92st SIBS Experimental Biology in the time and the space.
Research and scientific truth

Sassari, Italy, 2-4 December 2019
Journal of Biological Research
Bollettino della Società Italiana di Biologia Sperimentale

eISSN 2284-0230

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CHARACTERISTICS OF THE CELL MEMBRANE AND ION CHANNELS

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Biological membranes separate thermodynamically open systems in a highly selective way. ‘Selectivity’ in this view is provided by the regulation of permeation of molecules and atoms, binding of external ligands to membrane constituents and providing a large range of intra-membrane molecular transitions. The ‘Fluid-Mosaic Model’ of Singer and Nicholson (1972) has dominated the picture of processes manifested vertically across membranes for almost half a century. During the last decade however, the importance of a high level of lateral segregation and heterogeneity of membrane constituents, particularly the role of lipid composition and dynamics has gained an important role. Today the control of permeation across membranes regulated by integral membrane proteins can only be seen in a tight interplay with the highly dynamic components provided by the lipid bilayer. It has been found for example that principles behind lipid-protein interactions within the ‘signaling membrane’ of brain cells extend far into major functions of the brain such as homeostasis and psychological aspects including psychiatric conditions such as fear and depression [1]. Here our focus will be on the delicate interaction between voltage-sensitive channel proteins and lateral membrane effects exerted through polyunsaturated fatty acids (PUFAs) which seem to be play a key role regulating ion permeation through both, lipoelectric and lateral-pressure action on proteins (as recently reviewed by [2]). Beside their extensive work on a comparative view involving different animal models [3], Cocchi et al. have suggested a ‘symmetry breaking’ model in which low linoleic acid concentrations might operate at a boundary between ‘phase-transitions’ involving larger membrane patches which in turn can affect the gating and permeation state of ion channels [4]. In a recent pico-second time resolved molecular dynamics (MD) study of ion-states in the selectivity filter of Kv channels, we have found an ‘evolutionary optimized’ configuration with a very high sensitivity to perturbations from membrane components [5]. Together with the symmetry-breaking model of linoleic acid acting as a control parameter, these findings develop into a highly integrated view on membrane signaling in the brain with far reaching behavioral and psychological consequences.

REFERENCES


THE ROLE OF HUMAN ANATOMY IN THE BATTLE AGAINST PSEUDOSCIENCE

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Over the past few years there have been various instances in which newspapers and tabloids aggrandize the clinical applicability of some results published on questionable themes in scientific Journals considered reputable. Here I discuss three examples: 1) the definition of the interstitium as an organ; 2) the existence of lymphatics in the nervous system; 3) the presence of “mysterious channels” between the brain and the skull. Inaccurate information force mistaken conclusions and hopes. Indeed, this type of information can generate false expectations in the population other than causing confusion among students, physicians, and scientists who may be misdirected in their search of new knowledge. I also review some basic morpho-functional concepts related to these topics and underline the fact that making disproportionate and sensationalized claims on biomedical research findings may mislead patients (and their relatives) suffering from severe diseases and, also, create a climate of mistrust towards science in the general population.

CLIMATE CHANGE AND EXPERIMENTAL MARINE ECOLOGY

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A comprehensive understanding of how anthropogenic climate change is affecting the marine ecosystems is centrally important to the biology of the planet, yet efforts have been poorly developed. Recent studies indicate that rapidly rising greenhouse gas concentrations are driving ocean systems toward conditions that might drive fundamental and irreversible ecological transformation. The impacts of anthropogenic climate change so far include decreased ocean productivity, altered food web dynamics, reduced abundance of habitat-forming species, shifting species distributions, and a greater incidence of disease. In this scenario marine ecologists have an incredibly high responsibility to gain information to forecasting marine habitat distribution, tolerance of protected species to climate variation and the role of key stone species in the altered ecosystems. Examples of field and laboratory experiments on the effect of factors related to climate change on marine communities will be provided. Particular emphasis will be given to investigations on the impact of climate change by using multiple stressors experiments such as eutrophication, sedimentation and warming.

LINOLEIC ACID, CHOLESTEROL AND CELL MEMBRANE

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Some research studies have shown that there is a common
characteristic in ischemic cardiovascular disease and mood disorders: a reduction of Linoleic Acid in the platelet membranes (Cocchi, & Tonello, 2010; Cocchi et al. 2004; Cocchi, Gabrielli, & Tonello, 2013; Benedetti et al. 2014). This evidence, which significantly influences the adjustment of the mobility of the membrane, and that is mainly due to the characteristics of Linoleic Acid’s melting point, leads to reflection on the relationship between Linoleic Acid and Cholesterol, since the latter is a decisive element in the expression of membrane mobility. Given its greater molecular size among all other fatty acids, Linoleic Acid is the one that occupies the greatest space within the membrane lipid bilayer, and since the increasing number of cholesterol molecules within the membrane stabilizes the serotonin receptors, the same could apply to Linoleic Acid when it decreases. This is another possible hypothesis regarding the cooperation between cholesterol and Linoleic Acid (Cocchi, et al. 2015). In this regard, we should remember that cholesterol protects Linoleic Acid from membrane oxidation. An example (Mazari, Iwamoto, & Yamauchi, 2015) is shown in the phosphatidylcholine fraction, since it is one of the richest in Linoleic Acid among the various biological regions of the body. Another interesting form of interaction between cholesterol and Linoleic Acid regards the activation mechanism of endothelial cells triggered by Linoleic Acid (Lee et al. 2001). Also in this case, adequate amounts of cholesterol have the ability to control the effects of Linoleic Acid in endothelial cells through a mechanism of protection from oxidative and pro-inflammatory effects. It seems that this mechanism, which functions through signaling pathways that causes the activation of endothelial cells, is due to the amount of cholesterol. A moderate increase of cholesterol, therefore, appears to be beneficial. The low concentration of Linoleic Acid recorded in platelets and the deep involvement of these cells in coronary heart disease (Cocchi et al. 2004; Cocchi, Gabrielli, & Tonello, 2013) could mean, surprisingly, not that the low concentration of Linoleic Acid is an accidental marker of the phenomenon, but that, instead, it is a defense mechanism created by the body to reduce the activation phenomenon of endothelial cells by Linoleic Acid. Recently, another relationship between Linoleic Acid and cholesterol (HDL) has been demonstrated (Asselin et al. 2014) regarding the strong connection between the production of lipid peroxidation, 4HNE (4-hydroxynonenal), its potential precursor, i.e. Linoleic Acid, and HDL cholesterol. It seems that 4HNE increases proportionally with a reduction of HDL. These observations tend to show that the organism has a specific need to maintain a balance between cholesterol and membrane fatty acids where these two elements are critical for membrane mobility, the Gs protein, the cytoskeleton and the ion channels’ flow (Cocchi, Tonello, & Gabrielli, 2012; Tonello, & Cocchi, 2010; Cocchi, Tonello, & Rasenick, 2010).

REFERENCES

FROM GENES TO MECHANISMS TO THERAPEUTIC OPPORTUNITIES: THE SPECIAL CASE OF SARDINIA
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How complex traits are genetically regulated, what are the functional consequences of such regulation and how this information can be used to design new drugs represent central questions in human genetics, biology and translational medicine. The population of the Mediterranean island of Sardinia is particularly well suited to address these questions, as is witnessed by a series of successes in the mapping of complex traits and diseases. With this in mind, we have undertaken 4 integrated initiatives in the genetically isolated Sardinian population: (i) whole genome sequencing of >3,500 individuals yielded over 26,000,000 genetic variants that, through imputation methods, are interrogated in powerful genome-wide-genome association studies (GWASs) testing much larger samples of genotyped people with high-coverage array; (ii) sequencing of polyadenylated RNA from >1,000 individuals identified more than 30,000 sites that influence gene expression that provide a key resource for establishing the causal genes underlying the GWAS association signals; (iii) we assembled case-control sample sets of ~4,000 Sardinian multiple sclerosis cases, ~2,000 diabetes Type 1 cases, and ~4,000 controls, that allowed us to identify new autoimmune disease genes and variants; (iv) comprehensive profiling of circulating levels of different immune cells and soluble molecules in up to 7,000 general population volunteers and identification of numerous variants that regulate levels of these immune phenotypes and influence autoimmune disease risk. Overall, the findings generated through these initiatives highlight “experiments of nature” that point to disease mechanisms and new therapeutic targets that can be modulated therapeutically.

THE IMMUNE LANDSCAPE OF MYELODYSPLASTIC SYNDROMES
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Myelodysplastic syndromes (MDS) are a heterogeneous...
group of clonal hematologic diseases, characterized by dysplastic haemapoiesis and by a variable degree of peripheral cytopenia. Although several studies have highlighted the pivotal role of specific genetic lesions involving the stem cell compartment, clinical and laboratory findings suggest that different immune pathways could be deeply implicated in their pathogenesis. From the clinical point of view, a variety of autoimmune manifestations are reported in a large fraction of MDS patients. Moreover some patients are known to respond to immunosuppressive treatments such as for instance antithymocyte globulin (ATG). Noteworthy, aplastic anaemia, whose pathogenesis is driven by immune-mediated mechanisms, can evolve into a typical MDS with specific genetic lesions such as trisomy 8 or monosomy 7. Moving to the laboratory setting, several cell subpopulations, more often within the T-cell compartment, display abnormal distributions in MDS patients. Finally, both in vitro and in vivo findings clearly suggest that patient T-cells can be responsible of the functional inhibition of hematopoietic precursors.

EXTREMOPHILE MICROALGAE FOR BIOTECHNOLOGICAL APPLICATIONS

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To date, the attention to extreme environments and the microorganisms that live in them has increased because of the possible biotechnological applications. In this framework, the cultivation of extremophilic microorganisms has gained increasing interest also due to their ability to accumulate and produce high-value compounds. The extremophile microalga Coccomyxa melkonianii SCCA 04B was isolated from a river severely polluted by Neutral Mine Drainage, and its ability to adapt to a wide range of acidic and alkaline pH values was studied. Additionally, Coccomyxa melkonianii showed the capability of accumulating high amounts of lipids, particularly rich in palmitic, stearic, oleic, and linoleic fatty acids. For this reason, the strain has been selected together with other algae from the Sardinian Culture Collection of Algae (SCCA) for the COMISAR project. One of the main objectives of this project involves the optimization of high-value product extraction technology from sardinian microalgae. Microalgae, especially the extremophile ones, show enormous potential for a variety of applications in several strategic markets such as the nutraceutical, cosmetic and agribusiness sectors. The financial support of the COMISAR project (POR FESR 2014/2020 - Asse prioritario I “ricerca scientifica, sviluppo tecnologico e innovazione” Regione Autonoma della Sardegna, Italy) is gratefully acknowledged.

BIOARCHAEOLOGY OF URBAN AND RURAL SOCIETIES IN THE NORTH OF SARDINIA BETWEEN THE FOURTEENTH AND THE SEVENTEENTH CENTURIES

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The bioarchaeological analysis of human osteological remains from some urban and rural contexts, in the north of Sardinia, allowed to reconstruct the demographic trend, lifestyle, the possible subsistence activities and the pathogenesis of these samples, highlighting the differences between the two different types of contexts from the morphometric and paleopathological point of view and the ergonomic and entheseal similarities, although with considerable gender differences. These differences are probably related to the different context of relevance: skeletons from an agro-pastoral context allow marked enthesis, report traumas and are affected by inflammatory events localized mainly in the lower limbs, instead skeletons from urban sites are influenced pathologically, with an easier osteological and odontological diffusion of pathologies due to the high population density compared to a rural village.

DECIPHERING MECHANICAL AND ELECTROMAGNETIC PATTERNS FROM STEM CELL DYNAMICS: A NOVEL APPROACH TO REGENERATIVE MEDICINE

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Rhythmic oscillatory patterns sustain cellular dynamics driving the concerted action of regulatory molecules, microtubuli and molecular motors. We aim at disclosing novel perspectives in stem cell science, using physical energies, including electromagnetic fields and nanomechanical vibrations to govern (stem) cell fate. We will discuss our recent findings showing that properly conveyed radioelectric fields are able to: (i) enhance the differentiating potential of mouse embryonic stem cells, (ii) induce pluripotency in human adult stem cells, promoting their differentiation into cardiac, neural, skeletal muscle and endothelial cells, (iii) afford direct reprogramming towards the same lineages in human somatic cells (dermal fibroblasts), (iv) reverse human stem cell aging in vitro, (v) reprogram PC12 cancer cells into dopaminergic neurons, and (vi) optimize stem cell polarity. We will also focus on the use of atomic force microscopy (AFM) and hyperspectral imaging (HSI) to detect nanomotions generated by human adult mesenchymal stem cells (hMSCs) and induced pluripotent stem cells (iPS) during their growth and differentiation. By the aid of AFM and HSI, we are going to decipher vibrational signatures of cell commitment along multiple fates. We are currently developing “multifrequency mechanical transducers” to deliver such signatures to undifferentiated cells with the aim of promoting cell survival or targeted differentiation (i.e. cardiogenesis). Overall, we envision the use of physical tissue stimulation to target our stem cells in situ, boosting their rescuing potential without the needs for cell transplantation.
ORAL COMMUNICATIONS

THE GREEN MUMMY OF BOLOGNA: A MULTIDISCIPLINARY APPROACH TO THE STUDY OF MUMMIFICATION PROCESS

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The object of this study is the so-called “green mummy” of Bologna, a naturally mummified body that was found in the basement of an ancient mansion in Bologna in the twenties of last century. The body was found in a copper or bronze cist and for this reason both the skeletal and soft tissues are green colored. The purpose of this study was to know the degradation process of the body by means a multidisciplinary approach and to determine the tissue preservation degree. Several chemical and physical techniques such as FTIR spectroscopy, Raman spectroscopy, gas chromatography-mass spectrometry and microbiological analyses were used to analyze proteins, lipids and carbohydrates that form tissues with the aim to gain information about the biochemical degradation process. Histological analyses of soft tissues, carried out to determine their preservation degree, revealed that they are fairly preserved. FTIR analysis of hard and soft tissue, both green colored and non-colored, showed that the tissues lacking the green patina were better preserved their protein structure being minimally deteriorated, contrarily to what was observed in the green areas. Probably, the acidic liquids originating by the body decomposition caused the container corrosion, allowing the release of copper ions. It was hypothesized that copper ions might have caused the decarboxylation of the RCOO group of polypeptidic backbone favoring protein degradation. Therefore, copper did not favor the mummification process which was probably due to the environmental conditions, such as the low temperatures typical of the basement and the low availability of oxygen.

OSTEONEOCIC POTENTIAL OF ENGINEERED NANOMATERIALS UNDER MICROGRAVITY SIMULATION

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The potential application of nanomaterials could contribute significantly to the field of biology and nanomedicine. More recent decades have attended the development of large varieties of engineered nanomaterials in order to provide the osteoblastic/osteoclastic regeneration in bone loss human diseases. Osteoporosis is one of the major age-related disease revealing fracture and bone dysfunction that limit the quality of human life. Since osteoporosis diagnosis and treatment is a worldwide health concern, the use of the nanomaterial to improve the osteogenic differentiation could be very important. It is well known that microgravity (MG) induces bone loss in terms of a mineral density decrease about 2% in 30 days. Similar decrease occurs also in women affected by osteoporosis at least 12 months. In this context, we proposed the evaluation of osteogenic differentiation using nanomaterials under microgravity simulation. To evaluate the MG effects, the osteogenic potential of nanomaterials and their possible toxicity effect were evaluated using in parallel the Random Positioning Machine (RPM) to simulate microgravity condition (M, 0 x g). The mechanism of microgravity action on osteogenesis inhibition are still unclear and need to be clarified. However, nanomaterials have demonstrated their key osteogenic role providing an efficient osteogenic differentiation related to a bone formation. These results could contribute to investigate the osteogenic capabilities of several nanomaterials under MG condition, and how they can help to counteract the bone loss resorption and weightlessness, after space flight missions and human bone loss disease.

FROM NATURE TO MAN: ENVIRONMENTAL ANTHROPOLOGY IN THE ANTHROPOCENE

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The dramatic changes brought by the relationships between humans and their natural environments by different human activities such as the exploitation of natural resources and use of fossil fuels threatens humanity at large. Beside considerable disagreements on when Anthropocene began, it is considered an epochal transformation linked to deterioration of global ecologies; loss of biodiversity and environmental degradation. Environmental anthropologists are contributing both theoretical and by important ethnographic insights in analyzing and understanding the consequences of climate changes in socio-cultural systems worldwide. This article explores main theoretical contributions during the development of environmental anthropology as a discipline and the need to integrate Extended Evolutionary Synthesis (EES), how it can be deployed to stimulate research in climate changes consequences for a socio-ecological system.

SKIN STEM CELLS: ISOLATION , CHARACTERIZATION AND CULTURING CONDITIONS

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Skin provides the largest protective barrier for the organism from the environmental injuries. This tissue consists of two layers comprising different cells populations. Stem cells reside in a specific region called ‘niche’ in an undifferentiated state, until a specific stimulation induce their differentiation to maintain tissue homeostasis. This ability is influenced by cell senescence, being progressively slower along with age. Optimizing a method for the isolation of stem cells from skin, also preserving their features, as pluripotency, represent an important goal in regenerative medicine. Here we define novel experimental conditions to isolate and maintain stem cells from human skin. Skin biopsies of male and female patients were mechanically digested, and the resulting fragments were then left in culture. Cells migrating from the tissue were expanded, magnetically sorted, and then characterize by flow-cytometry. The preservation of the mesenchymal phenotype after culture and freezing was demonstrated by the capability of the cell to acquire an adipogenic and osteogenic phenotype under stimulation with specific culturing media. Culturing cells in the novel medium optimized specific features as cell growth, viability, and metabolic activity, as compared to culturing cells in a basic commonly used medium. Our results disclose a novel method for a finest isolation, expansion and culturing of mesenchymal stem cells from skin, unravelling an innovative tool for tissue regeneration and clinical applications.

INFRARED SPECTROSCOPY FOR THE STUDY OF THE BIOMOLECULES OF THE PAST

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The study of ancient and extant biomolecules has considerably improved the archaeological research in the last 20 years. Technological innovation combined with the study of the structural properties of ancient biological macromolecules has increased the reliability of the analysis in the archeological and physical anthropological fields and it has helped the interpretation and the understanding of molecular surviving in fossilized and mumified remains. Among the physical methodologies developed and tuned for the application in the field of biomolecular archaeology, Fourier Transform Infrared Spectroscopy (FTIR) represents a technique of excellence thanks to its minimally invasive character on the precious specimens to which it is addressed. Here we report the outline of the technique and the details of the preparation procedures for many types of samples. Some examples were extracted from the researches carried out in the recent years. Specimens were taken from a predynastic mummy (3680-3350 BC) and 10 embalmed heads (VI-XI dynasty) belonging to the Egyptian collection of the Museum of Ethnography and Anthropology of the University of Turin (Italy). The technique was applied also to the study of a case of a natural mumification, occurred in XVI-XVIII centuries, concerning a group of mumified bodies discovered in 2010 at Roccapelago (Modena, Italy). These and other examples highlight how FTIR can be considered a valuable analytical technique able to add new information to historical and archaeological knowledge and to contribute to the evaluation of the biomolecular deterioration state of the tissues, representing a powerful tool for conservation scientists and others engaged in the preservation of the cultural heritage.

CHARACTERIZATION OF VAPB AGGREGATES IN PBMC OF ALS PATIENTS

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A point mutation (P56S) in the gene encoding VAPB (vesicle-associated membrane protein-associated protein B), an endoplasmic reticulum (ER)-integrated membrane protein, leads to autosomal-dominant form of Amyotrophic Lateral Sclerosis (ALS) classified as ALS-8. VAPB-P56S forms ER-associated aggregates, leading to a complete reorganisation of ER structures, activation of cellular stress and finally to neurodegeneration. Numerous studies demonstrated VAPB involvement in sporadic ALS (sALS), however exact molecular mechanisms of pathogenesis are still lacking. ALS-8 is not so frequent and patients’ biopsies are not frequently available, thus careful analysis of PBMC could represent a good non-invasive option to study ALS. We isolated PBMC from Sardinian ALS patients, Parkinson’s disease (PD) patients and healthy control subjects (HC) to evaluate the VAPB expression through flow cytometry assay and its localisation by Immunofluorescence analysis. Immunofluorescence assay evidenced a specific ER alterations associated with VAPB in all ALS patients analysed. Furthermore flow cytometry analysis showed a reduction of VAPB expression in ALS patients compared to PD and HC. The data obtained seems to support the hypothesis that VAPB could represent a possible biomarker useful to ALS diagnosis and suggest that VAPB could be involved in ALS pathogenesis. Moreover the observation performed on PBMC revealed that these cells are a valid cellular model useful in ALS research.

THE INFLUENCE OF DISEASE COURSE AND GENDER ON METABOLIC RATES AND OXYGEN COST OF WALKING: A COMPARATIVE STUDY OF PEOPLE WITH MULTIPLE SCLEROSIS AND HEALTHY SUBJECTS

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Oxygen cost of walking (O2 Cw) and oxygen rate of consumption (VO2 rate) are increasingly employed to quantify walking impairment and gait-related fatigue in people with multiple sclerosis (PwMS). These metabolic indicators are elevated in PwMS with high and moderate disability, however data are more controversial in mildly disabled patients; moreover, gender differences regarding these metabolic aspects are still uncovered in MS. In a translational perspective, the aims of this research were to: 1) investigate differences in O2 Cw and VO2 rate depending on the MS course; 2) highlight gender-based differences within the experimental groups. To this end, we examined the respiratory exchange ratio, O2 Cw and VO2 rate during over-ground walking at comfortable and fast velocities in mildly disabled (EDSS ≤ 2) patients with different MS disease courses (relapsing remitting, RR; clinically isolated syndrome, CIS) and in matched, healthy control subjects. Results revealed that male patients with RRMS displayed significantly higher O2 cost of walking and metabolic rates than
DEEPEN THE KNOWLEDGE ABOUT SPIRAL VALVE AND ASSOCIATED LYMPHOID TISSUE (GALT) OF THE SMALL-SPOTTED CATSHARK SCYLIORHINUS CANICULA (LINNAEUS, 1758)

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Spiral valve intestine is a common feature of several fish species belonging to both Chondrichthyes and Osteichthyes classes. All the three subclasses of Chondrichthyes present this spirilized intestine. Spiral valve intestine is a morpho-functional adaptation that allows both (i) increasing intestinal absorption surface maintaining a small size and (ii) increase the permanence time of food within the gut. Being the gastrointestinal tract a potential pathogen invading way, the intestinal mucosa needs an high efficiency immunological surveillance. The present study had a double purpose and tended to explore the morphological, histochemical and immunohistochemical characteristic of both spiral valve mucosa and its associated lymphoid tissue. Tissue fragments from the spiral valve intestine of Scyliorhinus canicula (Linnaeus, 1758) caught in the Messina’s Strait were treated for histology and histochemistry (H/E, AB/PAS), lectin histochemistry (PNA, WGA), immunoperoxidase (Human TLR2, Human Integrin a5 1 and Mouse Laminin) and confocal immunofluorescence (Human TLR2, Human Langerin/CD207 and Bovine S100). Valvular let cells, differently positive to PAS, AB and AB-PAS, demonstrating the presence of different types of mucins. PNA and WGA Lectins, marked neutral and acid mucins involved in protection against pathogens invasions. Inteigrin a5 1 and laminin were expressed differently in enterocytes that apex, in lamina propria and in the muscularis mucosa. The spiral valve gut-associated lymphoid tissue (GALT) showed massive immune cells aggregates positive to TLR2, S100, Langerin/CD207. Intraepithelial lymphocytes within the columnar epithelium were present. The results of the present study wide the information about GALT of Elasmobranchii helping in understanding their immunology.

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vey, mothers are followed and measured during pregnancy. The weight of the newborn is correlated with all maternal variables, including gestational age, birth weight, pre-pregnancy weight, mainly weight gain at birth, except for mother’s stature. A genetic effect between mother and child is corroborated by significant positive correlation between the birth weight of the newborn and the mother.

THE IMPACT OF HUMAN GENETICS IN DRUG DISCOVERY
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Choosing the right biological target is the critical primary decision for the development of new drugs. Systematic genetic association testing of both human diseases and quantitative traits, along with resultant findings of coincident associations between them, is becoming a powerful approach to infer drug targetable candidates and generate in vitro tests to identify compounds that can modulate them therapeutically. Here, we discuss opportunities and challenges, and infer criteria for the optimal use of genetic findings in the drug discovery pipeline.

SPACE MEDICINE: USE OF EX VIVO HUMAN RESPIRATORY MUCOSA IN THE SURVEY OF THE EFFECTS OF MICROGRAVITY ON THE RESPIRATORY SYSTEM
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In the near future, the length and scope of space travel is set to increase significantly. The number of individuals who will have access to extra-terrestrial travels is also increasing. In view of the growing international interest towards manned long-term space exploration, possible effects of exposure to microgravity conditions affecting the respiratory system are subject of interest by major space agencies (NASA and ESA primarily). Our team has developed an advanced 3d tissue model of the human bronchial mucosa within a wide research project involving several universities and space agencies at international level. The model will be used to study the structural/functional alterations of the bronchial mucosa that may arise from prolonged exposure to reduced gravity conditions. Among the different modifications to be evaluated: development and performance of the pulmonary barrier; possible ciliogenesis modification due to its effects on fluid mechanics and mechanotransduction; formation of multi-cellular structures (Cell-Cell and ECM-Cell Interactions). The design and realization of experiments aboard the International Space Station (ISS) often clashes with greater difficulties than at ground level. Our work was to check the resilience of the model to the prohibitive environmental conditions present on board the vectors that transport the samples to the ISS, and to adapt the model to engineering requirements for proper functionality within the BIOLAB of ISS itself. To verify this, cell cultures were subjected to various boundary conditions: temperatures lower than growth optimum, reduced concentrations of CO2, restriction of gas exchange, prolonged starvation and storage of the culture medium at high temperatures. The bronchial mucosa cultures were analysed at the end of the treatments and their morphology was evaluated. We also used the monitoring of the Trans Epithelial Electric Resistance to evaluate the state of health of the cultures. The data obtained demonstrated how this culture model is able to overcome the critical phases of the journey to ISS and how it can conform to restrictive engineering requirements. It is possible to assert that in addition to the accurate reproduction of the bronchial human mucosa, the cell culture model possesses the characteristics necessary to be used in studies in an extreme environment such as the ISS, being able to provide data that could be relevant for future manned spaceflights.

CANCER EPIDEMIOLOGY IN ITALIAN MILITARY PERSONNEL DEPLOYED ABROAD (AGE LESS THAN 60 YEARS, 1996-2012). FROM THE DATA OF THE INQUIRY PARLIAMENTARY COMMISSION ON DEPLETED URANIUM AND VACCINES
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Since the 2000s the Ministry of Defense has conducted studies on Italian military personnel, identifying both excesses for Hodgkin’s lymphomas and unexpected reductions for all the tumors. Re-analyze the incidence and mortality for the tumors recorded in the 1996-2012 period by the Epidemiological Observatory of Defense (OED) and by the Inquiry Parliamentary Commission (2016-2018). Cancer cases diagnosed among male militaries aged between 20 and 59 (3,665 cases; 60% of total), enrolled in the 4 armed forces (FFAA) Air force, Carabinieri, Army and Navy were analyzed. The group of militaries involved in at least one mission abroad (“Missionaries”: No. 874; 23.8%) was compared with the “Non Missionaries” (N. 2,791; 76.2%). In the study period, 5,225,153 person-years were estimated (21.7% in the Missionaries). The incidence (SIR) and mortality ratio (SMR) standardized by age were calculated estimating also the 90% confidence interval. Among the “Missionaries” the SIRs were significantly increased for the overall 4 FFAA [874 cases; SIR = 112]; Army (n = 498; SIR = 116.6), Air force (n = 115; SIR = 126.7) and Carabinieri (n = 168; SIR = 152.8). The Navy appeared significantly “protected” (n = 93; SIR = 60.9). The excesses of risk in the set of Missionaries for both SIR and SMR seem to be consistent with the previous ones and with what was perceived by the military themselves. It is confirmed the need to acquire more complete and updated data on both the health of the military (non-neoplastic diseases, post-2012 diagnosis, aged over 60, women, children, spontaneous abortion, etc.)
Although these treatments are effective in many parts of the world, there is serious concern that malaria parasites are once again developing widespread resistance to this vital treatment. To date, artemisinin resistance has been confirmed in the treatment against Plasmodium falciparum malaria.

**OVEREXPRESSION OF THE CYTOKINE BAFF AND AUTOIMMUNITY RISK**

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Through a genome-wide association study (GWAS) using case-control samples from Sardinia we initially discovered an association with multiple sclerosis and systemic lupus erythematosus in TNFSF13, a gene encoding the cytokine BAFF. By means of multiple GWASs in Sardinian general population individuals, we then found that the same disease-risk-increasing allele at TNFSF13 was associated with upregulated humoral immunity through increased levels of soluble (s)BAFF, B lymphocytes, and immunoglobulins. Using fine mapping, transcriptomic and functional studies we ultimately identified and characterized the causal variant: an unusual insertional-deletion, GCTGT>A, where A is the minor, risk allele (“BAFF-var”) that creates a polyadenylation signal, yielding a shorter transcript. The shorter transcript, BAFF-var mRNA, was more actively translated than the wild-type, BAFF-WT mRNA, and led to higher production of (s)BAFF. BAFF-var mRNA was more actively expressed partly because it lacked the binding sites of repression for the microRNA miR-15a and the RNA binding protein NF90. Population genetic signatures indicate that BAFF-var was driven to high frequency in Sardinians by selective pressure, which was most likely due to Malaria infection. We can conclude that BAFF-var is a positively selected immune variant that increases autoimmunity risk.

**SYK INHIBITORS AS NEW ANTIMALARIAL DRUGS**

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Resistance to antimalarial drugs has spread rapidly over the past few decades, therefore to overcome it, WHO recommends Artemisinin based combination therapies (ACTs) for the treatment against Plasmodium falciparum malaria. Although these treatments are effective in many parts of the world, there is serious concern that malaria parasites are once again developing widespread resistance to this vital treatment. To date, artemisinin resistance has been confirmed in 5 countries of the Greater Mekong subregion. The development of resistance has highlighted the need for the search of novel antimalarial molecules. Recently, it was demonstrated that Syk inhibitors represent a new class of antimalarial drugs that suppress merozoite egress by inhibiting a host target that cannot be mutated by the parasite to develop drug resistance. Our purpose was to evaluate the antiplasmodial activity of different Syk inhibitors using in vitro approaches and to have more information on the mechanisms responsible for the protein-ligand recognition. A detailed understanding of Syk Kinase inhibition is the central focus, in order to understanding biology at the molecular level, with the future perspectives of discovering new class of drugs to obtain the highest efficacy of inhibition in infected RBCs.

**BIOLGEO IN ESA CAVES: AN UNDERGROUND MONITORING PROGRAM DEVELOPED FOR ASTRONAUT TRAINING**

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C.A.V.E.S. stands for Cooperative Adventure for Valuing and Exercising human behaviour and performance Skills. The three-week course prepares astronauts to work safely and effectively in multicultural teams in an alien environment, where safety is critical, with few resources, poor communication, and alteration of circadian rhythms. The teams venture into areas unexplored for them following the procedures applied in space, and perform various types of researches as in the ISS, this allows them to work and solve problems, but also to simulate life and coexistence in the International Space Station. It is precisely in this context that the new species found in the depths of the earth, are sampled and subsequently carefully analysed in the laboratory. The discovery of Alpironiscus sideralis carried out a molecular study of almost all the populations of the genus Alpioniscus present in Sardinia, high levels of genetic divergence among the populations were found. During their stay in the cave, the astronauts also carried out fieldwork for study the environmental microbiology. The bacterial diversity in the Su Bentu Cave in Sardinia was investigated by means of 16S rRNA gene-based analysis. Relative humidity approaches 100% and the measured mean annual cave air temperature is 14.8°C. The results of this study highlight that human impact is confined to locations that are utilized as campsites and that exploration leaves little microbial trails. Furthermore, we uncovered a highly specialized microbial, which is perfectly adapted to survive and thrive in an environment with low nutrient availability.

**META-ANALYSIS OF RNA-SEQ DATA TO GAIN INSIGHT INTO CROP RESPONSES TO HARSH ENVIRONMENTS**

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RNA-Seq analysis is a strong tool to decipher gene regulatory networks underlying environmental stresses in crops.
growing in harsh environments. Transcriptomic studies are usually conducted in a singular time, they do not provide any repetition across different seasons and frequently they encounter a high environmental variability because disturbing factors are frequently present. The identification of up- or down-regulated genes is often not enough to draw meaningful biological conclusions because it is hard to assign a clear role to each gene/protein belonging to specific signaling networks in host responses. Meta-analysis of transcriptomic data will discover commonalities and differences among differentially modulated genes and will allow screen which genes are good candidates for validation studies on gene-gene and protein-protein interaction networks. These analyses will allow delivering key knowledge on how a particular factors affect plant molecular responses to difficult environmental conditions. Meta-analysis of transcriptomic data represents a powerful tool to discover new candidate genes usable in marker-assisted selection. The objective of this work is to identify specific and common genes, proteins, gene sets, pathways linked to both abiotic and biotic stress resistances among key crops. The identification of genes involved in different stresses will improve diagnosis of an early “stress state” in response to stresses. Here, we will show results obtained by several meta-analysis of RNA-seq data related to plant responses to biotic and abiotic stresses. Common genes across species and type of stress usually encode key transcription factors, proteins involved in stress signaling and signal transduction, hormone-related pathways and other key gene categories have been identified. These analyses will help in monitoring stressed plants to start early specific management procedures for each stress and develop more sustainable therapeutic strategies through a fine-tuning of plant physiological responses.

TAXONOMIC DIVERSITY AND FUNCTIONAL TRAITS OF NEMATODE ASSEMBLAGES IN INTERTIDAL HABITATS ALONG ADRIATIC SEA COAST, ALBANIA

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Numerous studies have highlighted the importance of free-living nematodes in qualitative assessments of benthic ecosystems. Nematodes are considered as one of the most appropriate groups that best fulfills the characteristics of the indicator organisms. Recently, they are proposed as suitable for ecological quality evaluation of marine ecosystems, according to the Water Framework Directive (WFD). This study presents composition and structure of free-living marine nematode assemblages in five estuarine intertidal habitats along Adriatic Sea. Nematode assemblages from different sites are analyzed in term of diversity, life strategies and trophic structure, attempting to assess the anthropogenic pressure flowing from rivers into the estuaries, based on the state of nematode community succession. The comparison of different assemblages points out differences in taxonomic diversity and functional traits. The nematode assemblages were characterized by low trophic diversity and dominance of opportunistic genera, reflecting low ecological status.

EPIGENETIC PROFILING AND THERAPEUTICS IN LIVER DISEASE

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Epigenetic trait represents a stably heritable phenotype resulting from changes in a chromosome without alterations in the DNA sequence. Epigenetic modifications occur through strongly interlaced processes including DNA methylation, histone modifications, ncRNAs based mechanisms able to influence the gene expression. Disease susceptibility depends on a complex interplay between the individual genetic profile and epigenetic modulations so to aberrant DNA methylation and histone modification patterns caused by environmental stresses that may help to explain why identical twins do not always get the same disease. In Hepatocellular Carcinoma epigenetically induced genes were mapped to pathways driving cellular transformation, tumor growth and metastasis, while epigenetically repressed genes mapped to apoptosis, cell adhesion, and cell cycle progression. On the other hand, HBV and HCV viruses are recognized as drivers of epigenetic changes related to the risk of liver cancer after Sustained Virologic Respond to antiviral treatment of HCV. On the other hand, the association between nutrition, epigenetic modifications and metabolic disease is well established. In murine models a diet depleted of methyl donors, able to induce DNA hypomethylation, is related to steatosis development and genome-wide association studies in humans shown a general tendency to demethylation of genes associated with Non Alcoholic Fatty Liver Disease. The knowledge of epigenetic alterations in liver disease shows clinical and translational implications regarding variation in disease phenotype, better risk stratification for disease progression, development of new diagnostic/prognostic biomarkers and development of new drugs. Finally, lifestyle modification strategies seems to be useful to prevent unhealthy epigenetic traits development.

LOCALIZATION AND LEVELS OF HSP60 IN TISSUE SAMPLES OF NORMAL AND PATHOLOGICAL HUMAN STOMACH

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Heat shock proteins (HSP) are a family of proteins produced by cells in response to a stress condition, the increase in the concentration of these proteins does not only occur in relation to the stimulation of high temperature, but following a large number of stimuli such as infections, inflammatory cytokines, biomechanical forces, UV rays and heavy metals. The HSP are differentiated according to their molecular weight. The Heat shock important “non-canonical” functions in fact they are involved in various biomolecular cell mechanisms, such as DNA replication, gene expression, cell differentiation furthermore many studies have shown the relationship between chaperone activity and carcinogenesis. The purpose of the following experimental work was to evaluate, through immunohistochemistry and immunofluorescence, the localization and the expression levels of HSP60 and inflammatory markers (CD3, CD20, CD68) within biopsy samples of gastric mucosa of gastritis (mild to moderate), hyperplastic polyps and adenocarcinoma with a medium degree of differentiation (G2). The different biopsy specimens were selected from the archives of the Biotechnology Laboratory of Institute Euro-Mediterranean of Science and Technology (IEMEST). All selected adenocarcinoma samples were characterized by a
histopathologic diagnosis of type I (according to Lauren classification), with a moderate degree of differentiation (G2) and all sections selected showed simultaneously areas of normal mucosa, intestinal metaplasia and areas of adenocarcinoma. The sections were observed under an optical microscope (Leica DM 5000 B) connected to a digital camera (Leica DC 300F) for the evaluation of the immunohistochemical expression levels. The levels of expression of HSP60, CD-20, CD-3, CD-68 have been assessed in the epithelial cells (EC) and in the cells of the lamina propria (CLP) in the different types of biopsies. Hsp60 immunopositivity, seemed widespread at cyttoplasmatic level and, sometimes, with a granular aspect. The epithelial cells immunopositivity for HSP60, within the group of moderate gastritis, was estimated with an average of 33.5%, while the immunopositivity in the lamina propria cells was 20%. In the group of hyperplastic polyps, the HSP60 positivity achieved a value of 60% of the cells, while the immuno-positivity was 18.3% of the epithelial cells of the lamina propria. Finally, in the group of gastric adenocarcinomas, the immunopositivity to HSP60 has reached a percentage of 92% in the epithelial cells and 18.7% in the cells of the lamina propria. In conclusion, we can say that the HSP60 can play a role in stomach tumor pathogenesis. In the future, the study of HSP60 will open interesting perspectives on cancer molecularly therapies and individual diagnosis. A statistically significant difference has emerged in the expression levels of HSP60 of the three groups analyzed. In particular, the immunopositivity to HSP60 was elevated in adenocarcinoma samples compared to samples of hyperplastic polyps and chronic gastritis samples.

ALTERATION IN VESICLE TRAFFICKING IN PARKINSON’S DISEASE: FROM PATHOLOGICAL MOLECULAR MECHANISMS TO THERAPEUTIC OPTION
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Mutations in the leucine-rich repeat kinase 2 gene (LRRK2, PARK8) are the most frequent genetic causes of Parkinson’s. LRRK2 pathological mutations cause late-onset, autosomal dominant PD that is indistinguishable from idiopathic forms. LRRK2 is a member of Roco superfamily proteins, a novel multi-domain family of Ras-like G-proteins. Despite the apparent clinical association between LRRK2 mutations and PD, it remains enigmatic how LRRK2 pathological mutations may contribute to disease onset and progression. Different experimental results suggest an important role of LRRK2 in the control of vesicle trafficking, and alteration in synaptic vesicle trafficking seems a common pathological mechanism in both familial and idiopathic PD. We were able to demonstrate that LRRK2 plays an important role in the control of dopamine receptor trafficking (either DRD1 or DRD2) both in cellular and animal models. In our experimental conditions DRD1 internalization is significantly impaired in the presence of LRRK2 pathological mutant expression while DRD2 is accumulated in the Golgi areas. Moreover we have demonstrated that Leviteracetam, a compound largely used in human therapy for epilepsy treatment, is able to rescue different LRRK2 pathological phenotypes. LEV is able to regulate vesicle trafficking by SV2A binding strongly suggesting that the modulation of vesicle trafficking could be a relevant option for PD treatment.

IN-VIVO NEUROCHEMISTRY IN REAL TIME: THE USE OF ELECTROCHEMICAL MICRO- AND BIO-SENSORS
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In recent years, micro- and bio-sensors for the direct monitoring of neurochemicals within the brain tissues have become a very important tool for research in neuroscience. Their widespread use is mainly due to high sensitivity, owing to the electrochemical techniques used, but also to their miniaturization and low cost. Amperometric micro- and bio-sensors allow the conversion of a chemical signal, which occurs on the sensor surface, into an electrical detectable and measurable signal. Every device is the combination of the appropriate material that constitutes the transducer, the components that safeguard its selectivity (as well as the exclusion of the interfering species), the stability over time and, in the case of bio-sensors, the biocompatibility that guarantees its specificity. Micro- and bio-sensors have been used to monitor several neurochemicals owing to the energetic metabolism (as oxygen, lactate and glucose), neuromodulators, antioxidants as ascorbic acid, numerous neurotransmitters (as dopamine and acetylcholine) but also exogenous compounds as ethanol. These devices would allow to study the brain functionalities both in physiological and pathological conditions, both in physiological and pathological conditions, as well as to monitor the impact of pharmacological treatments. Micro- and bio-sensors have proven to be particularly useful for basic research on the major neurodegenerative diseases as Alzheimer’s and Parkinson’s disease, but also multiple sclerosis.

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INTEGRATIVE MARINE ZOOLOGY AS A TOOL TO FACE THE RUN TO EXTINCTION OF A MEDITERRANEAN FLAG SPECIES, THE FAN MUSSEL SHELL
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The fan mussel shell, Pinna nobilis (Mollusca), is a big infralittoral bivalve (up to 1m height), endemic to Mediterranean Sea, and thus considered one of its most iconic “flag” species. As a consequence of the decline of its populations during the 20th century, mainly due to human collection, the fan mussel has been included in a protection regime that led to the recovery of the species throughout the Mediterranean. Unfortunately, since 2016 a dramatic mass mortality event was
detected, which spread from the Spain coasts eastward. First epidemiological surveys suggested either or both Haplosporidium pinnae (Protozoa) and Mycobacterium sp. as etiological agents responsible of the disease. Under the light of these premises, the Integrative Marine Zoology (IMZ) represents a pivotal tool for an all-round investigation focused on the conservation of threatened species. In this case, IMZ aimed to infer the cause of the severe rate of mortality (until local extinctions) in Sardinia. We assessed the genetic variability of P. nobilis, and performed molecular diagnoses for the presence of potential pathogens. Phylogeny, dating, and phylodynamics assays were thus carried out on the pathogens found. Present results suggest a more complex scenario than expected, in which the status of a multifactorial disease seems to be the most plausible cause of this awful mass mortality event in Sardinia. Our findings also confirm the need to achieve a multidisciplinary approach, in which the integration of molecular, census and environmental data will ensure a deeper understanding of the multifaceted mechanisms that underlie this phenomenon, leading to a hopefully quick development of an effective action plan to face the run to extinction of P. nobilis, predicting the potential of survival of the species.

**ARTEMISININ INDUCED O$_2^-$ AND H$_2$O$_2$ SPECIES DETERMINATION IN OXIDIZED ERYTHROCYTES**

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The cornerstone of antimalarial treatment, artemisinin, has reduced malaria associated morbidity and mortality worldwide but our current understanding of its mode of action remain elusive. Despite tremendous research efforts on artemisinin since its discovery, there is still considerable debate concerning its mode of action on cancer cells too. The presence of the endoperoxide-bridge in Artemisinin and production of reactive oxygen species (ROS) is suggested as an important feature not only in antimalarial action but also in the fight against cancer cells. O$_2$ and iron constitute two of the major components of human erythrocytes responsible for the production of highly toxic ROS. Iron commonly mediates ROS production via Fenton reaction, where iron cycles back and forth between $^{+2}$ and $^{+3}$ states and in the process generates $^\cdot$OH free radical. In the erythrocyte, where $^\cdot$OH free radical will lead to Hb denaturation and further release of heme iron, this process can be autocatalytic, leading an ever increasing oxidative stress once it is initiated by the release of threshold amounts of free iron. We demonstrate by measuring production of ROS that previous exposure to Phenylhydrazine, a strong oxidant, activate ARTs.
POSTERS

ANALYSIS OF THE RECIPE OF A REPUBLIC AGE ROMAN FUNERARY BREAD FROM THE ANCIENT FORUM LIVII (PIAZZALE DELLA VITTORIA), FORLI, ITALY BY MEANS OF FTIR AND SEM TECHNIQUES

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A Roman funerary bread was found in a grave of the necropolis recently excavated in an extra-urban area of the ancient Forum Livii, along the ancient Via Aemilia; the area corresponding to the actual Piazzale della Vittoria, Forlì, Italy. Currently, there are only two contexts in Italy in which a bread was found in association with tombs: Forlì and Angera. The study of the grave goods allows to date the necropolis to the republic age between the I century B.C. and the II century C.E. The bread is a small loaf, 10 cm of diameter, with traces of burnt on one side. It is hypothesized it could be a votive offer and the present study aims to answer the questions: What were its ingredients? How was it prepared? And finally, how was the bread preserved? FTIR spectroscopy and Scanning Electron Microscopy analysis were performed on this sample. FTIR and EDS analysis showed that the bread contains high silicate concentration that could be characteristic of the cereal employed for the dough, such as millet, sorghum or barley. However it can arise from a small amount of clay that could have been deliberately included in the dough like in the case of the Panis Picenus. SEM analysis revealed that the starch retrogradation typical of staling of bread is the main process that has allowed the remain preservation.

CHRONICLES OF THE PARTHENOGENETIC MARBLED CRAYFISH INVASION IN THE SARDINIAN FRESHWATERS

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The marbled crayfish, Procambarus fallax f. virginalis, is a freshwater crayfish characterized by a parthenogenetic reproduction. It was identified for the first time in Germany in the 90s, and today it is considered as a highly invasive species as consequence of its ability to quickly create stable wild populations in different European areas. Due to various reports from southern areas of Sardinia, we collected 16 individuals in order to shed light on the diffusion of this species in the island. A portion of the subunit I of the mitochondrial Cytochrome c Oxidase gene (COI) was amplified and a single haplotype was found. The sequences obtained were identified using a BLAST analysis and showed a 100% identity to Procambarus fallax f. virginalis. The sequencing of a chitinase gene fragment was also used to exclude for samples of marbled crayfish from Sardinian to be carriers of the pathogenic oomycte which is potentially able to infect the crustaceans endemic to the island likely leading a plague-related mortality. All sequences of COI so far available on GenBank for the marbled crayfish (18) were added to our dataset obtaining a 647bp-long alignment including individuals from Florida, Italy, Germany, Sweden and Japan. The Network analysis performed on such a dataset depicted the relationships between haplotypes. Results pointed out the occurrence of an haplotype common to the 71% of the sequences which corresponded to the unique haplotype reported for Sardinian samples. Four further haplotypes were also found in some individuals from Florida and Germany, which diverge from the most common haplotype for few point-mutations.

CROSS-TALK BETWEEN FATTY ACID AND CYTOKINES IN ALS PATIENTS

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Amyotrophic lateral sclerosis (ALS) is a multifactorial neurodegenerative disease attributed to an intrinsic defect of upper and lower motor neurons. Growing evidences showed that also non-neuronal cells participate actively in the pathological process through different mechanisms. In this perspective fatty acid production and inflammatory process seem to play a role in the disease onset and progression. Here we quantified fatty acid composition and cytokines on ALS patients' plasma (n=34) and sex/age matched healthy controls (HC) respectively by GC-MS and cryptometric beads array (BCA) Becton Dickinson kits. Preliminary fatty acid analysis revealed a significative higher expression of C16:0 (HC vs ALS p=0.001) and its derivatives C16:1 n-7, C18:0, C18:1n-9 and 20:0 in ALS patients’ plasma compared to HC. Moreover by CBA analysis we observed an higher expression of pro-inflammatory cytokines TNF-α, IL-6 and IFN-γ and the presence of anti-inflammatory response mediated by an improve of IL-10 expression in ALS patient’s plasma compared to HC. Evidences, based on astrocyte cell lines analysis, revealed C:16 and C:18 induction to product IL-6 and TNF-α pro-inflammatory cytokines. Our data based on ex vivo analysis confirm this correlation. Moreover the IFN-γ high levels observed only in ALS patients could be directly correlated with the high C:20 concentration observed in ALS patients. However the increase in C:18:1 n-9 and IL-10, both known as an anti-inflammatory action, observed in ALS patients suggest the body’s attempt to defend itself from inflammatory process.

SMOKING INFLUENCE ON MALE AND FEMALE PHENOTYPES

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Sex and gender medicine focuses on differences and similarities in health and disease between sexes. In view of the fact that market access is often based on surrogate end points, biomarkers, or intermediate end points, the aim of our study is to assess the relationship between smoking and sex and gender on routine biochemical and haematological parameters to: a) evaluate potential male and female phenotypes; b) clarify the role played by smoking on male and female phenotypes; and c) describe the influence of body dimension on sex - gender differences. We enrolled 85 healthy young adult men (27 smokers and 59 non-smokers) and 85 age-matched women (32 smokers and 53 non-smokers). Haematological parameters were measured and data were analysed before and after body weight normalization. In non-smoking cohorts, there were numerous sex and gender differences and principal component analysis (PCA) distinguished two different phenotypes: male and female ones. The differences attenuated after body weight normalization, but the male and female phenotypes were still well evident. Smoking affected the studied parameters abolishing the male and female phenotypes. The current findings show that physiological factors are strongly associated with sex and gender and smoking habit. In non-smoking cohorts, only one phenotype is present. Our data suggest that sex and gender and smoking habit should be considered as a factor in clinical studies to be closer to real life.

OBLIGATORY ROLE OF ENDOPLASMIC RETICULUM IN BRAIN FDG UPTAKE

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The endoplasmic reticulum (ER) contains hexose-6P-dehydrogenase (H6PD). This enzyme competes with glucose-6P-phosphatase (G6Pase) for processing a variety of phosphorylated hexoses including 2-deoxyglucose-6P (2DG-6P). The present study aimed to verify whether this ER glucose-processing machinery contributes to brain FDG uptake. Effect of the H6PD inhibitor metformin on brain 18F-FDG accumulation was studied, in vivo, by microPET imaging. These data were complemented with the in vitro estimation of the lumped constant (LC). Finally, recticular accumulation of the fluorescent 2DG analogue 2-[N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino]-2-deoxyglucose (2NBDG) and its response to metformin was studied by confocal microscopy in cultured neurons and astrocytes. Metformin halved brain 18F-FDG accumulation without altering whole body tracer clearance. Ex vivo, this same response faced the doubling of both glucose consumption and lactate release. The consequent fall in LC was not explained by any change in expression or activity of its theoretical determinants (GLUTs, hexokinases, G6Pase), while it agreed with the drug-induced inhibition of H6PD function. In vitro, 2NBDG accumulation selectively involved the ER lumen and correlated with H6PD activity being higher in neurons than in astrocytes despite a lower glucose consumption. The activity of the recticular enzyme H6PD profoundly contributes to brain 18F-FDG uptake. These data challenge the current dogma linking 2DG/FDG uptake to the glycolytic rate and introduce a new model to explain the link between 18F-FDG uptake and neuronal activity.

STEM CELLS AND NUTRACEUTICALS: EXTRACTS FROM MYRTLE MODULATE OXIDATIVE STRESS AND SENESCENCE

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Amino acids and polyamines are metabolites that can be used to discriminate between different types of cancer from healthy controls. In this study, we conducted a metabolomic separation in a high-performance chromatography (UHPLC) using UHPLC coupled with the Orbitrap mass spectrometer are protagonists in protein synthesis. Many researches recognize polyamines as important cancer markers useful for early diagnosis and treatment. When the metabolic balance of this pathway is altered, the detection and identification of polyamine metabolism is useful as a solid starting point for the study of potential links between cancer and polyamines. The analytical method presented for the study of polyamines and amino acids in human plasma is based on chromatographic separation in a high-performance chromatography (UHPLC) associated with high resolution accurate mass (HRAM) without derivatization. The results highlight the possibility of discriminating between healthy and cancer patients.
Mesenchymal stem cells (MSCs) are multipotent stem cells that can differentiate into osteoblasts, chondrocytes, adipocytes, and myocytes if properly stimulated. Adipose tissue represents a good source for stem cell isolation. Human adipose tissue derived stem cells (hADSCs) have recently been proposed as a suitable tool for regenerative therapies, for their simple isolation procedure and high proliferative capacity in culture. Oxidative stress can cause damage and senescence in stem cells resulting in loss of regenerative potential of stem cells. The use of natural molecules could counteract the senescence of stem cells and restore their regenerative potential. Myrtus communis L. extracts obtained from fresh or industrial waste pulp and seeds were tested on mesenchymal stem cells from adipose tissue. In particular, we evaluated the effects of these extracts on the expression of specific genes involved in cell senescence. Mesenchymal stem cells derived from adipose tissue (ADSCs) were grown in a basic culturing medium. Cells were cultured in the presence or absence of freeze-dried myrtle extracts (from fresh or industrial waste pulp and seeds) for 12, 24 and 48 hours, and then incubated with H2O2 for 1 hour to induce oxidative stress and senescence. Our results demonstrate that pre-treating cells with the extracts could prevent the expression of specific genes involved in senescence commitment and the appearance of -Galactosidase staining, a marker of an established cell senescence phenotype. On the whole our results suggest that Myrtus extracts exert important antioxidant and protective activities to defend cells from stressful and harmful conditions.

**URINARY TRypsIN INHIBITOR (UTI) QUANTITYATION AND STRUCTURAL CHARACTERIZATION IN TYPE 1 AND TYPE 2 DIABETES**

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The Urinary Trypsin Inhibitor (UTI) is a chondroitin sulfate proteo-glycan that plays pleiotropic roles in many inflammatory processes. It consists of a small polypeptide of 147 amino acid residues, which carries an N-linked oligosaccharide at Asn⁴⁵ and a O-linked low-mannose chondroitin sulfate chain at Ser¹⁰. UTI levels increase up to tenfold in both acute and chronic inflammatory diseases and association with both type 1 and 2 diabetes mellitus (T1DM and T2DM) has been reported. These pathologies are a global health problem representing the fifth leading cause of mortality and a major risk factor for cardiovascular diseases. In this respect, the prompt preclinical diagnosis of the state is the key point for early intervention and disease management. We developed a method for UTI quantitation and structural characterization that was applied to the analysis of 39 samples from T1DM patients, 32 samples from T2DM patients and 52 samples from healthy controls matched for gender and age to patients. UTI was purified from urine by anion exchange chromatography, treated for chondroitin sulphate (CS) chain complete depolymerisation, and analysed for both UTI content and CS structure. UTI identification was performed by nano-LC-MS/MS analysis. Results showed increased UTI levels, as well as reduced sulphation of its CS moiety in both T1DM and T2DM, regardless age, medium-term glycemic control and albumin excretion rate, suggesting that UTI levels may represent a potential marker for the underlying inflammatory condition.

**REFERENCES**


**TESTING THE EFFECTIVENESS OF A NEW PROMISING ACETYLCHOLINESTERASE INHIBITOR FOR ALZHEIMER’S DISEASE USING DROSOPHILA MELANOGASTER TRANSGENIC MODEL**

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Alzheimer’s Disease (AD) is a progressive neurodegenerative disorder, it is characterized by short-term memory loss, shortened lifespan, locomotive defects and cognitive impairment. AD currently affects 50 million people around the world. The disease was firstly characterized by Aloise Alzheimer in 1907 after an autopsy on a 55-years-old woman named Auguste Deter, who died with progressive cognitive disorder. Henceforth three acetylcholinesterase (AChE) inhibitors have been approved by the FDA as treatment for AD. To model AD, we have over-expressed the amyloid peptide throughout the Drosophila melanogaster CNS. These transgenic flies recapitulated the clinical aspects of AD. Such induced defects were exploited to investigate the therapeutic effect of the newly synthesized AChE inhibitor XJP-1, which displayed dual binding activity for both the catalytic anionic site (CAS) and peripheral anionic site (PAS) of the AChE. XJP-1 also demonstrated the ability to ameliorate the AD symptomatology and slow the disease progression. Furthering this, we exploited the established AD Drosophila model to test all the FDA approved drugs for AD treatment, including NMDAR antagonist Memantine. The FDA approved drugs showed a modest effect in slowing the progression of AD, being more efficient on the symptomatology of the disease. Collectively, these data suggest that a dual binding activity on PAS and CAS mitigated amyloid aggregation thus delaying the neurodegenerative process.

**GAMMA-AMINOBUTYRIC ACID CONTENT IN FIORE SARDO CHEESE RELATED TO FREE AMINO ACIDS AND COMPOSITION**

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[page 14] [Journal of Biological Research 2019; 92:s2]
The bioactive compounds -aminobutyric acid (GABA) and the protein-free amino acids were measured in Fiore Sardo, a typical cheese produced in Sardinia. GABA is a non-protein amino acid which could have a beneficial impact on human health. The presence in the diet of this nitrogenous compound might have positive effects on metabolic disorders. A total of 45 cheese samples were collected from 6 dairy farms to evaluate GABA content and its relationship with free amino acids (FAA) and composition. The following were determined for each sample: pH, water activity, moisture, dry matter and NaCl. GABA and FAA were determined by HPLC-FL after derivatization with dansyl chloride. Great variability in the content of the nitrogenous compounds considered was found: the level of GABA ranged between 3 and 103 mg 100 g⁻¹. Overall 30% of the samples analysed have shown a content of GABA higher than that measured in other types of Italian typical cheeses. GABA content was positively correlated with FAA and pH (Ps0.01) whereas not significant correlation was found with α and humidity. To identify similarities and correlations between the samples of cheeses, the hierarchical cluster analysis (HCA) was performed using 37 objects and 6 variables. By mean of HCA was possible to assess that Fiore Sardo cheese characteristics differ from one producer to another; however, it seems quite constant within each firm over time.

METABOLIC RATES DURING DAILY LIVING ACTIVITIES IN PEOPLE WITH MULTIPLE SCLEROSIS

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Persons with multiple sclerosis (PwMS) exhibit lower values than healthy controls in peak aerobic capacity (VO2peak), ventilatory anaerobic threshold (VAT), peak respiratory exchange ratio (RERpeak), peak heart rate (HRpeak) and peak work rate (WRpeak). Whether they use more energy than healthy controls during submaximal activities is less clear. Moreover, no data are yet available on the metabolic rate of PwMS during daily living activities (ADL), which are basic tasks that must be accomplished every day for an individual to maintain independence and are used as a measurement of a person’s functional status. The present cross-sectional case-control study was aimed at measuring the energetic cost of a composite set of basic ADL, including dressing, toileting, transferring and mobility, as derived by validated rating scales. A portable, open-circuit gas analyzer system (MetaMax 3B, Cortex Medical, Germany) was employed to monitor the metabolic rate during 13 basic ADL assessed over 2 non-consecutive days in 10 PwMS (5 with moderate-to-severe disability; 5 with very mild disability) and in 5 age and gender-matched healthy control subjects. Activities were monitored for at least 5 minutes and followed by a 5-minute complete rest. Compared to controls and mildly disabled PwMS, more disabled individuals showed significantly higher VO2, VCO2 and HR, but not RER, values during dressing, car usage, doing laundry and climbing stairs. Interestingly, significant differences in metabolic rate were also found between mildly disabled PwMS and controls. Open-spirometry outcomes during every-day life may contribute to enhance our understanding of the pathophysiology of MS-linked fatigue.

LEVETIRACETAM TREATMENT AMELIORATES LRRK2 PATHOLOGICAL MUTANT PHENOTYPE

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Mutations in Leucine-Rich Repeat Kinase 2 (LRRK2) are the
most common genetic cause of Parkinson’s disease (PD). The LRRK2 physiological and pathological function is still debated. However different experimental evidence based on LRRK2 cellular localization and LRRK2 protein interactors suggest that LRRK2 may be part and regulate a protein network modulating vesicle dynamics/trafficking. Interestingly the synaptic vesicle protein SV2A is part of this protein complex. Importantly, SV2A is the binding site of the Levetracetam (LEV) a compound largely used in human therapy for epilepsy treatment. The binding of LEV to SV2A reduces the neuronal firing by the modulation of vesicle trafficking although by an unclear molecular mechanism. We have analyzed the interaction between the LRRK2 and SV2A pathways by LEV treatment. Interestingly LEV significantly counteracts the effect of LRRK2 G2019S pathological mutant expression in three different cellular experimental models. LEV rescues the negative effect of LRRK2 pathological mutant expression in the differentiation of primary neurons or PC12 cells. Moreover LEV rescues the effect of LRRK2 pathological mutant expression on dopamine receptor D2 (DRD2) localization/trafficking. Our data strongly suggest that LEV treatment may have a neuroprotective effect on LRRK2 pathological mutant toxicity and that LEV repositioning could be a viable compound for PD treatment.

**PLASTIC OCCURRENCE IN PELAGIA NOCTILUCA FROM THE STRAIT OF MESSINA**

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The number of studies showing the impact of microplastics (MPs) on marine organisms is constantly increasing. It is now known that, due to the small size, microplastics can be ingested by a wide range of organisms, such as zooplankton, invertebrates and fish. However, the occurrence of MPs in cnidarians is still little explored. The present study aims to assess their occurrence in the mauve stinger *Pelagia noctiluca*, collected from the Strait of Messina. In total 49 samples were examined. The specimens were divided into 4 pools based on their size; umbrella and oral arms were examined separately. Microplastic extraction was carried using chemical digestion. After digestion, the membranes were observed under stereomicroscope and MPs were visually identified and cataloged on the basis of shape, size and color. A total of 55 MPs in fibrous shape were found (44 black, 8 blue, 1 red, 1 light blue and 1 white). 62% of the microplastics were extracted from the umbrella, while 38% from the oral arms. Micro Raman and infrared spectroscopy were applied for the MPs identification. *Pelagia noctiluca* is the most abundant jellyfish species in the Mediterranean sea with a significant ecological importance. Our results confirm the ability of this species to uptake plastic particles, probably related to feeding behaviour. Therefore, this preliminary study suggest the potential use of this species as a sentinel organism for microplastic pollution.

**TDP-43 PHYSIOPATHOLOGY AND CYANOTOXINS EXPOSURE**

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Amyotrophic Lateral Sclerosis (ALS) is an adult-onset neurodegenerative disease, characterized by the progressive degeneration of upper and lower motor neurons. More then 20 ALS-causing genes, responsible for 10% of cases, were identified. In Sardinia, that represent a genetic isolate characterized by a higher frequency than expected of the rate of ALS (familial ALS), a high rate (about 30%) of TDP-43 A382T missense mutation has been reported. Environmental trigger may be involved in disease initiation and chronic exposure to the cyanotoxins L-BMAA it has been demonstrated to have a role in multiple neurodegenerative diseases including ALS. Cyanobacteria are ancient and photosynthetic ubiquitous microorganisms, able to cause Harmful Algal Blooms, especially in freshwater ecosystems. The interest in investigating the exposure to this non-protein amino acid as possible risk factors, particularly on vulnerable subpopulations of motor neurons, has significantly increased. The main objectives of our work are study the effects of cellular extracts of Cyanobacteria isolated from Sardinian lakes and reservoirs or purified L-BMAA, on cellular models of ALS focusing on models carrying TDP43-A382T mutation and investigate the effects of the exposure, via oral intake, to chronic BMAA on Drosophila models for ALS. At present we set up both cellular and Drosophila ALS models. Using neuronal SH-SY5Y cells expressing WT or pathological TDP-43 mutants, we investigated TDP-43 subcellular localization, cell viability and physiology through the analysis of different cell parameters, upon stimulation with different doses of BMAA.

**A NEW METHOD TO MEASURE REACTIVE OXYGEN SPECIES IN ERYTHROCYTE SYSTEM**

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Reactive oxygen species (ROS) are common by-products of normal aerobic cellular metabolism and play important physiological roles in intracellular cell signaling and homeostasis. Excessive amounts of ROS generate oxidative stress (OS), compromising cell health and contributing to disease development. Red blood cells (RBCs) are highly susceptible to oxidative damage due to the high cellular oxygen concentration and hemoglobin, a powerful promoter of the oxidative process. Biomarkers of OS can therefore be exploited as important tools in the assessment of disease status in humans and understanding ROS-dependent biochemical pathways. Several approaches such as fluorescence by
H$_2$DCFDA, HE, CBA, etc, are already available to detect ROS in eukaryotic cells. However, few fluorescence probes can be used in the erythrocyte system as a result of the complexity of RBCs. Consequently, we explored to establish a new method to measure ROS by Liquid Chromatography coupled to Mass spectroscopy (LC-MS). The LC-MS approach filters the target probe-radical adduct through a selective column, thus avoiding overestimation of ROS concentration and allowing specific radical determination. LC-MS method was successfully applied to erythrocytes for quantifying superoxide radicals and its reduced form hydrogen peroxide in the erythrocyte system under diverse conditions.

DETECTION OF THE DIFFUSION AND KNOWLEDGE OF ENERGY DRINKS, CONDUCTED THROUGH A STATISTICAL SURVEY ON PALERMO AREA

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In recent years energy drinks consumption has increased, due to their ability to improve physical and cognitive performance. Unfortunately, because of poor or incorrect information, people are not always aware of the harmful consequences of these drinks such as obesity, diabetes, hypertension, tachycardia until death. Therefore, we conducted a statistical survey in Palermo area, submitting a paper questionnaire to 1003 people. Data analysis shows that: only 29% of the audience never consumed energy drinks, while 71% tried them at least once in their life, especially in adolescence; 81% never or rarely drink them, 14% drink them a few times a month, while 5% drink them several times a week; energy drinks are mostly consumed in disco, sport and study contexts; 93% of respondents are aware of the risks caused by the excessive consumption of them; 91% know that it is not appropriate to associate them with alcohol, but 9% think it is better to mix them; 72% of interviewees know that people should never drink energy drink with alcohol, 13% do it only in the weekend, while 15% think it is appropriate to do whenever they want. In fact, 14% do not believe it is risky to take energy drinks together with alcohol; and 40% do not know whether it is risky or not, while 46% know it can be risky; 45% associate the idea of cigarettes with alcohol and energy drinks. In conclusion, it is appropriate to highlight their risks to prevent some fatal consequences.

STATISTICS OF DATA RELATED TO AMAZING SUBSTANCES ANALYZED AT THE “LABORATORY OF CHEMICAL INVESTIGATIONS” OF THE PALERMO SCIENTIFIC POLICE

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The substances analyzed mainly at the “Chemical Investigation Laboratory” of the Regional Cabinet of Palermo, in the period from 2013 to 2018, are heroin and cocaine. For analytical techniques, mass spectrometry techniques associated with gas chromatography were used. Statistical analysis revealed a general increase in both the distribution and the average percentage of active ingredient in heroin, a substance currently available on the illicit market at a lower price than in the past, often cut with substances of synthetic derivation. Comparison of heroin seizure data in western Sicily and throughout the national territory shows a growth trend; in particular, in 2017 there is an average percentage of active ingredient of 17.0% in the national territory and 25.7% in western Sicily. About the spread of cocaine, an increase in the average percentage of active principle (in 2017, 68.0% in the national territory and 75.6% in western Sicily) is observed in the data relating to the analyzes performed and in the use of cutting substances, with a maximum increase between 2017 and 2018, especially due to the presence of adulterants used in order to enhance the desired effect. The current panorama leads to the conclusion that “prevention”, implemented through a suitable information campaign, is the instrument of choice for containing and controlling the spread of these substances with an amazing action, especially among adolescents. In this context, professional figures such as those of the pharmacist can play a fundamental role.
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