. G-CSF-injected animals presented bone marrow (all sites) full of myeloid cells, namely neutrophilic myeloblasts, promyelocytes and mature neutrophils, with depletion of other hematopoietic series. Sometimes, these cells exhibited a sheath-like distribution along the vessels. Mitotic figures, GM cells, and vacuolated immature precursors were commonly seen. Liver and spleen were also involved, showing a clear expansion of their hematopoietic activity with many subcapsular myelomonocytic foci. Spleen also presented many more immature myeloid cells than controls, located especially inside some lymphoid follicles and along the trabeculae. In conclusion, G-CSF administration induces a huge and systemic expansion of the myeloid compartment, together with mobilization of immature progenitors and retraction of the other hematopoietic lineages.

O-008 IMMUNOLOCALIZATION OF EGF, IL-1α AND CSF-1 IN THE PERIODONTUM OF LOWER INCISOR OF RATS

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Tooth eruption is a multifactorial and complex process, whose cause and effect are not easily distinguished. In spite of many studies, it is still unclear which tissue/cells are associated with tooth eruption. The molecule that seems to initiate this event is the EGF, which activates other molecules such as IL-1α and CSF-1, indispensable growth factors in eruptive process. For this study, lower incisors of twenty adult males Wistar rats were divided transversally into five different regions, from the alveolar crest to odontogenic organ (named R1, R2, R3, R4, R5). Variable immunostaining for this growth factors was found in all regions (R1 to R5) in different tissues. With respect to the immunohistochemical of EGF, IL-1α and CSF-1 the highest intensity was observed in the dental follicle, being that, for the CSF-1 the marking in this tissue in the R5 was more intense than the observed for the other factors. Ameloblasts, stellate reticulum, intermediate stratum, peridontal ligament, and odontoblasts had also presented positive marking to the antibodies. These results are in agreement with the literature. Thus, they demonstrate the relevant physiological role of these growth factors (EGF, IL-1α and CSF-1) in the dental eruption, mainly when present in the dental follicle, which seems to be the responsible tissue for the dental eruption process.

O-008 IMMUNOLOCALIZATION OF EGF, IL-1α AND CSF-1 IN THE PERIODONTUM OF LOWER INCISOR OF RATS

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The crosslinking of the FcεRI results in mast cell degranulation. The initial events for signal transduction that lead to degranulation occur in lipid microdomains of the plasma membrane, called lipid rafts, characterized by the presence of gangliosides. The present work investigated the role of mast cell specific derivatives of the ganglioside GD3 on the modulation of the release of mediators from RBL-2H3 cells, a mast cell line. Cells were incubated for 1-24 hours with mAbAA4 that recognizes these derivatives of the GD3 gangliosides on the mast cell surface. In unstimulated RBL-2H3 cells the gangliosides are homogeneously distributed on the cell surface. With increased time of incubation with mAbAA4, the gangliosides aggregate and cap on the surface. When the cells are stimulated via FcεRI, the release of β-hexosaminidase decreases with the increase in time of incubation with mAbAA4. At 1 hr of incubation the release of β-hexosaminidase is the same in cells incubated with or without mAbAA4. However at 24hr, the release of β-hexosaminidase is decreased by 80% in the cells incubated with mAbAA4. This decrease in β-hexosaminidase was also correlated with the increase in the aggregation of the gangliosides. These results show a converse correlation between ganglioside aggregation on the cell surface and beta-hexosaminidase release via FcεRI. Financial support: CAPES, FAPESP, FAEPF.
Phospholipase D (PLD) activity is necessary for secretion in RBL-2H3 cells following FcεRI activation. RBL-2H3 cells were transfected with the active form of PLD2 (PLD2WT), the inactive form (PLD2CI) or only with the vector (PLD2F4). Living cells were examined by phase microscopy and for Differential Interference Contrast (DIC) microscopy cells were fixed with 2% glutaraldehyde prior to examination. Immunomicroscopy with mAb AD1 (anti-CD 63) was used to identify secretory granules and anti-GM130 to label the cis Golgi cisternae. By phase microscopy, all of the transfected cells appeared spindle shaped with a morphology similar to RBL-2H3 cells. However, the PLD2CI cells appeared more stellate and the PLD2F4 more elongated. DIC microscopy confirmed the basic morphology observed by phase microscopy, and suggested that the surface of the PLD2WT cells was ruffled. The presence of ruffles on the PLD2WT cells and the stellate shape of the PLD2CI cells were confirmed by SEM. Following stimulation via FcεRI, the PLD2WT and PLD2CI cells took longer to return to their unstimulated morphology than did the RBL-2H3 cells. Immunostaining suggests that the secretory granules in the PLD2CI cells may be reduced in size. These results demonstrate that PLD2 may be involved in regulating other cellular functions in addition to secretion of cytoplasmic granules.

Financial Support: FAEPa, FAPESP.

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The morphology of two ganglioside deficient cell lines (E5 and D1) was compared with that of RBL-2H3 cells. E5 cells variably express the α-galactosyl derivatives of the ganglioside GD1b on their surface and D1 cells do not express these gangliosides. By SEM, unstimulated RBL-2H3 cells are spindle shaped and their surface is covered with microvilli. Five min after stimulation, the cells spread and their surface is covered with ruffles. At 15 and 30 min, the cells have spread more and their surface is still covered with ruffles, at 45 min, the majority have not returned to their original morphology. The E5 cells have morphology similar to RBL-2H3 cells. By 5 min, the E5 cells have spread and their surface is covered with ruffles. At 15 and 30 min, the surface of the cells is covered with long ruffles at the equatorial zone. By 45 min, the E5 cells have returned to their original morphology. The majority of D1 cells are round, and their surface is dramatically altered. The D1 cells do not change their morphology in response to stimulation via FcεRI. The deficient cells had a reduced liberation of β-hexosaminidase. In conclusion, the mast cell-specific α-galactosyl derivatives of ganglioside GD1b may be important in cell activation and related morphological changes.

Financial Support: CAPES and FAPESP.
P-006 ANALYSIS OF PATHWAYS INITIATED IN ACTH AND FGF2 RECEPTORS IN RAT ADRENAL PRIMARY CULTURE CELL.
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Introduction and Goals: The mitogen-activated protein kinase (MAPK) cascades are a crucial cell-signaling pathway that has been used as a biochemical probe in the evaluation of mitogenic potential of hormones and growth factors. In this work we analyze MAPK pathways activation, ERK1-2, JNK/SAPK, p38, and PKA (p-CREB) in the trophic response of normal adrenocortical cells activated with ACTH (adrenocorticotropic) or/and with FGF2 (fibroblast growth factor). Methods: The cells used were Y1 mouse adrenocortical tumor cell line and rat adrenal primary culture cells, glomerulosa (G) and fasciculata/reticularis (F/R) cells. Primary culture cells were obtained from Wistar male rats through dissection, enzymatic and mechanical digestion, filtration and centrifugation. After that, cells were seeded in plates or in coverslips in DMEM medium with 10%FCS at 37°C and 5% of C0 2. After serum-starved for 24h the cells were treated with 10^-9M ACTH, 1ng/ml FGF2, A+F and 10^-9M AGII (angiotensin II) for different times. Cells then were fixed with formaldehyde 3,7% or solubilized in RIPA buffer for immunoperoxidase or Western Blot assays, respectively. Results and Conclusion: The analysis of CREB-p showed different kinetics of activation in G and F/R cells in a time depending manner; while F/R cells and Y1 cell line stimulated with ACTH present maximum of activation in 1h of treatment G cells showed maximum activation in 10min of treatment. Supported by FAPESP and PRP-USP.

P-007 ISOPRENALINE INDUCES SLOW CALCIUM SIGNALING IN SNAKE VENOM GLAND.
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The stimulation of beta-adrenoceptors present in Bothrops jararaca venom gland is important to trigger the venom production cycle. These receptors have a different pharmacological profile from those described in mammals. Despite atypical pharmacological characteristics, they are coupled to c-AMP signaling pathway (Yamanouye et al, Life Sci, 67, 217, 2000) as occurs in beta-adrenoceptors described in the literature. We are now interested in verifying whether beta-adrenoceptor in snake venom gland is coupled to other signaling pathway. In this study, we are evaluating the participation of calcium signaling. The intracellular calcium was measured by fluorimetric method, using fura-2-AM as a fluorophore. Isoprenaline was able to induce a slow increase in the intracellular calcium concentration in both free calcium medium (22.53±7.2%, n=7) and 2.5 mM calcium medium (56.61±11.13%, n=6) from the basal, and these values are significantly different (p<0.05). These results indicate that the stimulation of beta-adrenoceptor in snake venom gland mobilizes calcium from intracellular stores and extracellular calcium influx. In conclusion, beta-adrenoceptors in snake venom gland seems to be coupled to multiple signaling pathway. Supported by: FAPESP, CNPq, Fund. Butantan.

P-008 THE THYROMIMETIC SELECTIVE FOR THE THYROID HORMONE RECEPTOR β GC-1 INHIBITS THE PROLIFERATION OF OSTEOBLAST-LIKE CELLS AND STIMULATES THE GENE EXPRESSION OF OSTEOCALCIN.
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There are four isoforms of thyroid hormone receptors (TRs): TRα1, TRα2, TRβ1 and TRβ2. The functional roles of these isoforms in mediating the effects of triiodothyronine (T3) on the activity and proliferation of osteoblastic cells are not known. Our aim was to study (i) the growth cycle, (ii) the effect of T3 on cell growth and viability and (iii) the role of TRβ in mediating T3 actions on cellular growth and on osteocalcin (OC) mRNA expression of the ROS 17/2.8 osteoblast-like cells. The T3 analog selective for TRβ, GC-1, was used as a pharmacological tool. An equimolar dose of T3 and GC-1 (10^-8 M) significantly inhibited cellular growth. On the other hand, cellular viability was not modified by both hormones, suggesting that T3 and GC-1 did not induce cellular death but inhibited cellular proliferation. T3 stimulated OC mRNA expression in a dose- and time-dependent fashion. The GC-1 (10^-8 M/24 h) induction of OC mRNA expression was 1.9-fold vs. control, but was 2.9-fold lower than that of T3 and was not dose-dependent. Our findings suggest that TRβ mediates the inhibitory effects of T3 on cellular proliferation and that the induction of the OC gene expression by T3 depends on both TRα and TRβ mediation.

P-009 HEPATIC ALTERATIONS IN RATS TREATED CHRONICALLY WITH N^6-NITRO-L-ARGININE METHYL ESTER (L-NAME).
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Chronic treatment of rats with N^6-nitro-L-arginine methyl ester (L-NAME), an inhibitor of nitric oxide biosynthesis, causes cardiac and renal damage. In this work, we examined the changes in hepatic morphology in this model. Male Wistar rats received L-NAME (20 mg rat^-1 day^-1) in the drinking water for 2, 4 and 8 weeks, after which the livers were processed for histological analysis by light and transmission electron microscopy. Treatment with L-NAME caused hydropic degeneration, compaction of the sinusoidal lumen and deposition of connective tissue in the portal space. There was also a significant increase (*p<0.05) in arterial wall thickness. Electron microscopy showed damage to Kupffer cells, vacuolation, and changes in nuclear and sinusoidal morphology. PAS staining and direct quantification showed an increased glycogen content [5.58±1.03 (control) vs 10.09±0.78* (L-NAME), 4.46±0.93 vs 10.54±0.89* and 5.45±1.05 vs 11.27+1.28* mg glycogen/100 mg tissue, for 2, 4 and 8 weeks, respectively; n=5/group; mean±S.D.; *p<0.05]. There were no significant changes in tissue total cholesterol, HDL, LDL and triglyceride levels (n=5/group). These results show that chronic treatment with L-NAME caused hepatic alterations. However, ischemia, necrosis and fibrosis were less common than in cardiac and renal tissue. Financial support: FAPESP.
The aim of this study was to detect histopathological alterations in the reproductive organs after the hormonal replacement therapy (HRT) and to compare the results with phytoestrogens treated rats. Three groups of Wistar rats were isolated and submitted to HRT through gavage with: 1) 10 µg/ml of estradiol valerate and ciproterone acetate; 2) 46 x 10⁻³ µg/ml of soy protein; 3) distilled water (control group), during sixty alternating days. Breast and uterine samples were collected and processed for histological observation. The morphological results demonstrate that the treatment with estradiol valerate combined with ciproterone acetate was effective. The use of the isolated soy protein promoted similar effects, but the endometrium showed glands without dilatation, with cylindrical epithelium, and cells with eosinophilic cytoplasm, without vacuolization.

P-012 PIGMENT AGGREGATION IN SHRIMP CHROMATOPHORES.
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We propose a model for pigment aggregation by Red Pigment Concentrating Hormone, based on data from freshwater shrimp chromatophores. Like RPCH, A23187 and db-cGMP induce aggregation in 22 min. The minimum [Ca²⁺]ext that sustains RPCH-triggered aggregation is 10⁻⁷ M; Ca²⁺-channel blockers, verapamil and Mn²⁺, do not inhibit aggregation. Sarco-endoplasmic reticulum Ca²⁺-ATPase inhibitors, thapsigargin and cyclopiazonic acid, induce complete or ≈50% aggregation, respectively, while endoplasmic Ca²⁺-channel blockers, ryanodine and dantrolene sodium, do not cause aggregation, per se, but inhibit aggregation. IP₃, db-cAMP, forskolin, cADPR and enterotoxin, a guanylyl cyclase receptor stimulator, do not induce aggregation. The soluble guanylyl cyclase activators, sodium nitroprusside and SIN-1, induce ≈40% aggregation, while the soluble guanylyl cyclase inhibitors, protoporphyrin IX and LY83583, inhibit RPCH-induced aggregation ≈40%. Two signaling pathways may thus activate aggregation in caridean chromatophores: Ca²⁺ and cGMP. Cytoskeletal motor proteins are involved since actin polymerization inhibitors, latrunculin-A and cytochalasin B, completely inhibit RPCH-triggered aggregation, and A23187-induced aggregation by ≈70%; jasplakinolide, an actin polymerization inhibitor, inhibits RPCH-triggered aggregation ≈40%; the myosin ATPase inhibitor, butanedione monoxime, inhibits aggregation ≈30%. Colchicine, a microtubule polymerization inhibitor, does not affect aggregation, while EHNA, a dynein ATPase inhibitor, triggers ≈30% aggregation per se but inhibits RPCH-triggered aggregation ≈20%. These data suggest that actin microfilaments, myosin, and dynein, regulated by a Ca²⁺/cGMP transduction pathway may be responsible for pigment aggregation in shrimp chromatophores.

Financial support: CNPq, CAPES and FAPESP.

P-013 FAKE DEFICIENT MAST CELLS ARE DEFECTIVE INSECRETION AND CYTOSKELETON ORGANIZATION
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Mast cells play a critical role in allergic and inflammatory processes. In this study the responses of RBL-2H3 mast cells and two focal adhesion kinase (FAK) deficient variants, C5 and F5, to stimulation via FceRI were compared. C5 cells express 10% and F5 cells express 19% FAK compared to RBL-2H3 cells, but β-hexosaminidase release was decreased only in the C5 cells. By SEM, unstimulated RBL-2H3 cells are spindle shaped with their surface covered by microvilli. Five min after activation, the cells spread and their surface is covered with short ruffles. By 120 min, the cells have returned to their unstimulated morphology. The majority of C5 cells are round and covered with ruffles. By 30 min, the surface of C5 cells has pronounced ruffles and 2 hours after stimulation the cells have returned to their unstimulated form. F5 cells have a rounded-spindle shape with fine ruffles. When stimulated, F5 cells show some ruffling on the cell surface that becomes more pronounced at 45 min. By 120 min, F5 cells still have some ruffles. The organization of microtubules and actin filaments was disturbed in both C5 and F5 cells. Immunostaining also suggests that the secretory granules in C5 cells are clustered. These results suggest that FAK affects secretion and cytoskeletal organization.

Financial Support: CNPq, FAEP and FAESP.
P-014 NITRIC OXIDE SYNTHASE STIMULATION BY THE VEGETAL COMPOUND MAIS VIDA
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The aim of this work was to evaluate the effect of the vegetable compound Mais Vida on nitric oxide synthase (NOS) activity. The NOS was obtained by rat brain homogenization. A volume of homogenate containing 2 mg of protein was incubated with iNOS and eNOS inhibitors and different concentrations of the Mais Vida (0.26 – 78.00 mg/mL), composed by the ethanolic extracts of: leaves of babaçu (75%), bardana (4%), boldo (5%), espinheira-santa (5%), thuia (4%), white rose flowers (5%) and ipê-roxo stem (2%). The nitrite concentration was determined by the Griess method. The Mais Vida stimulated the NOS activity (dose-dependently). The Mais Vida concentrations of 0.26 – 6.50 mg/mL had no effect on NOS stimulation; the concentrations of 13.00 – 78.00 mg/mL increased NOS stimulation up to 247.59%. The Mais Vida concentrations of 0.26 – 6.50 mg/mL had no effect on NOS stimulation; the concentrations of 13.00 – 78.00 mg/mL increased NOS stimulation up to 247.59%. The Mais Vida concentrations of 0.26 – 6.50 mg/mL had no effect on NOS stimulation; the concentrations of 13.00 – 78.00 mg/mL increased NOS stimulation up to 247.59%. The results indicate that the Mais Vida stimulates the isoform iNOS. However, the Mais Vida seems to stimulate the NOS-independently nitrite production, as we do not achieve a total inhibition of Mais Vida in the presence of NOS inhibitors. The Mais Vida has been used for the treatment of cancer, so that its mechanism of action seems to include the cellular toxicity by the production of NO.

P-015 EFFECT OF BARDANA EXTRACT AND BOTHROPS VENOM ON NITRITE PRODUCTION BY RAT MUSCLE AND BRAIN
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The aim of the present work was to evaluate the effect of bardana extract on muscle regeneration and nitrite production in rat muscle and brain after muscle injury by Bothrops neuwiedi venom. We used Wistar rats divided into 3 experimental groups: control (venom-injured); bardana (no injury and treated with bardana) and treated (venom-injured and treated with bardana). The tibialis anterior was injured by intramuscular injection of 0.1 mg/kg of venom. The bardana extract was administered orally (500 mg/kg) during 7 days. The nitrite concentrations in muscle and brain homogenate were determined by the Griess method and histological analysis was also performed. The bardana extract increased the nitrite concentration in both muscle and brain and increased only slightly when associated with Bothrops venom (p<0.05). In the muscle the nitrite concentration obtained was: control: 0.2115; bardana: 0.2437 and treated: 0.2140 µg/µg protein and in brain, they were, respectively: 0.3759; 1.0194 and 0.5389 µg/µg protein. The bardana extract accelerated the inflammatory reaction and increased muscle regeneration. Our results indicate that the bardana extract increased muscle regeneration and nitrite production. Part of this nitrate could be produced by the stimulation of nitric oxide synthase enzyme.

P-016 ANALYSIS OF THE VITAMIN D RECEPTOR POLYMORPHISMS AND BONE MASS GAIN POST EXERCISE PROGRAMME.
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Bone mineral mass is affected by many variables such as genetics, environment and nutrition. Genetic factors account for 60-80% of the inter-individual bone mineral density variability. Amongst candidate genes with a potential role in determining bone mineral mass, several polymorphisms in genes of receptors directly involved in calcium homeostasis such as the Vitamin D receptor (VDR) has been studied. The VDR polymorphism found in the intron VIII changes a T>C and creates a Bsm I restriction enzyme site. The b allele is associated with significant better bone mineral density in post-menopause women. The aim of this work was to investigate if the genotype of this VDR polymorphism influenced the bone mass gain in a group of individuals. Sixteen young adult males underwent intensive exercise training programme during three months. Their bone mass was evaluated before and after the programme, showing significant increase. Genomic DNA from their peripheral blood leukocytes was extracted and used as a template for PCR reactions with specific primers, to analyze the polymorphic region of the VDR gene. We genotyped them by digesting with Bsm I restriction enzyme. No association was found between the Bsm I VDR polymorphism and the bone mass gain in these individuals, suggesting that the VDR influence alone is not directly responsible for different bone mass gain.

P-017 ANALYSIS OF THE EXPRESSION OF MUTANT CALCIUM-SENSING RECEPTOR
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The calcium sensing receptor (CASR) is a member of the G-protein-coupled receptors superfamily and plays a key role in extracellular calcium homeostasis. It regulates parathyroid hormone secretion from parathyroid cells and calcium reabsorption by the kidney. Extracellular calcium ion (Ca^{2+}) activates the calcium sensing receptor, leading to activation of phospholipase C-β via the Gq subfamily of G-protein, this increases phosphoinositide (PI) hydrolysis, which in turn causes release of calcium from intracellular stores. To examine the role of the specific amino acids on the transmembrane domain of the calcium sensing receptor, we transfected HEK-293 cells with a series of CASR cDNA with different point mutations in the transmembrane domain and a deletion of the third extracellular loop and analyzed their expression by Western blot. The receptor with the mutations L773Q, F788S, F806C, A835D and the deletion (∆ 827-838) showed levels of cell expression equivalents to the wild type receptor, indicating that change the polarity in these positions and deletion of the third extracellular loop have no impairment in receptor expression. In contrast, the receptor with the mutations A824S and A843T showed a significant reduction in expression in relation with the wild type, indicating that these positions are important to the receptor expression.
P-018 THYROID HORMONE INCREASES UROCORTIN-1 (UCN-1) IN CARDIAC MYOCYTES.
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Introduction: Ucn-1 is a peptide that belongs to the CRF family of peptides and with marked cardiovascular actions as cardiovascular protective role in response to an injury by hypoxia. Ucn-1 is localized in neurons of the central nervous system as well as in cardiac myocytes. However, the role of diverse hormonal stimuli closely related to cardiovascular changes, as thyroid hormone has not been evaluated yet. Materials and methods: Cultured neonatal rat myocytes were treated with different doses of T3 or T4, for 24 hours. After treatment with the hormones, the Ucn-1 presence was evaluated by immunoenchemistry, such labeling was quantified using fluorescence microscope (40X) and appropriated software. Results: Cardiac myocytes submitted to thyroid hormone treatment showed a significant increase on Ucn-1. The treatment with T4 (10^{-8} M) promoted a 3.0-folds increase and with T3 (10^{-8} M) a 4.7-folds increase compared to control group. Conclusion: The increase on Ucn-1 induced by elevated levels of thyroid hormone may be an important cardiovascular protective factor when the myocardium is subjected to hypoxia conditions as that promoted by hyperthyroidism.

P-019 RESEARCH OF THE ESTROGEN AND PROGESTERONE RECEPTORS, THROUGH IMMUNOCHEMISTRY IN HUMAN PLEOMORPHIC ADENOMAS (PA).
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The neoplasmy of salivary glands form an important group of neoplasms in the head/neck region, representing approximately 3% of the malignant and benign neoplasias of this region. The classical approach to the treatment of several neoplasies has been widely attributed to studies which detect the possibility of inhibition of a factor or its signaling way by specific drugs, that could be conclusive in the control of the neoplastic proliferation. Estrogen (RE) and progesterone (RP) receptors have already been appointed as neoplas promoters of PA, usually in the salivary glands. It is questionable the PA hormonal dependence because of its great incidence in females and similarities found between mama and salivary gland neoplasies. This study proposes to research the presence of RE and RP in human PA of salivary glands. Immunohistochemistry technique by the method of streptavidin-biotin peroxidase, in PAs embbeded in paraffin was analyzed, with monoclonal antibodies to RE and RP proteins. We found seven RE positive and eight RP positive of the 10 Pas analyzed, confirming that PAs could be hormone-dependent.

Q-001 PRECONCEPTIONAL INFECTION AND CONGENITAL TOXOPLASMOsis IN Calomys callosus Bellisa de Freitas Barbosa; José Roberto Miniéry; Deise Aparecida de Oliveira Silva; Eloisa Amâdia Vieira Ferro. 1 Laboratório de Histologia, ICBIM, UFU; 2 Laboratório de Imunoparasitologia, ICBIM, UFU. bellisasfb@yahoo.com.br

Toxoplasma gondii infects several cells of mamiferous and birds. There are two groups of high-risk individuals: the pregnant and the immunosuppressed patients. Calomys callosus, when acutely infected with T. gondii ME49 strain during pregnancy, transmits the disease for its offspring. There is a risk of fetal infection when the maternal infection occurs a few weeks prior to conception. This study aimed to verify temporally the acquisition of preconception maternal serocconversion on the risk of congenital toxoplasmosis. Twelve female C. callosus were inoculated with T. gondii and divided into three groups containing four animals that were coupleed after eight days (group 1), thirty days (group 2), and forty days (group 3). The animals were killed between the 17th and 20th day of gestation, when placentas and embryos were collected for morphological and immunohistochemical studies. Serum samples were also collected and evaluated in immunoenzymatic assays. All infected animals showed seroconversion, but the presence of parasites was found in placentas and embryos from females coupled after eight and thirty days of infection, but not after forty days of infection. In conclusion, in this study model, congenital toxoplasmosis can take place when maternal infection occurs at one month before conception, thus demonstrating the time of acquisition of resistance for congenital toxoplasmosis.

Q-002 ANALYSIS OF POTENTIAL TARGET TISSUES FOR ANTIHELMINTHIC DRUG ACTION IN MESOCOSTOIDS CORTI (PLATYHELMINTHES: CESTODA) BY CONFOCAL LASER-SCANNING MICROSCOPY
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Although platyhelminths are relatively simple organisms, parasitic species from this phylum present significant body architecture rearrangements during their complex life cycles, which must be considered in drug therapy attempts. Microscopy analyses of different developmental stages of parasitic platyhelminths allow the determination of body structures and/or regions which are more propitious to drug challenge. For that, Mesocostoids corti is a good model cestode, due to its easy experimental manipulation and close relationship to species of medical and/or veterinary importance, such as those from genus Echinococcus and Taenia. Whole mounted M. corti individuals, fixed at different developmental stages, were analysed by confocal laser-scanning microscopy, after staining with lectin WGA and phalloidin, to highlight tegument and muscle structures, and with DAPI, to visualize individual nuclei. Hematoxillin-eosin stained sections were used to follow the development of internal structures and tissues by light microscopy. Changes in tegument and muscle fibers arrangement during segmentation, somatic and sexual differentiation, and bud formation were visualized. Pools of cells, corresponding to possible proliferation and differentiation centers, were identified around suckers, along nerve cords and in developing proglotids, in regions of reproductive organs formation. The effects of different antihelminthic drugs (e.g. praziquantel and albendazole) on the identified tissues and structures are under evaluation. Supported by FAPERGS, CNPq, FAPESP, PROPESQ & RTPD Network (SIDA).
We have previously described in *L. amazonensis* a pore-forming protein (leishporin) that lyases cells, including macrophages. We have shown that pore formation occurs in two distinct steps: 1) binding of the cytolysin to the plasma membrane and 2) lysis itself, probably insertion and/or oligomerization of subunits. Neither surface proteins nor carbohydrates seem to be important for the binding of the cytolysin to cells. Aiming to determine the role of membrane lipids in binding leishporin, promastigote extracts were incubated with liposomes made of cholesterol and dipalmithoilphosphatidylcholine for 5 min at room temperature. After removal of liposomes, the lytic activity of the supernatant was determined. We verified that the liposomes totally remove the lytic activity of the parasite extract, indicating the binding of leishporin to one or to both lipids. The hemolytically inactive supernatant can be further activated by incubation with proteases or dissociating agents, which destroy or remove, respectively, an oligopeptide activated with classical stimulators (interferon gamma and interleukin-12). Our results suggest that Gal-3 plays a role in the hepatic granuloma formation and interferes in the inflammatory cell migration, as well as in situ proliferation and differentiation.

Galecint-3 (gal-3) has a proinflammatory activity inducing cell proliferation and differentiation, migration, adhesion and activation. It is expressed in macrophages, basophils, mast cells and neutrophils. Here we monitored the inflammatory cell populations from hepatic granulomas of galecint-3 knock out mice infected with Schistosoma mansoni. Schistosome-infected Galecint-3 KO and WT mice were studied at the chronic stage of disease (90 days). Histopathology: paraffin sections, hematoxylin/eosin, picro-sirius, sirius-red, and caspase-3 immunodetection. Flow Cytometry: cells harvested from garnulomas were stained with monoclonal antibodies for GR-1, Mac-1, CD4, CD5, CD8, CD19, B220, and e-Kit. Although no major granuloma modifications were observed, granulomas from KO animals were smaller than from WT animals (p=0.017), less fibrotic (p<0.05), with less eosinophils. The number of apoptotic cells (caspase-3+) was increased in KO mice. Flow cytometry showed that CD5+ cell numbers were diminished in KO animals, and CD4/CD8 ratio was altered, with increased CD4+ cells. B lymphocytes were increased in KO animals mainly due to B220+CD19+ population. Mac-1 population was not altered, although GR-1 subpopulation percentage tended to be higher in KO animals (p=0.06). Our results suggest that Gal-3 plays a role in the hepatic granuloma formation and interferes in the inflammatory cell migration, as well as in situ proliferation and differentiation.

**Q-005 INFLAMMATORY CELL DISTRIBUTION AND MOBILIZATION TO Schistosoma mansoni EGG-INDUCED HEPATIC GRANULOMAS IN GALECTIN-3 KNOCKOUT ANIMALS.**

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**Q-006 DISTRIBUTION OF EPITOPES OF TRYPANOSOMA CRUZI AMASTIGOTES IN CARDIAC MUSCLE OF Calomys callosus INFECTION WITH DIFFERENT SOURCES OF INOCULUM.**

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Distribution of the *Trypanosoma cruzi* stage-specific epitopes defined by monoclonal antibodies (Mab) raised against parasite forms were examined in the cardiac tissue in acute phase of the disease. *Calomys callosus* were infected with bloodstream trypanmastigotes (BT) and tissue culture trypanmastigotes (TCT) from the Y strain of *T. cruzi*. Sections of paraffin-embedded hearts were processed for confocal immunofluorescence microscopy. Different Mabs were used: Mab 2C2, reacts with a carbohydrate epitope in Ssp-4, a major surface glycoprotein of amastigotes; Mabs 1D9, 2B7, recognize epitopes on Ssp-4 different from Mab 2C2. Samples were double labeled with DAPI to visualize parasite’s kinetoplasts and nuclei. Mab 2C2 showed a fluorescence pattern distributed over the amastigote surface in animals infected with TCT, while amastigotes of animals infected with BT showed rare fluorescent spots in their surface. Mab1D9 presented plasma membrane and flagellar pocket region labeled in amastigotes of animals infected with BT but the reactivity in animals infected with TCT was very weak. Negative and flagellar pocket region labeling were observed with Mab 2B7 in amastigotes of TCT and BT, respectively. Differences in the expression of epitopes recognized by Mab 2C2, 1D9 and 2B7 suggest that molecules belonging the membrane surface changes when de inoculum are obtained from different sources (BT and TCT) changing the composition of surface antigens expressing in amastigotes.
Q-007 Leishmania Amazonensis PROMASTIGOTES CAN BE TARGETED TO PREFORMED COXIELLA BURNETII PHASE II PARASITOPHOROUS VACUOLES IN VERO CELLS.
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Parasitophorous vacuoles (PVs) containing a prokaryote and an eukaryote were built in CHO cells infected with Coxiella burnetii phase II and superinfected with Leishmania amazonensis amastigotes (Veras et al., Inf. Imm. 63, 3502, 1995). In those experiments chimeric vacuoles were first found after 6 h and increased in number at later time periods. In the present studies Vero cells, infected for 3 days with Coxiella promastigotes were superinfected with L. amazonensis stationary promastigotes at an MOI of 7:1. Colocalization of the organisms in PVs was first detected at 1 h of superinfection and recorded in video. At 3 h, 77% of the infected cells contained Coxiella vacuoles with an average of 5 tumbling promastigotes per vacuole. At 24 h, transitional forms of the parasites were found in the vacuoles. At 48 h doubly infected vacuoles contained an average of 15 amastigotes/PV. This growth kinetics was similar to that of cultures infected with L. amazonensis alone. When cultures were placed at 25°C for 24 h most of the amastigotes in the chimeric vacuoles were transformed into moving promastigotes; this was reverted when cultures were brought back to 37°C. Thus, stationary L. amazonensis promastigotes, at low MOIs are rapidly taken up by Vero cells and transferred into Coxiella vacuoles where they are able to differentiate and multiply as amastigotes.

Q-009 VIRAL INACTIVATION THROUGH INHIBITION OF VIRUS-INDUCED MEMBRANE FUSION
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Membrane fusion is an essential step in the entry of enveloped viruses into their host cells. Vesicular stomatitis virus (VSV) is a member of the Rhabdoviridae family, a group of enveloped virus. Both VSV binding to the cell surface and fusion between viral envelope and endosomal membrane are mediated by its surface glycoprotein, the G protein. Previously, we demonstrated that protein G His protonation was essential both for low-pH-induced conformational changes of VSV G protein and for the fusion reaction itself by modification of these residues with diethylyrocarbonate (DEPC). In order to investigate whether His modification affects viral infectivity, we evaluated the effect of VSV treatment with DEPC in BHK21, cells and primary cultures of CNS cells. Our results demonstrate that infectivity and viral replication were abolished by viral treatment with 0.5 mM DEPC as measured by 50% tissue culture infectious dose (TCID50) and Western Blotting analysis. To evaluate whether this inactivation approach is efficient for other viruses, the infectivity of the Mayaro virus, a member of the Togaviridae family, was measured by TCID50. The viral infectivity was abolished by modification with 10 mM DEPC. The results suggest that His residues protonation play a crucial role in VSV infection. Moreover, this method of inactivation can probably be used for other enveloped viruses. Supported by CNPq, FAPERJ and CABBIO.

Q-008 CAN ENUCLEATED CELLS BE INFECTED WITH SHIGELLA FLEXNERI?
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Invasion of cells by bacteria, often triggered by the activation of signaling cascades by pathogen-encoded virulence determinants, has been associated with extensive host cell transcriptional responses assumed to be neutral, protective, or to favor pathogen survival. Enucleation provides a tool to investigate whether His modification affects viral infectivity, we evaluated the effect of VSV treatment with DEPC in BHK21, cells and primary cultures of CNS cells. Our results demonstrate that infectivity and viral replication were abolished by viral treatment with 0.5 mM DEPC as measured by 50% tissue culture infectious dose (TCID50) and Western Blotting analysis. To evaluate whether this inactivation approach is efficient for other viruses, the infectivity of the Mayaro virus, a member of the Togaviridae family, was measured by TCID50. The viral infectivity was abolished by modification with 10 mM DEPC. The results suggest that His residues protonation play a crucial role in VSV infection. Moreover, this method of inactivation can probably be used for other enveloped viruses. Supported by CNPq, FAPERJ and CABBIO.

Q-010 SURVIVAL OF THE POPULATION OF NITRERGIC MYENTERIC NEURONS IN THE COLON OF BALB-C MICE INFECTED WITH Trypanosoma cruzi
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NADPH-diaphorase staining was performed on whole mount preparations of the muscularis externa of the distal end of Balb-c mouse colon taken from control animals and animals acutely infected with the Y strain of T. cruzi. Ganglia of acutely infected mice displayed an irregular distribution: the neurons stained intensely, but they were not altered in morphology. The number of NADPH-diaphorase positive neurons in the myenteric plexus was counted by a systematic random sampling method. In the control animals, there were 63,156 ± 6,366 stained neurons in the distal part of the colon. There was no significant difference in the neuron numbers between the control and the chagasic animals. Measurements of size showed small, medium and large neurons. The results suggest that the infection caused by T. cruzi affected only the large neurons. The density of NADPH-diaphorase positive varicose axons in the myenteric plexus and in the muscularis appeared to be slightly greater in the infected animals. These results provide evidence that the majority of the population of the myenteric NADPH-d positive neurons of the Balb-c mouse colon, survive in the T. cruzi infection.
Natural and synthetic polycationic proteins, such as protamine, have been used to reproduce the tissue injury and epithelial permeability caused by positively charged substances released by polymorphonuclear cells during inflammation. In this work, we examined the influence of protamine on paracellular barrier function and TJ structure using two strains of the epithelial Madin-Darby canine kidney (MDCK) cell line (“tight” TJ-strain I and “leaky” TJ-strain II). Protamine induced concentration-, time- and strain-dependent alterations in transepithelial electrical resistance (R_t) only when applied to apical or apical+basolateral monolayer surfaces. In MDCK II cells, protamine 50 µg/ml caused a significant increase in R_t, however, protamine 250 µg/ml significantly decreased the R_t after 30 min. In contrast, treated MDCK I monolayers showed a significant decrease in R_t after treatment with protamine at both concentrations. The protamine-induced decrease in R_t was paralleled by an increase in the phenol red basolateral-apical flux in both MDCK strains, suggesting disruption of the paracellular barrier. Marked changes in cytoskeletal F-actin distribution and a significant reduction in the junctional expression of the tight junctional proteins occludin and claudin-1 but subtle alterations in ZO-1 were observed following protamine-elicited paracellular barrier disruption. In conclusion, protamine induces alterations in the epithelial barrier function of MDCK monolayers that may involve the cytoskeleton and TJ-associated proteins.

Cell coupling and adhesion mediated by intercellular junctions play a crucial role in the insulin secretion process in the endocrine pancreas. Neonatal pancreatic islets display a decreased insulin secretion in comparison with adult islets. In this work we investigated the cellular expression and localization of the adherens junctional protein β-catenin and the gap junctional connexins Cx36 and Cx43 in endocrine pancreas of neonatal and adult rats. As revealed by immunoblotting and immunocytochemistry, adult rat islets showed a relatively high expression of β-catenin, associated to the cell-cell contact region, and of Cx36, that has been shown to form channels between B-cells, in comparison with neonatal islets. However, we observed no differences in expression and localization of Cx43 between neonatal and adult pancreatic islet cells. The increased expression of these junctional proteins during development may indicate a relationship between in vivo maturation of the insulin secretory machinery and cell coupling mediated by gap and adherens junctions.

Financial Support: FAPESP, FAEP/UNICAMP

Gap junctions (GJs) have active participation in the regulation of different processes as cellular proliferation and differentiation in hematoipoiesis. GJs are intercellular structures that allow passing through small molecules of until 1kDa. They are formed by 6 proteins called connexins (Cxs). The Cx in the bone marrow (BM) is the Cx-43. Our objective is to evaluate the expression of the Cx-43 in the BM after treatment with the 5-Fluourouracil (5-FU) that is a myelossuppressive agent. Control (0.9% saline) and treated animals (mouse C57/BL6) with 5-FU (150mg/kg) were used. Bone were fixed, decalcified in EGTA and embedded in wax. The primary antibody was anti-Cx-43, the secondary was goat anti-rabbit antibody Alexa Fluor 488-conjugated and the nucleus was stained with DAPI. The images were captured in the confocal microscope Carl Zeiss LSM 510. Some authors observed the increase of Cx-43 expression in nucleate cells in BM of mouse treated with 5-FU. However, we observed precise labeling of Cx-43 in non-nucleate cells as well as in nucleates cells. It is known that after the treatment with 5-FU occurs an increase of platelets. It is probable, that the non-nucleate cells that presented label for Cx-43 are the platelets. This result is very important in cellular physiological responses in which Cx-43 is increased.

Ora serrata is the transition region between the retina and ciliary body. The aim of this study was to describe and localize the intercellular junctions in the ora serrata region of albino and pigmented rabbit eyes. It was used 18 eyes of adult male rabbits, 12 albino and 6 pigmented, fixed and processed for transmission electron microscopy. Semithin sections stained with toluidin blue were examined and documented in the light microscope and ultrathin sections contrasted with heavy metals, in the electron microscope. The ora serrata region showed adherens, gap and tight junctions in the retina side, in the properly transition and in the ciliary body side in the eyes of albino and pigmented rabbits. The adherens junctions are plentiful, forming the zonulae adherens, appearing tight junctions between Müller’s cells and the photoreceptors, in the retina side of the ora serrata region. In the ora serrata proper or transition region, adherens and focal tight junctions attach the pigmented cells apical membranes with no undifferentiated glial cells. In the ciliary side of the ora serrata region, there are adherens, gap and tight junctions. The adherens junctions, desmosomes and gap junctions were found between the apical and apicolateral membranes of the ciliary epithelium cells. The tight junctions appear as zonulae occludens in the non pigmented cells apicolateral membranes and as focal tight junctions between pigmented and non pigmented cells apical membranes. There were no differences between albino and pigmented rabbits intercellular junctions types and distribution.
R-005 EXPRESSION OF GAP JUNCTION GENE CONNEXIN 43 IN HEPATOCARCINOMA FUSED TO GREEN FLUORESCENT PROTEIN REPORTER SYSTEM.

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Gap Junctions were defined morphologically as specialized contacts between cells that mediate the direct exchange of small molecules needed to coordinate multicellular system functions. They are composed of proteins called connexins that are assembled in a family of genes that differs each other on molecular weight. The abolishment of connexin expression is also related to many cases of tumor progression. In order to investigate the role of connexin 43 in living cells, we used an enhanced green fluorescent protein to construct Cx43-GFP. To this end, we isolated Cx43 gene from Rattus norvegicus clone fused its carboxi-terminal to GFP by PCR. The use of lipofectin showed to be an important step for efficient transfection in rat hepatocarcinoma cell line (HTC). The request of GFP fused to specific target has been reported to be a useful tool, even to localize membrane molecules. HTC cell line showed to be deficient in Cx43 expression therefore it is a relevant system of study. The confocal microscope cell imaging showed gap-junction-like staining at interfaces of positive Cx43-GFP study. The confocal microscope cell imaging showed gap-junction-like staining at interfaces of positive Cx43-GFP study. The confocal microscope cell imaging showed gap-junction-like staining at interfaces of positive Cx43-GFP study. The confocal microscope cell imaging showed gap-junction-like staining at interfaces of positive Cx43-GFP study. The confocal microscope cell imaging showed gap-junction-like staining at interfaces of positive Cx43-GFP study. The confocal microscope cell imaging showed gap-junction-like staining at interfaces of positive Cx43-GFP study. The confocal microscope cell imaging showed gap-junction-like staining at interfaces of positive Cx43-GFP study. The confocal microscope cell imaging showed gap-junction-like staining at interfaces of positive Cx43-GFP study. The confocal microscope cell imaging showed gap-junction-like staining at interfaces of positive Cx43-GFP study. The confocal microscope cell imaging showed gap-junction-like staining at interfaces of positive Cx43-GFP study. The confocal microscope cell imaging showed gap-junction-like staining at interfaces of positive Cx43-GFP study. 

S-001 FISH OIL MODULATES THE FATTY ACIDS SYSTEM.

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Dietary rich in mono or polyunsaturated fatty acids (PUFAs - ω3) are recommended due to several benefits to human health. Besides the studies recognizing the therapeutic effects of PUFAs - ω3, additional facts can be added with focus on the dynamics of PUFAs in several organs. The dynamics of fatty acids (FA) in hepatic tissue of rats receiving diets with fish and pork lipids at different levels was studied. After 13 weeks, one half of the animals receiving experimental diets with lipids were killed, whereas others continued the assay for four more weeks, receiving only the control diet. Total lipids from liver was isolated and the fatty acid composition was determined through gas chromatography. Independent of its presence in diets, C22:6n3-DHA was found in hepatic tissue of rats receiving all treatments. In contrast, C20:5n3-EPA was identified only in liver of rat receiving diets with fish oil. Even after four weeks of intake control diet, animals that had received diets with fish oil presented higher levels of DHA. It was also observed that higher concentrations of arachidonic acid levels in hepatic tissue. The profile of fatty acid in hepatic tissue may be modulated by lipid origin in diets. This was supported by the different fatty acid profiles found in liver of rats that received diets with fish and pork lipids. 

Due to the nutraceutical properties of polyunsaturated fatty acids (PUFA), it is important to establish an experimental model for their study, correlating intake with tissue-specific dynamic and effects. A trial was run with five groups of male rats receiving fish oil or pork lipids at 4 % and 10 % supplied in diets. Experimental diets were given for 13 weeks, when rats were killed and other tissues were collected. At this point, the remaining animals start to intake a normolipidic diet, until the 17th week, when a second tissue collection was carried out. Lipids were extracted from adipose tissue, with the fatty acids (FA) composition determined by gas chromatography. Adipose tissue FA concentration reflected the pattern of diets. It seems that neither origin nor concentration of lipids in diet changed the FA profile, denoting its stability in adipose tissue. Linoleic acid maintained the same concentration in both visceral collections, and its tissue concentration showed the same profile in both diets. Eicosapentaenoic and docosahexaenoic acids of fish oil diets showed a special profile, the first did not appear in adipose tissue, and the second was detect in the first sample collection in low concentrations, disappearing at the second tissue collection. Considering its low dynamic, adipose tissue is not a good model to study PUFAs.

S-002 DYNAMIC OF POLYUNSATURATED FATTY ACIDS IN ADIPOSE TISSUES OF RATS.

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Creatine is an alimentar supplement used by high-impact athletes aiming an increasing in muscular mass. In this study we evaluate if the creatine oral supplementation causes modifications in intestinal wall. Male Wistar rats either received the standard diet for 8 weeks (control), or were treated with the standard diet for 4 initial weeks and then with diet plus supplement (2% creatine monohydrate) for the 4 final weeks (treated). Some animals were submitted to physical training in belt conveyor five times per week, during sixty minutes, in 8 weeks. The animals were divided into four groups: sedentary (S), sedentary supplemented with creatine (SCr), trained (T) and trained supplemented with creatine (TCr). After the sacrifice the duodenum was collected and processed for paraffin embedding and HE staining, for morphometric studies of crypts, villus and intestinal wall. A significant reduction was verified in the intestinal wall in group TCr when compared to groups SCr and T. The results were: muscular tunic thickness : SCr=100,3±23,2; T=113,2±19,4; TCr=50,8±8,4; crypts deepness : SCr=265,8±9,5; T=272,7±7,9; TCr=222±19,2 and villus height: SCr=494,1±70,8; T=462,0±25,9; TCr=362,6±38,2. Despite exercise increases translocation of glycosis and create transporters in striated muscle, it is possible that the combination between exercise and creatine supplementation could be inducing creatine transporters saturation and inhibition of glycosis transporters(GLUT) in enterocytes leading to a decreasing of energetic availability causing reduction of the intestinal wall.

S-003 CREATINE ORAL SUPPLEMENTATION ASSOCIATED TO PHYSICAL TRAINING DECREASES THE DUODENAL WALL.

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Creatine is an alimentar supplement used by high-impact athletes aiming an increasing in muscular mass. In this study we evaluate if the creatine oral supplementation causes modifications in intestinal wall. Male Wistar rats either received the standard diet for 8 weeks (control), or were treated with the standard diet for 4 initial weeks and then with diet plus supplement (2% creatine monohydrate) for the 4 final weeks (treated). Some animals were submitted to physical training in belt conveyor five times per week, during sixty minutes, in 8 weeks. The animals were divided into four groups: sedentary (S), sedentary supplemented with creatine (SCr), trained (T) and trained supplemented with creatine (TCr). After the sacrifice the duodenum was collected and processed for paraffin embedding and HE staining, for morphometric studies of crypts, villus and intestinal wall. A significant reduction was verified in the intestinal wall in group TCr when compared to groups SCr and T. The results were: muscular tunic thickness : SCr=100,3±23,2; T=113,2±19,4; TCr=50,8±8,4; crypts deepness : SCr=265,8±9,5; T=272,7±7,9; TCr=222±19,2 and villus height: SCr=494,1±70,8; T=462,0±25,9; TCr=362,6±38,2. Despite exercise increases translocation of glycosis and create transporters in striated muscle, it is possible that the combination between exercise and creatine supplementation could be inducing creatine transporters saturation and inhibition of glycosis transporters(GLUT) in enterocytes leading to a decreasing of energetic availability causing reduction of the intestinal wall.
Violacin, a pigment isolated from Chromobacterium violaceum in the Amazon river, Brazil, exhibits several biological properties, including a potential antitumor activity against several cell types. Our early reports have shown that this indole derivative induces apoptosis as indicated by cell bleb formation, chromatin condensation, caspase cascade activation and alteration in the expression of Bcl-2 oncprotein. Recently, studies have indicated the ability of violacin to modulate the activity of protein phosphatases. The reversible phosphorylation of proteins plays a crucial role in regulating signaling pathways that control cellular growth, differentiation, and activity. This study examines the effects of violacin on the activity of human blood serum tartrate resistant acid phosphatase (TRAP). Phosphatase activity was measured as p-nitrophenylphosphate released after incubation for 30 minutes with 0.1 mol/L substrate in 100 mmol/L acetate buffer, pH 5.5. The effect of violacin on TRAP activity was time- and dose-dependent, with an IC50 value of 5 µmol/L, after 20 minutes of incubation. This observation on violacin effect on TRAP activity raises the possibility for its application in the treatment of bone disorders such as osteoporosis, consistent with the documented key role for TRAP in osteoclastogenesis. Supported by FAPESP, PRONEX and CNPq.

We have investigated the effects of different water extracts from plants on metalloproteinases (MMP-2 and -9) activities. Extracts from two plants infusion with deionized water were lyophilized extract. The inhibition results are based on the disappearance of MMPs bands at zymogram compared to the control gel. 100% of MMPs bands disappeared in our samples. The lyophilized extract of plants can be used as MMPs inhibitor. The process was specific for the amino acid residue involved in the new site able to establish desorption ionization time-of flight mass spectrometry) analysis induced vesicle fusion. MALDI-ToF-MS (Matrix-assisted laser desorption ionization time-of flight mass spectrometry) analysis of the modified protein showed the participation of Lys27 as amino acid residue involved in the electrostatic interaction of cytochrome c with lipid bilayers, at pH 7.4. In this work, we demonstrated that after association with PCPECL (phosphatidylcholine, phosphatidylethanolamine and cardiolipin) vesicles at pH 7.4, the decrease of the pH up to 6.2 led to vesicle fusion due to the ionization of Lys residue situated at the opposite side of site A. This phenomenon was analyzed via turbidity and photon correlation spectroscopy measurements. The analysis of pH curves for vesicle fusion indicated that the phenomenon was dependent of one ionizable group with pKₐ= 6.95 ± 0.1. The process was specific for cytochrome c since albumine and protamine failed to induce vesicle fusion in a pH-dependent manner. Furthermore, cytochrome c N-acetylated and modified by DEPC did not induce vesicle fusion. MALDI-ToF-MS (Matrix-assisted laser desorption ionization time-of flight mass spectrometry) analysis of the modified protein showed the participation of Lys27 as the amino acid residue involved in the new site able to establish electrostatic interaction with acidic phospholipids. Supported by CNPq and FAPESP.
T-002 ULTRASTRUCTURE OF THE SPERMATOOZA OF DOMESTIC DUCK (Anas platyrhynchos).

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The ultrastructure of the spermatozooa of the domestic duck (Anas platyrhynchos) was analyzed by transmission electron microscopy and compared with the results obtained in preliminary studies involving other non-passeriform birds. The spermatozooa were characterized by presence of a short head, short midpiece and long principal piece. Each spermatozoon was long, narrow and cylindrical. The head was formed by a reduced acrosome that contained moderately electron-dense homogenous material. The implantation fossa was observed between the base of the nucleus and the proximal centrosome. The midpiece contained electron-dense material associated with the proximal centriole and nuclear membrane, and a long distal centriole surrounded throughout its length by a whole of 11 to 12 elliptical mitochondria. A dense annulus separating the midpiece from the principal piece was seen. Posteriorly to the annulus, the axoneme was formed surrounded by a dense fibrous sheath, representing the principal piece or flagellum, which represented a long segment with a smaller diameter in relation to the midpiece. The spermatozoon of the domestic duck resembles that of other non- passerine birds, corresponding to a basic type of spermatozoon.

T-003 ULTRASTRUCTURAL STUDY OF RAT DIAPHRAGM AFTER 4 WEEKS OF DENERVATION

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Morphological changes of muscle fibers from denervated diaphragm were studied. Four male, adult, albino rats (Rattus norvegicus) with 200 g weight and 60 days old were used. Phrenic neurotomy was performed on the left side in accordance with the Vital Brazil technique (1965). The right side of the diaphragm was used as control. The animals were sacrificed 4 weeks after surgical procedures. The muscles were removed and fragments of presetted areas from both sides were took out. The blocks were fixed with glutaraldehyde (2.5%) in PBS and processed for TEM. Morphological changes were found in some fibers along the denervated muscles. These fibers exhibit focuses of loosely arranged myofibrils with disarranged and/or faded Z lines. The nuclei are centrally positioned and show folded membranes which give them fragmented profiles. Pool of mitochondria with different degeneration grades and small myelin-like structures are seen in these muscle fibers too. These altered muscle fibers aside of morphologically normal ones confirm that muscle fibers with different metabolic profiles and morphology respond in a proper way at the denervation.
**T-005 DETECTION OF PARAMYXOVIRUS IN SMALL INTESTINE OF PASSERIFORM BIRD (DOUBLE-COLLARED SEEDEATER – Sporophila caerulescens). ULTRASTRUCTURAL STUDY.**

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Wild avian are important disseminators and viral reservoirs due to the natural migratory condition. Therefore, they house different types of viruses, which may affect other specimens, including domestic and commercial avian many times causing significant economic losses. Paramyxovirus transmission occurs by means of a direct airborne contact between avian, through dust particles in food and water. The New Castle Disease is caused by type I avian paramyxovirus being highly infectious and affecting SNC. Also respiratory alterations, circulatory disorders and severe diarrhea may occur. Paramyxovirus family, are pleomorphic, and filamentous or rounded shape containing a double lipid envelope with lipoprotein capsomers that may be surrounded by a nucleocapsid protein sometimes shaped like a "herring bone". They are simple strand RNA virus, from 18 to 20 kb, with cytoplasmic replication, measuring from 100 to 500 nm diameter. During illegal commercialization of Brazilian birds, among others an adult passeriform bird (Double-collared seedeater – Sporophila caerulescens) was apprehended and being forwarded to the CRAS, Tiete Ecological Park. In the next day, without presenting any symptomatology the animal died. Fragments of the small intestine were sent to the Electron Microscopy Laboratory of the Biology Institute of São Paulo to investigate viral agents being processed by negative staining techniques (rapid preparation) and resin-embedded and measuring from 100 to 500 nm diameter could be observed by means of the negative staining technique. Ultrathin sections revealed in the epithelial and in the cells of muscular layer, the presence of deformed nuclei with a marginalized chromatin, containing electrondense inclusions bodies formed by helical nucleocapsids. These inclusions bodies were also observed in the cytoplasm. Electronondense inclusions bodies containing granular or thread-like material and others with lower electrondensity were also visualized. Mature virus particles containing envelope covered with fine surface projections or spikes measuring from 100 to 250 nm and non-enveloped immature particles were observed inside the vacuoles and scattered all through the cytoplasm. Viral particles budding from the cytoplasmic membranes were also observed. Cellular organelles were altered.

**T-006 SOME ENZYMEOLOGICAL FEATURES OF SALIVARY THORACIC GLAND IN Polistes versicolor (OLIVIER, 1791) HYMENOPTERA: VESPIDAE**

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The Polistes versicolor salivary thoracic gland is composed by pseudodini. One this is constituted by a central cell (type A), surrounded by parietal cells (type B). The ducts 1, which arise from pseudodini, lead to the formation of ducts 2 and the fusion from these ducts forms the duct 3. The duct cells (type C) constitute the epithelium of those ducts and they appear differentiated in each one. Associated to the duct 2 we found some cells, named associated duct cells (type D). These ones may also add glycoconjugates to the secretion, produced by the central cell. There are no morphological differences among the casts. The enzymatic reactions, ATPase, SDH, NADH-TR, phosphatase, show some positively granules in the cytoplasm. These enzymes are located in the mitochondria and membranes and are involved in oxidative processes. The parietal cells, duct 1 cells and associated duct cells present a strongly reaction, indicating that these cells may act modifying the secretion, regulating the cell volume and the concentration of solutes. In P. versicolor, the development of the salivary thoracic gland is not dependant on the degree of ovariian development. This gland is related to foraging activities, adult-adult and adult-larvae trophalaxis, larval feeding and construction of the nest. These activities are performed by wasps of different ages, both subordinated and dominant females.

**T-007 EVALUATION OF THE ACTIVITY OF DIFFERENT FLAVONOIDS IN THE OXIDATIVE BURST OF NEUTROPHILS AND PHAGOCYTOSIS ASSAY.**

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The polymorphonuclear leukocytes (PMNL) have been referred to as professional phagocytes and are very efficient in internalizing particles. The phagocytosis are associated to the generation of chemically reactive molecules, such as superoxide anions, hydrogen peroxide, singlet oxygen as a result PMNLs of respiratory burst activation, which is essential for host defense against microorganisms. However, large amounts of reactive oxygen species (ROS) are suggested to be responsible for many diseases. In this work we evaluated the effect of flavonoids (Miricetin, Kaempferol and Galangin) on the generation of ROS by rabbit neutrophils (PMNLs) stimulated with immune complexes (IC-IgG). ROS production was measured by luminol and lucigenin enhanced chemiluminescence assay (CLlum and CLluc). The ultrastructural evaluation of PMNLs phagocytosis upon stimulation with IC-IgG colloidal gold complexes, in the presence of flavonoids was also assessed employing electronic microscopy. The obtained data demonstrated that flavonoids inhibited CLlum and CLlue production by PMNLs upon stimulation with IC-IgG, but did not interfered with these ICs phagocytosis. These observations suggest that these compounds may modulate the responses of PMNLs to IC-IgG, which are very important in an exacerbate inflammatory reactions, like autoimmune diseases.

**T-008 ULTRASTRUCTURE OF SILK GLANDS IN ADULT FEMALES OF SPHECID WASPS Microstigmus spp (HYMENOPTERA)**

João Naves de Ávila.

The ultrastructure of silk glands in adult females of sphecid wasps is described. Individual glands are scattered in the posterior portion of the metasoma, being formed by an enlarged secretory unity with one conducting canal. Each secretory unit contains a large extracellular lumen and numerous secretory granules, delimited by membrane and filled with middle electron dense content of homogeneous aspect, which are exocytosed into the extracellular lumen. Cell cytoplasm is filled with rough endoplasmic reticulum, polyribosomes and Golgi apparatus profiles. The basal plasma membrane has many short infoldings and is lined by a thin basement membrane. The apical membrane is invaginated to form the boundary of the extracellular lumen, containing scattered short microvilli. The cells are mononucleated and the nucleus is pleomorphic containing disperse chromatin. The extracellular lumen is separated from the receiving canal by a convoluted sponge-like wall forming many villi, which in their tip are lined by a thin single layered cuticle. In the basal region of the villi a cuticular covering is lacking. In the conducting canal secretion acquire two different electron densities. A thin cuticle lines the lumen of conducting canal, the cell cytoplasm is scarce and the organelles are represented for few rough endoplasmic reticulum and polyribosomes. These results suggest that sphecid’s silk glands have a unique structural organization when compared with silk glands in others insect order.
In crickets, the most common strategy employed by males to attract females for mating is to emit a calling song; however, some crickets’ species have lost their acoustic signaling ability and have evidently developed other communication modes. The genus *Eidmanacris* (Phalangopsidae) is an example of this kind of differentiation; the males of this genus possess metanotal glands that are thought to be the source of the sexual pheromone. The aim of this work is to analyze the ultrastructure of these glands; the adult males thorax of *E. corumbatai* was dissected, fixed with glutaraldehyde, post-fixed in osmium tetroxide, contrasted with uranyl acetate, according to the electronic microscopy technique. The ultrastructural analysis of the sections showed that the tip of these glands is destroyed during the mating by the female to receive hemolymph. The cellular composition of these glands included pigmentary cells, sensory cells and class III gland cells. The glandular cells exhibit typical components related to the synthesis of protein and lipid; these components must be involved in the production of pheromone, because the female accepts the mating when the male raises his tegminas and shows the metanotal glands. The sensory cells can be related with specific recognition and/or the release of haemolymph to ensure the mating. The haemolymph ingestion by the female during copulation has an effect on post-mating behavior of this female.

U-001 ADENOSINE TRANSPORT IN ENDOTHELIUM FROM FETUSES WITH INTRAUTERINE GROWTH RESTRICTION.
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Adenosine activates the L-arginine/nitric oxide (NO) signalling pathway in human fetal endothelium (HUVEC). In HUVEC from intruternue growth restricted fetuses (IUGR) activity of L-arginine/NO pathway is reduced. Since extracellular adenosine level is maintained mainly by the transport capacity of endothelium through system hENT1, we studied whether adenosine transport was altered in HUVEC from IUGR. [3H]Adenosine transport (4 µCi/ml, 20 s, 37°C) and [3H]NBMPR binding (0.01–2 nM, 4 µCi/ml, 30 min, 37°C) were determined in HUVEC in culture (from Clinical Hospital of the Pontificia Universidad Católica de Chile), in absence or presence of nitrobenzylthioinosine (NBMPR, 0.1–10 µM), hENT1 protein was determined by western blot and hENT1 mRNA was quantitated by real time RT-PCR. IUGR reduced NBMPR-sensitive adenosine transport associated with reduced maximal transport capacity (ie. \( V_{\text{max}}/K_p \)). IUGR also reduced the maximal binding sites for NBMPR, without altering the apparent Kd. Preliminary experiments show that hENT1 protein is unaltered by IUGR, but hENT1 mRNA number of copies is reduced (~90%). Thus, IUGR reduces adenosine transport capacity in HUVEC due to changes in the expression or availability at the plasma membrane of hENT1 nucleoside transporters.

Supported by FONDECYT 1030781 & 1030607 (Chile).

U-002 HYPOXIA REDUCES EXPRESSION OF EQUILIBRATIVE NUCLEOSIDE TRANSPORTERS 1 AND 2 IN HUMAN UMBILICAL VEIN ENDOTHELIAL CELLS.
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Hypoxia alters transport of nucleoside in several cell types. Membrane nucleoside transport occurs via the human equilibrative membrane transporters (ENTs) in human umbilical vein endothelial cells (HUVEC). The aim of this study was to determine whether changes in the level of O2 alter expression of hENT1 and hENT2 in primary cultures of HUVEC. Primary cultures of HUVEC from normal, full term pregnancies (from Clinical Hospital of the Pontificia Universidad Católica de Chile) were cultured for different periods of time (0-24 h) under different levels of O2 (21-1%). hENT1 and hENT2 mRNA was quantified by real time qPCR, using 28S mRNA as housekeeping gene. Protein level was determined by western blot. hENT1 and hENT2 mRNA expression was reduced in 2% O2 compared with 10% O2, but hENT2 protein level was increased in summary hENT1 and hENT2 gene expression are down-regulated by low O2 tension, an effect that could lead to alterations in nucleoside transport in human fetal endothelial cells.

Supported by FONDECYT 1030781 & 1030607 (Chile) and Dirección de Investigación, Escuela de Medicina, Pontificia Universidad Católica de Chile.

V-001 ACID PHOSPHATASES ACTIVITY IN MOUSE PERITONEAL MACROPHAGES TREATED WITH THE HOMEOPATHIC MEDICATION CANOVA
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Canova is a Brazilian homeopathic immune modulator used in several diseases where the immune system is disturbed. Morphological and biochemical results previously obtained in our laboratory show that Canova acts on macrophages, modulating its functions. This assay aims to evaluate modifications in the activity of lysosomal acid phosphatase (LAP) and tartrate resistant acid phosphatase (TRAP) in mouse peritoneal macrophages. Acid phosphatases are usually used as lysosomal system markers, catalyze the hydrolysis of phosphate esters and their presence is a biochemical indicator of activated macrophages. Ultrastructural and cytochemical assays were used to detect acid phosphatase activity. In optical microscopy, TRAP and LAP activities were detected in mouse peritoneal macrophages interacting or not with yeast by using a Sigma’s Kit. Electrondense reaction products of enzyme activity were observed as a weak positive reaction in the control group when compared to macrophages treated with Canova. The vesicles at early endosome region of this treated group show a strong electrondense reaction product. These results were confirmed by Sigma’s kit. Macrophages treated with Canova showed enhanced LAP activity, but a decrease was observed in TRAP activity. The same results were obtained when macrophages and yeast were allowed to interact. CONCLUSION: the acid phosphatase enhanced activity observed with electron and optical microscopy shows that macrophages are activated by treatment with Canova.

Financial support: CAPES, Paraná Tecnologia.
**V-002 MOBILIZING ANNEXIN 1 IN HUMAN LARYNX SQUAMOUS CARCINOMA CELL LINE (HEP-2)**

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The anti-inflammatory protein Annexin 1 (AnxA1) has been described as a mediator of glucocorticoid-regulated cell growth. In cancer, this protein is implicated in the pathogenesis of malignant neoplasms of different origins. In the present study we used a human larynx squamous carcinoma cell line (Hep-2) to investigate the expression of AnxA1 during treatment with the glucocorticoid dexamethasone and the N-terminal region of AnxA1 (peptide Ac 2-26). Addition of dexamethasone (1µM) and AnxA1 (2.5µg/ml) caused cellular growth inhibition in 7-day-old-cultures. The presence and localization of AnxA1 in Hep-2 cells was investigated by immunocytochemistry, using an antibody raised against the N-terminal region of AnxA1. The Hep-2 cells (7 days) showed immunoreactivity to AnxA1. A significant increase (p<0.05) in AnxA1 immunoreactivity was detected in both the nucleus and cytoplasm of AnxA1-treated cells in relation to dexamethasone-treated and control cells. The cells incubated with dexamethasone also revealed a significant increase of the protein content (p<0.05) in relation to the control cells. The data suggests a mechanism through which AnxA1 may regulate cell proliferation in tumor cultured cells. Supported by FAPESP

**V-003 THE Rel FAMILY TRANSCRIPTION FACTOR RelB IS LOCALIZED IN HUMAN MITOCHONDRIA**

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NF-kappaB is a dimeric transcription factor formed by Rel family proteins: NF-kappaB1 (p50/p105), NF-kappaB2 (p52/p100), RelA (p65), RelB and c-Rel. NF-kappaB is usually found inhibited in the citoplasm due to its association with an inhibitor protein, I-kappaB. The classic activation pathway occurs by I-kappaB phosphorylation followed by proteasome degradation, which releases NF-kappaB dimmer to nucleus. We have investigated the subcellular localization of all Rel family proteins. For this purpose, we used the PSORT program package for prediction of subcellular localization (http://www.psort.org), which showed that all human Rel family proteins, except RelB, present a nuclear-citoplasmatic localization. According to this analysis, RelB has a great probability to be in mitochondria (43.5%). The mitochondria targeting sequences prediction program (MitoProt; http://ihg.gsf.de/ihg/mitoprot.html) was used to corroborate this suggestion. To confirm this observation, we carried out an indirect immunofluorescence assay using an antibody anti-RelB together with a specific dye to mitochondria (MitoTracker ™) in HeLa cell line. We observed that RelB resides exclusively in mitochondria, suggesting that this distribution may reflect novel roles to the NF-kappaB members, beside its role in transcription.

**V-004 HUMAN LEUKOCYTE FORMIN PROTEIN ASSOCIATES WITH Akt, AN IMPORTANT CELL SURVIVAL PATHWAY**

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The very large family of Formin proteins is involved in processes such as morphogenesis, embryonic differentiation, cell polarity and cytokinesis. Formins bear a formin homology–1 (FH1), FH2 and FH3 domains. A novel human gene from the Formin family, denominated human leukocyte formin gene, was cloned in our laboratory, and found to be expressed almost exclusively in hematopoietic cells. Western blotting analysis demonstrated that the protein encoded by this gene is overexpressed in lymphoid malignancies, cancer cell lines and peripheral blood leukocyte from chronic lymphocytic leukemia (CLL) patients. About the interactions of this protein, we also found out that this protein associates with Akt, a critical survival regulator in many different cell types. The association was observed in a protein extract of Jurkat cell line and in peripheral blood leukocytes from CLL patients (n=5). Furthermore, cotransfections in 293 cells, using an expression vector pEGFP containing FH2 or FH3 domains, and an expression vector of pCMV-HA containing the full Akt showed that both FH2 and FH3 domains are involved in the association with Akt. In conclusion, we report that human leukocyte formin protein, expressed in lymphoid malignancies, is associated with Akt, an important pathway for cell survival.

**V-005 PROTECTIVE EFFECT OF ANNEXIN 1 IN A MODEL OF RAT EXPERIMENTAL PERITONITIS: ROLE OF THE MAST CELLS AND LEUKOCYTES**

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Mast cells (MC) can release an array of mediators, including histamine, tumoral necrosis factor alpha (TNF-α) and annexin 1 (ANX-1), which can induce both a rapid and a delayed inflammatory response, measured by leukocyte (PMN) recruitment. To analyze this reaction, we investigated the ANX-1 effect in rat mesentery after inflammatory stimulus. Rats were treated with ANX-1 i.v. at dose of 20 µg/kg. Five minutes later, experimental peritonitis was induced i.p. injection of 1.5 mg/kg of carragenin. Rats were sacrificed one or four hours later by CO₂ exposure. Fragments of mesentery were fixed (4% paraformaldehyde; 0,5% glutaraldehyde), embedded in LRGold and stained with toluidine blue 1% in borax 1%. In control rats, several MC presented intact (1.67±0.23) and a few degranulated (1.12±0.20), as well as a few PMN (0.40±0.08). One hour after carragenin administration, there was an increase in the MC (4.81±0.45); about 69.69% of these cells presented a degranulation process. Four hours after, an increase in the PMN number (6.18±0.20) was noticed. Treatment with ANX-1 for one hour restored the level of MC (1.69±0.16) and, for four hours, the PMN number (0.25±0.03). ANX-1 seems effective in the regulation of MC number and PMN accumulation in the inflammatory response, and as a target for the development of novel anti-inflammatory therapies.

Financial support: FAPESP
W-001 A NOVEL PrPrC PARTNER, VITRONECTIN, PROMOTES AXONAL OUTGROWTH IN DORSAL ROOT GANGLIA

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The cellular prion protein (PrPc) is the normal isoform of the infectious protein (PrPsc) involved in a number of neurodegenerative diseases. The physiological functions of PrPc include oxidative stress protection and cellular signaling. It has been also shown that its interaction with laminina promotes neurite outgrowth and maintenance. We evaluated the association of PrPc with vitronectin, another extracellular matrix protein whose neurotrophic properties include neurite growth, motoneuron and cerebellar granule cell differentiation.

We characterize PrPc binding to vitronectin in vitro with a Kd of 12 nM and mapped the interaction sites in PrPc between residues 105-119 and in vitronectin between residues 309-322. In mouse embryos, both proteins are expressed in the brain, medulla and dorsal root ganglia (DRG). Furthermore, we show that vitronectin and its peptide 309-322 can promote axonal growth in DRG, a phenomenon that is abrogated in PrPc null DRG and is inhibited by anti-PrPc antibody. We conclude that PrPc is able to bind the extracellular matrix protein vitronectin through specific binding sites. In addition, both proteins have the same distribution in DRG and mediate axonal growth.

Supported by FAPESP.

W-002 INVOLVEMENT OF STI1, A CO-CHAPERONE PROTEIN, IN THE HIPPOCAMPAL NEUROGENESIS MEDIATED BY CELLULAR PRION PROTEIN

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Prions, the etiological agents for infectious degenerative encephalopathies, act by inducing structural modifications in the cellular prion protein (PrP*). We have verified that PrP* binds ST11 (stress inducible protein 1) and this complex induces neuroprotective signals through MAPK and PKA. Immunohistochemistry and immunoblotting assays demonstrate that PrP* and ST11 are highly expressed in neurons. Herein, we sought to evaluate the role of PrP* – ST11 interaction in neurite outgrowth in hippocampal neurons from wild-type (Prnp<sup>+/+</sup>) and PrP* ablated (Prnp<sup>−/−</sup>) mice embryos. ST11 and ST11 peptide<sub>230-245</sub> (which contain ST11-PrPc binding site) were able to elicit neuritogenesis in Prnp<sup>−/−</sup> hippocampal neurons which can be inhibited by anti-PrP* and anti-ST11 antibodies. However, a ST11 molecule where the PrP* binding site was deleted (ST11<sub>1230-245</sub>) failed to extend neurites. Furthermore, no neuritogenesis was observed when cells from Prnp<sup>−/−</sup> animals were plated in the presence of ST11 or ST11 peptide<sub>230-245</sub>. Thus, we provided evidence for the ability of ST11 to bind PrPc and mediate neuritogenesis through MAPK pathway.

W-003 VASODILATORS IMPROVE MUSCLE FIBER REGENERATION IN DYSTROPHIC Mdx MICE

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Mdx mice are deficient in dystrophin and also show a reduction in the components of the dystrophin-glycoprotein complex, including the neuronal nitric oxide synthase (nNOS). nNOS produces NO, a signaling molecule with important functional roles in muscle, such as regulating blood flow and activating the myogenic satellite cells. Since muscle regeneration ability is decreased in mdx mice, we examined whether the administration of two types of vasodilators, which are NO and non-NO donors, could help muscle regeneration. Tibialis anterioris muscle of C57Bl/10 and mdx mice were injected with lidocaine to induce muscle degeneration. The animals received intraperitoneal injections with isosorbide dinitrate (NO donor) or verapamil hydrochloride (non NO donor). After 12 days of treatment, the total number of muscle fibers and the central-nucleated fibers were counted to obtain a regeneration index (RI). In the mdx, RI was significantly higher in the NO-donor treated group (0,93±0,01), than in the verapamil group (0,23±0,02). In controls, the RI was not so increased as in the mdx mice (isosorbida, 0,12±0,01; verapamil, 0,16±0,08). It is suggested that vasodilators NO-donors improve muscle regeneration more efficiently, with little contribution of their action as vasodilators.

Support: FAPESP, CAPES, CNPq, FAEP-UNICAMP.

W-004 QUANTITATIVE ANALYSIS OF IMUNOREACTIVE MYOSIN-V MYENTERIC NEURONS OF ILEUM AND PROXIMAL COLON OF ADULTS WISTAR RATS (Rattus norvegicus).

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The protein myosin-V is used as a neuronal marker and can be found in pre-synaptic ending, organelles surrounded by membrane and linked to membranes seems to being related to the membranes dynamic (exocytosis and endocytosis), axoplasmatic transport and neurotransmitter releasing. Similar to neurons of the Central Nervous System, the neurons of the Enteric Nervous System, responsible for local controlling of intestinal motility, blood flow and secretion, are myosin-V immunoreactive, in this way, the total neuronal population can be inferred. This population can range according to specie, age or gastrointestinal part, being this number higher in large intestine than in the small intestine. The aim of this study was estimate the number of myosin-V immunoreactive neurons of the myenteric plexus, through ileum and proximal colon whole mount preparation of rats with 360 days. It was quantified the number of neurons in 80 microscopic fields in antimesenteric and intermediary regions of ileum and antimesocolic and intermediary regions of proximal colon. Heterogeneous neuronal cellular bodies in size and intensity of stained, grouped in ganglions, besides nervous fibers with typical arrangement were observed. In relation to the neuronal density it was not verified differences statistically significant (ileum: 16.060 neurons/cm<sup>2</sup> e proximal colon: 14.922,2 neurons/cm<sup>2</sup>).
W-005 QUANTITATIVE STUDY OF THE MYENTERIC PLEXUS OF THE JEJUNUM OF RATS WITH STREPTOZOTOCIN-INDUCED DIABETES TREATED WITH ASCORBIC ACID
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The aim of this study was to verify the effect of the supplementation with ascorbic acid on the density of the myosin-V myenteric neurons in the jejunum of the chronically diabetic rats. The study was performed 4 months after inducing experimental diabetes with streptozotocin (35 m/Kg). Three groups with 5 rats (Rattus norvegicus) each were compared, i.e., nondiabetic (group ND), diabetic (group D) and diabetic treated with ascorbic acid (1 g/L/day) (group DA). The rats of groups D and DA showed an increase of glycemia and glycated hemoglobin (p < 0.05) confirming the diabetes condition. The whole-mounts from jejunum were stained with the technique of hemoglobin (p < 0.05) confirming the diabetes condition. The study was performed 4 months after inducing experimental diabetes with streptozotocin (35 m/Kg). Three diabetic rats. The study was performed 4 months after inducing experimental diabetes with streptozotocin (35 m/Kg). Three


The Ciliary Marginal Zone (CMZ) is located at the meeting between the Retinal Pigmented Epithelium and the neural retina and contains neural stem cells that are capable of proliferating and differentiating into different retinal cell types. Since stem cell differentiation and proliferation are often controlled by the same pathways relevant for embryogenesis, we propose to investigate the role of TGF-β in the CMZ. During embryogenesis, regulation of the TGF-β pathway plays a decisive role in determining the formation of nervous or epithelial tissue from neuroectoderm. Activation of TGF-β pathways induces epithelial tissue, whilst inhibition by antagonists such as noggin and follistatin allow the formation of neural tissue. Thus, we hypothesized that the balance of TGF-β ligands and antagonists in the CMZ is also relevant in determining the fate of the stem cells located there. To verify this, we processed eyes from 5 days old chick for in situ hybridizations for noggin and follistatin mRNA. We observed that the expression pattern of these TGF-β antagonists differ significantly. In the central retina, noggin and follistatin were found in the ganglionar nuclear layer (GCL). While Noggin levels at the GCL were constant throughout the retina, follistatin levels decreased significantly at the periphery. Both TGF-β antagonists were present in the peripheral INL but not in central INL. Overall distribution of noggin and follistatin was broader at the peripheral than central retina.

W-007 CELLULAR ALTERATIONS IN Gallus domesticus CEREBRAL CORTEX AND CEREBELLO EXPOSED TO LEAD ACETATE.
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Lead acetate (Ac2Pb) can cause in the organisms morphological, biochemical and behavioural alterations, which persist even after metal exposition ceases. The present study intended to evaluate the cellular morphology and apoptosis in animals exposed to Ac2Pb. Gallus domesticus embryos were treated on the fifth embryonic day (E5) with two Ac2Pb doses (18,75µg or 37,5µg), diluted in saline (1 ml). Control group was not treated and all embryos and young chickens were monitored. On the eighth day (D8) the brains were prepared for light microscopy. For layers characterization HE was used, for lead deposition analysis, Timm autometallography was used and apoptose was identified with Hoechst technique. In two dosages, there was Ac2Pb deposition in the blood vessels; in cerebellum labeling was more evident in Purkinje cells and in the molecular layer at 18,75µg dose. It was diffuse at 37,5µg dose, mainly labeling nervous fibers. In cerebral cortex the nervous fibers show diffuse labeling, and it is not possible to detect difference between the two doses. At 18,75µg, blood vessels, molecular and Purkinje layers showed an expressive number of apoptotic cells; at 37,5µg, apoptotics cells were seen in meninges, blood vessels and diffuse in all neural tissue. Results indicate that Ac2Pb deposits in nervous tissue at both doses and lead to apoptotic process suggesting the harmful action of this metal. CAPES

W-008 DUAL EFFECT OF PrPc LIGANDS VITRONECTIN AND STI-1: DIFFERENCES BETWEEN EMBRYONIC AND ADULT NEURONAL CELLS
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Prions are infectious agents responsible for transmissible spongiform encephalopathies. The prion protein is an abnormal isoform of the cellular prion protein (PrPc) which is a protein present mainly in neurons. Some of the PrPc physiological functions are already known: participation of PrPc on copper uptake, protection against oxidative stress and cell death. Our group showed that PrPc can interact with laminin, vitronectin and STI-1 (a co-chaperonin). In 12 day-old mice embryos these interactions stimulated neurogenesis of neurons from the dorsal root ganglion (DRG). After evaluating these proteins role during neurite formation in the embryo, we sought to investigate its functions in neurite regeneration. In order to do that we used adult DRG culture. When adult injured DRG neurons were treated with laminin, vitronectin and STI-1, neuroregeneration occurred only in laminin treated cultures. From these data we conclude that STI-1 and vitronectin stimulate neurite growth ex vivo only in embryonic DRG neurons. In counterpart, laminin promotes neurite growth ex vivo in both embryonic and adult DRG neurons. Supported by FAPESP and CNPq.
W-009 DISTRIBUTION OF FERRITIN AND TRANSFERRIN IN THE CHOROID PLEXUS OF THE GUINEA PIG

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The choroid plexus (CP) is constituted by a layer of epithelial cells line on an axis of richly vascularized connective tissue (pia-mater), found in all vertebrates. The aim of this study was to verify the distribution of the ferritin and transferrin in the choroid plexus. Iron is essential for normal neurological function. Biochemical processes in the brain that are dependent on iron include neurotransmitter synthesis, myelin production and basic cell functions such as energy production. The animals were anaesthetised with Ketamina and Xilasina, perfused transcardiacally with physiological saline followed by 4% paraformaldehyde (200ml) in phosphate-buffered saline. The brains were removed, cryoprotect in 15-30% sucrose. For immunohistochemistry technique, sections were rinsed and endogen peroxidase was inhibited with metanol (95ml), H2O2 30% (5ml); then, BSAT 1% (Triton X-100 0,4%) and NGS 3% each one for 30 minutes. Antisera used were ferritin (1:1000), transferrin (1:200) overnight at 4°C; IgG (1:50) and PAP (1:500) for 2 hours at room temperature. Ferritin and transferrin immunoreactivity was observed in cytoplasm of the choroid plexus epithelial cells preferentially in their luminal borders. The influx of iron into brain is regulated by transferrin receptors and the storage of iron is largely dependent upon availability of ferritin.

Supported by CNPq and FFCCMPA

W-010 DISTRIBUTION OF ELASTIC FIBER SYSTEM IN THE RAT PINEAL GLAND.

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The pineal (PG) is a neuroendocrine gland that produces melatonin. Incomplete connective septa extend from the outside capsule limit groups of cell types, pinealocytes and glial cells. In this study, the distribution of the elastic fiber system (elastic-EF, elaunin-EL and oxytalan fibers-OF) were analyzed in rats (postnatal ages - P 21, 45, 96, 106 and 330). Paraffin sections were stained by orcein, Verhoeff’s hematoxylin and Weigert’s elastin. Incomplete connective septa extend from the outside capsule limit groups of cell types, pinealocytes and glial cells. In this study, the distribution of the elastic fiber system (elastic-EF, elaunin-EL and oxytalan fibers-OF) were analyzed in rats (postnatal ages - P 21, 45, 96, 106 and 330). Paraffin sections were stained by orcein, Verhoeff’s hematoxylin and Weigert’s elastin. Magnetic fields may be modulated by the treatment with cyclosporin A.

Supported by CNPq and FFCCMPA

W-011 ASTROCYTES DEPICTED IN NORMAL AND DISEASED TISSUES.

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Astrocytes, the most numerous glial cells of the SNC, perform most of the brain functions including information processing. GFAP (glial fibrillary acidic protein)-labelled astrocytes were investigated in normal SNC, in spontaneous diseases, and in ethidium bromide (EB)-induced demyelinated lesions. Normal and cyclosporin A-immuno-suppressed Wistar rats were used to observe astrocytic responses in spontaneous and experimental SNC lesions. Adult male Wistar rats were used in three groups: normal rats (n=2); rats injected with 0,1% EB (n=24) or 0,9% saline (n=8) into the cisterna pontis; rats injected either with 0,1% EB (n=16) or 0,9% saline (n=4) into the cisterna pontis and immunosuppressed with 10 mg/kg of cyclosporin-A daily for four weeks. Animals were perfused with 10% buffered formalin from 24 hours to 31 days after surgery. Brainstem slices were embedded in paraffin and GFAP immunostaining was performed using the avidin-biotin method. In normal SNC, GFAP positive astrocytes were seen under the pia and round blood vessels, under ependymal cells, round neurons and scattered within the neuropil. Within EB-induced demyelinated lesions, remaining astrocytes were strongly immunoreactive to GFAP round the periphery of the lesions. In EB- demyelinated cyclosporin A-immunosuppressed rats, GFAP immunoreactivity seemed less conspicuous than in normal demyelinated rats suggesting that, although conservative, GFAP expression may be modulated by the treatment with Cyclosporin A.

W-012 EFFECTS OF CNTF ADMINISTERED LOCALLY OR SYSTEMICALLY AFTER SCIATIC NERVE LESION.

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Ciliary neurotrophic factor (CNTF) is a valuable agent for rescuing lesioned motoneurons, but systemic administration has dose-dependent side effects. We administered CNTF subcutaneously or applied locally on the transectioned sciatic nerve of neonatal rats (P2). The right sciatic nerve was transected and gelfoam containing either 0µg of CNTF (L;n=7) or PBS (C;n=5) was applied on the proximal stump. Rats with the same lesion received CNTF (1.2µg/g) (S;n=5) or PBS (C;n=5) once a day for 5 days. Body weight (BW) was registered from P2 to P7. At P7 the rats were sacrificed, the lumbar enlargement was embedded with paraffin and serial transverse sections were stained with cresyl violet. Motoneurons of the ventrolateral group were counted and the ratio between the number of motoneurons in the operated and control sides (NSR) was calculated. NSR of animals treated with CNTF local (0,6±0,01) or subcutaneously (0,77±0,02) was higher than controls (C;n=0,53±0,03; p<0,03; C;n=0,53±0,02; p=0,001). NSR of the group L was lower than that of the group S (p<0,001). BW of the group L was similar to its control from P2 until P7, whereas the animals of the group S exhibited a BW 32,5% lower than their control at P7. The local administration of CNTF provides relevant neurotrophic action and not is related to side effects, which validates its use in traumatic nervous injuries. (FAPEP/CAPES)
W-013 HuC/D ANTIGENS IN DORSAL ROOT GANGLION CELLS OF THE SOUTH-AMERICAN OPOSSUM (Didelphis albiventris)
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Immunohistochemistry was used to demonstrate HuC/D antigens, which play a crucial role in the vertebrate nervous system as RNA-binding proteins for determining the levels of expression of a large number of genes. Their presences were never investigated in the Opossum. Dorsal root ganglia (DRG, C3, L5) from 4 male, adult opossums were processed for light microscopy after Karnovsky solution perfusion. Sections were incubated for 1h in a solution of 0.1% Triton X-100 and 1% heat-inactivated normal goat serum and then left overnight at room temperature with the primary antibody anti-HuC/D (1:200). After washing, sections were processed with the Vectastain ABC kit, with the substrate BCIP/NBT and mounted with Entellan. HuC/D immunolabeling showed expression of the two antigens in all Opossum DRG neurons. High-resolution imaging demonstrated a typical cytoplasmatic localization of HuC/D antigens. In about half of the neurons, immunostaining was also detectable at the nuclear level. These results demonstrate the presence of the HuC/D antigens in Opossum DRGs as observed in other vertebrates suggesting a conservative evolutionary pattern. In addition, these proteins are specific neuronal markers, which may be used as a useful tool for investigating the early stages of DRG neurogenesis during Opossum development.

W-014 ULTRASTRUCTURAL STUDY OF MUSCLE FIBERS AND NEUROMUSCULAR JUNCTIONS OF THE THYROARYTENOID MUSCLE
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Some mammals muscle groups, as the laringeal ones, present tonic muscle fibers aside the fast twitch muscle fibers. The later are supplied by single en plate neuromuscular junctions (NMJ) and the former by multiple NMJ. This work aimed to analyze some structural differences between tonic muscle fibers and fast twitch muscle fibers. The later present a strong type of synaptic clefts with central and lateral invagination while the former are simpler. The fast twitch fibers were present only in NMJ were found. These NMJ present the axon terminals fit in the synaptic clefts which have variable depths. The sarcosomal folds are not homogeneously arranged along the NMJ cleft. The Schwann cell bodies and their cytoplasmatic projections are present covering the axon terminals. The SEM study revealed empty synaptic gutters with irregular distribution of junctional fields inside. In some NMJ the axon terminals were not removed and are present filling up the synaptic cleft.

W-015 QUANTITATIVE EVALUATION OF THE POSITIVE NADH-D MYENTERIC NEURONS FROM ILEUM OF RATS TREATED WITH MONOSODIUM GLUTAMATE
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Glutamate is an excitatory neurotransmitter in the central nervous system. The administration of the monosodium glutamate (MSG) in neonatal rodents induces a loss in number of neurons in this system, that may lead to obesity. Current studies show that glutamate has neuroprotective effect also in the enteric system. The current study aims to evaluate the effects of the neonatal treatment with MSG over NADH-d myenteric neurons. Male Wistar rats were used (Rattus norvegicus), and received 5 doses of saline (4 mg/g of body weight) (control group) or equivalent volume of MSG (MSG group) in days 1, 3, 5, 7 and 9. The animals were weighed and killed at day 100, being the epididimal and retroperitonial fat taken and weighed. Samples of ileum were collected, prepared as whole-mounts for neuronal evidenciation by histochemical method of NADH–d enzyme. In each whole-mount preparation, 80 microscopic fields were counted in antimesenteric and intermediate regions of the intestinal circumference. The obesity induction in group MSG was measured through significant differences in Lee-index and in the epididimal and retroperitonial fat. It was observed positive NADH–d neurons of several sizes grouped in ganglia and the number of these neurons (control: 15042.7 neurons/cm²; MSG: 12126.62 neurons/cm²) did not change significantly between groups.

W-016 IMMUNOLABELLING OF NEUROFILAMENT, TNF-α, AND iNOS DURING THE EXPERIMENTAL AUTOIMMUNE NEURITIS.
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Experimental Autoimmune Neuritis (EAN) is an animal model of the human peripheral demyelinating disorder Guillain Barré Syndrome. Inducible nitric oxide synthase (iNOS) and pro-inflammatory cytokine TNF-α may contribute to nervous fibers damage in EAN. Alterations of axonal cytoskeletal proteins have been less studied. We investigated the neurofilament (NF) phosphorylation state and the presence of TNF-α and iNOS expressing-cells in rat sciatic nerve during EAN. EAN was induced in Lewis rats by injection of an antigenic fraction of protein P2 into hind footpads (50µg.s.c). Animals were sacrificed at the onset of the clinical symptoms (stage1), at the acute phase (stage2) and at the recovering phase (stage3). Sciatic nerve sections were processed for phosphorylated NF, TNF-α and iNOS immunolabelling and analyzed under confocal microscope. At stage1, the NF showed continuous Immunolabelling. Some cells were positive for iNOS or TNF-α, similar to that observed in naive rats. At stage2, intense NF labelling fragmentation was accompanied by iNOS+ or TNF-α+ cell population increase. At stage3 only few cells were positive for iNOS or TNF-α. However, many axons were not labelled and some showed continuous but slenderer labelling for NF. Our results suggest that the increase of local NO production by iNOS+ cells during the acute phase of EAN might be related to axonal injury evidenced by neurofilament dephosphorylation. (CAPES/FAPESP).
W-017 MYOTOXIC AND NEUROTOXIC ACTIONS OF A PHOSPHOLIPASE A2, BtiL-2, FROM Bothrops alternatus VENOM, ON Skeletal Muscle in vitro.
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Local and systemic skeletal muscle degeneration is a common consequence of envenoming following snakebites. Phospholipases A2 (PLA2) are important myotoxic components in snake venoms, with most of them producing a similar pattern of muscle degeneration. In this study, we isolated a new PLA2 (BtiL-2) from Bothrops alternatus venom using gel filtration on Superdex G-75 followed by reverse phase HPLC. The molecular mass of the purified protein was 14 kDa based on SDS-PAGE. In chick isolated biventer cervicis muscle preparations, BtiL-2 (0.1, 0.5, 1, 2, 10, 50 and 100 µg/ml) significantly inhibited the twitch-tension responses (result not showed). The histopathological alterations seen in skeletal muscle involved a complex series of degenerative events, including hypercontraction and myofibril disorganization. These findings indicate that BtiL-2 is neurotoxic and myotoxic in chick isolated biventer cervicis muscle.

W-018 INFLUENCE OF NORMAL NEURONS ON LAMININ ORGANIZATION ON THE GliOBlastoma CELLS STABILIZED IN CULTURE.
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Introduction: Recently, intense research revealed the variety of biological characteristics in gliomas. Glioma cells modify the extracellular matrix (ECM) organization during progress of malignancy. Objectives: In order to understand neuron-glia cell interactions, we have developed a coculture model to study the effects of neurons on the expression of glioblastoma ECM elements

Methodology: Neurons obtained from the cerebral cortex of E18 and P0 rats were plated onto human glioblastoma cells. Coculture was maintained for 24 hs in serum free medium. Immunocytochemistry was performed using an anti-laminin (LN) antibody, a protein of the ECM. Results: A dense fibrilar LN network was detected on the surface of glioblastoma cells. However, when E18 and P0 neurons were simultaneously cultured onto tumoral cells, we observed a rearrangement in the LN antibody, a protein of the ECM. Results: A dense fibrilar LN network was detected on the surface of glioblastoma cells. This network was found at the cell surface of the tumor cell. Conclusion: Our results suggest that neurons may modulate the LN expression on the glioblastoma ECM.

Supported by: PRONEX, FAPERJ, CNPq, FUJB.

W-019 GLUTAMATE INDUCTION OF GFAP GENE PROMOTER IS MEDIATED BY TGF-β1 AND INVOLVES MAPK/PI-3-K PATHWAYS
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We recently demonstrated that cortical neurones induce the GFAP gene promoter of cerebral cortex transgenic astrocytes by inducing TGF-β1 secretion. In this study we aim to characterize glialneuron interaction in GFAP gene promoter activation, inducing TGF-β1 secretion by astrocytes. We report that glutamate activates GFAP gene promoter of transgenic astrocytes derived from cerebral cortex, midbrain and cerebellum. To identify TGF-β1 direct effect in astrocyte cultures, neutralizing antibodies against human TGF-β1 were added to transgenic astrocytes cultured in presence of neurons, TGF-β1 or glatamate. Addition of anti-TGF-β1 antibody fully prevented neuronal, TGF-β1 or glatamate effects, demonstrating that GFAP gene expression induction is mediated by TGF-β1, independently of glutamate. The mGlu2/3R antagonists (MCPG) attenuated astrocyte differentiation in primary cultures containing neurons, TGF-β1 or glatamate. This effect was also reduced by a MAPK pathway inhibitor (LY294002) and a PI-3-K pathway inhibitor (LY294002). These results suggest that glial mGlut2/3R activation induces astrocyte differentiation by MAPK and PI-3-K pathways activation, leading to TGF-β1 induction. In conclusion, we demonstrate that TGF-β1 and glatamate abilities to induce astrocytes differentiation, modulating the GFAP promoter. We now report that activation of glial group-II mGluR enhances the TGF-β1 synthesis through MAPK and PI-3-K pathways activation. Our work reveals an important role for neuron-glia interactions in astrocyte development and strongly implicates the involvement of glutamate and TGF-β1 in this event.

W-020 STI1 BINDING TO CELLULAR PRION PROTEIN MODULATES GLIOMA PROLIFERATION
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Malignant gliomas are aggressive and highly invasive. In glioblastoma, median survival ranges from 9 to 12 months. Mechanisms of tumorigenesis include disregulation of both proliferation and programmed cell death, and therefore the identification of trophic systems with action upon tumor cells may be of importance. Previous studies identified stress-inducible protein 1 (STI1), a co-chaperonin, as a cellular prion protein (PrPc) ligand, that triggers neuroprotection in retinal explants. The present work examines the effect of STI1 upon the proliferation of a human glioblastoma cell line (A172). STI1 expression was detected by Western blot analysis and the presence of PrPc at the cell surface of this tumor cell was detected by cytofluorimetry. Conditioned culture media assayed by Western blot showed that STI1 is also secreted. To investigate the effect of STI1 upon tumor proliferation we assayed radioactive thymidine uptake in cell cultures treated for 24 hours either with or without mouse recombinant STI1 protein. These experiments show an increased thymidine uptake when treated cells were compared with those untreated. The effect of STI1 was abolished by simultaneous treatment with an anti-PrPc antibody. These results support the hypothesis that the increase in cell proliferation induced by STI1 depends on endogenous PrPc. In conclusion, our data show that STI1 modulates the proliferation of a human glioblastoma, upon engagement of PrPc, possibly through an autocrine/paracrine mechanism. Financial support: CNPq; FAPERJ; FAPESP; FUJB.
The prion protein PrPc has been the centre of intense research due to its implication in transmissible spongiform encephalopathies (TSE). Post-translational modifications of the endogenous cellular form, PrPsc, by unclear mechanisms lead PrPsc accumulation in the brain during TSE. Recently, a new molecular partner of PrPc, Stress Inducible Protein 1 (STI1), was identified. This 66 KDa protein interacts with PrPc in a specific and high-affinity manner. Thus, we investigated the role of STI1 in the peripheral nervous system in events such as axonal transport and regeneration. In order to investigate if STI1 is axonally transported, we initiated our study using the sciatic nerve test section/accumulation model in rodents. One-D western blot analysis showed an increase in the signal intensity in the proximal segment compared with the basal one. This is consistent with a 24h accumulation and may suggest that STI1 is anterogradely transported. In our regeneration studies, we crushed the sciatic nerve of rodents and 4 to 21 days after, we analyzed the STI1 expression. It was observed an increase in STI1 signal in nerve segments proximal and distal to the crush site. This could indicate a role of STI1 in the peripheral nervous system regeneration.

Supported by FAPESP, Ludwig Institute and GIS “Infections a prions”.

W-023 INVOLVEMENT OF THE CELLULAR PRION PROTEIN IN THE ASTROCYTE DIFFERENTIATION
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The physiological functions of the cellular prion (PrPc), a pivotal participant in prion diseases are under intensive investigation and new approaches are trying to characterize its role during brain development. In vitro studies have shown that PrPc interacts with glial fibrillary acid protein (GFAP). Astrocyte differentiation is regulated by a transition program during brain development involving vimentin-GFAP expression. The increased expression of GFAP and the down-regulation of vimentin direct astrocyte maturation. In the present work, we investigated the role of PrPc in the astroglial differentiation using astrocyte cultures from E17 cerebral cortex from wild type (PrP+/+), PrPc gene ablated (PrPuc0/0) and PrPsc over-expressing mice (Tg-20). Morphological analysis showed no differences between cultured astrocytes from PrP+/+, PrPuc0/0 and PrPsc0/0 and Tg-20 mice. However, immunoblotting assays demonstrated that in astrocytes from Tg-20 animals the GFAP expression is higher than in PrP+/+. Conversely, GFAP expression is lower in astrocytes from PrPsc0/0 mice when compared to wild type animals. Vimentin expression was lower in astrocytes from Tg-20 mice and higher in PrPsc0/0 mice cells when both groups were compared to PrPuc0/0 animals. These preliminary results suggest that PrPc is involved in the regulation of vimentin-GFAP expression and astrocyte maturation.

Supported by FAPESP.
The giant synapse has been studied in three main species of squid: *Loligo pealii* (north Atlantic Ocean), *Loligo opalescens* (Pacific Ocean) and *Loligo forbesae* (Mediterranean Sea). The synapse is so large that it is possible to see its pre and postsynaptic terminals with the aid of only a dissecting microscope. Glass microelectrodes can be inserted into the presynaptic and postsynaptic terminals simultaneously recording both activities during synaptic transmission. The large axonal diameter eases access to the molecular presynaptic machinery for direct injection of pharmacological agents, as well as biochemical markers to label and associate the distinctive presynaptic events for transmitter release. Most of the current knowledge of the human nervous system was distinctive presynaptic events for transmitter release. The EM observation of the synaptic active zones of the stellate ganglia in the local squid show the terminals is finer making easier the identification and dissection of the ganglia. EM observation of the synaptic active zones of thestellate ganglia in the local squid show the terminals proportionally much larger than *Loligo pealii*. Some axoplasmic proteins as p65, and the neurofilament, as expected, are equivalent to those described in the other species. Supported by FAPESP, FUNDAMAR, S. Sebastião, SP and FAEP, RP,SP.

W-028 EM CRIO-IMMUNOLABELING OF SYNAPTIC PROFILES IN THE FASCIA DENTATA INNER MOLECULAR LAYER (IML) OF HUMAN HIPPOCAMPUS.

The temporal lobe epilepsy (TLE) is most common of the human epileptic syndromes. One of the main histopathological findings of the human ELT is the hippocampal sclerosis. This sclerosis is characterized by specific neuronal death and mossy fiber sprouting. The synaptic terminals of mossy fibers contain ionic zinc in the interior of the synaptic vesicles, that can be identified by the autometallographic (AMG) reaction of neo-Timm. The behavioral and histopathological characteristics of the ELT was modeled in experimental animals with systemic injection of pilocarpine, a muscarinic agonist. Pilocarpine induces status epilepticus that, in turn, causes recurrent spontaneous seizures in the animals. Hippocampal sections examined after the neo-Timm AMG show mossy fiber sprouting in the animals that had suffered status epilepticus and no alteration in the control animals. At the electron microscope, nanocrystals of silver were observed in the interior of synaptic vesicles in aberrant synaptic terminals in the internal molecular layer of the dentate gyrus (DG) of epileptic animals. Immunogold labeling of the NMDA scaffold protein PSD-95 and the AMPA receptor subunit GluR2 detected their presence in the aberrant sprouted terminals. Financial support: CAPES, FAPESP, FAEP.

W-029 DETERMINATION OF THE INDEX AND DURATION OF THE CELL CYCLE IN GRANULAR NEURONS OF RAT CEREBELLUM (*Rattus norvegicus*) AFTER ALCOHOL ADMINISTRATION.

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The cerebellum is formed by two germinative zone: the primary zone that coats the tect of the IV ventricle and the external granular layer (EGL) that coats the cerebellar cortex during the development. The present study aim to analyze the effect of alcohol unique dose on the pups at P3 on the total time of the cellular cycle (Tc), the label index (LI) and S-phase time (Ts) of external granule cells after one and two intraperitoneal injection of BrdU. The LI of granular cells in the treated group (0.266) was increased in the number when compared with the control group (0.193). The total quantity of granular cells after one or two injection of BrdU showed statistically significant differences between treated and control group. No Statistically significant differences was observed in the S-phase time (p=0.463) and in the total cycle (p=0.602) between control and treated groups. It was noted an increased on time in the S-phase of treated group when compared with control (7.588 and 5.352 hours respectively). The same was observed with the total cycle time: treated group had an average of 31.982 hours when compared with the control group that had an average of 25.896 hours, suggesting that alcohol changes the cell cycle kinetic.
Carboxypeptidases (CPs) perform important functions including protein/peptide digestion as well as selective biosynthetic roles. The A/B subfamily of metalloCPs, includes CPB1, plasma CPB and CPA1-A4. Recently three new members were added: CPA5, CPA6, and CPO. The specificity of CPA6 hasn’t been experimentally determined, but modeling suggests that CPA6 cleaves aromatic/aliphatic residues. Because the CPAs have overlapping enzyme substrate specificities, their unique location is the key issue relating to their function. The purpose of this study was to determine CPA6 distribution to gain insights towards its function. RT-PCR was used to determine which tissues express CPA6 mRNA. In situ hybridization was used to map its location. Both RT-PCR and in situ hybridization showed CP6 mRNA expressed in the brain, with the highest expression found in the olfactory bulb. CPA6 mRNA was abundant in both main and accessory olfactory nucleus. The hybridization signal was mainly over the mitral cell layer as well as the granular cell layer. No signal was found in the periventricular area or the olfactory nerve layer. Other areas of the brain such as the septal nucleus, cingulated cortex, pontine and over the inferior olivary nucleus showed a less intense labeling. Taken together, these data suggest a role in the processing of protein/peptides related to olfactory bulb neurons stimulation, such as pheromone signaling.

**W-031 DISTRIBUTION OF BRAIN MYOSIN V (BM-V) IMMUNOREACTIVITY IN THE SPINAL CORD AND FOREBRAIN OF THE RAINBOW TROUT, Onchorhynchus mykiss**

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Synemin, an intermediate filament (IF) first described in avian erythrocytes and muscle, was also documented in a subset of mouse astrocytes, during development. Recently, we have demonstrated synemin expression in normal and pathological human lens cells. Synemin is expressed together with other IF, as desmin or vimentin. Here, we used antibodies against three isoforms of synemin, the 180, 150 and 40 kDa, which have been obtained by alternative splicing of the pre-mRNA. By immunocytochemistry, we analysed these isoforms expression together with other IF in cultures from human or rat CNS. Confocal microscopy analysis showed a cell-specific distribution pattern either in neurons or astrocytes. The large isoforms were mainly associated with GFAP in glial cells, whereas the small isoform was co-expressed in neurons, identified with the neuronal markers β-III tubulin and NeuN. Similar distribution was observed either in rat or human cells. These data suggest that, as a cytoskeletal protein expressed during development, the regulation of the synemin genes could modulate cell phenotype and function. Moreover, synemin could play a role of an additional cell marker during development, by this cell-specific distribution pattern. Supported by: CAPES-COFECUB, AFM, CNPq, FAPERJ, FUJB-UFRJ.

**W-032 INTERMEDIATE FILAMENT PROTEIN SYNEMIN ISOFORMS ARE EXPRESSED SELECTIVELY IN ASTROCYTES AND NEURONS**

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Adenoviral vectors are able to provide gene transfer into the CNS, achieving both neuronal and glial cells. Using increasing hAd5 vector concentrations, we first examined the tropism of the hAd5 vector, by intracerebral injections in rat brains and on primary neural cells in culture. Interestingly, we demonstrated that hAd5 vectors display a markedly dose-dependent differential tropism both in vivo and in vitro. Astrocytes were selectively transduced at low concentrations, whereas neurons were transduced only at higher concentrations of vector particles. To get more insights in the characterization of adenoviral cellular receptors involved in this specific cell tropism, we used mutated Ad vectors, referred as CAR-ablated vectors, by modifications of the fiber proteins. We investigated the consequences of point mutations in the fiber knob domain. Our results showed that the impairment of CAR-binding abolished neuronal tropism both in vivo and in vitro, suggesting the involvement of CAR as the major receptor for hAd5 binding in neurons. Moreover, we significantly demonstrated distinct transduction patterns for neurons and astrocytes. Supported by: CAPES-COFECUB, AFM.

**W-033 GENE TRANSFER INTO THE CENTRAL NERVOUS SYSTEM USING WILD-TYPE AND MUTATED ADENOVIRAL VECTORS**

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The present study was conducted to determine the location, morphometry and the morphology of NADPH-diaphorase positive neurons in the cardiac ganglia of mice. NADPH-diaphorase staining was performed on whole-mount preparations of the atria of mice. The number of neurons ranged from about 39 to 79 neurons (mean: 57; S.E.M.:4). The sizes of the neurons (area of the maximal profile) ranged from about 80 µm² to about 260 µm ². The estimated 57 ganglion neurons per transverse section were situated cranial to the pulmonary veins, (2) caudally to the venous return, and (3) in the atrial groove. These types of neurons could be distinguished on the basis of their shape: monopolar cells, bipolar cells and cells with three large processes (multipolar neurons). On the other hand the number of neurons stained with NADH-diaphorase method was 530 ± 23 (mean ± S.E.M.). Therefore, the NADPH-diaphorase positive neurons of the heart represent 10% of the total number of cardiac neurons. The present results provide anatomical evidence that selective activation of the neurons may affect discrete regions and or specific cardiac functions.

Previous studies in guinea pig hearts have shown the presence of specific atrial granules, which secrets an activator of sodium and water excretion and consequently blood pressure reduction. This hormone, called atrial natriuretic factor (ANF), are secreted and released by the arterio-aortic complex under stimului of hypervolemia or blood pressure increase. The endocardial surface plays a fundamental role in the transport and activation of ANF. The objective of this paper was to analyse the myoarchitecture, angioarchitecture, endocardial surface and ultrastructure of the guinea pig atrio-aortic complex, and their role in the ANF function. Hearts from 20 guinea pigs were used. The results showed that the muscle architecture in the atricles are more complex than in the atria. The arterial arrangement of the atria shows a vascular net relatively uniform and dense. In the atrile, the arterial net is denser and tortuous. The scanning electron microscopy of endocardial surface has shown irregularities on the auricular surface, with the atrial surface showing a uniform arrangement. The findings related to the atrial-aortic complex are very useful, since it has been observed in studies related to cardiac surgeries that removal of the auricles, and consequently reduction of circulating ANF, causes acute and chronic abnormalities in the body fluid control.

Isolated tumoral cells from primary malignant brain tumors of glial origin (glioma) show a diffuse cellular infiltration into the brain parenchyma. These invasive characteristics generally turn the best available multimodal therapy uselesful. Here we aim to investigate the contribution of normal brain cells to this invasive tumoral phenotype. We first analyzed the in vitro effects on glioma cells migration and invasion of conditioned-medium (CM) obtained from different rat primary neural cell cultures, as well as the thyroid hormones T3 and T4. We analyzed the ability of these CM and hormones to induce: i) tumor cells migration in the Boyden chamber experimental system; ii) the expression of specific markers of migration (MMP-2, brevican) by RT-PCR. Preliminary results showed that the transmigration of A172 glioma cell line in the Boyden chamber is significantly increased when these cells were pre-treated during 48 hours with 50 nM T3 or T4. This could be of particular interest if considering that activity of type II-desiodase, in part responsible for the homeostasis of bioactive TH levels in the brain, is induced around invasive mass glioma. The in vivo invasive characteristics of glioma cell lineages stereotaxically implanted in adult immunosuppressed rat brain are also investigated in our laboratory at the present time.
W-038 T3 EFFECT ON MYOSIN 5A EXPRESSION IN THE CENTRAL NERVOUS SYSTEM
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Myosin 5a (M5a) acts in cell migration and intracellular vesicular transport, which are events regulated by thyroid hormones (TH) during Central Nervous System (CNS) development. Previous studies show that T3 appears not to regulate M5a synthesis in astrocytes, however it is not know the T3 effect on these cells. In this work, we analyzed the T3 effects on M5a expression in the CNS trying to verify its participation in structural and functional alterations induced by TH using for this purpose three experimental protocols: (1) brain extracts and synaptosomes purified from normal and hypothyroid adult Wistar rats, (2) culture of cortical neurons and astrocytes from newborn (P0) normal and hypothyroid rat, and (3) U373 cells, a human astrocytoma expressing GFAP, T3-treated. In hypothyroid rats, M5a expression in the brain, neurons, synaptosomes and astrocytes was reduced (~50%, 20%, 20% and 30%, respectively). T3 increased M5a level in neurons, synaptosomes and astrocytes from newborn (P0) normal and hypothyroid rat, and U373 cells. Moreover, tumorigenic changes did not affect M5a expression on T3 action.

CAPES, PROEX, FAPERJ, CNPq.

X-001 EFFECT OF 17β-ESTRADIOL (E-2) AND THE ESTROGEN ANTAGONIST ICI182,780 (ICI) ON DNA CONTENT AND CHROMATIN TEXTURE OF HUMAN BREAST EPITHELIAL CELLS
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MCF-10F immortalized human breast epithelial cells treated twice a week for two weeks with E-2 develop characteristics indicative of neoplastic transformation similarly to the effects promoted by the carcinogen benzo[a]pyrene (BP). We have previously reported changes accompanying BP-induced tumorigenesis in nuclear parameters of the MCF-10F cells as assessed by image analysis. It is thus expected that changes in nuclear characteristics occur in E-2-treated cells similarly to those induced by BP. The effect of the estrogen antagonist ICI182,780 isolatedly on MCF-10F cells is not known, although it does not abrogate the proliferative activity and invasiveness induced by estradiol in these cells. In the present study we compared DNA amounts and geometric and textural parameters in Feulgen-stained MCF-10F cells treated with different doses of E-2, ICI and BP, followed by treatment with hormone- or drug-free medium, using image analysis procedures. E-2 was found to induce decrease in DNA amounts as a hormone dose-dependent response, and chromatin reorganization not necessarily associated with DNA loss. These results possibly result from different hormone dose-dependent target pathways. ICI induced nuclear changes assumed to be associated with the activation of apoptotic pathways. (CNPq; NIH R01 CA67238, PHS, DAMD 17-00-1-0229/0249, FAPESP)

X-002 NUCLEAR IMAGE PROPERTIES OF MCF-10F CELLS GROWN ON SLIDE SUBSTRATES DIFFERING IN NATURE AND SIZE
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The immortalized human breast epithelial cell line MCF-10F is an important tool for studies on experimental tumorigenesis induced by drugs, transfected oncogenes, and hormones. Apoptotic ratios for MCF-10F cells cultivated on glass have been found to be lower than those obtained for cells grown on plastic under same available slide surface size and working medium volume; the opposite has been found when these cells were transformed by benzo[a]pyrene. Considering that many relevant data have thus far been established only for MCF-10F cells cultivated on glass, DNA content and nuclear parameters as assessed by image analysis (Zeiss/Kontron equipment; KS400 software) were compared here for cells grown on plastic and glass substrates differing in chamber surface sizes and working culture medium volumes (areas: 9.4 and 0.5 cm²; culture medium volumes: 2.5-4.5 and 0.2-0.4 ml, respectively).

It was concluded that for slides with the larger chamber, plastic substrate was more advantageous than glass, since the former did not affect the cell nuclear sizes significantly. Chambers with the operational volume of 0.2-0.4 ml revealed inadequate for cell nuclear image analysis studies on account of the variable geometric, densitometric and textural results produced and a very slow growth rate generated. (CNPq, NIH R01 CA67238, PHS, DAMD 17-00-1-0229/0249, FAPESP)

X-003 CHANGES IN CHROMATIN ORGANIZATION AND EXTENSIBILITY IN ADULT MOUSE HEPATOCYTES FOLLOWING STARVATION AND REFEEDING
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Since starvation and refeeding following starvation are stress agents in several organisms, their effects on chromatin organization and extensibility deserve investigation. The effect of a 48 h food deprivation and of 48 h of refeeding subsequent to starvation in adult mouse hepatocytes was studied here with topochemical assays, image analysis, polarization microscopy and the gravity action. Starvation increased the chromatin packing states, especially in areas of non-condensed chromatin, and induced a drastic decrease in the level of nuclear matrix glycoproteins and in the frequency of nuclei with chromatin extensibility under gravity. The changes in chromatin packing were not accompanied by alterations in the DNA amount. In refed mice the extent of chromatin unpacking was greater than that in well-fed controls, but the frequency of nuclei with chromatin extensibility, and the amount of glycoproteins reactive to Con-Br were lower than those in controls. Additionally, a sensible increase in DNA amounts was observed after refeeding. The duration of refeeding was probably insufficient to re-establish the stereorearrangement of the chromatin-nuclear matrix and to restore the chromatin fluidity to the level seen in well-fed mice. These changes may be associated with the silencing and reactivation of genes involved with the nutritional metabolism. (CNPq, CAPES, FAPESP)
X-004 KARYOTYPE CHARACTERIZATION OF THE SPECIES PIMELODUS MACULATUS (PISCESES, PIMELOIDIDAE) FROM SOUTH OF MINAS GERAIS, BRAZIL.

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The Pimelodidae family is a heterogenous fish assemblage comprising over 300 species, and has been historically diagnosed by the lack of specializations seen in other Neotropical catfish families. The genus Pimelodus, belonging to this family, has about 24 species distributed in Central and South America (north to southern most Mexico). The objective of the present study was to analyze the karyotype of Pimelodus maculatus from the Coqueiro stream, Alfenas, MG, Brazil. Chromosome analysis was carried out on 240 metaphases of Pimelodus maculatus (8 specimens: 4 females and 4 males). A diploid number of 56 chromosomes was observed in all specimens and karyotype was 40 metacentric/submetacentric and 16 subteloacentric/acrocentric chromosomes, with a fundamental number (FN) of 106. One pair of NOR-bearing subteloacentric chromosomes was characterized with the NOR-sites located at a telomeric position on the long arm. No heterochromatin was visualized after C-banding. Our data and that of literature confirmed that P. maculatus has some conservative chromosome characteristics, such as the diploid and fundamental number, and the NORS, that were always present in a pair of subteloacentric chromosomes. Financial support: FAPEMIG and UNIFENAS.

X-005 MOLECULAR CYTOGENETICS IN FIVE HETEROTERPAN SPECIES

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The amount and location of heterochromatin in Largus rufipennis, Dysdercus albofasciatus, D. chaquensis, D. ruficollis and Jadera sanguinolenta are analyzed by C, and DAPI/CMA banding techniques. These five species are cytogenetically characterized by the possession of holokinetic chromosomes and a pre-reductional type of meiosis. C-banding reveals C-positive bands at the telomeric regions in all chromosomes of L. rufipennis and small C-positive bands in D. albofasciatus and J. sanguinolenta. DAPI/CMA banding indicate that the five species lack repetitive sequences either AT or GC-rich, except for the NOR in L. rufipennis and D. albofasciatus. Total genomic DNAs are extracted and cut with restriction endonucleases; the diffuse smear detected and the absence of banding restriction should be due to the small amount of highly repetitive DNA or the missing of target sites recognized by the tested endonucleases. It is noteworthy the appearance of four bands during the electrophoresis of total genomic DNA of J. sanguinolenta even without endonucleases digestion. One of the DNA fragments hybridizes principally on the telomeric regions of the X. The present genome analysis reveals a great heterogeneity with respect to heterochromatin DNA composition and chromosome organization within Heteroptera. Further studies are required to get a better understanding of heteropteran holokinetic chromosomes.

X-006 CHROMATIN EXTENSIBILITY IN SPERMATOZOA OF THE HONEY BEE

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Chromatin and DNA flow from liver cell nuclei of the mouse after treatment with concentrated saline and detergent solutions and under the action of gravity, thus forming extended chromatin fibers (ECF). Chromatin packing states and ECF formation in these cells are affected by starvation such that an increase in chromatin condensation is accompanied by decrease in frequency of chromatn extensibility (Moraes AS – Masters’ dissertation, Unicamp 2004). Considering that a highly packed chromatin state is found in spermatozoa and that in the honey bee, particularly, the sperm cell nuclei unusually bear a very lysine-rich histone H1, typical for somatic cells, we analyzed the ECF formation in these cells in comparison with liver cells from well-fed mice. Freshly prepared smears of semen from Apis mellifera drones under fixed and unfixed conditions were subjected to lysis protocols followed by gravity action, stained with toluidine blue at pH 4.0, and examined with polarized and unpolarized light. The protocol that originated abundant ECF production in the mouse liver cells induced ECF formation in part of the bee sperm cells; the protocol that originated less ECFs in the former did not induce ECF in the latter. The results are in agreement with the chromatin higher-order packing state and presumed histone composition involved in the bee spermatozoa. (CNPq, FAPESP)

X-007 DNA CONTENT AND CHROMATIN CONDENSATION IN POLYPLOID NUCLEI OF THE STINGLESS BEES, MELIPONA QUADRIFASCIATA AND M. RUFIVENTRIS

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Species of the stingless bee Melipona genus have been discriminated into two different groups regarding their heterochromatin content (Rocha MP & Pompolo SG, Genet Mol Biol 21: 41, 1998). M. quadrifasciata and M. marginata for instance pertain to the group with low heterochromatin content (I), whereas M. seminigra and M. scutellaris pertain to the group with high heterochromatin content (II). Unpublished data by Pompolo suggest that M. rufiventris is a member of the group II. The above-cited classification has been based on C-banding data as applied to mitotic chromosomes. In this study we investigated the Feulgen-DNA content and chromatin condensation as assessed by image analysis in polyploid nuclei of larval Malpighian tubules of species assumed to differ in their chromosome C-heterochromatin content, M. quadrifasciata and M. rufiventris. Feulgen-DNA values corresponding to more than one ploidy class were found in both species. However, the Feulgen-DNA values which basically define each ploidy degree were higher in M. rufiventris, although M. quadrifasciata and M. rufiventris have the same number of chromosomes. This is probably due to different responses to the Feulgen hydrolysis in these species on account of differences in their chromatin condensation, agreeing with the idea that M. rufiventris contains more heterochromatin than M. quadrifasciata. (CNPq, FAPESP)
The salivary gland complex of the triatomines, vector hematophagous insects of Chagas' disease, is composed of three pairs of well-differentiated glands: the anterior (D1), median (D2) and posterior (D3). The secretions produced by these glands have many proteins and enzymes that facilitate the digestion of the ingested blood. In order to identify nuclei complexes, we studied the structure of the interphase nuclei (euchromatin, heterochromatin and nucleolus) of salivary glands cells of adult insects males and females of Panstrongylus megistus. The glands were removed from insects, fixed in acetic acid (45%) and lactic acid (50%), squashed and submitted to different cytochemical methods: lacto-ace tic orcein; impregnation with silver nitrate; Feulgen reaction; Toluidine Blue (pH 4.0); Variant method of Critical Electrolyte Concentration (CEC) and C-band ing. The preparations were examined with a photomicroscope (Zeiss - Jenaval) and photographed. In all cells (D1, D2, D3) of males and females, the results evidence: the presence of polyploid nuclei; a clear association between the nucleolar and heterochromatic corpuses; intense cytoplasmatic metachromasy and many secretion vesicles in cytoplasm. Such characteristics are associated with intense synthesis activity to produce the gland salivary secretions. It is suggested these cytochemical characterizations are essential to maintain the hematophagy of triatomines.

Financial support: CNPq/PIBIC

Glycoproteins reactive to Con-A and assumed to be nuclear matrix components stable even under acetic acid-ethanol fixation have been found in liver cell nuclei of well-fed mice. These proteins are broken down under starvation but their synthesis is recovered following refeeding (Moraes AS – MSci dissertation, Unicamp 2004). On assuming that nuclear Con-A-reactive glycoproteins may also occur in other cell types, we investigated here the Con-A response in fibroblast nuclei of well-nourished animals. These results indicate that cells from malnourished animals are more susceptible to environmental damage.

Replication of circular DNA faces numerous topological obstacles that need to be timely solved to allow complete duplication and separation of newly replicated molecules. Small bacterial plasmids provide a perfect model system to study the interplay between DNA helicases, polymerases, topoisomerases and the overall architecture of partially replicated molecules. Recent studies demonstrated that partially replicated circular molecules have an amazing ability to form various types of structures (supercoils, precatenanes, knots and catenanes) that help to accommodate the dynamic interplay between duplex unwinding at the replication fork and DNA unlinking by topoisomerases. The topological changes that take place as a replicon replicates are just beginning to unravel. For the moment it seems that during replication all the possible topological forms RIs can adopt play some role. The topological cycle of a replicon appears to involve supercoiling, precatenation, knotting, catenation and decatenation. Whether or not these changes observed for small plasmids apply also for the large topological domains of bacteria and linear eukaryotic chromosomes remains to be shown.
The silver impregnation technique, which is used to detect the active NORs-bearing chromosomes, is very important, considering that its employment permits, for example, to establish phylogenetic relationship and the plasticity of these regions among taxonomically related species. In the present work males of *Goniosoma speleuaim*, *Gonyleps curticornis*, *Ilhaia cuspidata* and *Progonyleptidelius striatus* were analyzed to determine the NORs pattern. The chromosomal preparations of these species were obtained from testicular cells and submitted to the technique of sequential staining Giemsa-silver impregnation. In these four species, NORs were observed in the pair 1 chromosomal elements short arm, occupying the proximal region in *G. curticornis* (2n = ±88), *I. cuspidata* (2n = ±88) and *P. striatus* (2n = ±86) and distal region in *G. speleuaim* (2n = ±109). Additionally, *P. striatus* showed multiple NORs on pair 1. Taking into account that pair 1 chromosomes of these four species possess NORs, a certain conservativeness seems to exist in relation to the position of this region and these chromosomes can be considered prominent markers in the Opiliones order evolutionary analysis. However, the NORs distal position in *G. speleuaim* and the multiple NORs in *P. striatus* suggest that chromosomal rearrangements are acting in the ribosomal clusters of these species.

**X-013 COMPARATIVE CYTOGENETICS OF ANURAN SPECIES: Barycholos ternetzi AND Eleutherodactylus binotatus (LEPTODACTYLIDAE)***

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Barycholos and Eleutherodactylus are closely related genera, sharing extensive morphological similarity. Taking into account that karyological data may be important to taxonomy and systematics, we start cytogenetic investigation in *B. ternetzi* (4 males from Gurinhatã, MG) and *E. binotatus* (2 females from Biritiba Ussu, SP). The karyotypes have 2n = 22, with eight pairs of biarmed-chromosomes (pairs 1 to 7, and 9), showing differences in size and in the centromere position in both species, and three pairs of uni-armed chromosomes, corresponding to the 8th, 10th, and the 11th. The coincident position of these chromosomes in the two karyograms represents a sinapomorphy. The species have centromeric heterochromatin, but *E. binotatus* presents some interstitial C bands, two of which are very conspicuous, located in the long and in the short arms of the chromosomes 7. In *E. binotatus*, Ag-NORs are in chromosomes 1, whereas in *B. ternetzi* they are located in the chromosomes 9 and 10. In the sample of this later species, four distinct patterns, with 1, 2, and 3 stained Ag-NORs were observed. More resolutive cytogenetic analyses, based on fluorochrome staining and BrdU incorporation banding, are under way, in order to elucidating the relationship of the two genera. FAPESP, CNPq

**X-015 CHARACTERIZATION OF THE NUCLEOPLASMIC RETICULUM BY ELECTRON MICROSCOPY IN CHO CELLS.***

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Nuclear calcium (Ca^{2+}) regulates functions that are distinct from the effects of cytosolic Ca^{2+} signals. We recently identified a novel reticular network of nuclear Ca^{2+} stores with the capacity to regulate Ca^{2+} signals in localized subnuclear regions. This structure was denominated the nucleoplasmic reticulum. The presence of such machinery provided a potential mechanism by which Ca^{2+} can simultaneously regulate multiple independent processes within the nucleus. However, further studies about the structure of this reticular network remained to be done. Therefore, the aim of this work was to investigate the structure of the nucleoplasmic reticulum. CHO cells were cultured in plate inserts, Millicell, and prepared for TEM. After thin sections examinations, the nucleoplasmic reticulum was visualized as tubules of double-membrane invaginations of the nuclear envelope into the nucleoplasm. The cells also presented abundant and complex double-membrane ring structures with pores. Additionally, the lumen of these tubules was continuous with cytosol and contained organelles such as mitochondria. Our results are in agreement with our previous functional data and suggest that the nucleoplasmic reticulum architecture may allow deep and complete communication between different cellular compartiments.

**X-014 ASSOCIATION BETWEEN PERIODONTITIS AND POLYMORPHISM***

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The periodontal destruction leads to clinical findings such as increased of periodontal probing depth and involves a complex interaction between bacterial and host immune system. TNF-β, an immune modulator, is encoded by the major histocompatibility complex (MHC). Our study aimed to analyze the association between periodontitis and TNF-β gene polymorphisms. Were evaluated 105 individuals, which were separated, according clinical attachment loss, in A group, 45 health individuals, and B group, 60 subjects with chronic periodontal disease (PD). DNA samples were obtained from the individual’s epithelial cells through scraping of the buccal mucosa. Polymorphism in the TNF-β gene was analyzed by PCR, followed by Ncol restriction endonuclease digestion (RFLP). Frequency of TNF-β gene showed significant differences between A and B groups (p<0,05). The genotype homoziogote was prevalent in the health patients (42%), and the genotype heterozygote in the PD group (31%). The susceptibility seemed to be increased either in the homozygote genotype (OR = 8; 95%-IC = 1.7-36.3) than in the heterozygote (OR = 6; 95%-IC = 2.4-15.9). The Ncol polymorphism of the TNF-β gene are the associated with clinical attachment loss due periodontal disease, in the studied population. These findings suggest that TNF-β gene genotype might be a risk indicator for the susceptibility to chronic periodontal disease.
Y-001 ULTRASTRUCTURAL IDENTIFICATION OF LYSOSONES IN Leishmania (L.) chagasi.
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Species of the L. (L.) mexicana complex are causative agent of cutaneous human diseases and present in their amastigote forms abundant membrane-bound structures, called megasomes. These organelles have lysosome properties, like arylsulfatase activity, and are rich in cytosine proteinases, which are involved in virulence and intracellular survival of Leishmania. However, megasomes have not been reported in visceralizing species of Leishmania which present low cytosine proteinase activity. The present study focused on lysosomal compartments in promastigote and amastigote forms of L. (L.) chagasi, a visceralizing specie. Transmission electron microscopy of serial sections and tree-dimensional reconstruction allowed visualization of large structures in amastigote forms of L. (L.) chagasi similar to megasome and multivesicular tubule (MVT)-lysosome structure in promastigotes. Morphometric data showed that the relative volume occupied by these structures are 5% in amastigotes and 3.2%, in promastigotes. Cysteine proteinase were detected by immunolabeling in the amastigote form whereas the lysosomal content of amastigote and promastigote was confirmed by arylsulfatase cytochemistry. The present study demonstrated for the first time the existence of megasome and (MVT)-lysosome structures in amastigote and promastigote forms of L. (L.) chagasi, respectively. The megasome may be involved in the survival of the amastigote in the macrophage host cell, whereas the MVT-lysosome may be related to the infectivity of promastigotes.

Y-002 VACUOLES CHARACTERIZATION OF Colletotrichum acutatum GERMINATED CONIDIA BY ACID PHOSPHATASE DETECTION
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In fungi, intravacuolar digestion is made by a variety of hydrolytic enzymes, including Acid Phosphatase. This enzyme hydrolyses phosphate esters and has been used as a vacuolar and lysosomal marker. It is observed inside spores, hyphas and appressoria vacuoles, as well in fungi cell wall. Previous studies with Colletotrichum acutatum demonstrated conidia vacuolization during germination. In the present study, in order to characterize these vacuoles, cytochemistry reaction for Acid Phosphatase was realized during different germination stages. The optical cytochemistry was done by using Naphthol AS-BI phosphate as substratur and the diazonium dye Fast Garnet-GBC as receptor (Sigma Kit). After 24 hours of germination, medium size vacuoles stained by Acid Phosphatase reaction were observed inside germinated conidia, germ tubes and appressoria. Our results show that, as germination moves forward, the conidium vacuoles become larger. The same happens to the vesicles in the appressorium. After 48 hours, the vacuoles and vesicles appear extremely marked by Acid Phosphatase reaction. In this time, the cell wall also show an intense enzyme reaction. The wide distribution of this enzyme during the germination of C. acutatum, suggests that it plays an important role in this process. Therefore, the comprehension of its intracellular distribution and modifications throughout the germinative process can bring useful informations for the development of strategies in the control of germination.

Y-003 EFFECTS OF ALCOHOLIC GARLIC EXTRACT IN Colletotrichum acutatum CONIDIA GERMINATION
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Colletotrichum acutatum is the major causal agent of strawberry anthracnose. Conidia cytoplasm is replete with lipid bodies that function as a storage site for neutral lipids playing an essential role during the germination process. The fungicides used to combat these organisms are very toxic, expansive and often inefficient. Natural products, with low toxicity, represent a very attractive way to control fungi germination. In this work, activity of garlic (Allium sativum L.) extract on the Colletotrichum acutatum conidia has been investigated. Conidia were germinated and stained by Red Nile for lipid detection. A laser scanning confocal microscope – Radiance 2001 (Bio-Rad®) coupled to an Eclipse E-800 (Nikkom®) – with a 60X/1.4 NA oil plan-apochromat objective lens was used for fluorescence and differential interference contrast microscopy (DIC) analysis of fungal cells. The controls showed ungerminated conidia full with lipid bodies, which decrease during germ tube and appressorium formation. Alcoholic extract were very effective and inhibited 100% of germ tube or appressorium formation. Confocal analysis demonstrated complete lipid bodies depletion, suggesting a great interference of the garlic compounds in conidial metabolism. The precipitate of alcoholic extract promoted abnormal structures formation, exhibiting a large vacuoles, without apressorium formation. Altogether, these results demonstrated that the garlic substances inhibited a normal germinative process. Supported by CNPq and Paraná Tecnologia

Y-004 PHOSPHATE UPTAKE INCREASES MITOCHONDRIAL H2O2 RELEASE
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The mitochondrial membrane has a proton gradient that includes two components: the electric potential (∆Ψ) and the pH gradient (∆pH). It is well established that the ∆Ψ regulates mitochondrial hydrogen peroxide (H2O2) release secondary to the dismutation of starting from superoxide radicals formed by monoenergetic oxygen reduction at intermediate points of the electron transport chain. We used the main intracellular membrane permeable anion phosphate (Pi) to alter ∆pH and verify the effects on H2O2 release. As expected, Pi decreased mitochondrial ∆pH, measured using the pH-sensitive fluorescent dye BCECF, and increased ∆Ψ, estimated by following safranine O fluorescence. H2O2 release, measured using Amplex Red/horseradish peroxidase, was stimulated by Pi. Acetate, another membrane-permeable anion which decreases ∆pH and increases ∆Ψ, did not stimulate mitochondrial H2O2 release, suggesting the Pi effect is independent of ∆pH. The stimulatory effect promoted by phosphate was prevented by the presence of CCCP, which decreases the transport of phosphate across the inner mitochondrial membrane, suggesting Pi must be in the mitochondrial matrix to stimulate H2O2 release. In conclusion, we found that Pi stimulates mitochondrial reactive oxygen species generation in manner independent of its effect on ∆pH and dependent on Pi uptake. This increment in reactive oxygen generation may be a cause of oxidative stress under conditions such as ischemia, in which intracellular Pi concentrations increase.
A number of natural toxins inhibits mitochondrial respiratory chain complexes. Recent studies showed that veratrine inhibits oxygen consumption by both NADH and FADH₂-linked substrates and dissipation of electrical membrane potential in isolated mitochondria from rat skeletal muscle. Veratrine, a commercial extract of Shoenocaulon officinale (Liliacea), is a mixture of alkaloids, mainly represented by cevadine and veratrindine. These alkaloids cause activation of sodium channels leading to hyperexcitability and depolarization of the excitable membranes. In this work, the ultrastructural alterations induced by veratrine on isolated mitochondria from rat skeletal muscle and its toxic effects on respiratory chain enzymes through cytochemical methods were investigated. Adult Wistar rats were sacrificed through cervical dislocation before hind limbs muscles were removed for the isolation of mitochondria. The mitochondrial pellets were incubated with 250 and 500 µg/ml veratrine concentrations and submitted to cytochemical reactions for detection of cytochrome oxidase, NADH and succinic dehydrogenases activities. Subsequently, the pellets were routinely processing for transmission electron microscopy. The isolated mitochondria showed morphological alterations and negative reaction for dehydrogenases and cytochrome oxidase enzymes after incubation with veratrine. Our results suggest that the toxic effects of veratrine on isolated rat skeletal muscle mitochondria can be related to an inhibitory action on respiratory chain. The veratrine action also can be the result of a non-specific alteration of the mitochondrial inner membrane permeability.

Support: CAPES, FAEP, FAPESP.

Z-001 DUFOR GLAND SECRETORY PATTERN IN EUSOCIAL BEES: Apis mellifera, Bombus terrestris, Melipona bicolor and Scaptotrigona postica (HYMENOPTERA: APIDAE).
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The Dufour gland is an accessory gland of the bee female reproductive apparatus and in eusocial bees its function is not established yet. Although this gland presents a very simple morphological pattern, its secretory mechanisms are very complex in eusocial bees. In A. mellifera, the worker glandular cells are synthetically inactive, since they uptake from the haemolymph the hydrocarbons of the secretion and do not seem to process this substances intracellularly. In queens, the morphology analysis support absorption capacity of exogenous substances by the glandular cells, besides there is intracellular synthesis of esters, presenting the cells a well-developed smooth endoplasmic reticulum network. In B. terrestris and M. bicolor part of the secretion may also come from haemolymph, since the glandular cells present morphological structures indicative of this. The physogastric queen Dufour glands in S. postica differ significantly from the other taxa studied. The glandular cells do not present morphological cues of intracellular absorption capacity, but clearly are able to uptake substances through the intercellular spaces, which do not cross the cells. The substances seem lipids, which may be selected from the other haemolymph substances through the complex double-layered basal lamina that surround the gland.

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The mandibular glands of the Hymenoptera in general are structures associated with the mandibles and are part of the salivary glands system. The mandibular glands are located on each side of the head and open at the mandibles. The histological studies performed by Pavon & Camargo-Mathias (2001) in workers of Atta sexdens rubropilosa revealed that these glands are divided into two portions: a secretory portion and a reservoir. The present work had the object of studying the ultra-structure of the mandibular glands of small and medium-size workers and soldiers of the ant Atta sexdens rubropilosa through the use of techniques of transmission electron microscopy (TEM). Our results revealed that the glands in all three castes studied consisted of a reservoir, composed by a pavement epithelium, and by secretory cells of rounded shape and with nuclei of the same morphology. The secretory cells, mainly in small workers and soldiers, were rich in smooth endoplasmic reticulium. The medium-size workers and soldiers presented mandibular glands with cells rich in mitochondria of varied morphology. Well-developed Golgi complexes were found in soldiers. The secretory cells of the three castes studied presented granules of a lipidic secretion, thus justifying a possible pheromonal function for the secretion. These cells were provided with collecting intracellular canaliculi that were connected to the reservoir through their extracellular portion. Supported by FAPESP (Proc. n° 03/00553-8)

Z-003 THE HYPOPHARYNGEAL GLAND OF THE LEAF-CUTTING ANT (Atta sexdens rubropilosa).
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The social behavior of ants is controlled by means of the dynamic interactions between the environment and the individuals, mainly through the pheromones. Among the exocrine gland is the hypopharyngeal gland (HG), located laterally over the pharyngeal plate. Few works concerning the HG in ants, and these have focused mainly on the relationship between glandular development and the division of labor. Therefore, the present work aimed to describe and compare the HG in the different castes of the leaf-cutting ant Atta sexdens rubropilosa. The ants collected in the field were dissected in fixative solution in order to extract the HG, which was then submitted to routine procedures for histological, histochemical, and electron microscopy. The HG of different castes showed similar morphology varying only in relation to the secretory cell number. The secretory intracellular reservoir presented positive reaction to Xyline Ponceau and P.A.S, indicating the presence of protein and polysaccharides in the secretion. These structures were stained by phosphoin-FITC and analyzed under confocal microscopy. 3D reconstructions were performed to understand the HG morphological organization. Therefore, we suggest that the function of this gland would be the production of digestive enzymes and/or some sort of mucus, which, together with the secretions produced by the salivary glands of the thorax, would be related to the production of saliva.

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Recent morphological and ultra-structural data show that the convoluted gland, umbrella-shaped structure found inside the reservoir of the venom gland of wasps and ants, performs a secretory role in the Hymenoptera that possess it. The cells of this gland would be responsible for a high enzymatic activity that would probably alter the precursory compounds produced in the external filaments, prior to the liberation of the final venom into the reservoir. The present work had the object of studying the ultra-structure of the recently described convoluted gland of workers of the ant Pachycondyla striata through the technique of transmission electron microscopy (TEM). For this study, the specimens were dissected in order to extract the venom gland, which was immediately processed according to routine procedures for TEM. The cytoplasm of the cells of the convoluted gland presented several elements distributed therein, such as ribosomes and polyribosomes, lipid droplets, and protein inclusions in the shape of crystals, which probably represent a form of protein storage for posterior use. This observation lead us to suggest that the convoluted gland not only adds substances to the venom stored in the reservoir, but it may also extract from its substances such as proteins that would remain stored in the cytoplasm of the convoluted gland.

Supported by FAPESP

Z-005 INFLUENCE OF AN ARTIFICIAL DIET ON THE SECRETORY ACTIVITY OF THE HYPOPHARYNGEAL GLANDS OF Apis mellifera (HYMENOPTERA, APIDAE).

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The hypopharyngeal glands of Apis mellifera possess a high secretory activity in nurse workers, while these glands appear degenerated in the foraging workers. The present study verified the influence of different diets (proteic or energetic – candy) in the normal cytochemical pattern of the secretory process of the hypopharyngeal glands of workers of Apis mellifera with 15 and 30 days of age through the detection of acid glycoconjugates (Alcian Blue), neutral glycoconjugates (PAS), and total protein content (Bromophenol Blue and Xyline-Poncet). Regular nurse and foraging workers were also analyzed for comparison. Individuals with 15 days of age and fed with the proteic diet presented a secretion with a lower protein content in relation to the nurse worker, in which the material secreted was stained with a maximum intensity, thus indicating the high amount of proteins. Under the same conditions, the glandular tissue of workers with 30 days of age did not present a secretion, due to the advanced degree of degeneration of the hypopharyngeal gland at this age. Bees with 15 days of age and fed with candy lacked all of the proteic components of the secretion in relation to the nurse workers. This diet also caused a delay in the elimination of the secretion, which occurred only at the 30th day of age.

Supported by FAPESP (99/04818-9).

Z-006 EARLY DEFENSIVE MECHANISMS OF RAT PROSTATE EXPOSED BY BACTERIAL INFECTION.


Inflammation of the prostate gland constitutes an important problem in urology. We are interested in analyzing defensive mechanisms in response to an experimental infection. In a model of bacterial prostatitis, we evaluated the stroma reaction, the morphology of epithelial cells, and the expression of secretory proteins as galectin-1 and Prostatic Binding Protein (PBP), both with capacity to inhibit chemotaxis and phospholipase A2 activity. Wistar rats 3 month-old were inoculated with PBS or with a suspension of E. coli (109 UFC) beneath the ventral prostate capsule. Animals were sacrificed 24, 48, 72 hours after inoculation. Ventral prostates were processed for light and electron microscopy. The expression of galectin-1 and PBP in prostatic fluid was evaluated by ELISA. Besides of a great interstitial infiltrate, at 24 hours there was a characteristic hypertrophy and hyperplasia of muscular cells enveloping the acini around the basal membrane. Also epithelial cells exhibited a marked hypertrophy, and PBP and galectin-1 expressions was significantly increased by immunocytochemistry and ELISA. Besides, numerous cells undergoing apoptosis, appeared phagocytosed by macrophages invading the epithelium between 24 and 48 h post inoculations, as determined by TUNEL technique. The present results demonstrate an early activation of unspacific strategies against infection and inflammation in ventral prostate gland. While the stroma developed a physical barrier of hypertrophic muscle cells, proliferated epithelial cells overexpressed anti-inflammatory proteins and responded to apoptotic signals probably as a protective mechanism.

Z-007 DIFFERENT BEHAVIOUR OF BRONCHIOLAR CLARA CELLS UNDER ALLERGENIC AND PHARMACOLOGICAL STIMULI: MORPHOLOGICAL AND FUNCTIONAL ANALYSIS.

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Non ciliated bronchiolar Clara cells secrete CC16, an antiinflammatory and immunomodulatory protein that could play a key rol in the control of inflammatory pathologies, such as asthma. Female BALB/c mice were used to study Clara cells response under two regulatory conditions: A) an inflammatory process induced by ovalbumin (OVA) and B) exposition to an inhaled corticosteroid. Group “A” was sensitized with OVA and then aerosolized for 7 days with 1% OVA (30min/day). Group “B” mice were treated for 20 days with an aerosol containing 45ug/mL budesonide (20min/day). Statistical analysis consisted in an ANOVA/Tukey test. Two different patterns of Clara cell response were observed; allergic inflammation induced a hyperactivated state with marked hypertrophy and characteristics of goblet cells containing big secretory granules co-localizing CC16 and mucoprotein. The number of immunoreactive Clara cells/mm of basal membrane and CC16 expression in bronchoalveolar lavage (BAL) were significantly increased. After budesonide treatment, Clara cells number/mm basal unit was increased showing a state of extreme differentiation with prominent apical domes containing abundant mitochondria; however CC16 levels in BAL were similar to control. We conclude that Clara cells present two different responses depending on the nature of stimuli: trans differentiation to goblet cell induced by allergy, or complete differentiation exerted by corticoids, which evidences an additional mechanism of corticoids to control inflammation.
NAD(P)H oxidase activity, H$_2$O$_2$, O$_2$ effects on mouse macrophages oxidative metabolism through (ONOO$^-$). Our findings provide an explanation of how further insight into the possible mechanisms that trigger cells development of anti-inflammatory therapeutics. In order to gain NAD(P)H oxidase but no increase in H$_2$O$_2$ production, in addition to increased NO production suggest that before - is reacting with NO producing peroxinitrite ($\text{O}_2^\cdot$- and nitric oxide (NO) generation. Treatment with Canova leads to an increase in NAD(P)H oxidase activity. There were no differences in H$_2$O$_2$ and O$_2$ production, but we found an up-regulation of NO production by treated macrophages. The over expression of NAD(P)H oxidase but no increase in H$_2$O$_2$ production, in addition to increased NO production suggest that before dissmutation, O$_2$ is reacting with NO producing peroxinitrite (ONOO$^-$). Our findings provide an explanation of how treatment with Canova may enhance immune functions, providing a therapeutic approach in tumoral, infection and inflammatory diseases.

Financial support: CAPES and PARANÁ TECNOLOGIA
Z-012 ACUTE AND SUBACUTE TOXICITY OF HERBICIDE 2,4-D IN GILLS EPITHELIUM OF FISH
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The toxicity of the herbicide 2,4-D (2,4-dichlorophenoxyacetic acid) on Poecilia vivipara was investigated in acute and subacute toxicity tests. Groups of fishes were exposed to different concentrations and periods, 1 and 2ppm were observed 48 hours to subacute toxicity, 3 and 4ppm for 24 hours to acute toxicity, both to detect the 2,4-D effects on gills epithelium. Behavioral changes were observed. The citochemicals analysis of gills epithelium were done with PAS, Alcian Blue and lectins selective stained. In PAS were observed blood-vessel swelled and mucous cells types I and II at 1ppm; at 2ppm had less quantity of mucous cells and gills hypertrophy and hyperplasy; at 3ppm the epithelium was all damaged, plenty of bigger mucous cells types I, II and III; and at 4ppm was observed higher quantity of mucous cells type III and hyperplasy and tumors tissue. The classical citochemistry showed that at 4ppm were presented higher quantity of carboxilated-glycoconjugates and the others concentrations presented sulphatated-glycoconjugates. 2,4-D exposure led to ionoregulatory impairment and mucus secretion, although chloride cell hypertrophy was induced. The changes in mucus secretion suggest a compensatory response to respiratory surface reduction in gills (tissue damaged) in order to maintain oxygen transference from water to the tissues, allowing the fishes to survive during the experiment.

Z-013 HEPATOBIILIARY FUNCTION AND TRANSPORT PROTEINS IN OBESE ZUCKER RATS.
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The obese Zucker rat (ZR) is a widely used experimental model for fatty liver disease. Although livers from ZR are more susceptible to various type of injury, basal bile secretory function has not been systematically assessed in the ZR. Aim: To study bile secretion and expression of main hepatobiliary transporters in obese ZR. Methods: Bile flow and biliary secretion of lipids and glutathione were determined. Histological studies were performed. Protein mass and mRNA levels of the Na+taurocholate cotransporting polypeptide (Ntcp), Bile salt export pump (Bsep) and Multidrug Resistance-associated Protein 2 (Mrp2) were assessed by Western and Northern blot, respectively. Results: Decreased bile flow, biliary lipid and glutathione secretion as well as reduced hepatic transport of both taurocholate and bromosulphthalein were found in obese ZR. Hepatic Mrp2 protein mass was markedly reduced in obese rats while Ntcp and Bsep protein levels were similar to lean rats. Down-regulation of Mrp2 seems to involve both transcriptional and post-transcriptional mechanisms. Conclusions: Livers of 14-week-old obese ZR showed significant steatosis without inflammation. Obese ZR exhibit an impaired bile secretory function with significant functional and molecular alterations. The marked reduction in the expression of Mrp2 in obese ZR may explain the reduction in biliary glutathione excretion and suggests the existence of a decreased hepatic ability to excrete endo and xenobiotics in these animals. (Fondecyt 1020641)

Z-014 ALTERED BILE SECRETORY FUNCTION IN EXPERIMENTAL NON-ALCOHOLIC FATTY LIVER DISEASE.
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Non-alcoholic fatty liver disease (NAFLD) is an increasingly important clinical entity. Fatty liver infiltration is considered as a predisposing condition for various types of liver damage. Impaired biliary excretion may be a contributory factor for this increased susceptibility in fatty livers. Our aim was to study basal bile secretion and protein mass of two of the main biliary canalicular transporters in experimental NAFLD. Bile secretory function was determined by conventional techniques in Sprague-Dawley rats control and fed with a choline-deficient diet (CDD) during 8 weeks. Protein mass of the Bile Salt Export Pump (Bsep) and Multidrug Resistance-associated Protein 2 (Mrp2) was assessed by immunoblot. CDD induced a marker fatty liver and biochemical evidence of hepatocellular injury. An impaired bile secretory function was found in CDD as reflected by reduction of bile flow and biliary secretion of bile acid and organic anions. Bsep and Mrp2 protein mass did not change in this experimental model. In conclusion, an impaired bile secretory function with cholestatic features is present in experimental NAFLD. A preserved protein mass of Bsep and Mrp2 with decreased biliary bile salt secretion suggests the presence of a functional impairment of these transporters in CDD fed rats. [Fondecyt 1020641]

Z-015 QUANTITATIVE EVALUATION OF GILL MUCOUS CELLS RESPONSE IN THE EURHILALE FISH (Poecilia vivipara) UNDER STRESS OF SALINITY VARIATIONS
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There are 4 subtypes of mucous cells in the gills of guppy responsible for the production of the glycoconjugate layer that recovers epithelial surface. In the present work it was evaluated the behavior of these mucous cells in the fresh-water (FW) and blackish sea-water (SW) living fishes submitted to stress by variations of salinity concentration (0 to 20ppm and 20 to 0ppm, respectively). A panel of 12 lectins was used to label the mucous cells in the histological sections and their distribution and incidence in the gills were quantitatively evaluated. The mucous cells subtype I, II, III and IV were selectively stained by UEA, Con A, WGA and PNA, respectively. Quantitative evaluation performed by image analysis system (Imagelab) and statistical treatment (ANOVA and Tukey Test) showed that fishes submitted to variations of salinations increased the number of subtypes I and II mucous cells at 0 and 10ppm, while the subtypes III e IV were higher at 15 and 20ppm. This behavior of mucous cells seems to be one of mechanism related to adaptive capability of guppies as eurhaline fish. Supports: PIBIC-CNpq, FUNAPE, FAPESP.

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The presence of antibodies in birds’ semen has been described, however, the origin and role of these immunoglobulins (Ig) are not known. A great number of plasma cell-like cells were recently identified within the epithelium of the phallus of tinamous and drake. Plasma-cells are commonly found in the connective tissue, but rarely within the epithelium. Therefore, this study aims to confirm the identification of these intraepithelial cells by characterizing and quantifying possible subtypes of immunoglobulins secreted. For this purpose, phalli of drake were processed for histology and immunohistochemistry and semen was processed for ELISA-assay. The results showed the presence of numerous intraepithelial plasma-cells in the fixed tubular portion of the drake phallus, identified by positivity for the immunoglobulins IgM, IgA and IgY. The intraepithelial plasma-cells showed variation in frequency depending on the immunoglobulin subtype. Plasma-cells secreting IgM were more abundant (46.5%) than those secreting IgA and IgY (32.0% and 6.3% respectively). The ELISA-assay confirmed the results showing that IgM is the most abundant immunoglobulin in the semen. Once IgA is the predominant immunoglobulin in mammalian and avian mucosa immune systems, the results are suggestive of a peculiar specialization of the avian copulatory organ.

Supported by FAPEMIG, CNPq.

Z-017 ACID AND ALKALINE PHOSPHATASES IN THE MIDGUT OF THE BEE MELIPONA QUADRIFASCIATA ANTHEMIS (HYMENOPTERA, APIDAE)

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Acid phosphatases has been associated with many metabolic functions in the insect midgut such as lysosomes and intracellular digestion in the digestive cells, while alkaline phosphatases are present in the brush border of these cells associated with absorption. This work investigated the occurrence of phosphatases activities in the midgut of Melipona quadridasciata antheroides to test the hypothesis that these enzymes play a role in digestion and absorption processes in bees. Midguts of nurse and forager workers and queens were fixed in Zamboni’s solutions, following standards histological procedures. Sections 3 µm thick were submitted to Gomori’s histochemical test to detection of acid and alkaline phosphatases. Alkaline phosphatase was restricted to brush border of the middle and posterior midgut region of nurse workers and queens, which have pollen grains inside the midgut lumen, but it was absent in forager workers, which fed on nectar. On the other hand, acid phosphatase was present only inside some pollen grains in the midgut lumen. Lead sulphide deposit was observed in the spherocrystals also in control sections, therefore without relation with enzymatic activity. Results suggest that alkaline phosphatase may play a role in final digestion and absorption in the brush border, while acid phosphatase activity in the midgut may be exogenous.

Z-018 EFFECT OF AN ARABINO GALACTANA (ARAGAL) FROM THE GUM OF Anadenanthera colubrina (ANGICO BRANCO) ON PERITONEAL MACROPHAGES FUNCTIONS AND TUMORAL CELLS.

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In order to obtain insights on the biological effects of ARAGAL, an arabino galactana from the Anadenanthera colubrina, its immunological properties on macrophages and tumoral cells were evaluated. In vitro and in vivo exposure to ARAGAL increased the occurrence of activated macrophages in a time-, and a dose-dependent pattern. In vitro exposure (300 µg.mL-1) showed ~ 91 % of activated macrophages and in vivo treatment (250 mg.kg-1) showed ~75% of activated cells. Treatment of animals with 50, 100 or 200 mg.kg-1 of ARAGAL increased peritoneal exudate cell (PEC) numbers by ~18, 44 and 88% respectively. The phagocytic ability was increased with 25 µg.mL-1 of polymer. O2- production by macrophages from ARAGAL-treated mice was increased in presence or absence of PMA, and TNF-α production increased 26 fold by peritoneal macrophages from treated animals. Structures of S-180 cells were observed inside the macrophage cytoplasm after treatment with ARAGAL, with100 mg.kg-1 a growth inhibition of 39% of S-180 solid was detected and 66% to ascitic form. The increase of TNF-α production could be responsible with the antitumor activity of ARAGAL, which can be a possible biological response modifier.

Z-019 SUPEROXIDE DISMUTASE IN PERIPHERAL BLOOD OF SHIFTFWORKERS

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The impact of shift and night work on health is a complex problem. There is a high inter- and intra individual variability related to circadian asynchrony, psychophysical condition, performance efficiency, mental and physical health. Many studies indicate the possibility that the stress affects biochemical parameters, and that these elements should be responsible for hormone and biochemical alterations, which impair the circadian rhythm. For the working population, daytime sleeping may be unfavorable, though it shows how workers cope with their conditions. The aim of this study was to analyze the superoxide dismutase in the blood serum of shiftworkers. For biochemical analyses we used the peripheral blood of twenty-four students who are shiftworkers at Centro Universitário do Planalto de Araxá. Students that work during the day were taken as controls. The superoxide dismutase was determined by the method of Nitroblue tetrazolium reduction. We observed that superoxide dismutase was 25% higher in the shiftworker students. The results suggest that the shiftworking stress probably affect cellular biochemical parameters.
Ana Cristina Prado Veiga-Menoncello; Albertina Pimentel Lima; Shirlei Maria Recco-Pimentel.

Ana CT Palei, Débora C Souza-Costa, Talita Zerbini, Lívia F Lopes, José E Tanus-Santos, Raquel F Gerlach.

Ana Flávia Vigário, Giorgia Lay-Ang, Janaina de Souza Menezes, Rodolfo Carvalho, Simone Maria Teixeira Sabóia-Morais

Ana Maria Galvan Custodio, Guilherme Paladino de Jesus, Rejane Maira Góes, Sebastião Roberto Taboga

Ana Paula Azambuja; Carla Wanderer; Cloris Ditzel Faraco; Silvio Marques Zanata

Ana Paula Duarte, Andréia Otake and Roger Chammas.

Ana Paula Marques de Mendonça Lopes, Karina Teixeira Naves, Cristina Pacheco Soares, Newton Soares da Silva

Ana Paula S. Faloni; Estela Sasso-Cerri; Eduardo Katchburian; Paulo S. Cerri

Ana Rita Sousa Coutinho; Camilla Mota Mendes; Maria Lucia Zaidan Dagli; José Antônio Visintín; Mayra Elena Ortiz Assumpção.

André Gustavo Oliveira; Luiz Telles; Irina Geraldo; Germán Mahecha; Cleida Oliveira

André Luiz Cândido da Silva; Ariani Corrêa Barbosa; Ruth Janice Guse Schadeck

André Luiz Pasqua Tavares and Gregory Thomas Kitten

André R Mampumbu; Silvia G Pompolo; Maria Luiza S Mello

Andrêia Aparecida Oliveira, Roberta Beatriz Abdala, Cibele Marli Cação Paiva Gouvêa, Gilberto Antonio de Oliveira

Andrêia Averci Canalli, Nicola Conran, André Fattori, Sara T. Olallia Saad, Fernando F. Costa.

Andréia Benedita Poletto, Robson Francisco Carvalho, Alexandre Azevedo, Adriane Pinto Wasko, Claudio Oliveira, Fausto Foresti, Cesar Martins.

Andressa Antunes Prado de França; Suelen Nogueira Desssaune; José Eduardo Serrão; Clóvis Andrade Neves

Ángela C. Ito; Marta B. Leonardo; Marta V. Medeiros; Gerson R. Campos; Stephen Hyslop; Maria Alice da Cruz Höfling

Angelica de Oliveira Gomes; Marcos Silva; Eloisa Amália Vieira Ferro.

Angélica Soares; João Paulo Ferreira Schoffen; Maria Raquel Marçal Natali; Elsa Maria de Gouveia

Angelo Luiz Cortelazzo; Maria Izabel Gallão

Antonella Sachsida Braga Villela and Benedicto de Campos Vidal

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Antonio Sesso, Joyce T. Kawakami, Elizabeth S. Hito, Flávio Taniguchi, Márcia H. V. Leosvaldo and Maria de L. Higuchi

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Linhares, Tatiana Hochgreb, Chao Yi Yan, Brad Davidson and José Xavier-Neto

Marcus Alexandre Mendes Luz; Maria Julia Marques; Humberto Santo Neto

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Martha Silveira e Costa; Marcelo Faulhaber ; Maria Cristina Marcucci; Martin Metzger; Sergio Paulo Bydloki ; Ana Carolina Oliveira; Estela Maria Novak.

Mattos, GE, Lotfi, CFP

Max Pereira Gonçalves, Mauricio Resende, Tânia Maria Gomes de Pinho, and Edmilson Amaral de Souza

MC Leal & LR França

Melissa Bilibio; Vera Haas; Sérgio Porto; Esdras Escobar; Rubens Rodrigues.

Melissa Markoski; Edvaldo Trindade; Alice Laschuk; Arnaldo Zaha; Helena Nader; Henrique Ferreira.

MF Coelho, LCH Machado, MSA Costa, RGP Ramos
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