ALLOXAN-INDUCED DIABETIC MICE: A MODEL TO STUDY THE THYMUS INVOLVEMENT IN HUMAN DISEASE

VENTURINI J. (1), MARCELINO, A. A. (1), DIAS N. S.(1) MORENO T . G. (1), BRITO V. N. (2), ARRUDA M. S. P. (1)

(1) Department of Biological Sciences, Faculty of Sciences, UNESP, Bauru, São Paulo State, Brazil, (2) Lauro de Souza Lima Institute, Bauru, São Paulo State, Brazil.

Induction of diabetes by alloxan cause damage to the pancreatic β-cells and produces typical diabetes signs together with complications involving several organs such thymus. We have used this diabetic model to sought the importance of thymus on susceptibility infection, one of the typical aspect in this condition. We observed that the usual alloxan dosage (75 mg/kg) induced a marked number of deaths in a short period (< 15 days). However, with 60 mg/Kg, the mice were alive for more than 30 days, enough time to evaluate the chronic infectious aspects. With the possibility of this drug acting directly on the thymus, before the installation of diabetes, we investigated this organ in BALB/C mice treated with alloxan (60mg/kg) at the following times: 3, 6, 12 and 24 h. The drug did not show significant effects in these periods on thymus weight, celulalirity, viability of thymocytes and thymus architecture, as well as the percentual of peripheral T-lymphocytes when compared to values of control group. Nonetheless, to analyze the functional aspect of thymus on diabetes condition, we weighed the organ, observed thymus reticulin and architecture in diabetic mice induced by alloxan at 6, 24, 48 hours and 7 days (48 h *p.i.*). After 6 h, we observed a marked decrease of relative thymus weight, a great loss of cortico-medullary definition and disorganization of extracellular matrix. However, a gradual recovery of these parameters was observed along the experimental times with a total recovery on the 7th day. Our results suggest that alloxan-induced diabetic experimental model is an option for studies that involve thymus in this pathophysiology, since there is no detrimental effect of this drug on the thymus. Moreover, we suggest that thymus alterations observed in the initial times are consequence of diabetes condition and could be considered as immunological complications with possible impairment in infectious course of these patients.

KEY WORDS: alloxan, diabetes, thymus.

FINANCIAL SUPPORT: FAPESP

CORRESPONDENCE TO:

Maria Sueli Parreira de Arruda, Departamento de Ciências Biológicas, UNESP, Bauru, SP. Email: sueli@fc.unesp.br

ALTERNATIVE METHODOLOGY FOR IMMUNOLOGY LAB CLASSES THAT USES ANIMAL TESTS: DEVELOPMENT AND UTILIZATION OF SIMULATION.

CUBO NETO F. (1), GURGUEIRA A. P. (1), GALEMBECK E. (1), COSTA F. T. M. (2)

(1) Laboratory of Educational Technology, State University of Campinas – UNICAMP, Campinas, Brazil, (2) Laboratory of Immmunology and Parasitology, State University of Campinas – UNICAMP, Campinas, Brazil

The computer uses in education is being extremely useful due to the possibility of using animations, images and simulation resources, absents in printed textbooks. In biology education, animals are frequently used in teaching labs. Nowadays, studies are carried out to find alternative teaching methods that avoid using animals unnecessarily. Several classes that have made use of animals can be replaced, but keeping the same educational learning objectives. Research papers have reported that elementary and High School students, using computer simulations have learnt equally or even better than others taught by the traditional ways. The aim of this work was to develop a computer simulation to replace the immunology class entitled "Lymphoid organs collection and cell separation" addressed to the Biology undergraduate students. The developed computer simulation can be used for both traditional and web-based distance education courses. The role software contains four videos showing the animals handling procedures. Images, animations, tutorials showing details of the process of dissection, organs collection and cells separation and highlight same major steps. A simulation of the cell counting using a virtual Neubauer Chamber generates random images with the cells to be identified and counted. The software was designed using the Macromedia Flash. The developed software allows the students to simulate the experiment as many times as they consider necessary until the complete comprehension of the content, it is not possible in the traditional lab classes.

KEY WORDS: Educational software, Neubauer chamber, simulation.

FINANCIAL SUPPORT: CNPq

CORRESPONDECE TO:

Francisco Cubo Neto, Laboratório de Tecnologia Educacional – Departamento de Bioquímica, Universidade Estadual de Campinas – UNICAMP. Email: cneto.francisco@gmail.com

ALTERNATIVE METHODOLOGY FOR IMMUNOLOGY PRACTICAL CLASSES THAT USES LABORATORY ANIMALS: VIDEO DEVELOPMENT AND UTILIZATION

GURGUEIRA A. P. (1), CUBO NETO F. (1), GALEMBECK E. (1), COSTA F. T. M. (2)

(1) Laboratory of Educational Technology, IB, State University of Campinas – UNICAMP, Campinas, Brazil (2) Laboratory of Immunology and Parasitology - IB, State University of Campinas – UNICAMP, Campinas, Brazil.

Finding alternative ways of using animals in both experimental and educational fields has been demanded to the researchers and educators. Dissection, for example, rarely involves more than observation and memorization; students are not challenged with forming hypotheses or collecting and interpreting data. By using video and multimedia, it is possible to aggregate value to the traditional dissection classes, bringing the students to work in a higher cognitive level than memorization requires. The purpose of this project is to develop videos that can be used during Immunology classes, in both traditional and distance education, offering the students a high level didactic and to suppress dissection classes. The Immunology class, which is formerly offered in Biologic Sciences study at the State University of Campinas, had one of the practice classes experiment filmed. The title of this class is “Laboratory animals handling - lymphoid organs collection and cell separation”. The first video shows the anesthesia stage and its effects. The second one demonstrates white bleeding process, which enabled the animal’s blood collection. The third video exposes the dissection process and lymphoid organs (spleen, thymus and lymph node) collection, which will be utilized in another stage. The fourth and last video shows the collected spleen being sliced with tweezers and shears. Subsequently, it is macerated for the obtainment of a substrate that contains blood cells, which will be utilized in a second step for cellular viability counting using the Neubauer Chamber. The use of videos in the mentioned teaching situation, followed by the use of a Neubauer Chamber computer simulation were field tested and showed to be useful as a teaching tool.

KEY WORDS: Educational video, alternative ways, immunology.

FINANCIAL SUPPORT: CNPq

CORRESPONDECE TO:

Andréia Pimentel Gurgueira, Laboratório de Tecnologia Educacional – Departamento de Bioquímica, Universidade Estadual de Campinas – UNICAMP

Email: dehiabio@yahoo.com.br

ANIMAL-FREE TEACHING OF REPRODUCTIVE PHYSIOLOGY: VIDEO REPLACEMENT OF GONADECTOMY, HORMONAL TREATMENT AND NECROPSY IN RATS

CHELINI M. O. M. (1), CASTILHOS L. R.(1) CORTOPASSI S. R. G. (2) KUGELMEIER T. (1), OLIVEIRA C. A. (1), SOUZA N. L. (3)

(1) Laboratory of Hormones Dosage, Department of Animal Reproduction, (2) Department of Surgery, (3) Department of Pathology, School of Veterinary Medicine and Animal Science, University of São Paulo.

In light of the growing awareness of the need to improve animal welfare and to refine, replace, and reduce the number of animals needed for demonstration purposes, we produced a video to replace gonadectomy, hormonal treatment, and necropsy of rats traditionally performed by veterinary students during classes on reproductive physiology. The first module of this video, set in a laboratory environment, demonstrates restraint and intraperitoneal injection, inhalation anaesthesia, surgical preparation of the patient, main surgical procedures, postoperative recovery and hormonal treatment. In the second module, the physiological response to hormonal treatment is shown and discussed by the way of animated diagrams. Covered topics include reproductive anatomy, hormonal function, hypothalamus-pituitary-gonadal axis regulation, and surgical technique. The 20-minutes DVD was filmed and edited by interns at University Multimedia Studio, which resulted in low cost but high profissional quality. Using such an alternative resource we are now able to save many rats from pain, suffering, and death. Moreover, the always short class time can be better employed for fruitful exchange of views between teachers and students about the main theme of the class: physiology. This alternative didactic resource was well accepted by the students.

KEYWORDS: vídeo-class, replacement, rat, reproductive physiology.

CORRESPONDENCE TO:

Marie Odile Monier Chelini, LHD, VRA, FMVZ, USP, Av. Prof Dr Orlando Marques de Paiva, 87, São Paulo, 05508-000. Email: marodile@usp.br

CYTOGENETIC STUDY OF THE UCh RAT LINES – VOLUNTEER ETHANOL DRINKER: PRELIMINARY RESULTS

SPONDA C. J. (1), SANTILONI V. (1), ALMEIDA-FRANCIA, C. C. D. (2), MOTA L. S. L. S. (1)

(1) Department of Genetics, Institute of Biosciences, UNESP, Botucatu, São Paulo, Brazil; Department of Anatomy, Institute of Biosciences, UNESP, Botucatu, São Paulo, Brazil.

It is known that the alcohol metabolism in women is different from men. If it is given two individuals of opposite sex the same dose adjusted in according to body weight, the woman will present higher alcoholic levels in the blood. The fragility of the alcohol inebriate effects in women is explained by the greater amount of fat tissue, by variation in alcohol absorption during menstrual cycle and by differences between the two sexes in the gastric concentration of alcoholic desidrogenase (a crucial enzyme for alcohol metabolism). In several studies about alcoholism, the use of genetically specific animal lines has shown to be a powerful tool to examine the genetic influence of alcoholic dependence. The aim of this research is to analyze cytogenetically the animals of UChA and UChB lines, volunteer ethanol drinker, through of standard Giemsa staining, GTG-, CBG- and Ag-NOR banding patterns in order to evaluate possible chromosome alterations. We used 28 female rats divided into 7 groups: A and B) UChA (only water) at 30th and 100th days of experiment, respectively; C) UChA of volunteer ethanol drinker at 10% (ethanol + water); D and E) UChB (only water) at the 30th and 100th days of experiment, respectively; F) UChB of volunteer ethanol drinker at 10% (ethanol + water); G) female rats Wistar as control. The material, bone marrow, of the C, F and G groups will be collected at the 100th day of experiment. So far we analyzed about 40 cells of each animal in standard Giemsa staining of A, C, D, E and F groups. The preliminary results show a great percentage of aneuploids cells – 38, 27, 17, 34 and 47% in the A, C, D, E and F groups, respectively. Later, the chromosome banding patterns to clarify the rearrangements will be used.

KEY WORDS: chromosome alterations, *Rattus norvegicus*, ethanol.

CORRESPONDENCE TO:
Lígia Souza Lima Silveira da Mota, Departamento de Genética, Instituto de Biociências, UNESP, Botucatu, SP, Brazil. Phone: +55 14 38116229. Fax +55 14 38153131. Email: lmota@ibb.unesp.br

DIURETIC AND HIPOTENSOR ACTIVIY OF AQUEOUS EXTRACT OF BRAZILIAN PARSLEY SEEDS (*Petroselium sativum* Hoffm.) IN RATS.

ALVES M. J. Q. F.(1), CAMPOS K. E.(2), BALBI A. P. C (3)

(1) Laboratory of Kidney and Biomembranes, Departament of Physiology, Biosciences Institute, UNESP/Campus de Botucatu-SP – São Paulo Estate, Brazil; (2) Laboratory of Experimental Research of Gynecology and Obstetrics, Department of Gynecology and Obstetrics, School of Medicine of Botucatu – São Paulo State University (Unesp) – São Paulo State, Brazil; (3) Departament of Physiology, School of Medicine of Ribeirão Preto, São Paulo University (USP) – São Paulo State, Brazil.

Purpose: the vegetal specie, *Petroselium sativum* Hoff., known as parsley, is widely used in the Brazilian folk medicine as diuretic. The objective of this study is to study if Brazilian use of parsley aqueous extract has similar effects with investigations that show a diuretic effect of *P. sativum* in rats. Methods: 19 rats were anesthetized and we canulate the trachea, left carotide artery (for arterial pressure measurement) and urinary bladder (to collect urine). After 40 minutes of adaptative surgery conditions, anesthetized rats were administered as related with their group: control (CON), oral administration with 1.0 mL of filtered water, and treated group (AE), oral administration with aqueous extract of seeds of parsley 20% (AE). Urine was collected three times (30 minutes each) and then this material was used for sodium and potassium determinations, to evaluate the amount excreted of these ions. Arterial pressure was measured by mercury manometer for 9 times. All data were statistically evaluated. Results and conclusion: in the analyzed parameters, CON group did not show any differences; but AE group showed an increased of urinary flow and sodium and potassium amount excreted, and also decreased arterial pressure. All the parameters presented these modifications after 30 minutes of administration of AE ($p<0.05$). These results show that the treatment with the AE lead to a natriuretic and hypotensor effects in anesthetized Wistar rats, confirming the use of Brazilian population of this herb as diuretic.

KEY WORDS: parsley, *Petroselium sativum*, rat, diuretic, arterial pressure.

FINANCIAL SUPPORT: CNPq

CORRESPONDENCE TO:

Maria José Queiroz de Freitas Alves, Laboratory of Kidney and Biomembranes, Department of Physiology, Biosciences Institute, UNESP/Campus de Botucatu-SP, São Paulo State, Brazil Phone: + 14-38116251. Fax: + 14-38116251. Email: zeze@ibb.unesp.br

EFFECT OF ESSENTIAL OIL FROM *Citrus aurantium* L. ON MDX MICE'S MUSCULAR REGENERATION

SOUZA P. A. T.(1), MARQUES M. J. (2), HIRUMA LIMA C. A. (3), MATHEUS S. M. M.(4)

(1) Student of Biology (Lic.) IB/Unesp/Botucatu, (2) Department of Anatomy/IB/UNICAMP, (3) Department of Phisiology-IB/Unesp/Botucatu, (4) Department of Anatomy IB/Unesp/Botucatu.

Duchenne muscular dystrophy (DMD) is a severe X-linked recessive disorder. This disease is an inflammatory myopathy characterized by muscular fiber and adjacent tissue degeneration and by dystrophin absence. Dystrophin in skeletal muscle is a subsarcolemmal cyto-skeletal protein. Mdx mutant mice show a marked deficiency in dystrophin. The aim of this research was to observe the possible effect of essential oil obtained from *Citrus aurantium* bark as anti-inflammatory action in the mdx muscular fiber. We used ten mdx mice (014/06-CEEA). The animals, after weaning, were brought from Unicamp. The mice were divided into control and citrus treated groups (50 mg/kg/weight/day). After 60 days of treatment the mice were anaesthetized with hypnol 2%. One ml of blood was taken to evaluate creatine-kinase activity (Moura, 1982). The diaphragm, sternomastoideus, anterior tibial and gastrocnemius muscles were removed and processed by histological routine. Serial transverse sections of these muscles were stained with HE and the central nuclei were counted by Axiovision 4.0 program. The serum creatine level showed 6168.5 μ/l in the control group and 1288.4μ/ l in the treated group. By the histological studies no remarkable morphological differences were observed between treated and control groups. In spite of that, the anterior tibial and gastrocnemius muscles of the treated group showed some regeneration signals based on central nuclei number. Therapeutic intervention using several therapies is important for many patients with muscular dystrophy. It could allow to live more and to be more active, while genetic therapies have not been developed yet. As a new therapeutic resource, phytotherapy has become more and more popular among people around the world. Future researches with citrus specific components must be carried out for better definition of their effects, mainly as anti-inflammatory.

KEY WORDS: MDX, *Citrus*, muscular fiber.

CORRESPONDENCE TO:

Selma M. M. Matheus, Departamento de Anatomia do Instituto de Biociências da UNESP, Botucatu. Email: micmath@ibb.unesp.br

EUTHANASIA METHODS IN ANIMALS

AGUIAR A.J.A. (1)

(1)Department of Veterinary Surgery and Anaesthesiology, Faculty of Veterinary Medicine and Animal Science, São Paulo State Univesity, Botucatu, Brazil.

ABSTRACT: Euthanasia is a complex subject involving many ethical concerns and behavioral aspects. The word has a Greek root and means: *"good death"*. The aim of euthanasia is the sudden interruption of natural life without promoting any pain or stress response during the process. Among the several methods that can be used to produce euthanasia, physical and chemical techniques are the most frequently performed. These can be considered adequate, acceptable only under specific conditions or unacceptable. Euthanasia is a procedure employed in biological sciences and has well defined clinical purposes. The selection of method depends on the species, number of animals euthanazed, associated diseases and clinical conditions, personal skills, hazard of accidents, time to achieve unconsciousness, reliability, and irreversibility of technique. An important matter is that unconsciousness should be obtained quickly, before any unpleasant physical or emotional experience could be perceived.

KEY WORDS: euthanasia, bioethics, analgesia, anaesthesia in animals.

CORRESPONDENCE TO:

Antonio J. A. Aguiar, DCAV-FMVZ, Unesp, Distrito Rubião Júnior, s/nº, Botucatu-SP, CEP 18.618-000, Brazil. Phone: + 55 1838116252-25.

Email: ajaguiar@fmvz.unesp.br

EXPERIMENTAL INFECTION OF AIRmin MICE BY INTRAPERITONIAL ROUTE WITH ENTEROPATHOGENIC *Escherichia coli* (EPEC)

VULCANO A. B. (1), AMARAL J. A. (1), CABRERA W. H. K. (1), TINO DE FRANCO M. (1), RIBEIRO O. G. (1), ALVES P. C. P. (1), CARBONARE C. B. (2); CARBONARE S. B. (1)

(1) Laboratory of Immunogenetics, Butantan Institute, São Paulo/SP; (2) Laboratory of Mucosa Immunology, ICB-USP

Enteropathogenic *E. coli* (EPEC) are important agents of infantile diarrhea, able to produce attaching and effacing lesions on the enterocyte membrane. The interaction between EPEC and host cells is simulated by *in vitro* models of bacterial adhesion to cultured cells, resulting in localized adherence. As the *in vitro* models are limited, we intend to search an *in vivo* model for this infection. AIRmin mice were selected for minimal inflammatory response and showed low resistance in experimental *Salmonella* infection. Our aim was to obtain an intraperitonial (i.p) infection model to EPEC in AIRmin mice and to set up the LD_{50} for these bacteria. Groups of four AIRmin mice, aged 4 weeks were infected by intraperitonial route with various doses of live EPEC (saline, 10^7, 10^8 and 10^9cfu) in 200µL. The same experiment was performed with mice aged 8 weeks. Both experimental groups were observed for 45 days to calculate the mortality and the LD_{50} by the Reed & Müench method. Serum and feces were collected from all animals before infection and afterwards form the surviving animals. The values of DL_{50} were 4.21 x 10^7 bacteria to the mice aged 4 and 3.1 x 10^7ufc to the mice aged 8. IgG serum antibodies analyzed individually raised during 3 weeks after infection and showed a great variability between animals. Fecal IgA antibodies analyzed as a pool of each group, showed a peak 2 weeks after infection in the two groups. Our study established the infection model of AIRmin mice aged 4 or 8 weeks inoculated by i.p route and determined the LD_{50} values. In future studies, we will compare these results with the ones obtained in oral infection experiments. Both approaches can be used in future studies of infection and protection by passive immunotherapy or vaccines against *E.coli* infections.

KEY WORDS: *Escherichia coli,* EPEC, LD_{50}, AIRmin mice.

FINANCIAL SUPPORT: FAPESP, CNPq

CORRESPONDENCE TO:

Vulcano, A.B., Immunogenetic Laboratory, Av. Vital Brasil, 1500, Instituto Butantan, São Paulo, Brazil. Phone: (11) 3726-7222 (2146). Email: amandabardella@butantan.gov.br

EXPERIMENTAL INOCULATION OF ARMADILLOS WITH *Mycobacterium leprae*: PRELIMINARY RESULTS

ROSA P. S. (1), BELONE A. F. F (1), SILVA E. A. (1), PEDRINI S. C. B. (1), PINKE C. A. E. (2)

(1) Lauro de Souza Lima Institute, Bauru, São Paulo, (2) UNESP, Botucatu, SP.

Experimentally infected *Dasypus novemcinctus* armadillos have been an important source of *M. leprae* bacilli. Despite the innumerous reports showing that about 90% of the animals develop disseminated infection, this species has not been thoroughly studied in Brazil and in two moments the experimental infection resulted in positive inoculations. The aim of the present study was to evaluate the viability of inoculating armadillos as an alternative source of large amounts of bacilli. Nine *D. novemcinctus* were inoculated with *M. leprae* suspension containing 10^8 bacilli/ml by subcutaneous and intravenous routes. The suspension was obtained from skin biopsies of one untreated multibacillary leprosy patient. All animals were previously tested with the intradermal Mitsuda antigen as an indicative of susceptibility. In order to eliminate the possibility of natural infection, animals were adapted to life in captivity for several months, being clinically evaluated every two months. None of them showed signs of natural infection. Prior to inoculation, the sera of the animals were tested for detection of anti-PGL-I antibodies using an ELISA test and results were negative. Thirteen months post-inoculation with *M. leprae*, 5 animals presented nodules at the site of subcutaneous inoculation. One animal died 20 months after infection because of dissemination of the disease. This animal showed presence of large amounts of whole alcohol acid resistant bacilli in the skin, liver, spleen and lymph nodes. Two other animals died around 30 months after inoculation and the remaining 2 animals with lesions are alive and clinically well despite the presence of the nodules on skin. Until now, the other 4 animals have not developed any signs of infection after inoculation which suggests they are resistant animals.

KEY WORDS: armadillos, *M. leprae*, *Dasypus novemcinctus*

CORRESPONDENCE TO:
Patrícia Rosa, Instituto Lauro de Souza Lima, Rodovia Cmte João Ribeiro de Barros Km 225, Bauru, SP, 17034-971, Fone: 55-14-3103-5911, Email prosa@ilsl.br

HEART FAILURE AFFECTS MYOD AND MYOSIN HEAVY CHAIN EXPRESSION IN RAT DIAPHRAGM MUSCLE

PACAGNELLI F. S. (2), CARVALHO R. F. (1), CAMPOS G. E. R. (2), SUGIZAKI M. M. (1), PADOVANI C. R. (1), NOGUEIRA C. R. (1), CICOGNA A. C.(1), SILVA M. D. P. (1)

(1) Department of Morphology, Clinics and Biostatistics, UNESP, Botucatu, Brazil, (2) Departments of Cell Biology and Anatomy, UNICAMP, Campinas, Brazil, (3) Department of Physiotherapy, UNOESTE, Presidente Prudente, Brazil.

Diaphragm myopathy has been described in patients with heart failure (HF), atrophy and increased expression of type I myosin heavy chains (MHC). The pathways that regulate the expression of MHC during HF have not been described and myogenic regulatory factors (MRFs) may be involved. The purpose of this investigation was to determine whether MRF mRNA expression during heart failure is associated with changes in MHC expression levels in the diaphragm. Methods*:* Diaphragm muscle from both HF and control Wistar rats was studied when overt HF had developed twenty-two days after monocrotaline administration. MyoD, myogenin and MRF4 content were determined by RT-PCR, and MHC isoforms by polyacrylamide gel electrophoresis. Results: HF animals presented decreased MHC IIa/IIx protein isoform and MyoD gene expression content, without altering MHC I, IIb, MRF4 and myogenin. Conclusion: Our results show that in HF, alterations in MyoD mRNA expression may in part explain alterations in MHC IIa/IIx content.

FINANCIAL SUPPORT: FAPESP

KEY WORDS: Heart failure, myogenic regulatory factors, myosin heavy chain, diaphragm muscle, Wistar rats.

CORRESPONDENCE TO:

Maeli Dal Pai-Silva, Departamento de Morfologia, UNESP, Botucatu, 18618-000, São Paulo, Brasil. Email: dpsilva@ibb.unesp.br

HEART FAILURE ALTERS MATRIX METALLOPROTEINASE GENE EXPRESSION AND ACTIVITY IN RAT SKELETAL MUSCLE

DARIOLLI R. (1), JUSTULIN Jr L. A .(2), FELISBINO S. L. (1), SUGIZAKI M. M. (3), OKOSHI M. P. (3), CICOGNA A. C. (3), CARVALHO R. F. (1), DAL PAI-SILVA M. (1)

(1) Department of Morphology, UNESP, Botucatu, São Paulo State, Brazil, (2) Department of Cell Biology, UNICAMP, Campinas, São Paulo State, Brazil, (3) Department of Clinics, UNESP, Botucatu, São Paulo State, Brazil.

Heart failure is associated with a skeletal muscle myopathy with cellular and extracellular alterations. The hypothesis of this investigation is that extracellular changes may be associated with enhanced mRNA expression and activity of matrix metalloproteinases (MMP). We examined MMP mRNA expression and MMP activity in Soleus (SOL), extensor digitorum longus (EDL), and diaphragm (DIA) muscles of young Wistar rat with monocrotaline-induced heart failure. Rats injected with saline served as age-matched controls. MMP2 and MMP9 mRNA contents were determined by RT-PCR and MMP activity using electrophoresis in gelatin-containing polyacrylamide gels in the presence of SDS under nonreducing conditions. Heart failure increased MMP9 mRNA expression and activity in SOL, EDL, and DIA and MMP2 mRNA expression in DIA. These results suggest that matrix metalloproteinase changes may contribute to the skeletal muscle myopathy during heart failure.

KEY WORDS: Heart failure, metalloproteinases, extracellular matrix, skeletal muscle, Wistar rats.

FINANCIAL SUPPORT: FAPESP

CORRESPONDENCE TO:

Rafael Dariolli, Departamento de Morfologia, Instituto de Biociências da UNESP, Botucatu, 18618-000, São Paulo, Brasil. E-mail: rdariolli@gmail.com

HIGH DOSE OF ANABOLIC STEROID IMPAIRS CARDIAC FUNCTION IN RATS

TANNO A. P. (1), CUNHA T. S. (1), ROSA K. T. (2), GIORDANO F. C. L. (1), CALIL C. M. (1), NOVAES P. D. (1), IRIGOYEN M. C. (2), MARCONDES F. K. (1)

(1) Department of Physiological Sciences, Dental School of Piracicaba, State University of Campinas, Piracicaba, SP, Brazil; (2) Hypertension Unit, Heart Institute, School of Medicine FMUSP, SP, Brazil.

Human studies on the effects of androgenic anabolic steroids (AAS) present some limitations, regarding the control of pattern and amount of AAS administration because of ethical considerations. Since AAS abuse is frequent, animal models are useful in order to evaluate AAS side effects. Based on this, we designed this study to investigate the isolated and combined effects of AAS and resistance training on cardiac morphology and function. Male Wistar rats were divided into 4 groups: sedentary vehicle (SV), trained vehicle (TV), sedentary nandrolone (SN) and trained nandrolone (TN). Training was performed by jumping into water (50-70% body wt-load, 5 days/week, 6 weeks). Two days after the last training session, rats were anesthetized and submitted to echocardiography, or killed and heart was analyzed for collagen infiltration. The data were compared by two-way ANOVA and Tukey test ($p<0.05$). Both physical training and AAS treatment induced left ventricular hypertrophy, and this effect was more pronounced in TN group, as demonstrated by the increase on left ventricle mass/body weight ratio (SV:1.78±0.06 = TV:1.98±0.06 < SN:2.07±0.05 = TN:2.27±0.05) and on relative wall thickness index (SV:0.38±0.01 < TV:0.46±0.01 = SN:0.51±0.02 < TN:0.56±0.02). AAS by itself or combined with resistance-training decreased cardiac output index (SV:213±11 = TV:204±19 < SN:184±18 = TN:151±14 mL/min/g) and increased cardiac collagen content (SV:2.93±0.22 = TV:6.48±1.01 < SN:10.32±0.75 < TN:34.34±2.77 μm^2). These findings showed that the association between high dose of nandrolone and physical training induced cardiac concentric hypertrophy and deleterious alterations in the cardiac muscle structure and function.

KEY WORDS: cardiac hypertophy, nandrolone, resistance-training.

FINANCIAL SUPPORT: FAPESP, CNPq

CORRESPONDENCE TO:

Fernanda Klein Marcondes, Department of Physiological Sciences, Faculty of Dentistry of Piracicaba, State University of Campinas, Piracicaba, SP, Brazil. Phone: +55-19-3412-5380, Fax: +55-19-3412-5212. Email: fklein@fop.unicamp.br

IMMUNOMODULATORY EFFECT OF *Agaricus blazei* SS. HEINEM IN EXPERIMENTAL INFECTION WITH *Paracoccidioides brasiliensis.*

GAMEIRO M. C.(2), MARTINS P. R.(3), CASTOLDI L. (3), ROMAGNOLI G. G. (3), PINTO A. V. S. F.(3), SOARES A. M. V. C. (1), KANENO R. (1)

(1) Department of Microbiology and Immunology, IBB-UNESP; (2) Department of Tropical Diseases and Image Diagnosis, FMB-UNESP; (3) Department of Pathology, FMB-UNESP, Botucatu, SP, Brazil.

The medicinal mushroom *A. blazei* have been studied due to immunostimulatory properties of it compounds. In this study we aimed to evaluate the *in vivo* effect of a fraction of *A. blazei* (ATF) on the experimental infection of mice with *P. brasiliensis* (Pb18). BALB/c mice (*n*=8) were experimentally infected with Pb and treated by i.p. administration of 250 µg of a fraction ATF *(*3, 4 and 5 days post-infection). Animals were sacrificed after 14, 28 or 56 days for evaluation of permanence of infectious units in the spleen, liver and lung (CFU), evaluation of peritoneal macrophages activity and the cytokine profile of their spleen cells by ELISpot technique. We observed that infection induces increased number of IL-4 and IL-6-producing cells and suppresses the number of IFN-γ-producing cells, during the early period (14 days). Treatment with ATF enhances the number of IL-10, IL-12, TNF-α and IFN-producing cells and decreased the number of IL-4-producing cells (28 and 56d). Treatment enhances H_2O_2 (14, 28, 56d) and NO (56d) production by macrophages but was not able to protect animals against the infection, since the same number of colony-forming units of Pb was isolated from target organs. Data showed that administration of ATF was able to enhance the fungicidal activity of peritoneal macrophages following *in vitro* challenge with Pb (76.80% ± 55.42 vs 11.03% ± 22.23 and 14.85%± 2.29 vs 8.60 ± 1.63). Our data indicate that the ATF, although increases the fungicidal activity of macrophages and modifies the profile of cytokines produced in reply to the infection, does not shorten the permanence of fungus in the target organs.

KEY WORDS: *Agaricus blazei*, *Paracoccidioides brasiliensis*, macrophage, cytokines.

FINANCIAL SUPPORT: FAPESP, CAPES

CORRESPONDENCE TO:

Ramon Kaneno, Department of Microbiology and Immunology, IBB-UNESP, CEP: 18618-000 – Botucatu – SP, Brazil. Phone: +55 14 3811-6058. Fax: +55 14 3815-3744. Email:rskaneno@yahoo.com.br

ROAD-KILLED WILD ANIMALS: USE IN MOLECULAR ECO-EPIDEMIOLOGY OF FUNGAL PATHOGENS

RICHINI-PEREIRA V. B. (1), GRIESE J. (2), BOSCO S. M. G. (1), SILVA R. J. (2), BAGAGLI E. (1)

(1) Department of Microbiology and Immunology, Institute of Biosciences UNESP-Botucatu, São Paulo; (2) Department of Parasitology, Institute of Biosciences UNESP-Botucatu, São Paulo.

ABSTRACT: A great number of road-killed wild animals have been observed, most of them due to the presence of road in their habitat, interfering with natural trail and the availability of food along the road that may act as an attraction for fauna. These animals may offer new opportunities for eco-epidemiological studies of pathogens, because it attends the São Paulo State Law n. 11.977 that restricts the use of animals in research and prioritizes alternative methods. Besides, the use of road-killed animals has several advantages over conventional methods, because they do not need to be anesthetized or killed, an important fact in terms of conservation biological aspects. Although culturing of tissues samples is not possible, the use of molecular tools represents an alternative method for pathogen detection. The wild animals were collected by the Highway Department of São Paulo State (DER), and were conditioned and identified according to species and geographical location. Until the present, a total of 23 animals was collected (3 *Cavia aperea,* 6 *Cerdocyon thous,* 2 *Dasypus novemcinctus,* 1 *Dasypus septemcinctus,* 2 *Didelphis albiventris,* 1 *Eira barbara,* 2 *Galictis vittata,* 2 *Procyon cancrivorus,* 3 *Sphiggurus spinosus,* 1 *Tamandua tetradactyla*). A necropsy was carried out and tissue samples were collected for DNA extraction. Preliminary results, using Nested-PCR with specific primers, indicated that some animals are naturally infected with *Paracoccidioides brasiliensis*, an important fungal pathogen with ecological aspects poorly understood. The approach employed herein will contribute to detecting environmental occurrence of fungal pathogens, as well as determining natural reservoirs in wild animals and facilitating understanding of the host/pathogen relationships. This project was authorized by IBAMA and CEEA.

KEY WORDS: road-killed animals, fungal pathogens, molecular tools.

FINANCIAL SUPPORT: FAPESP, FUNDUNESP

CORRESPONDENCE TO:

Eduardo Bagagli, Depto de Microbiologia e Imunologia, Instituto de Biociências UNESP-Botucatu. Email: bagagli@ibb.unesp.br

SEARCH FOR *Mycobacterium leprae* AND OTHER MYCOBACTERIA IN WILD ARMADILLOS

PEDRINI S. C. B. (2), ROSA P. S. (2), BAGAGLI E. (1), LOPES C. A. M. (1)

(1) Department of Microbiology and Immunology - Botucatu, Institute of Biociences, UNESP, (2) Animal Facility - Lauro de Souza Lima Institute, Bauru, São Paulo State, Brazil.

Leprosy is still a worldwide public health problem. Brazil, in addition to India, shows the highest prevalence rates of the disease. Natural infection of armadillos *Dasypus novemcinctus* with *Mycobacterium leprae* has been reported in the United States, Mexico, Argentina and Brazil, in the state of Espírito Santo. Potentially pathogenic and ambiental mycobacteria have been isolated from these animals. Identification of bacilli is difficult, particularly because of its inability to grow *in vitro.* The use of molecular tools represents a fast and sensitive alternative method for diagnosis of mycobacteriosis. In the present study, the diagnostic methods used were bacilloscopy, culture and PCR using specific *primers* for *M. leprae* repetitive sequences. The PCR was performed using genomic DNA extracted from liver, spleen, lymphnodes and skin of 21 *D. novemcinctus, Euphractus sexcintus* and *Cabassous tatouay* armadillos from the Western region of the state of São Paulo. From those, 17 samples were taken from the DNA Bank of the Fungal Biology Laboratory at Department of Microbiology and Immunology – IBB, UNESP. Standardization of the technique was done in *M. avium* experimentally infected hamsters and *M. leprae* experimentally infected *D. novemcinctus* tissue from the Laboratory Animal House at Lauro de Souza Lima Institute, Bauru-SP. No one of the wild armadillos showed natural mycobacterial infection. Only the armadillo inoculated with material collected from untreated multibacillary leprosy patient was PCR positive. The genomic sequencing revealed 100% identity with *M. leprae.* Based on the method we may conclude that wild armadillos seem not to play an important role on epidemiology of leprosy in the Western region of the state of São Paulo.

KEY-WORDS: *Dasypus novemcinctus, Mycobacterium leprae,* mycobacteria, PCR, wild armadillos.

CORRESPONDENCE TO:

Silvia Barbosa Pedrini, Instituto Lauro de Souza Lima, Rodovia Cmte João Ribeiro de Barros Km 225, Bauru, SP, 17034-971, Phone: 55-14-3103-5886. Email silviapedrini@yahoo.com.br

SMALL RODENT CARCASSES AS EXPERIMENTAL MODELS IN FORENSIC ENTOMOLOGY

MORETTI T. C. (1), RIBEIRO O. B. (2), THYSSEN P. J. (1)

(1) Department of Parasitology, Biosciences Institute, UNESP, Botucatu, São Paulo State, Brazil; (2) Department of Parasitology, Biology Institute, UNICAMP, Campinas, São Paulo State, Brazil.

Although studies of the *post-mortem* fate of human corpses are of forensic interest, and in natural environments large animals become available to insect colonization soon after death, the fate of the vast number of small carcasses in some habitats, and the parameters that control this process, have not been objective of important investigations. Due to this situation, decomposition studies of small rodent carcasses were conducted in a secondary wood area within the Campus of UNICAMP in the municipality of Campinas (Brazil), from August 2003 to June 2004, to analyze the composition of the local carrion visiting and colonizing invertebrate fauna. Four laboratory mouse carcasses (*Mus musculus*) and four rat carcasses (*Rattus norvegicus*) were exposed in each season, during the set period. The carcasses were placed in an iron-mesh cage, which was adequate to collect adult and immature insects. We collected 6514 specimens (820 adults and 5694 immatures) of 53 arthropod species from the families Sarcophagidae, Calliphoridae, Muscidae, Fanniidae, Syrphidae, Richardiidae, Sepsidae, Micropezidae, Otitidae, Drosophilidae, Phoridae, Dolichopodidae, Anthomyiidae, Asilidae and Lauxaniidae (Diptera), Formicidae, Ichneumonidae, Encyrtidae and Apidae (Hymenoptera), Staphylinidae (Coleoptera) and Gonyleptidae (Opiliones). The most abundant species breeding on the carcasses were *Lucilia eximia* (Wiedemann, 1819) (Diptera: Calliphoridae) and some Sarcophagidae species, such as *Peckia (Pattonella) intermutans* (Walker, 1861) and *Sarcophaga (Liopygia) ruficornis* (Fabricius, 1794), which are rarely seen breeding on carcasses of large animals. For the adult insects, the size (rat or mouse) of the carrion ($F= 5.59$; $P< 0.0221$) was statistically significant for the dependent variable frequency, which also happened with the immature insects ($F= 9.45$; $P< 0.0106$). The significance level was $\alpha = 0.05$.

KEY WORDS: Forensic entomology, animal carcasses, Diptera, mice, *Rattus norvegicus*.

CORRESPONDENCE TO:

Thiago de Carvalho Moretti, Department of Parasitology, Biosciences Institute, UNESP, Botucatu, São Paulo State, Brazil. Email: tcmoretti@yahoo.com.br

THE ROLE OF *Slc11a1* GENE IN *Paracoccidioides brasiliensis* INFECTION IN MICE SELECTED FOR ACUTE INFLAMMATORY REACTION

TRINDADE B. C. (1), CAVALHEIRO J. S. (1), BALDERRAMAS H. A. (1), SORGI C. A. (2), FACCIOLI L. H. (2), DE FRANCO M. (3), OLIVEIRA S. L. (1)

(1) Department of Microbiology and Immunology, IBB, UNESP, Botucatu, Brazil; (2) Department of Clinical, Toxicological and Bromatologic Analyses, FCFRP, USP, Ribeirão Preto, Brazil, (3) Laboratory of Immunogenetics, Butantan Institute, Brazil.

Mice selected for the maximum (AIRmax) or minimum (AIRmin) acute inflammatory reaction show different susceptibility degrees to the intracellular parasite multiplication, being useful as an experimental model to *Paracoccidioides brasiliensis* infection. These lines show distinct patterns of leukocyte infiltration and *R* and *S* allele frequency disequilibrium of the *Slc11a1* gene. In this study, we investigated the interaction of the *Slc11a1 R* and *S* alleles with the inflammation modulating Quantitative Trait Loci (QTL) during paracoccidioidomycosis, in the homozygous AIRmax^{RR}, AIRmax^{SS}, AIRmin^{RR} and AIRmin^{SS} lines. Male mice were i.t. infected with 1x10^{6} yeast cells of *P. brasiliensis* (Pb 18) and sacrificed 2 and 7 days after infection, in order to evaluated the influx of PMN in the bronchoalveolar lavage (BAL), IL-1 production by ELISA and CFU counts in lungs homogenates. Increased CFU numbers was observed only in AIRmin^{RR} line at day 2 after infection. At day 7 after infection, the AIRmax lines showed a better infection control than the AIRmin lines by CFU counts but did not show interline difference R/S. The absolute number of PMN was significantly higher in BAL from AIRmax^{RR} than AIRmin^{RR} and AIRmin^{SS}. Between AIRmax^{RR} and AIRmax^{SS}, a tendency of a higher influx of PMN in BAL of AIRmax^{RR} was found. IL-1 levels at day 2 after infection were low in lungs from AIRmin^{RR} line and the same was observed in AIRmax^{RR} at 7 days after infection. These data suggested a participation of *Slc11a1* gene modulating the experimental paracoccidioidomycosis in lines of mice selected for acute inflammatory reaction together with other factors involved in the AIR selection.

KEY WORDS: inflammation, paracoccidioidomycosis*, Slc11a1.*

CORRESPONDENCE TO:

Bruno Caetano Trindade, Department of Microbiology and Immunology, Biosciences Institute, São Paulo State University, Botucatu, São Paulo State. Phone: +55 014 3811 6058. Email: brunocatrin@yahoo.com.br

THE STATE OF ART IN THE PRODUCTION OF LABORATORY ANIMALS

MATTARAIA V. G. M. (1), RODRIGUES U. P. (1), GHIURO E. J. V. (1), ROCHA A. P. (1), MOREIRA V. B. (1), TÁVORA M. F. C. L. F. (1).

(1) Central Animal Facility – Butantan Institute, São Paulo – SP, Brazil

Animal experimentation dates back from the ancient Greeks and Romans, but it was during the nineteenth and eighteenth century that it slowly progressed from an uncommon practice to a scientific focus. In the twentieth century the debate about animal ethics got stronger. The institutions that produce immunobiologicals and biopharmacols have a need for animals free of specific patogens to be used as control subjects in researches, because the final product, by demand of the WHO manual, has to be tested *in vivo*. The goal of this work is to share with the scientific community, the efforts developed by biotechnical researchers in developing sanitarial and genetical animals according to the new concepts of ethics and animal well-being. We started by developing a project for physical adequation in the breeding areas with defined fluxes, following the introduction of sanitarial control, handling techniques, physical and chemical contention, and establishing concepts and criteria for the understanting of the operation principles that allowed us to get the maximum from the animals inside a high ethical standard. The possibility of working with sanitary and genetically-defined animals help us attend the growing demand of Butantan Institute products without a proportional growth in the number of animals used in tests. It happens due to the constant involvement and intense trainning of all production researchers of the Institution working under the 3R principle. A program that is very stimulating is the training of technicians with theorical and practical classes that teaches them to avoid suffering and stress. We understand the need for animal testing but we do not accept the manipulation of these animals without a scientific and a high human condition.

KEY WORDS: animal experimentation, ethics, sanitarial control.

CORRESPONDENCE TO:

Vânia Gomes de Moura Mattaraia, Divisão Biotério Central – Instituto Butantan, São Paulo, Brasil. Email: vmoura@butantan.gov.br