

# Journal of Biological Research

**Bollettino della Società Italiana di Biologia Sperimentale**



**87<sup>th</sup> SIBS National Congress  
on Peoples, Environment, Health**

**Turin, Italy, 5-6 December 2014**

ABSTRACT BOOK

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# Journal of Biological Research

Bollettino della Società Italiana di Biologia Sperimentale

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FRIDAY 5 DECEMBER

## QUAGLIARIELLO LECTURE (INVITED)

**ISCHEMIC AND PHARMACOLOGICAL POSTCONDITIONING OF THE HEART: PROTECTIVE ROLE OF APELIN-13**

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If reperfusion follows an ischemia long enough to produce a myocardial infarction, a worsening of the damage takes place with increase of the infarct size, a more severe loss of contractility, the appearance of myocardial contracture and a greater incidence of arrhythmias. The overall injury is defined ischemia/reperfusion (I/R) injury. Reperfusion injury may be more severe than ischaemia injury and is characterised by impaired nitric oxide (NO) synthesis and increased reactive oxygen species production. The heart may be protected against I/R injury by ischemic pre- (IPre) or post-conditioning (IPost). While the former is obtained with repeated 2-3 min coronary occlusions before the infarcting ischemia, the latter is performed with repeated 10-15 s occlusions starting a few seconds after the beginning of reperfusion. In protection a pivotal role is played by NO. The protection of the heart is also obtained with compounds which can mimic IPre or IPost. Apelin, an endogenous peptide, is produced in various tissues and organs. Out of the different isoforms, apelin-13 is the most active one on cardiovascular system, where it displays positive inotropic effect and vasodilation. Apelin prevents I/R injury if given in the very early phase of reperfusion, but not before ischemia, *i.e.* it mimics IPost but not IPre. The activity of apelin is displayed via the G protein coupled receptor APJ. Apelin-induced protection includes infarct size limitation, a better recovery of contractility with reduction of contracture and prevention of arrhythmias. Apelin protects via RISK pathway elicited by PI3K-Akt system.

## ORAL COMMUNICATIONS

**SYK INHIBITORS AS POTENTIAL ANTIMALARIAL DRUGS: FIELD STUDY IN VIETNAM AND UGANDA**A. Pantaleo, M.C. Pau, T.H.G. Phan, F. Turrini, P.G. Pippia  
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Despite intense world-wide effort, malaria is still a major cause of mortality and morbidity, especially in third world countries. The intracellular parasite *Plasmodium falciparum* is the most lethal of the malaria species. Resistance to current anti-malarial therapies is spreading, and can conceivably be circumvented by targeting the host erythrocyte. An integral step in parasite egress includes weakening of the host cell membrane via modification of host membrane proteins, including the integral erythrocyte membrane protein band 3. Over 75% of band 3 is lost by the end of egress, which is critical since the band 3-ankyrin interaction constitutes the major connection linking the membrane to the cytoskeleton. Curiously, phosphorylation of band 3 tyrosines by syk kinase also induces release of band 3 from the cytoskeleton, leading to membrane fragmentation. Since tyrosine phosphorylation of band 3 is prominent during the later stages of intra-erythrocytic parasite development, we treated infected red blood cells with Syk inhibitors to assess their abilities to block parasite egress. Within 48 hours of syk inhibitor addition, parasitemia was found to decline by >95%, with many infected red blood cells (RBCs) still containing entrapped parasites. Not surprisingly, syk inhibition also prevented tyrosine phosphorylation of band 3, allowing retention of band 3 in the RBC membrane and preventing membrane fragmentation. To determine whether syk inhibition might constitute a resistance-proof antimalarial therapy, blood was collected directly from infected Vietnamese and Ugandan patients and was treated with syk inhibitors. Blood analysis revealed the same reduction in parasitemia seen with laboratory strains of *P. falciparum*.

**INSULINE RESISTANCE AND TYPE 2 DIABETES IN THE AMERICAN INDIAN POPULATIONS**

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Pathological predispositions exist in different populations as a result of genetic differences. The Amerindians, for example, are more susceptible to alcoholism and type 2 diabetes. A startling statistic on alcoholism among the Amerindians, provided by the Indian Health Service (IHS) in 2005, revealed that of the slightly more than one million Native Indians served by the IHS in 33 states, the alcoholism mortality rate was 33.9 per 100.000 population, approximately 5.3 times the rate of the general US population. This can be explained by the absence of two particular enzymes: aldolase and alcoholase, which render Amerindians incapable of metabolizing alcohol in the same way as Europeans. Another

major problem is type 2 diabetes. Statistics provided by the IHS in 2005 reported that the rate of type 2 diabetes, including all the health complications that accompany it (glaucoma, heart disease, strokes, obesity, kidney disease, circulatory disorders affecting the arms and legs, etc.) was higher than 75% in the Indian Reservations. Fortunately this alarming trend can be reversed through proper diet and education, which must be provided urgently to ensure the survival of the Amerindian population.

### **A PROTEOMIC APPROACH TO STUDY THE EFFECTS OF MICROGRAVITY AND OXIDATIVE STRESS ON SIGNAL TRANSDUCTION IN HUMAN T LYMPHOCYTES**

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The aim of this research was to study the protein expression and post-translational modifications of human T cells undergone to microgravity and oxidative stress. During spaceflights the weightlessness leads to several modifications of physiological cellular processes such as proliferation, differentiation, signal transduction, cytoskeleton and gene expression. Experiments in real and simulated microgravity conditions have shown that immune system function is altered in about 50% of space crew members. The effect of microgravity in T cells on cytoskeleton, IL2-IL2R system, apoptosis and Rel/NFκB pathway is well known, while little is known about changes of phosphorylation and their functional consequences. It has the potential to be a valuable indicator of the impact of microgravity on cellular metabolism. As a result of the whole study, clinical problems related to disturbed immune cells functions of astronauts during long-term space flights could be anticipated and prevented. Many pathologies, as leukemia and lymphoma, appear to be directly associated to oxidative phenomena, which influence protein phosphorylation changes a probable molecular switch in the pathways that regulate cell proliferation and carcinogenesis. In this work T cells response in extreme conditions was evaluated to better clarify the chemical complexity of lymphocytic proteome. Particularly, we focused the attention on the Spleen Tyrosine Kinase family components already known for their behaviour in pathological states. Our experiments demonstrated a link between cysteine oxidation and tyrosine phosphorylation changes in T cells and a novel role of Syk inhibitors in exerting their anti-inflammatory activity through the inhibition of a response initiated by reactive oxygen species.

### **ECOSYSTEM BASED APPROACH APPLIED TO DISEASE MANAGEMENT IN AGRO-ECOSYSTEM**

S. Sozzo,<sup>1,2</sup> G. Gilardi,<sup>1,2</sup> A. Garibaldi,<sup>2</sup> M.L. Gullino<sup>1,2</sup>

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Basil (*Ocimum basilicum* L.) is an economically important crop grown in northern Italy, as well as in other Mediterranean regions. *Genovese Gigante* is the only cultivar used for industrial production of *Denominazione di Origine Protetta (DOP) pesto*. Basil cultivation on the Riviera Ligure

can be considered as a small agro-ecosystem within the Mediterranean ecosystem. Mediterranean-type ecosystems are vulnerable to desertification; when an ecosystem community has high biodiversity the risk of disease decreases, this pattern, is called the *dilution effect*. The biodiversity and the composition of species in a community is the true indicator of higher and lower rates of disease. In spite of this, the agro-ecosystem species are able to survive, but their handling and management is becoming more and more difficult because of an increasing spreading of pests. Recently, the spreading of downy mildew on basil caused by *Peronospora belbahrii* has become economically relevant in the Liguria Region. The economic evaluation and environmental impact assessment in the Mediterranean ecosystem with basil crop system and diseases caused by this pathogen, are still complex. The main target of this study is the characterization of the different indicators associated to *P. belbahrii* based on interactions observed between the ecosystem and the basil agro-ecosystem.

### **RARE TUMORS AND CLINICAL TRIALS**

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Rare cancers belong to the group of rare diseases that are normally defined as diseases with a prevalence of less than 50 out of 100,000. Even when defined more conservatively by taking into account some peculiarities of natural history and prognosis, rare cancers represent about 20% of all cases of malignant neoplasms, including all cancers affecting children and teenagers and many affecting young adults. There are significant variations in incidence and mortality rates for different types of rare cancers. Information about rare cancers, their treatment options and where to obtain appropriate treatment is in many cases not readily available to patients. Sub-optimal treatment outcomes are common for rare cancers due to a lack of medical expertise in the management of rare cancers, poor referral rates from general practitioners and pathologic misdiagnosis. Outcomes for a diverse range of rare cancers could be improved through the establishment of reference networks or centres of expertise. Current methodologies and regulations require the benefit of new treatments to be proven in a large number of patients. Given the inherently low numbers of rare cancer patients, large studies are not feasible. The risk of not building enough evidence to gain approval of new agents and the high cost of small trials may discourage industry from developing drugs for most rare cancers.

### **EXPRESSION OF ADHESION MOLECULES IN INTERMEDIATE EXTRAVILLOUS TROPHOBLAST IN NORMAL AND PATHOLOGICAL CONDITIONS**

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Extravillous trophoblast (EVT) represents a particular type of fetal trophoblast that normally invades maternal decidua. Three subtypes of EVT could be identified: i) superficially invading decidua; ii) deeply invading decidual stroma and myometrium; iii) invading the vascular wall of the spiral arteries. A lot of complex and intertwined mechanisms are involved in the regulation of distribution and proliferation of

EVT. In particular, the adhesion molecules (E-cadherin) and the anti-adhesion molecules (Mel-CAM) interaction seem to have a significant role. Some pathological conditions probably represent the effect of a deregulation in this control system: placenta accreta-increta, *post-partum* hemorrhage, lack of spontaneous afterbirth and superficial placental adhesion. Another rare pathology of placenta involving mainly the villous growth and maturation is indicated as *superficial or defective implant*. Recently it has been postulated that the villous abnormalities were related to an inadequate/abnormal placental site. In these cases we were able to point out a well preserved villous growth potential in spite of poor developed villi by using two specific immunohistochemical stainings (ki67-cells proliferation marker and p63-cytotrophoblastic cells marker). In the present work we investigate adhesion molecules expression in 10 selected cases diagnosed as placentas with *defective implant*. In 9 out of 10 cases a remarkable reduction of both molecules was demonstrated. The 20 control cases did not show a similar reduction, with the unique exception of a case of pre-eclampsia. These results strongly emphasize the role of adhesion molecules in the regulation of EVT in pathological implants (overgrowth) but also in all conditions of inadequate or insufficient implants.

## DENTAL HEALTH IN THE PAST: RESEARCH AND PERSPECTIVES FROM PALEOPATHOLOGY

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This paper reviews the current status of dental research in paleopathology. Diseases and injuries of teeth and jaws are important topics in the study of ancient human population because they provide a wealth of information on past lifestyle, diet, illness and so are valuable indicators of environmental changes. Teeth, generally, survive well to chemical and microbial degradation in archaeological soils and they are routinely used in anthropological and paleopathological research.



## SATURDAY 6 DECEMBER

## ORAL COMMUNICATIONS

**EFFECT OF DIETARY SUPPLEMENTATION OF PROBIOTICS AND PALM FRUITS EXTRACTS ON INNATE IMMUNE RESPONSE OF SEA BASS (*DICENTRARCHUS LABRAX*)**

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The great development in aquaculture sector in recent decades has increased the interest in study of performance of fish growth through to enhance of the fish immune system by manipulating diets. Alternative methods of disease prevention have been sought as immunostimulants and probiotics, prebiotics and symbiotics, as additives in the diet. Thus, the aim of the current study was to evaluate the potential *in vivo* effects of dietary supplementation of probiotics and palm fruits extracts on the humoral (level of total IgM antibodies, peroxidase and complement activities) and cellular innate immune response (respiratory burst, peroxidase and phagocytic activities) of the sea bass (*Dicentrarchus labrax*). Bacterial probiotic (*Shewanella putrefaciens*, Pdp11, 10<sup>9</sup> cfu g<sup>-1</sup>) and palm fruit extracts (100 g kg<sup>-1</sup>) from Tunisian dates were orally administered alone or in combination to sea bass specimens in a commercial pellet food. Fish were sampled after 2 and 4 weeks of treatment and serum and head kidney leucocytes were collected from specimens. Innate immune parameters were evaluated. This study showed for the first time in sea bass a immunostimulation in the of most activities evaluated in animals fed with the addition dates extracts or this substance in combination with Pdp 11 probiotic, mainly after 4 weeks of treatment. These results suggested that a dietary supplementation of palm fruit extracts administration alone or in combination with Pdp 11 probiotic has beneficial effects on the innate immune parameters of *D. labrax*, therefore these extracts could be considered as immunostimulant food additive in fish farming.

**ANTIOXIDANT PROPERTIES AND POLYPHENOLIC COMPOSITION OF LEAVES OF CALABRIAN *LAVANDULA MULTIFIDA***

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*Lavandula multifida* (Lamiaceae) is a short-lived plant which spontaneously grows along the Mediterranean coast, on poorly-evolved limestone soils, in hot-arid climatic conditions. In Italy it has been found in Calabria (Capo dell'Armi

and in Sicily (Capo S. Alessio, M. Pellegrino and Brucoli). Since *L. multifida* populations appear to be reduced and fragmented due to the human impact on its natural habitat, this plant has been included in the *Regional Red Lists of Italian Plants* under the status IUCN of *critically endangered* in Calabria region. Some metabolites and essential oils obtained from *L. multifida* leaves show antimicrobial and antifungal properties, thus increasing the economic value of this species. The aim of this work was to evaluate the antioxidant properties and polyphenolic composition of Calabrian *L. multifida*, in comparison with the commercial species *Lavandula angustifolia* which, in contrast to *L. multifida*, is widely distributed and so easy to be found and collected. For this purpose, extracts from fresh leaves of the two plant species were tested for enzymatic and non-enzymatic antioxidants, in order to evaluate the antioxidant potential of the extracts; moreover, polyphenolic composition was determined by HPLC analysis. During the last few years the exploitation of native lavender species has increased also for a renewed interest in the use of naturally derived compounds. Due to its rarity and the necessity of preserving its genetic heritage, Calabrian *L. multifida* needs to be valorized also for its wide possibilities of applications in pharmaceutical, cosmetic and food industries.

**DETERMINATION OF THE DIFFERENT MEAT LIPID FRACTIONS BY GAS-CHROMATOGRAPHY**

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The lipid profile of meat has always had a negative connotation about the secondary effects on human health. In particular, cholesterol and saturated fatty acids of meat have been related to the onset of different cardiovascular diseases; however these lipid components have been recently re-evaluated thanks to a better knowledge of the meat lipid composition and to the unexpected positive effects of these components on human health. The aim of this work was to evaluate the lipid composition of main lipid classes in different meats, to provide an overall picture about the meat composition as related to cardiovascular diseases risk and human health. *Longissimus dorsi lumborum* from beef, pork, lamb, wether and rabbit, as well as thigh and breast from chicken and turkey, were analyzed. The total fatty acid and main lipid classes' composition were determined by gas chromatography (GC) and Fast GC coupled to mass spectrometry, respectively, in both intramuscular and subcutaneous fat. In general, the subcutaneous fat was mainly composed by unsaturated fatty acids. Free cholesterol (13.90 mg/100 g tissue, which correspond to 2.54-4.25% of total lipid matter) was mainly detected in intramuscular fat. The diglyceride content was higher in white meat than red ones, due to a higher enzymatic hydrolysis in white meat, thus, leading to a reduction of its shelf-life. In conclusion, the aforementioned

analytical determinations of meat lipid composition could be used to better define the quality and nutritional value of the most widely consumed meat products.

### MILK FAT GLOBULE: BIOLOGICAL AND NUTRITIONAL ASPECTS

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The milk fat globule is one of the most complex and sophisticated component that can be found in nature. Its particular structure has been thoroughly investigated in the last 30 years and have been elements extremely similar to a biological cell. The milk fat globule presents a structure and a secretion system unique in nature. In fact, the globule is composed by two membranes: the external plasma membrane is very similar to a one of a normal biological cell and a monolayer of phospholipids that protects the inner core of triglycerides. Its plasmatic membrane is characterized by tri-layer structure and present many lipoprotein that can help to configure the journey of the lipid droplet from endoplasmic reticulum to the apical membrane. The present work has the objective to update the knowledge about the origin of the milk fat globule inside the mammary cell related to the role of the Golgi and the cytoskeleton.

### EDUCATE PEOPLES FOR THE ENVIRONMENT AND HEALTH: NOURISH THE PLACET-ENERGY FOR LIFE

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This research is based on the education of the environment to preserve the planet and create a *public awareness* to: i) nourish the earth, ii) promote energy useful to human life and its healthy development, iii) guarantee good health. This study began in Valle Argentera Municipal district of Sauze di Cesana. This environment, pastures, production of *noble milk*, this atavic people's lifestyle made homogeneous through adaptation to the alpine ecosystem, give results useful for the divulgation of *good practices*. The Sauze community in the inter-living, propose new courses of nourishing and nourishing oneself. Stock raising in the area's total essence, spirituality, its background, states and together with the outcome of faith has sent forth for over a thousand years, from San Restituto, the imposing spiritual and cultural fortress in the heart of the Valley. For this population, land in both material and universal goods to be defend and cultivated with knowledge and respect. From here, comes the first indication of *territorial health*, from unpolluted water to pastures, how attention to origins and self-nourishment is balanced, thanks to the honest use of resources. Human life, land, water, livestock are one the same. Products are the result of knowledgeable balance. In this district, there are families of antique stock: Berton, Perachon, Manzon, Merlin, Prin Abeil, Prin Clari, Prin Derre. This innovation of this research consists in education *good practices* to promote the wheel of life.

### HEALTH STATUS OF WORKERS EXPOSED TO DIFFUSE EMISSIONS FROM AN ELECTRIC ARC FURNACE IN TRENTO (ITALY)

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Objective of the study was to evaluate if the workers probably exposed to many pollutants in a foundry with electric arc furnace (Trentino, Italy) had a higher mortality and morbidity than expected. We used retrospective cohort study and comparison of health fee exemptions. A cohort of 331 exposed workers was studied from 1979 to 2009 (mean follow-up 20.7 years): the standardized mortality ratio (SMR) was calculated for all causes and major diseases. The group of exposed workers was compared to the general population and to internal control group. Rates of exemption from health fee of 235 workers were compared to the total number of all health fees exemptions in the Province of Trento (PT) and the Community of Valsugana and Tesino (CVT). An excess mortality due to any cause was found in the blue collar workers as compared to the general population (SMR 1.13; 29 observed) and to the internal group (relative risk 2.34; 16.6 avoidable deaths). The mortality rate due to specific causes was increased for all types of cancers (SMR 1.36; 14 cases), for lung cancer (SMR 3.35; 8 cases), for ischemic heart disease (SMR 1.27; 4 cases), for chronic liver disease (SMR 1.16; 2 cases) and for *injury and poisoning* (SMR 1.55; 7 cases). The rate of exemption from health fees of the exposed workers was found to be superior to the rate of the PT and CVT for all seven diseases studied. The risk was statistically significant in diabetes mellitus, hypertension with organ damage and rheumatoid arthritis. Working environment including diffuse emissions are the most likely cause of the increased prevalence of diseases observed among workers.

### SLEEP HOMEOSTASIS AFTER THE INDUCTION OF A TORPOR-LIKE STATE IN THE RAT

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During the recovery period from sleep deprivation Delta power during NREM sleep increases *homeostatically*, i.e. in proportion with the duration of previous wakefulness. A large increase in Delta power was also observed at the exit of a state of torpor/hibernation, suggesting that the time passed in torpor/hibernation is a period of sleep deprivation, despite the little brain activity. While one experiment has shown that the after-torpor Delta increase is apparently homeostatically regulated, strengthening the hypothesis that consider the torpor bout as a period of sleep deprivation, two different experiments have found the opposite, suggesting that Delta enhancement may just be the consequence of a non-specific reactivation of the cortical functions. Since a powerful increase in NREM sleep Delta power was also observed after the induction of a torpor-like state in a non hibernator like the rat, we tested whether a homeostatic response to a sleep-deprivation period at the end of the torpor bout was present. Twelve male Sprague Dawley rats (300-350) were used. Animals were induced into a torpor-like state and sleep deprived (n=6, 6 h) by gentle handling at the end of the torpid

period or let recovery untouched ( $n=6$ ). Animals exposed to the sleep deprivation protocols showed a powerful increase in NREM sleep Delta power at the end of the deprivation period ( $281 \pm 13\%$ ), larger than the one observed in the control group ( $237 \pm 19\%$ ). These data support the hypothesis that the period passed in torpor can be considered as sleep deprivation.

### ELECTROENCEPHALOGRAPHIC CHANGES DURING THE INDUCTION OF A TORPOR-LIKE STATE IN RATS

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The periods of torpor are typically followed by the occurrence of high-Delta power NREM sleep (NREMS). In the present study, this phenomenon was investigated in rats through the induction of a torpor like state by the pharmacological inhibition of the rostral ventromedial medulla (RVMM), a key area in the central nervous control of metabolism, promoting thermogenesis. Twelve male Sprague-Dawley rats (300-350 g) were used, surgically implanted, under general anaesthesia (Diazepam, 5 mg/kg, i.m., ketamine, 100 mg/kg, i.p.), with electrodes for chronic EEG recording, a thermistor for the detection of the hypothalamic temperature (Thy), a catheter for arterial blood pressure recording, and a microcannula for drug delivery within the RVMM. Two groups of animals were microinjected within the RVMM (1 injection/hour, for 6 h) with the GABAA agonist muscimol [1 mM, 100 nl; Group 1 ( $n=6$ )] or with saline [0.9%, 100 nl, group 2 ( $n=6$ )]. In Group 1 a deep hypothermia was observed during the 6-h microinjection period (Thy fell to  $22.80 \pm 0.8^\circ\text{C}$ ) which was accompanied by a decrease of EEG activity and a progressive shift of the EEG spectral power towards the low-frequency region. After the end of the injection period, EEG activity showed a progressive increase and the EEG spectral power shifted towards higher frequencies. Soon after the recovery of normothermia, a powerful intensification of Delta power in NREMS was observed, which lasted about 6 h. The dynamics of EEG changes during and after the torpor-like state induction were similar to those described in natural torpor.

### PELAGIA NOCTILUCA (CNIDARIA: SCYPHOZOA) AND ITS VENOM. THREE DECADES OF RESEARCH IN ITALIAN LABORATORIES AND FUTURE PERSPECTIVES

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Recurrent outbreaks of *Pelagia noctiluca* and health problems consequent to stings were recorded during the last decades. This phenomenon forced some Italian University laboratories to study this cnidarian. The first studies concerned the distribution, the biochemical composition and the morphology of nematocysts of *Pelagia noctiluca*. The discharge mechanism of nematocysts was defined starting from early 1980s when enzymes, cations, anions, and pH were observed to have an influence on this process. Notably, trypsin, extreme

pH values, some anions ( $\text{I}^-$ ,  $\text{Cl}^-$ ,  $\text{SCN}^-$ ), and thioglycolate were seen to induce, while  $\text{La}^{3+}$  and  $\text{Gd}^{3+}$  to prevent, nematocyst discharge. The discharge of both *in situ* and isolated nematocyst was found to be  $\text{Ca}^{2+}$  dependent. Furthermore, *Pelagia noctiluca* nematocysts were seen to retain their discharging capacity in distilled water. The toxicological evaluations were carried out mainly using the crude venom from *Pelagia noctiluca* because, unfortunately, to date the composition is not known. Hemolytic and cytotoxic properties of crude venom have been evaluated on erythrocytes and cultured guinea-pig fibroblasts, mouse fibroblasts, and cancer (neuroblastoma) cells. The activity on other cnidarians has been also assessed. The crude venom induced apoptosis by reactive oxygen species generation and decrease in mitochondrial transmembrane potential, loss of mitochondrial integrity, and alteration of cell membrane permeability. A pore-forming action mechanism on mitochondrial membrane, with oxidative damage was also suggested. In addition, the protective activity of some compounds has been evaluated. Future challenges will concern the attempts to characterize the venom and to perform a wider screening of cytotoxicity induced to normal and cancer cells.

### NEUROTOXINS IN CNIDARIA AND CONVULSIONS IN ZEBRAFISH AND IN SEVERE MYOCLONIC EPILEPSY OF INFANCY

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Severe myoclonic epilepsy of infancy (SMEI) is an epileptic cerebropathy characterized by drug resistant seizures of different type (tonic-clonic, clonic, absences, myoclonus), starting at 6-7 months of life, and electroencephalography (EEG) initially normal, but subsequently abnormal, with interictal spikes and waves, polyspikes, slow waves. SMEI is a genetic cerebropathy (Dravet) due to SCN1A gene deletion, causing voltage-gated sodium channels non-inactivation. In SMEI not all symptoms may be attributable to gene mutation, namely the repetitive seizures, the status epilepticus, the onset age reported in the disease. We have observed a typical case of SMEI which presented 3-4 seizures every week, myoclonus and status epilepticus, abnormal EEG. Baraban et al. reported zebrafish with genome similar to humans and zebrafish with a mutation in a voltage-gated sodium channel (scn1b mutant) recapitulating the SMEI symptomatology. Cnidarian *Nematostella vectensis* is a carnivore producing Nv1 neurotoxin, binding to a receptor of voltage-gated sodium channels, present in central nervous system and acting in crustaceans, in zebrafish and in mammals. In zebrafish the neurotoxin I produces repetitive convulsions and paralysis. Also some peptides constituent of the neurotoxin I cause the same symptoms (Wankle). In SMEI and in other epileptic disorders the repetitive seizures and the status epilepticus are attributed to a production of cytokines (polypeptides) as interleukin  $1-\beta$  and  $1-\delta$  and other polypeptides. On the basis of the analogy of activity of neurotoxin I on sodium channels, we hypothesize that the production of peptides during seizures analogues to Nv1 neurotoxin may cause status epilepticus and repetitive convulsions in SMEI, acting on the voltage-gated sodium channels receptors.

## ECOLOGY OF *TIGRIOPUS FULVUS* FISCHER, 1860 (COPEPODA: HARPACTICOIDA): METAPOPULATIONS AND ENVIRONMENTAL FACTORS

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*Tigriopus fulvus* (Fischer, 1860) is a supralittoral harpacticoid copepod usually found in Mediterranean Sea splash-pools which has many biological applications (aquaculture, ecotoxicology, including genomics). Aim of the present paper is to contribute to the ecological knowledge of the *Tigriopus fulvus* populations of Genova Nervi studying the main environmental parameters in relation to the metapopulation structure and distribution. Beside its well-known resistance to short-term environmental fluctuations, less is known about climate change effects on this species, as already recorded and studied in *Tigriopus californicus*. A first analysis related to population and new environmental conditions has been done in order to understand the possible behavioural and physiological response to the climatic change.

## EVALUATION OF BIMANUAL PERCUSSION PERFORMANCE IN NORMAL SUBJECTS AS A METHODOLOGICAL APPROACH FOR INVESTIGATING THE PROFESSIONAL PATHOLOGY IN DRUMMERS

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Several studies showed that musculoskeletal disorders present a significant problem for musicians. In particular, for drummers can occur two types of drumming injuries: over-use injuries like inflammations due to continual impact, muscular contraction and gravitational force, and traumatic injuries due to a single accident. In this study we developed an original computer-aided method for evaluating bimanual percussion performances using two different types of drumstick grip (modern and traditional grips) in 72 non-professional subjects of both sexes aged from 13 to 56 years were studied. Statistical analysis of quantitative data in terms of temporal precision and intensity of these percussion performances (efficacy, efficiency) and of qualitative data in terms of perceived satisfaction was carried out. We consider that this method may be useful for investigating the professional pathology in drummers.

## FROM THE BALTIC SEA TO THE PADANA PLAIN. TRACES OF MEDIEVAL MIGRATIONS IN NORTHERN ITALY REVEALED BY UNIPARENTAL MARKERS AND DEEP-ROOTED PEDIGREES\*

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Social and cultural factors had a critical role in determining the genetic structure of Europe. Therefore, socially-stratified populations may help to focus on specific episodes of European demographic history. In this study we use uniparental markers to analyse the genetic structure of *Partecipanza* in San Giovanni in Persiceto (Northern Italy), a peculiar institution whose origins date back to the Middle Ages and whose members form the patrilineal descent of a group of founder families. From a maternal point of view (mtDNA), *Partecipanza* is genetically homogeneous with the rest of the population. However, we observed a significant differentiation for Y-chromosomes. In addition, by comparing 17 Y-STR profiles with deep-rooted paternal pedigrees, we estimated a Y-STR mutation rate equal to  $3.90 \times 10^{-3}$  mutations per STR per generation and an average generation duration time of 33.38 years. When we used these values for tentative dating, we estimated 1300-600 years ago for the origins of the *Partecipanza*. These results, together with a peculiar Y-chromosomal composition and historical evidence, suggest that Germanic populations (Lombards in particular) settled in the area during the migration period (400-800 AD, approximately) and may have had an important role in the foundation of this community.

\*Full paper in: Traces of medieval migrations in a socially stratified population from Northern Italy. Evidence from uniparental markers and deep-rooted pedigrees. Heredity (Edinb). 2014 Sep 10. doi: 10.1038/hdy.2014.77. [Epub ahead of print]

## THE REPOPULATION OF GIAGLIONE (TO), ITALY AFTER THE PLAGUE OF 1629-30: AN ANALYSIS WITH THE FREQUENCIES OF SURNAMES

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The epidemic plague between 1629-1631 caused a dramatic contraction in the size of north Italian populations. The effect has been similar to a genetic bottleneck that reduced genetic variability in populations. Because of random genetic drift some alleles may be lost, while others can be successful, originating a genetic pool very different from the previous. An estimation of the genetic change in the ancient populations can be obtained by a comparison between the structure of the surnames before the plague and after the bottleneck. In this paper we studied the population of Giaglione (Susa Valley), one of the first Italian communities who was affected by plague from summer 1629. Before the disaster the population was constituted by 1250 inhabitants; after the plague there was only 400 persons. The effects of the bottleneck were calculated using the distribution of burial's surnames, for the period between 1604-1644. The modality of repopulation were studied by the censuses of 1680, 1718 and 1799. After the plague has been observed a flow of migrants that have temporarily enriched the community of new surnames. These new alleles have not been successful and the repopulation of Giaglione occurred largely from the descendants of the survivors to the plague. Therefore, the surnames have remained the same, but with frequencies completely changed compared the beginning of the sixteenth century. The trend of surnames' structure has been studied using the relationship index of Chen & Cavalli-Sforza and the multidimensional scaling analysis.

## TRANSHUMANCE BETWEEN HIGHLAND AND LOWLAND SARDINIANS: A SURNAME DISTRIBUTION ANALYSIS

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The isonymic method was used to analyze the relationships among Sardinian populations from five villages in the historical-geographical zone of Barbagia di Belvì, a mountainous area traditionally devoted to sheep-rearing and the point of departure of transhumance toward lowland areas. The current surname structure of each population was determined from telephone directories and the structure in the first half of the 19<sup>th</sup> century from *Status Animarum* records. The data were compared with the current surname distribution, obtained from telephone directories, in lowland villages with a different environmental, historical-cultural and economic background, final destination of transhumance. The spread of surnames in Sardinia may have occurred by means of transhumance, occurring every year along precise routes from the pastoral mountain zones to the agricultural plains. The standardized index of Chen and Cavalli-Sforza was used to calculate the isonymic relationships among the five villages of Barbagia di Belvì (Aritzo, Belvì, Desulo, Gadoni and Tonara). Application of non-metric Multidimensional Scaling to the isonymy matrices showed that the villages of Barbagia di Belvì constituted a group that was largely homogeneous in time and space. Transhumance routes have been studied using the Spatial Autocorrelation (Moran's I), applied to surnames distributed in the historical-geographical zone in which Sardinia is historically divided. The main aim is to find out if there was appreciable admixture between the Sardinian populations of the southern lowlands and those of the central mountains, with the isonymic method used to simulate the genetic relationships.

## THE HUMAN POPULATION IN THE ALPINE ECOSYSTEM: THE EXAMPLE OF BELLINO, VAL VARAITA (CN), ITALY

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The biological characteristics of human communities that live isolated for many generations due to their environment, have always interested anthropologists to understand the phenomenon of adaptation. These are the reasons that have led in the seventies of the last century some researchers of the University of Turin to take on an interesting project aimed to a multidisciplinary anthropological reconstruction of some human isolated groups that lived in mountain. Specifically were examined the residents of Bellino, a locality placed in Varaita (CN) in the north of Italy in Piedmont, considered as a more than valid example for his history and geography. The local population was constituted by 432 inhabitants. The research was conducted on 267 individuals, 127 women and 140 men. This paper is a synthesis of this past research about genetic variability, demographic and social data and wants to think about events that certainly have an impact on the socio-anthropology of those places also today.

## FRIDAY 5 DECEMBER

## POSTERS

## MESSINA SIBS BRANCH

**FROM CULTURE TO APPLICATION. AGAR FROM GRACILARIA GRACILIS OF GANZIRRI LAGOON (SICILY, ITALY)**

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Cultivation of *Gracilaria* genus is a diffused practice in many Southeastern Asian countries and South American countries. Interest in these algae is due to the possibility of agar extraction from thalli. Agar is a polysaccharide of high economic value used as a natural gelling agent in foods, pharmaceutical compounds, industrial applications and many other topics. Considering the excellent results of our experience in the culturing of *Gracilaria gracilis* in Ganzirri lagoon (Oriented Natural Reserve of Capo Peloro, Messina, Sicily, Italy) with a growth rate of 952% in only 70 days, we tried to close the cycle from culture to application with agar extraction and application in microbiology with setting-up Petri's plates for bacteria culture. Our cultured *Gracilaria* has been sent to Java Biocolloid (Indonesia) to evaluate agar quality. Results were very satisfactory with presence of agar of 13.2%, purity 57.5% (high) and gel strength of 880 g/cm<sup>2</sup> (common is 700 g/cm<sup>2</sup>). Agar extraction was also carried out in our laboratories, resulting in agar of 9.4%. We are currently trying to optimize our extraction protocols to obtain a highest yield. This experience shows the immediacy and easy applicability of agar. This could be an important challenge in our future. Agar used in Italy is imported from extra-European countries and there are no industries in EU states extracting this basic polysaccharide.

Acknowledgments: study supported by INNOVAQUA Project PON02\_00451\_3362185-CUP B61C12000880005.

**STRUCTURAL AND IMMUNOHISTOCHEMICAL STUDY OF PHOTOPHORES IN THE BODY OF THE DRAGONFISH, CHAULIODUS SLOANI**

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*Chauliodus sloani* is a deep sea fish that lives in the Messina's Strait (Sicily, Italy) at depth between 300 and 1000 m. At these depths the only available light present derives from the bioluminescence. The bioluminescence found in 42 families of Osteichthyes is a phenomenon by which living organisms emit light through chemical reactions leading to chemical energy that is converted to light. Bioluminescence is found in symbiotic bacteria (extrinsic) or in epidermal

derivates such as glands (intrinsic). These particular pluricellular glands are known as photophores, and embedded in the dermis. *C. sloani* has intrinsic bioluminescence. The photophores map consist of two subocular and a ventral series of 63-70. The specimens were collected in particular weather condition (strong tidal currents and wind from SE) in the north Messina's coast, and the skin samples from the lateral and ventral body walls fixed for immunohistochemistry. Some sections were stained with routinary histological methods using trichrome stainings and AB-PAS methods, and others were subjected to double immunolabeling techniques using confocal immunofluorescence to study the innervation patterns of the photophores. The photophores are composed of a luminous epithelium and lens epithelium. In these epithelia two functional cell types, are found, namely, the photocytes that are delimited by a reflector, and the lens cells or filter cells. A layer of melanophores forms a capsule surrounding the reflector. The photocytes are associated with adrenergic and nitrergic nerve fibers we have detected using antibodies to TH and nNOS with colocalization methods. Previous studies have pointed to the effects of adrenaline-induced light emission. The role of NO in the modulation of light emission is, however, not fully clarified.

**BIOLOGICAL ACTIVITIES OF POLYSACCHARIDES FROM MARINE ALGAE**

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Marine organisms have proven to be rich sources of structurally diverse bioactive compounds with valuable biomedical potential. Marine algae are important for phycocolloids like agar, carrageenan and alginate in the industrial uses, but in recent years, polysaccharides have emerged as an important class of bioactive natural products. Algal polysaccharides show different interesting properties. They are reported to have blood anticoagulant, anti-tumor, anti-mutagenic, anticomplementary, immunomodulating, hypoglycemic, antiviral, hypolipidemic and anti-inflammatory activities. Among these, their anticoagulant activity has high scientific interest and has been well investigated by numerous researches. Biological activities of polysaccharides derived from two algal species, *Ulva* sp. (Chlorophyta) and *Agardhiella* sp. (Rhodophyta) has been here evaluated. Species were collected in two brackish lakes (Faro and Ganzirri) forming a complex ecosystem (Cape Peloro Lagoon) in Northeastern Sicily. Aqueous algal extracts were tested at two different concentrations (10 and 20 mg/mL) and their cytotoxicity were evaluated by trypan blue test and haemolysis test. Emo-coagulation parameters were furthermore tested by evaluating partial thromboplastin time (PTT), prothrombin time (PT). Our results show that both algal extracts has no cytotoxic effects and that they prolong PT and PTT value suggesting an inhibiting activity on extrinsic and intrinsic coagulation pathways. In conclusion, this study suggests a possible exploitation of investigated macroalgae as potential resources in biomedical scopes.

## PALERMO SIBS BRANCH

**NEW 5-DIMETHYL-3-HETEROARYL-1H-4-ONN-AZOXYCYANIDES: SYNTHESIS AND ANTIMYCOTIC ACTIVITY**S. Aiello,<sup>1</sup> F. Venturella,<sup>2</sup> M. Giammanco<sup>1</sup><sup>1</sup>Physiology and Pharmacology Unit, Department of Legal, Social and Sports Sciences, University of Palermo;<sup>2</sup>Department of Biological, Chemical and Pharmaceutical Sciences and Technology, University of Palermo, Italy

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In the field of infectious diseases, in the last years an increasingly high importance is ascribed to mycosis as causes of illness and mortality in particularly susceptible patients: leukemia, organ transplant, AIDS and immunosuppressed patients, creating clinical and epidemiological problems. Although several drugs are available, antifungal spectrum is still limited especially for invasive infections, which often have fatal results. We previously reported the antifungal activity of a series of products, in which the 1,5-dimethyl-4-(cyano-NNO-azoxy) pyrazol-3-yl and 1,3-dimethyl-4-(cyano-NNO-azoxy) pyrazol-5-yl moieties were linked to pyridine, pyrazole, isoxazole, thiophene and the furan rings. All compounds displayed interesting antifungal activity against *Candida krusei* and *Candida glabrata*, two fungal species resistant to azoles, is noteworthy. The presence of the cyano function appeared essential for activity. The need for novel antifungal agents lead us to synthesize and to investigate the antifungal activity of new ONN-azoxycyano derivatives in which the 3 position is replaced with quinolinic and benzenic rings. The title compounds tested *in vitro* for antifungal activity against *C. glabrata* and *C. krusei*, displayed remarkable antifungal activity, (MIC=0.25 and 0.5 µg/mL) that means that some of the title compounds were 16 and 32 fold more potent than Amphotericin B and Fluconazole, respectively. These results suggest that, depending on the heterocyclic molecule bound to the 3 position of 1H-4-ONN-azoxycyanides, it is possible to modulate the antifungal activity of these new class of derivatives. Synthesis and *in vitro* biological test of title compounds will be reported.

**IL-1 $\beta$  MAINTAINS THE DNA HYPERMETHYLATION OF ANTI-INFLAMMATORY IL10 GENE IN A HUMAN INTESTINAL EPITHELIAL CELL LINE**

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Intestinal inflammation is a natural process crucial to maintain gut integrity, but its deregulation is involved in the pathogenesis of severe intestinal disorders. Intestinal epithelial cells play a crucial role in the inflammatory response, modulating the immune cell exposure to antigens and by their ability to secrete many inflammatory mediators. IL-1 $\beta$  represents a pivotal player: secreted by infiltrated leucocytes, it induces the expression of several pro-inflammatory genes. Also the anti-inflammatory IL-10, whose function is to terminate the inflammatory process, modulates the intestinal physiology. Recent clinical reports showed that patients with ulcerative colitis in remission phase have significantly higher *IL10* gene expression in mucosa compared with active patients and controls. Moreover, in the latest years aberrant epigenetic mechanisms

were put in binomial relationship with chronic inflammatory diseases. Previously, we described a demethylation of pro-inflammatory *IL6* and *IL8* genes in human colonic Caco-2 cells differentiated into an enterocyte-like phenotype and exposed to the inflammatory action of IL-1 $\beta$ . In the present study we evaluate whether the IL-1 $\beta$  treatment affected the methylation status of the anti-inflammatory *IL10* gene, in the same *in vitro* model. Our results showed that IL-1 $\beta$  treatment did not change the hypermethylation status of the *IL10* promoter. Moreover, in cell lysates from IL-1 $\beta$ -treated Caco-2 cells, we observed a dose-dependent increase of DNMTs activity and, surprisingly, a decrease of DNMT3b expression. These findings put in evidence the complexity of relationship between IL-1 $\beta$  and DNMTs, and may suggest a potential role of IL-1 $\beta$  as pleiotropic modulator of DNA methylation in Caco-2 cell line.

**METHYLATION DECREASE OF BECN1 GENE INDUCED BY PHYTOCHEMICAL INDICAXANTHIN IN CACO2 CELLS: AN EPIGENETIC HYPOTHESIS OF AUTOPHAGY**

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Autophagy is a highly conserved catabolic process that degrades and recycles intracellular components through the lysosomes. The role of this process in tumor genesis and tumor progression is controversial: in the early stages, it can block tumor growth and conversely it can promote its progression in the later stages. The tumor suppressor *BECN1* gene, encodes the protein Beclin 1, a marker of autophagy down-regulated in several types of cancer, such as colorectal cancer. There are a lot of both genetic and environmental risk factors for colorectal cancer, including diet: for this reason, in accordance with epidemiological studies, consumption of foods rich in phytochemicals is widely promoted. The betalainindicaxanthin (Ind) is a phytochemical from the *Opuntia Ficus-Indica* fruit having several biological activities, such as antioxidant, anti-inflammatory. It showed anti proliferative and proapoptotic effects in colorectal adenocarcinoma (Caco2) cells where was able to regulate gene expression through modulation of methylation state of DNA at CpG islands. For the first time, using Methylation-Sensitive Restriction Endonuclease PCR (MSRE-PCR), we report that Ind (50 e 100 µM) decreases the methylation of *BECN1* promoter in Caco2 cells, to the same extent as 5-azacytidine (Zcyd, positive control). Interestingly, colorimetric detection of DNA Methyl transferases activity, indicates that Ind reduced the activity of these enzymes, like Zcyd did. These preliminary data, indicating that Ind is able to decrease the methylation of *BECN1* gene, allow us to propose an epigenetic hypothesis of autophagy regulation in Caco2 cells.

SATURDAY 6 DECEMBER

## POSTERS

## MESSINA SIBS BRANCH

**COPPER-DEPENDENT REGULATION OF DIFFERENT PROTEINS INVOLVED IN IRON METABOLISM**L.M. Di Bella,<sup>1</sup> R. Alampi,<sup>2</sup> F. Biundo,<sup>2</sup> M.R. Felice<sup>2</sup><sup>1</sup>Interuniversity National Group of Marine Sciences, Rome;<sup>2</sup>Department of Biological and Environmental Sciences, University of Messina, S. Agata (ME), Italy

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Copper and iron are two essential elements for all the organisms because are indispensable for basic cellular processes. It is known that there is a close correlation between the metabolism of iron and copper, and ceruloplasmin fully represents the correlation between these two elements. Moreover, recent studies have suggested that alteration of copper metabolism is one of the pathogenetic mechanisms of Alzheimer's disease and Wilson's disease in which was observed an increase of iron accumulation inside cells and organs. Different studies were carried out in copper deficiency conditions in which expression of different proteins involved in iron metabolism were considered but the effect of this, combined with inflammation conditions, was never considered. In the light of these knowledge, the aim of the present work was to investigate the effect of copper deficiency, obtained by a specific chelating agent (BCS), on the expression of some proteins involved in iron metabolism such as Ceruloplasmin, Ferroportin, Transferrin Receptor 1, Divalent Metal Transporter 1, in normal conditions and in combination with the pro-inflammatory cytokine interleukin-6, using, as experimental model, human hepatocellular carcinoma cell line, HepG2. The results obtained have highlighted that the expression of these proteins are influenced by the different treatments, suggesting a more complex effect of copper deficiency on cellular iron metabolism.

**PLATELET-ACTIVATING FACTOR INDUCED INFLAMMATION: IN VIVO STUDY**A. Silva-Herdade,<sup>1</sup> G. Andolina,<sup>2</sup> C. Faggio,<sup>2</sup> C. Saldanha<sup>1</sup><sup>1</sup>Institute of Microvascular and Inflammation Biology, Institute of Molecular Medicine, Lisboa, Portugal; <sup>2</sup>Department of Biological and Environmental Sciences, University of Messina, S. Agata (ME), Italy

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Leukocytes are key players in the inflammatory processes; they are passively transported inside the bloodstream and interact with proteins of the endothelial glycocalyx. These interactions are responsible for the mediation of an anti-inflammatory response. The arrangement of selectins and integrins regulate their action. In this study, using an animal model of inflammation, we intend to quantify the leukocyte-endothelial cell interactions as well as the erythrocyte deformability and nitric oxide (NO) quantification. To

achieve the animal model of inflammation we have used Lys-EGFP-ki mice, whose granulocytes are fluorescent. Mice were divided in two groups: control and inflammation. The inflammation was induced by an intra-scrotal injection of  $10^{-6}$ M platelet activating factor (PAF). Using an intravital microscopy approach the results were analyzed 1h post-inflammation. We have verified that PAF induces an increase in the number of rolling leukocytes as well as a decrease in the rolling velocities, which is characteristic of an inflammatory response. The erythrocyte deformability of the inflamed mice is decreased at low values of shear stress and those mice present a greater amount of NO efflux from erythrocytes when compared to the untreated mice. In conclusion, the quantification of the leukocytes-endothelial interactions and the lower erythrocyte deformability are characteristics of an inflammatory response. The increased efflux of NO from erythrocytes constitutes a factor of the nitrogen stress associated to inflammatory states.

**ENHANCED ERYPTOSIS FOLLOWING SULFORAPHANE EXPOSURE**S. Calabrò,<sup>1</sup> K. Alzoubi,<sup>2</sup> F. Lang,<sup>2</sup> C. Faggio<sup>1</sup><sup>1</sup>Department of Biological and Environmental Sciences, University of Messina, S. Agata (ME), Italy; <sup>2</sup>Department of Physiology, University of Tübingen, Germany

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Suicidal erythrocyte death (eryptosis) is characterized by cell shrinkage, cell membrane blebbing, and cell membrane phospholipid scrambling with phosphatidylserine exposure at the cell surface. Sulforaphane, an isothiocyanate from cruciferous vegetable, counteracts malignancy. The effect is at least in part due to the stimulation of suicidal death or apoptosis of tumour cells. Mechanisms invoked in sulforaphane-induced apoptosis include mitochondrial depolarization and altered gene expression. Even though they lack mitochondria and nuclei, erythrocytes may, similar to apoptosis of nucleated cells, enter eryptosis, a suicidal cell death characterized by cell shrinkage and phosphatidylserine translocation to the erythrocyte surface. Cell volume was estimated from forward scatter, phosphatidylserine exposure at the cell surface from annexin V binding and  $[Ca^{2+}]_i$ ; from Fluo-3 fluorescence. A 48-hr treatment of human erythrocytes with sulforaphane (50-100  $\mu$ M) significantly decreased forward scatter, significantly increased the percentage of annexin V binding cells and significantly increased  $[Ca^{2+}]_i$ . The effect of sulforaphane (100  $\mu$ M) on annexin V binding was significantly blunted but not abrogated by the removal of extracellular  $Ca^{2+}$ . Sulforaphane (100  $\mu$ M) significantly increased ceramide formation. In conclusion, sulforaphane stimulates suicidal erythrocyte death or eryptosis, an effect at least partially due to the stimulation of  $Ca^{2+}$  entry and ceramide formation.



## POSSIBLE ROLE OF THE MELANOCORTIN SYSTEM IN THE REGULATION OF FEAR RESPONSE AND ANXIETY

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Melanocortins are small peptides encoded by a complex precursor called proopiomelanocortin. They are mainly composed by the adrenocorticotrophic hormone and melanocyte-stimulating hormones. These peptides exert their physiological actions by binding to five melanocortin receptors that positively couple to the production of c AMP (MC1R-MC5R). Atypically, melanocortin signaling is regulated also by endogenous antagonists, agouti-signaling protein (ASIP) and agouti-related protein (AGRP) that compete with the melanocortin peptides by binding to MCRs. ASIP is expressed mainly in the ventral skin where inhibits melanogenesis by binding to MC1R. AGRP binds MC3R and MC4R in the brain for the regulation of energy balance and growth. AGRP is expressed mainly in the lateral tuberal nucleus where blocks the inhibitory action of the MSH on the MC4R, thus stimulating the intake. The comparative study of brain transcriptome of transgenic fish overexpressing ASIP1 vs wildtype fish showed more than two thousand genes differentially expressed. Recent studies have demonstrated that Kiss1 is involved in the regulation of fear response and anxiety. Kiss1 is expressed only in the ventral habenular nucleus (Hv). Our transcriptomic results show that central expression of Kiss1 is repressed in transgenic fish suggesting that the melanocortin system could regulate both behavioral aspects throughout the Kiss neuronal system. In addition, we demonstrate that MC4R is expressed profusely in the Hv. The possible involvement of melanocortin system in the regulation of fear response and anxiety will be discussed.

## SAPONIN, A TRIGGER OF ERYPTOSIS, THE SUICIDAL ERYTHROCYTE DEATH

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Saponins, naturally occurring glycosides and triterpene glycosides in plants, are considered useful in the prophylaxis and treatment of several disorders, including malignancy. The effect of the substances is partly attributable to induction of both apoptosis and necrosis. Saponins are known to trigger hemolysis. The present study explored whether saponin triggers eryptosis. A 24 h exposure to saponin (15 µg/ml) resulted in a significant increase of phosphatidylserine exposure (estimated from annexin V binding) and a significant stimulation of hemolysis (estimated from hemoglobin release), but did not result in a significant decrease of cell volume (from forward scatter). Saponin (15 µg/ml) further increased  $[Ca^{2+}]_i$  (from Fluo3-fluorescence) and ceramide formation (utilizing specific antibodies). Annexin V binding was significantly blunted but not abrogated in the nominal absence of extracellular  $Ca^{2+}$ . The concentration required for both, eryptosis and hemolysis (15 µg/ml), is within the range of concentrations previously employed *in vitro* and *in vivo*. In conclusion, saponins trigger cell membrane scrambling,

an effect partially due to entry of extracellular  $Ca^{2+}$  and ceramide formation. Saponins have been proposed for the treatment of a variety of diseases including cancer, obesity and diabetes and osteoporosis. According to the present observations, stimulation of eryptosis by saponins may, at least in theory, lead to anemia and thrombosis. The therapeutic use of saponins may thus be limited by their effects on erythrocyte integrity.

## STIMULATION OF SUICIDAL ERYTHROCYTE DEATH BY NITAZOXANIDE

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Nitazoxanide, a drug effective against a variety of pathogens and used for the treatment of gastrointestinal infections, triggers apoptosis and is thus considered to be employed against malignancy. Similar to nucleated cells, erythrocytes may undergo an apoptosis-like suicidal cell death or eryptosis. Hallmarks of eryptosis include cell shrinkage and phospholipid scrambling of the cell membrane with translocation of phosphatidylserine to the erythrocyte surface. Stimulators of eryptosis include increase in cytosolic  $Ca^{2+}$  activity ( $[Ca^{2+}]_i$ ). The  $Ca^{2+}$ -sensitivity of eryptosis is also increased by ceramide. A 48-h exposure to nitazoxanide (1-50 µg/ml) did not significantly modify  $[Ca^{2+}]_i$  but significantly increased ceramide formation, decreased erythrocytes volume (10 µg/ml), increased the percentage of phosphatidylserine exposure (10 µg/ml) and, at higher concentrations (20 µg/ml), stimulated haemolysis.  $[Ca^{2+}]_i$  was estimated from Fluo3-fluorescence, cell volume from forward scatter, phosphatidylserine exposure from annexin-V-binding, ceramide abundance utilizing fluorescent antibodies and haemolysis from haemoglobin release. Treatment of erythrocytes with nitazoxanide in the nominal absence of  $Ca^{2+}$  significantly blunted the phosphatidylserine translocation. However,  $Ca^{2+}$  removal blunted, but did not fully abrogate nitazoxanide-induced cell membrane scrambling, an observation pointing to the involvement of additional mechanisms. The effect is at least partially due to ceramide formation. As shown in previous studies, ceramide could sensitize the cell membrane to  $Ca^{2+}$ , and thus, ceramide could trigger cell membrane scrambling and eryptosis even at constant  $[Ca^{2+}]_i$ . In conclusion, nitazoxanide triggers eryptosis, an effect at least partially due to stimulation of ceramide formation.

## FORMULATING POLYPHENOLS IN HYDROPHILIC POLYMERIC SOLID DOSAGE FORMS AND PHARMACOKINETIC CHARACTERIZATION BY MATHEMATICAL MODELING

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Food is an excellent supply of active ingredients and nutraceuticals that can exhibit beneficial effects on human body functions for well-being and for keeping a healthy status. They have been recognized for their potential ability in disease risk reduction, and they have been associated to a possible health care costs reduction for their preventive therapeutic action. Examples of these are polyphenols which are of acknowledged nutritional value for the treatment of chronic diseases. These compounds are receiving great attention since they show different biological effects on the immune, cardiovascular and gastrointestinal systems. Many of these have been formulated in solid dosage forms for oral administration. The present work describes the kinetic modeling of nutraceuticals from modified release solid dosage forms. Mathematical models (e.g. zero order, first order, Higuchi model and Korsmeyer-Peppas) are important tools to describe the pharmacokinetic profile of a nutraceutical, to estimate its bioavailability and bioequivalence. Hydrophilic polymers have been used for the production of two types of systems (polymeric matrix versus cylindrical tablets), loaded with selected polyphenols, and the release profile of these has been characterized using dependent model methods and independent model methods. The hydrogel layer of the polymer is considered the modulator of the polyphenol release kinetic. The dissolution and release of polyphenol from hydrophilic polymers can be described by several mathematical models, however the selection of a suitable mathematical model depends on physicochemical parameters, such as type of nutraceutical and polymer, molecular size of the compound and its solubility, pH and temperature.

#### FUNCTIONALIZED NANOSTRUCTURES DERIVED FROM SELF-ASSEMBLED POLYPHENOLS FOR TARGETED DELIVERY AGAINST CANCER

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Polyphenols (e.g., theaflavins, theaflavin 3-O-gallate, theaflavin 3-O-gallate, theaflavin 3,3'-O-gallate, epigallocatechin gallate, epicatechin gallate, catechins, 2 quercetin glycosides, quinic acid, gallic acid and caffeine) are found in a variety of foods and beverages and have already demonstrated their effectiveness in the prevention of malignancies in humans. Plant polyphenols exhibit anti-oxidant activity, vasodilatory, anti-inflammatory, anti-fibrosis and anti-apoptosis properties, which activate pro-survival cellular pathways. Others, such as flavonoids, resveratrol, and curcumin, retard fat storage, reduce lipid levels, blood pressure, blood glucose, and insulin resistance, contributing thereafter to span life quality. The purpose of this work has been the development of nanostructures derived from molecular self-assembled polyphenols with functional properties to target cancer cells. Matrix nanostructures, composed of functional monomers or oligomers, have been produced and loaded with selected polyphenols by covalent or non-covalent chemistry links. Molecular self-assembly nanostructures were found to be pH dependent and were characterized by circular dichroism, static vs dynamic light scattering, transmission elec-

tron microscopy, Raman spectroscopy, Fourier transformed infrared, differential scanning calorimetry and X-ray diffraction. For targeted delivery, the nanostructures may be surfaced-functionalized with specific targeting ligands for folate receptors which are known to be over-expressed in many cancer cells. The proposed strategy is expected to improve the current alternatives for cancer therapy, based on natural compounds. Further studies are currently ongoing to map cytotoxicity and genotoxicity of the proposed nanostructures.

#### ANTIVIRAL AND IMMUNOMODULATORY EFFECTS OF BIOPOLYMERS BY MARINE EXTREMOPHILIC BACILLI AGAINST HERPES SIMPLEX VIRUS TYPE-2

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Herpes simplex virus type 2 (HSV-2) has now to be responsible of the most common and continuously increasing viral infections in humans. Due to the appearance of resistance to the available treatments, new biomolecules exhibiting different mechanisms of action could provide novel agents for treating viral infections. The immune response against HSV-2 involves intricate and multifactorial aspects to whom viruses have developed various mechanisms to evade them. Biopolymers (BPs) from two novel thermotolerant *Bacillus horneckiae* strains (APA and SBP3) isolated from shallow vents of Panarea Island (Italy), were evaluated for their antiviral and immunomodulatory effects against HSV-2. The BPs were used at the concentration of 400 µg mL<sup>-1</sup> that resulted not-cytotoxic towards human peripheral blood mononuclear cells (PBMC) and WISH cells. Both BPs hindered the HSV-2 replication in PBMC, showing 60 and 80% of viral inhibition for BP-SBP3 and BP-APA, respectively, but they did not in WISH cells. To verify if the antiviral activity was related to the immune response involved in controlling viral replication, the production of cytokines was evaluated on PBMC. Higher levels of Th1-type cytokines (INF-γ, INF-α, TNF-α, IL-12 and IL-18) were detected in PBMC treated with BP-APA than in those treated with BP-SBP3. Th2-type cytokines (IL-4 and IL-10) were not induced by BP-APA. Therefore, the antiviral effects of BPs were related to the pattern of cytokines induced. As stimulators of Th1 cell-mediated immunity, BPs from the two novel thermotolerant bacilli could be considered powerful sources for the development of prophylactic and therapeutic strategies against herpes virus infection.

#### ULTRAVIOLET RADIATIONS RESISTANCE OF SPORES FROM TWO NOVEL BACILLUS HORNECKIAE STRAINS OF SHALLOW MARINE HYDROTHERMAL ORIGIN

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Spores from *Bacillus* are able to survive to different environmental stressors, including the ultraviolet radiations (UVR) exposition. *Bacillus horneckiae* is a novel radioresistant,

extremophile isolated from a Phoenix spacecraft assembly clean room able to resist to UVR. The aims of the present study were: i) to evaluate the UVR resistance of spores from two novel thermotolerant *Bacillus horneckiae* strains (APA and SBP3), isolated from shallow marine vents of Panarea Island (Italy); and ii) to explore the external catalase activity, as putative resistance mechanism against UVR exposition. After purification from vegetative cells, spores were exposed to UVA ( $\lambda=365\text{nm}$ ) and UVC ( $\lambda=253\text{nm}$ ). Catalase activity was spectrophotometrically determined by measuring the decomposition of  $\text{H}_2\text{O}_2$  (0.05% v/v). The spores from both strains showed UVA resistance to  $80\text{ J/m}^2$ . Spores from strain SBP3 were more resistant to UVC (up to  $480\text{ J/m}^2$ ) than those from strain APA ( $240\text{ J/m}^2$ ). After UVA exposition (10 min), spores from both strains, surviving to  $\text{H}_2\text{O}_2$  treatment, possessed a major catalase activity (34% from APA and 38% from SBP3) than unexposed spores. These results suggest that in addition to the coat structure, catalase could play an important role in spores-UVR resistance, inactivating the damage of reactive oxygen species, derived by radiations on vital biomolecules (proteins and nucleic acids). Radioprotective core component and the external catalase of the novel bacilli represent new models to investigating the responses to environmental oxidative stressors. Catalase from these bacilli could provide useful applications for industrial (cosmeceutical, food, pharmaceutical) as well as environmental and biotechnological purposes.

## PIEMONTE ORIENTALE SIBS BRANCH

### RESVERATROL INDUCES INTRACELLULAR $\text{Ca}^{2+}$ RISE IN MALIGNANT MESOTHELIOMA: A POSSIBLE THERAPEUTIC APPROACH

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Malignant pleural mesothelioma (MPM) is an aggressive cancer with poor prognosis, strictly correlated to asbestos exposure. Current therapies are unsatisfactory and there is an urgent need for novel approaches. Intracellular  $\text{Ca}^{2+}$  is known to play an essential role in cancer development and growth. Aiming at finding anticancer polyphenols able to modulate MPM cell  $\text{Ca}^{2+}$ , we have studied here the effect of the grapes stilbene resveratrol (Res). This compound is known for various possible beneficial effects, including its ability of inhibiting proliferation and inducing apoptosis in cancer cells. The effect of Res on MPM REN cells was studied by means of microspectrophotometry microscopy after cell loading with the fluorescent probe fura-2. Cells transiently exposed to 1, 3, or 10  $\mu\text{M}$  Res showed increasing peaks of  $[\text{Ca}^{2+}]_i$ . No effect was visible in  $\text{Ca}^{2+}$ -free medium, while the  $[\text{Ca}^{2+}]_i$  peak was reduced by non-selective ( $\text{Ni}^{2+}$ ) and highly selective (NNC 55-0396) T-type  $\text{Ca}^{2+}$  channel blockers. Dose-dependent curves of Res-induced  $[\text{Ca}^{2+}]_i$  peaks showed a rightward shift for normal MeT-5A mesothelial cells ( $\text{EC}_{50}=4.9\ \mu\text{M}$ ) with respect to MPM REN cells ( $\text{EC}_{50}=2.7\ \mu\text{M}$ ), indicating higher sensitivity of tumor cells to Res. Accordingly, incubation with 3 or 10  $\mu\text{M}$  Res for 7 days resulted in cell growth inhibition for REN cells but not for mesothelial cells. The complex of data indicates that Res induces a  $\text{Ca}^{2+}$  influx that is possibly mediated by T-type  $\text{Ca}^{2+}$  channels. The effect shows selectivity towards MPM cells, suggesting its possible use in the clinical treatment of mesothelioma malignancies.

## PARMA SIBS BRANCH

### FOURIER TRANSFORM INFRA RED INVESTIGATION OF THE INFLUENCE OF HYDRATION AND NON-ENZYMATIC GLYCATION ON THE STRUCTURAL PROPERTIES OF COLLAGEN

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Collagen is the major structural protein found in connective tissues of higher organisms. The structure of the macromolecule is stabilized by opportune conditions of temperature, ionic strength, pH. But a key role is undoubtedly played by water which regulates chain flexibility and assures the water mediated H-bonds favoring the fiber alignment. Water subtraction and non-enzymatic glycation reactions, accompanied by the formation of intermolecular cross-links, are recognized as the main causes of ageing, inducing protein solubility decrease and thinning and stiffening of the fibers. With the aim to investigate these effects, Fourier Transform Infra Red Spectroscopy (FTIR) measurements were performed to study the hydration role on the structure of type I collagen (rat tail, SIGMA) prepared as dry film on  $\text{CaF}_2$  windows, assembled in a dry box with salt solutions (water activity in the range 0.06-0.97). The technique was applied as well to the study of the interaction of collagen incubated with carbohydrates (glucose and ribose), for 15 days at  $37^\circ\text{C}$ . The carbohydrate/protein compounds were lyophilized, prepared as pellet with KBr and measured in transmittance. Amide I region ( $1600\text{-}1700\text{ cm}^{-1}$ ) is used to evaluate secondary structure changes occurred to the collagen as a consequence of the hydration/dehydration and glycation treatments. The results are matched with those extracted from the Amide III ( $1200\text{-}1350\text{ cm}^{-1}$ ) band analysis. The OH-stretching vibration region ( $3500\text{-}3300\text{ cm}^{-1}$ ) provides information on the properties of water structured around the protein at different hydration degree. The absorption region between  $1000\text{-}1100\text{ cm}^{-1}$  monitors the collagen glycation reaction.

### SEM MICROSCOPY OF MUMMIFIED SKIN

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The skin is a tissue composed of different layers. Each of them carries out a specific function. Epidermis, mainly constituted of keratinized cells, guarantees protection from dehydration and pathogen agents whilst dermis, composed in particular of collagen fibers provides resistance to tensile strength and compression. Analytical technique were more often applied to archaeological remains to characterize the constituting materials and their state of preservation. The knowledge of the modification mechanisms of the skin during time represents a fundamental condition for setting defined protocols of museum conservation of a particular class of archaeological remains: Mummies. The skin of some Egyptian mummies, natural and embalmed, from the collection of the Museum of Anthropology

and Ethnography G. Marro of the University of Turin and of some natural mummified remains from Roccapelago were analyzed by means of Scanning Electron Microscope. The samples differ for their nature, origin and dating. The powerful technique used enabled us to identify the main constituting structures as stiffened and curled collagen fibers and the lacking and/or the transformation of the different layers of the skin and its preservation state. In addition, traces of the embalming materials and fungal infestations were observed.

## PALERMO SIBS BRANCH

### 3,5-DIODO-L-THYRONINE-INDUCED MODIFICATION IN PITUITARY-THYROID AXIS IN RATS FED HIGH-FAT DIET. A PRELIMINARY REPORT

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Experimental observations highlight that the administration of 3,5-diodo-L-tyronine (T2) may decrease the body weight and the plasma levels of cholesterol and triglycerides and may prevent the onset of hepatic steatosis in rats fed diets rich in lipids (HFD). On the basis of these findings we have carried out some *in vivo* studies to evaluate the effects of increased levels of T2 on pituitary thyroid axis function in HFD rats. Fifteen Wistar male rats were divided in 3 groups. The first group (N) was fed with a standard diet. The second group (G) was fed with a diet high in fat (HDF), while the third group (GT2) was additionally administered intraperitoneally with T2 (70 µg/100g body weight) for 3 days a week up to 4 week. Blood samples from animals were collected and stored at -20°C until 3<sup>rd</sup> generation and TSH, T3, T4, ACTH, triglycerides, cholesterol, glucose ALT, AST, Alkaline Phosphatase were assayed. Furthermore, rat liver from rats underwent histological examination to assess the degree of steatosis. The administration of T2 (70 µg/100 gr body weight 3 times a week up to 4 weeks suppressed TSH secretion in HDF rats. Unlike observed in the liver of rats from group N and group GT2, the histological examination of the liver from G group rats showed the presence of hepatic steatosis. These preliminary data highlight that the administration of 70 µg/100 b.w. of T2 inhibits TSH secretion and prevent the onset of hepatic steatosis in HFD rats.

### BINGE DRINKING: CONSUMPTION PRACTICES AMONG YOUNG PEOPLE. ANALYSIS OF THE QUESTIONNAIRES COMPILED BY STUDENTS OF PALERMO CITY AND PROVINCE INVOLVED IN THE PREVENTION AND INFORMATION PROJECT

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The term *binge drinking* is used in the northern countries of

Europe, to point out the consumption of great intoxicating quantities of alcohol, until feel bad, for the pure and simple desire to get drunk. Conventionally, binge drinking is the consumption of at least six glasses of alcoholic drinks, different ones as well, in a single occasion and one after the other. A survey has been conducted through administration of questionnaires with the purpose to have an idea about the territorial diffusion of such practice among the students of Palermo and province. In the months of April and May 2014, this survey affected 30 classes of different schools in 10 cities and province, involving about 740 students. The number of the students was inferable from the questionnaires compiled before the formative action. The sample results representative enough among teenagers (14/18 y.o.), and the female part is predominant. 36% declared to spend Saturday night in pubs, while 18% in discotheques. 25% of consulted people replied to connect the time spent in pubs to alcohol. Other important factors are: 36% abuse of alcohol just in the week-end (factor confirming the alarming Binge practice); 30% declare to get in the car with a bit drunk driver (13% with a totally drunk driver). 65% declare to have an adequate knowledge of potential alcohol effects. The question about motivations convincing a teenager to take drugs gave the following result: 40% desire of transgression; 30% desire to conform with the group; 23% the research of an easy wellness. This data convince more and more to understand the importance of the prevention and information role. All this to avoid that trends and lifestyles sacrifice teenagers for the enormous economic interests staying behind the sale of alcohol, and prepare as society more and more a salve of consumption and abuses even lethal such as alcohol.

### MONITORING AND USE OF ANTIMYCOTICS FOR SYSTEMIC USE SUPPLIED BY THE STATE HOSPITAL PHARMACY OF MARSALA (TP), ITALY: MYCAMINE® (MICA FUNGIN)

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Micafungin is an antimycotic drug and represents an important addition to the available therapies for the treatment of systemic fungal infections. Micafungin is used in the treatment of invasive candidiasis, oesophageal and prophylaxis of *Candida* infections. It inhibits, in a non-competitive way, the synthesis of 1,3-β-D-glucan, a component of fungal cell wall and is rapidly distributed into the tissues. It has a high-rate bond with respect to plasma protein; the above mentioned bond is independent from the concentration of the drug. It is metabolized through the liver, being not subject to intense metabolic transformations until the excretion. There is no evidence of systemic accumulation after repeated use and the steady-state is reached in 4-5 days. Medical records examined at the State Hospital Pharmacy of Marsala, highlights that, from 01.06.2014 to 01.08.2014, in this hospital 12 vials were used by the patients hospitalized in the department of Intensive Care: 8 patients are aged between 75 and 83 and have a body weight higher than 40 kg; 3 patients are aged between 40 and 60 and have a body weight higher than 40 kg and 1 patient has an age equal to 17 years and has a body weight equal to 40 kg. Two patients need a dose increase, for the others 10 patients the first dose resulted sufficient. Mycamine was used for the treatment of hypovolemic post-operative shock. The most frequently recorded adverse

reactions were anaemia, hypokalaemia, hypomagnesaemia, phlebitis, nausea, liver problems. Given the different weight of the subjects, the dosage was different.

### MONITORING OF ATYPICAL ANTIPSYCHOTIC DRUGS SUPPLIED BY THE PHARMACY OF FRATELLI PARLAPIANO HOSPITAL, RIBERA (AG), ITALY

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Mental disorders are among the worst and widespread health problems, they are associated with important repercussions on the psychosocial sphere and to high economic costs. Antipsychotic drugs used in treatments of these pathologies belong to different chemical classes, but associated to biochemical effects, pharmacological, and in part super imposable. A retrospective survey has been led on external patients, they were under antipsychotic drug treatments already in the archive and arrived to the attention of the Pharmacy of the F.lli Parlapiano Hospital, Ribera (AG), Italy in 2013. For each patient have been extrapolated: gender, date of birth, place of residence, ICD-9CM, pharmacological therapy, and prescribing centre. Collected data have been calculated through Microsoft Office Excel to notice: prevalent diagnosis; prescribed drugs; prevalence by age group; gender, and off label prescribing in patients suffering from dementia. This survey is composed of 535 patients: 463 suffering from mental disorders and 72 suffering from degenerative diseases of SNC (especially Alzheimer). The distribution of psychic and neurological disorders in the two genders is balanced and the average age is 61.55. 64.7% live in the province of Agrigento and are treated at CSM of Ribera. 434 patients suffer from non organic psychosis; 14 patients suffer from organic psychosis; 70 patients suffer from a moderate form of Alzheimer, 2 suffer from other forms of dementia, while for 25 patients no diagnosis has been noticed as the medical prescription had been done by prescription SSN. Prescribed drugs have shown the following percentages: olanzapine 33.04%; quetiapine 33.19%, risperidone 17.97%; aripiprazole 7.4%; clozapine 4.02%; asenapine 1.69%; paliperidone 1.27%, ziprasidone 0.42%. It is a common opinion in the international scientific literature that a wise use of antipsychotic drugs of second generation gives important advantages in long term treatment of psychotic disorders.

### BIOMARKERS OF OXIDATIVE STRESS AS AN EARLY WARNING SYSTEM TO DETECT *IN VITRO* XENOBIOTICS' TOXICITY: EFFECTS OF SUB LETHAL DOSES OF BIS (2-CHLORO-1-METHYLETHYL) ETHER IN CELL CULTURE

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Bis (2-chloro-1-methylethyl) ether (BCMEE) is a  $\beta$ -haloether,

produced as a by-product from the manufacture of propylene glycol and oxide. It has been demonstrated that BCMEE can induce liver cancer in male mice and lung cancer in female mice. Furthermore, supporting the concern regarding the carcinogenicity of BCMEE, haloethers with structural homology to BCMEE have shown evidences of carcinogenicity. The aim of this work was to determine the effects of sub lethal doses of BCMEE on cell lines, focusing on oxidative stress markers, due to the well know crosslink between xenobiotics exposure, oxidative stress and chronic diseases, such as cancer. Fibroblasts and hepatoma cell line (HS-68, HEP-G2), were exposed to increased concentration of BCMEE (1.5-150  $\mu$ g/100 mL) for 72 h. Toxicity was assessed by MTT test while oxidative stress was assessed by measuring the MDA (Malondialdehyde) levels and by the detection of intracellular ROS. The results show that, on both cell lines, BCMEE induces a significant vitality reduction, up to the 85%, in a dose-dependent manner in respect of the control ( $P < 0.05$ ); a significant increment of ROS (200 vs 50 relative fluorescence) and MDA (0.5 vs 1.5  $\mu$ g/g T.P.) was also detected compared to the control ( $P < 0.05$ ), that positively correlate with vitality reduction. In general, the overall results show that, in cellular system, BCMEE at sub-lethal levels can generate oxidative stress, confirmed by the increase of ROS and MDA. Considering the link between ROS and chronic diseases, such as cancer, BCMEE can represent a significant potential for environmental contamination.

### SALINITY STRESS RESPONSE IN THREE SPECIES OF SPARIDAE CANDIDATE FOR ORGANIC AQUACULTURE

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*Diplodus puntazzo*, *D. vulgaris* and *D. sargus* are three fish species common in the Mediterranean and already used in polyculture; nowadays these species are considered under a biological aquaculture perspective. In this study the response to sudden salinity variations was evaluated, in order to assess their feasibility to grow, under extensive conditions, in salt-works. The markers assessed cover both primary and secondary biomarkers of stress response and biochemical stress markers related to quality of the products. For each species, fishes were distributed into three tanks: control (37‰ salinity), salinity stress (50‰ salinity) for 3 h (50-3), salinity stress for 6 h (50-6). The cortisol and glucose, among the hematic parameters, showed a significant increase in stressed organisms, depending from both the exposure time and the salinity level. In the muscle, the lactate showed significant differences ( $P < 0.05$ ) in all the species, confirmed by the levels of Hsp70 protein, depending from the exposure period, although the highest levels were found in *D. puntazzo*. The water muscular percentage content did not showed significant variations, suggesting the ability to modulate the ionic balance under the tested conditions. Overall, the results indicate that according to the survival and the biochemical responses, the three species evidenced a certain grade of adaptation to transitory salinity variations.

## REGGIO CALABRIA SIBS BRANCH

### EVALUATION OF THE SENSITIVITY OF DIFFERENT KITS USED IN ANCA'S DETERMINATION

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Small and medium vessel vasculitis are associated with the presence of anti-neutrophil cytoplasmic antibodies (ANCA) in sera of patients affected by these immuno disorders. ANCA antigen targets have been described as a range of proteases, found in neutrophils, including PR3, MPO, neutrophil elastase (NE), cathepsin G, BPI, lysozyme and lactoferrin. The combination of indirect immunofluorescence (IIF) analysis with enzyme linked immunosorbent assays (ELISAs) is internationally recommended for ANCA detection. However the screening of ANCA remains a challenge for the scientific community because of the presence of highly homologous sequences within the ANCA antigens and the lack of standard methods. This study aimed to evaluate the performances of commercially available assays (IIF, ELISA, immuno-dot assays) for ANCA detection in terms of specificity and sensitivity. Sera of 60 patients were tested for ANCA by IIF and ELISA. The results obtained, both IIF and ELISA methods, suggesting that both methods are good for approximately 50 samples; however a great variation has been observed for 10 samples tested by four ELISA kits, two IIF and one immuno-dot assay. Based on this evaluation, we can confirm that the specificity and sensitivity of commercially available kits for the detection of ANCA vary considerably. The major challenges for academia and industry remain the developments of i) antigen-specific assays with high sensitivity, and ii) internationally validated calibrators. Currently, it is recommended to test the same clinical sample with various methods and kits produced by different manufacturers to address these issues.

### EVALUATION OF DIAGNOSTIC EFFICACY OF A NEW TEST, ZNT8, USED IN THE SCREENING OF DIABETES MELLITUS 1

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Diabetes Mellitus is a chronic disease associated with an increase in mortality rate all over in the world. The identification of risk factors and the early detection of the pathology have the aim to develop effective strategies to prevent or delay the clinical onset. Diabetes Mellitus type 1 (DM1) is a form of diabetic disease of autoimmune aetiology with increasing incidence rates. The objective of this study was to evaluate the efficacy of an innovative method of investigation, aimed at finding ZnT8 autoantibodies, in combination with other diagnostics tests [Glutamic Acid Decarboxylase (GAD-IA2) and Islet Cell Antibodies (ICA)] used in the screening of DM1. The anti-ZnT8 are autoantibodies directed against the C-terminal portion of isoform 8 of the zinc transporter (ZnT8). ZnT8 autoantibodies, anti-GAD-IA2 and anti-ICA were measured in the sera of 40 patients 17 of them aged between 6 and 20 years. The detection was carried out through ELISA

(enzyme-linked immunosorbent assay) and IIF (indirect immunofluorescence) using kits provided by various companies. The results obtained showed that the ZnT8 is present in 45% of sera tested; anti-ZnT8 were found in combination with anti-GAD-IA2 in 60% of samples and samples with high levels of GAD-IA2 exhibited similar high levels of ZnT8. Based on these data, it was established that the combination of ZnT8, GAD-IA2 and ICA leads to an increase in the diagnostic sensitivity and specificity; in addition, the ZnT8 is a test which can replace tests such as ICA without loss of sensitivity and specificity, and represents a reliable diagnostic tool for the autoimmune diabetic pathology.

## SASSARI SIBS BRANCH

### FEMORAL BONE MICROMORPHOLOGY: A COMPARATIVE STUDY BETWEEN WILD (OVIS ARIES MUSIMON) AND DOMESTIC SHEEP (OVIS ARIES ARIES)

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The mouflon (*Ovis aries musimon*) and the sheep (*Ovis aries aries*) are the wild and domestic subspecies of the same species. In spite of some different phenotypic features, such as coat color and horn morphology, their skeletons are very similar, so distinguishing bones between these two subspecies is not easy. In this work, mouflon bone micromorphology was studied for the first time and compared to the domestic subspecies, in order to highlight the role played by the lifestyle on bone with special regard to secondary osteon morphology and morphometry. Area, perimeter, minimum and maximum diameter of more than 200 secondary osteons and Haversian canals were measured in cross sections of four adult femurs from each subspecies. Qualitative investigation of bone histology revealed plexiform and irregular Haversian tissue in both subspecies, as previously reported for Ruminants. In addition, the mouflon showed the presence of several secondary osteons clustered in small groups in many regions, which may be consistent with the definition of dense Haversian tissue. Quantitative analysis demonstrated that mouflon secondary osteons are larger than in the sheep and made of a higher number of lamellae (5-7). The wide areas characterized by dense Haversian tissue frequently found in mouflons, as well as the larger dimensions of secondary osteons may be consequent to the habits of wild life typical of that subspecies.

## TURIN SIBS BRANCH

### VERTEBRAL MORPHOMETRY FOR STATURE ESTIMATION IN FORENSIC ANTHROPOLOGY BASED ON DEXA IMAGING: A PRELIMINARY REPORT

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Incomplete remains are a common issue in forensic investigation. Identification of these remains suggests to perform the anthropological profile, assessing attributes as sex, age,

ethnicity and stature from bones. If long bones, commonly used for stature estimation, are fragmented or missing, the measurements made on the vertebral column are considered reliable. On this purpose, a method based on a population similar to the remains recovered is required. In this preliminary study we measured heights of the vertebral bodies in a Caucasian Italian population, evaluated by images of dual energy X-ray absorptiometry (DEXA) morphometry in the living investigated in diagnostic routine. DEXA utilises two X-ray beams with different energy levels for calculating the different absorptions of soft tissues and bone and it is considered to be

the most reliable densitometric technique as for spatial resolution, precision, and accuracy. It is also a quantitative method to identify osteoporotic vertebral fractures and anatomical anomalies, based on the measurement of vertebral bodies. In this preliminary study, thoracic and lumbar segments of the spine were measured in 209 females (mean age: 65.5 years; mean stature: 155.8 cm) and statistical analysis was performed, obtaining regression formulae for estimated living stature from T6-T12, L1-L4 and T6-L4 spinal segments. The range of standard deviation of the calculated stature varied from 5.32 to 5.7.

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