BIOCELL
2009, 33(3): A225-A241

ISSN 0327 - 9545
PRINTED IN ARGENTINA

UNESCO CHAIR IN CRIOBIOLOGY

Centro Binacional de Criobiología Clínica y Aplicada
UNESCO Chair in Criobiology
Facultad de Ciencias Bioquímicas y Farmacéuticas
Universidad Nacional de Rosario
Suipacha 570 (S2002LRK) Rosario
Argentina

Lectures and Abstracts from the

3er WORKSHOP IN CRYOBIOLOGY APPLIED TO MEDICAL SCIENCES

May 5-7, 2009

Rosario, ARGENTINA

The lectures and abstracts from ***3er Workshop in Cryobiology of Medical Sciences***
have been revised and evaluated by a scientific committee

Board of the *3er* Workshop in Cryobiology Applied to Medical Sciences

President *Prof. Dr. Edgardo E. Guibert*
Vicepresident *Prof. Dr. Joaquín V. Rodriguez*
Secretary *Dra. Alejandra B. Quintana*
Sub-Secretary *Dra. María Eugenia Mamprin*
Tresurer *Dra. María Gabriela Mediavilla*

Collaborators

Lic. María Dolores Pizarro
Dra. Valeria Sigot
Dra. Cecilia Mansilla

Scientific Committee

Dr. Blas Melissari (Uruguay)
Dra. Alejandra Quintana (Argentina)
Dra. María Eugenia Mamprin (Argentina)
Dra. María Gabriela Mediavilla (Argentina)
Dra. Valeria Sigot (Argentina)

Governmental and Private sponsors

- ✓ Agencia Nacional de Promoción Científica y Tecnológica
- ✓ Secretaría de Ciencia, Tecnología e Innovación Productiva - SECYT
- ✓ Universidad Nacional de Rosario
- ✓ Gobierno de la Provincia de Santa Fe, Sub-Secretaría de Ciencia y Técnica
- ✓ UNESCO Chair in Cryobiology
- ✓ Cámara de Comercio Italiana de Rosario
- ✓ Consulado de Italia
- ✓ Centro Studi Fegato, Trieste, Italy
- ✓ AGA
- ✓ Claudio Lamelza S.R.L.
- ✓ Titania Viajes y Turismo
- ✓ Alfa Uno
- ✓ Bio Esanco S.A.
- ✓ Lovob Científica

LECTURES OF THE MASTERS

LM-1.
TOLERANCE IN THE LABORATORY AND IN NATURE
Fuller BJ.
Royal Free & UCL Medical School; University Department of Surgery; Royal Free Campus; UCL; London NW3 2QG; UK, & UNESCO Chair in Cryobiology
E-mail: b.fuller@medsch.ucl.ac.uk

Organ transplantation is the treatment of choice in many countries for end-stage diseases, but now demand for donor organs outpaces the supply, and better cold preservation for organs is again an important question. Hypothermic organ preservation has developed since the 1970's via two main strategies, based on laboratory experimentation. However, we have come to learn that these overlap many of the natural strategies evolved in organisms living in extreme environments. The two methods are (1) hypo-metabolism induced by cold hypoxic flush (HCF) and (2) metabolic support by hypothermic perfusion preservation (HPP). In HCF, solutions have been developed which reduce the energy demand required for essential homeostatic controls in the cold organs, by suppressing metabolism over and above the reduction resulting from cooling alone. HPP exploits a different survival mechanism using a low continuous perfusion of the organ's vascular bed in the cold, supplying small amounts of oxygen which support ongoing aerobic metabolism and maintain mitochondrial integrity. This review will discuss the development and current practices for both HCF and HPP in clinical organ preservation. Parallel observations to natural cold tolerance will be drawn. The balance between usage in the clinic for HCF and HPP has waxed and waned over the past 30 years, with HCF seen as simpler and less expensive. Never-the-less, in the clinic, the continuing shortage of suitable organs for transplantation has driven the need to access organs from donors previously considered unsuitable (the so-called 'expanded criteria' donors and non-heart beating donors) where organ function may already be compromised before cold preservation starts. In this situation, HPP has a role to play by supporting or even repairing organ metabolism during the storage period. Additional clues about the importance of aerobic metabolism can be found using oxygen carriers (perfluorocarbons) in the cold without perfusion. For the future, we will need a much greater understanding of how to control the signalling mechanisms involved in cell survival at low temperatures in non-adapted mammals (man), which we may learn from re-evaluating studies on cold tolerance in the natural world.

LM-2.
SOME LIKE IT COLD. HOW PERIPHERAL BLOOD STEM CELLS SURVIVE CRYOPRESERVATION
Sputtek A.
Institut f. Transfusionsmedizin, Univ.-Klinikum Hamburg-Eppendorf, Martinistr 52, 20246 Hamburg, Germany.
E-mail: sputtek@uke.uni-hamburg.de

There is a growing interest in how to harness the natural ability of living cells to survive the unfriendly treatment of freezing. Cryopreservation of somatic and reproductive cells, tissues, and organs has a wide range of applications in biotechnology, biomedicine, agriculture, forestry, aquaculture, biodiversity conservation and – transfusion medicine. The cryopreservation of blood cells can be regarded as a classical field of development and application of low temperature biology. Cellular therapies based on the use of hematopoietic stem cells either from bone marrow or peripheral blood have become a standard therapy for a variety of diseases. Some transfusion medicine-based institutions were fortunate and clever enough not to miss this train. Cryopreserved autologous peripheral blood progenitor cells (PBSC) in combination with high dose chemotherapy and/or irradiation have become a "standard" blood component for the treatment of more than 10,000 patients in Europe per year. These patients suffer from various malignant diseases, e.g. lymphoma, myeloma, leukaemia, and germ cell tumours.
Usually PBSC are cryopreserved using dimethyl sulphoxide (DMSO) with or without hydroxyethyl starch (HES). The original technique involves 10% DMSO and protein levels of 2-4% (addition of albumin or autologous plasma), slow, controlled rates of cooling (1-2ºC/min, controlled rate freezers) and storage in the vapour phase over liquid nitrogen. An alternate cryopreservation technique with 5% DMSO and 6% HES has also shown to be effective when uncontrolled cooling is performed in mechanical freezers at about –80ºC. We have studied the interaction of different cooling rates and different mixtures of the cryoprotectants [DMSO/HES]. Our results clearly indicate that optimum results compared with the pre-freeze values for numerical recovery, membrane integrity (trypan blue exclusion) and colony forming potential (CFU) are achieved at cooling rates from 1 to 5ºC/min with at least 5% DMSO being present. For short-term storage of cryopreserved PBSC (e.g. weeks), mechanical freezers with sufficiently low temperatures (-80ºC) may be acceptable. However, we could demonstrate that storage of PBSC in a mechanical refrigerator at –80ºC lead to a significant loss of cell membrane integrity and clonogenic potential already after 3 months compared to storage for an equivalent amount of time in the vapour phase over liquid nitrogen (<-170ºC). So for reliable long-term storage (e.g. years) we recommend temperatures well below -120ºC, which usually requires the use of liquid nitrogen. Thawing is usually performed at the patient's bedside and vital signs are taken throughout the transfusion. In most centres no post-thaw washing step is performed to remove the cryoprotectant DMSO and debri, because of the cell loss and the danger of clotting. If thawing does not take place at the patient's bedside, storage time after thawing and temperature become important quality parameters. We found that already within a few hours after thawing, cell counts, membrane integrity and viability in terms of colony forming potential decreased significantly.

LM-3.

DETERMINATION OF VITREOUS PHASE IN GRAFT CRYOPRESERVATION

Melissari B[1], Deri E[1], Lantero JC[1], Pérez Campos H[2], Saldías M del C[2], Alvarez I[2]
[1]Laboratorio de Biomecánica. Instituto de Ensayo de Materiales. Facultad de Ingeniería. Montevideo. Uruguay.
[2]Instituto Nacional de Donación y Trasplante de Células, Tejidos y Órganos. Montevideo. Uruguay .
E-mail: blas@fing.edu.uy

Introduction: In cardiovascular tissue cryopreservation processes, a controlled freezing is important to avoid cellular damage produced by osmotic effects, ice formation and physicochemical action of solute concentration growth in the final step of solidification.
Experimental knowledge of the process advises that tissue viability is probably better achieved by vitreous models. In mixed solutions with cryoprotectant agents added, complex solid state are achieved under predetermined cooling programs, with both vitreous and crystalline configurations in variable partitions. In previous works, crystallized fraction was quantified by measuring the cooling curve area changed by heat rejected during crystallization. Direct methods for determination of physical structure of resulting solid are not frequently available in technical literature for these materials. In this paper, a method to determine vitreous fraction of solid complex is established for different concentrations of cryoprotectant by densitometric analysis of their radiographic images.
Materials and Methods: Samples of single RPMI 1640 media and different Dimetilsulfoxide solutions from 10% to 50% v/v with RPMI 1640 in 4 cc criovials were analyzed. They were cooled in a Gordiner Electronics Inc. model 9000 system with TIRO 2000 cooling program at mean rate of -1ºC/min down to -142ºC, and subsequently stored in liquid Nitrogen vapors.
The samples were RX exposed during 0.6 s in a 10 mA Toshiba XRM equipment, obtaining the corresponding electronic radiographic images. The images were analyzed by means of an image processing program to determine density in longitudinal and radial sections.
Results and Discussion: Longitudinal and radial densitometric profiles were obtained from the cryopreservant samples.
The measured density decreases for increasing concentration between 0% to 50% of DMSO, resulting in an increase of the vitreous fraction of the solid, manifested through an increase of the transparency.
Conclusions: the methodology applied to determine the structure of the solid allows the quantification of the vitreous fraction, which relates directly with its capacity to be used effectively in cryopreservation of tissue, in terms of its survival.
Key words: Biomechanics, Cardiovascular tissue, *in vitro*.

L-1.

LAW AND CRYOBIOLOGY: THE INTERSECTION BETWEEN BIOLOGICAL AND BIOGRAPHICAL TIMES

Tau JM.
Asesor Jurídico de C.U.C.A.I.B.A.
E-mail: josemtau@fibertel.com.ar

At the beginning of the XXI century, while visiting this city of Rosario, the Lovaine´s University very well known philosopher, lawyer and bioethics specialist, Professor Jan Broekman, pointed out that the metaphor of the Euclidian space definitively belongs to the juridical evidence since the Roman Empire Law. This was explained by the idea that the juridical identity is well established through the corporeity, and by assuming that the development of the juridical situations related to genetics respond also to the same metaphor as their main reason to be. Also the HuGo Project belongs to this type of idea and allowed for the plain development of the anatomic map once conceived in the XVI century by Vesalio. But the metaphor seems to be not strong enough when the juridicity is challenged by those aspects related to the beginning of human life or when the temporal dimension is incorporated in the medical techniques of cell and tissue cryopreservation. Beside the references related to the gestational time in the Civil Code –in relation to the "nasciturus" recognition of rights- the biological time was practically outside the juridical positive order deeply influenced by the continental- European way of thinking. By the second half of the XX century, there was a need to confirm the real moment of death in order to immediately have the dead body available. In this way and to legally move into the correct diagnosis of neurological death, an especially new biological time was established that mainly considered its confirmation by clinical and instrumental signs as well as by the permanence of the body.

The moral and juridical conflicts associated to the cryopreservation of cells and tissues with the aim of their future transplantation, include not only the processes of procurement, manipulation, cryopreservation and thawing of these elements for their further use, but also the precise meaning and the consequences of the biological time of things such as those "extra-commercium" that by direct or presumptive wish of the donor, are objects of the juridical relation developed through the procurement act, in which the State has an important participation as a regulator and auditor, and also as a direct player of the system.

The *in vitro* fertility techniques that have been around for more than thirty years usually include the cryopreservation of germinal cells, pro-nucleated ovocytes, zygotes and also non implanted embryos. In this last case it has also been debated, in judicial instances, the controversy of the moral and juridical status of embryos and the special category of pre-embryos adopted by some legislation in order to make reference to the blastocyte in the period before implantation. In juridical systems like the Argentine, that lack a related specific law, these procedures take place only under the general considerations of the practice of medicine and the cryopreserved embryos depend only in the best technical and ethical knowledge and commitment of the professionals in charge of these activities in the different centres.

It was only since their purification and culture from embryos generated *in vitro*, around November 1998, that the " Human Primordial Stem Cells" shacked the ethical debate almost in a proportional way as the great hopes generated by this so called "biology of hope", but finally the great debate only took place in the United States after a recent definition of its president to overcome the republican veto to the use of federal public funds for research with these cells obtained from the internal masses of blastocytes derived from assisted fertilizations.

The moral status of the blastocyte is also in the intersection of the temporal dimension. The essential debate is considered by two main philosophical conceptions: the genetic or pre-developmental and the gradualist. This last one current of thinking, mainly in its evolutionist or epigenetic format, moves to the paradoxical idea in which the cryopreserved pre-embryos could have their biological and biographical times dissociated from each other, taking in account the adopted philosophical position.

In this "post-genomic" era, the main question that could be addressed by the only subject directly involved, is the right to live. This dilemma cannot forget an important issue so basic for the genetic and biological sciences such as the objectivity and variability of the genetic information coded in all the human chromosomes. The answer no necessarily implies the recognition of the personality of the embryo, but accepts the fundamental concept that further regulation of cell differentiation depends basically on information programs inherent to it. But also by denying these characteristics of all zygotes or embryos in an early phase, and beside the intense debate about the juridical legacy of the medical practice that can imply its lost or elimination, the philosophical and juridical tradition of the continental-European way of thinking, wouldn´t be completely cancelled and the principle of precaution could find a limit for their generation, cryopreservation and destruction for research purposes.

By considering more than those strictly scientific, philosophical, medical or juridical aspects and also by projecting our ideas to the bio political sphere, all these points, that were just only lightly considered here, require a multidisciplinary approach as that one provided by the Bioethics. Anyway, they will probably keep being a real challenge to whom, by trying to overcome the figurative level, in benefit of the formal thinking, consider the human dignity as a great value and by the necessity of a "real" limit (not only strategic-discursive) to guarantee the efficacy of the law in order to protect life, under the emptiness of the regulatory scenario, the expectations of the society on knowledge and the pressures of the global market. mario@bernaski.com.ar

L-2.
STEM CELLS IN MEDICINE: CAUTIOUS HOPE
Peralta J.
Laboratorio de Metabolismo del Oxígeno. Hospital de Clínicas José de San Martín. Universidad de Buenos Aires.
E-mail: jorghighpear@hotmail.com

Stem cells are called "mother cells" in Spanish language and a very ancient and popular tango song tells us that there is only one mother and though we often forget that, life finally teaches us that we have to go back to this love. Japanese investigators love argentine tango and reprogrammed fibroblasts to IPC cells (induced pluripotent cells) and through bypassing ethical or religious issues went back to mother´s love. Recently the argentine ministry of Science, Technology and Innovative Production created a Regenerative Medicine Comission that deals with basic stem cell research. But when physicians want to implant therapeutically any type of human stem cells to human beings they must submit a research experimental protocol to INCUCAI to be approved. INCUCAI is the national agency which regulates transplantation of cells, tissues or organs and reports to the Ministry of Health. In Argentina there is no specific legislation yet about human *in vitro* fertilization technics or the use of embryonic stem cells in therapeutics. As we know embryos need a gestant mother to develop a human being which cannot evolutionate from an isolated blastocyst . Endometrial implantation often occurs during the first week after oocyte fertilization. As ectodermal neural streak develops around the 14^{th} day, this point is regarded as the end of pre-embryo state (Warnock comission inform) in which embryonic stem cells can be obtained from the inner cell mass in some european countries such as Spain. Besides the universally accepted bone marrow transplantation for hematological diseases, most of published clinical studies with somatic stem cells were carried out in cardiac regenerative therapy in the postischemic myocardium. The trials raised high expectations but none addressed the mechanisms involved in the differentiation of transplanted cells and led to transient clinical benefits not exceeding two years. In the neurological field only well designed works are just now being initiated. In our country many trials are often proposed without previous animal experimentation. Today the results of more than a dozen trials worldwide are already in the public domain but we still do not have a single piece of solid data documenting whether any of the approaches used is capable of regenerating functional cells in the microenvironment (niche) in which are implanted. Mononuclear cells from bone marrow can be administered inmediately after their obtention or like human embryonic stem cells and umbilical chord blood cells (being increasingly used as an alternative stem cell source) can be cryopreserved. The technics are conventional cryopreservation or trough programmable or vitrification methods. Vitrification seems to result in the highest attachment and recovery rates compared with the other two methods. Finally we can say that there is only one mother as the letter of the tango stated but stem cells or mother cells are diverse such as those coming from zygotes, clonotes or partenotes and represent a promise to the future. But for the moment and as a given mother advise we have to take this message: stem cells in medicine represent a cautious hope.

L-3.
A DESCRIPTION OF THREE FROZEN INCA MUMMIES KNOWN AS THE CHILDREN OF LLULLAILLACO
Bernaski M.
E-mail: mario@bernaski.com.ar

Main objectives: the cryopreservation complex was created because of the necessity of preserving, studying and put three pre-Columbian frozen bodies on display in the High Altitude Archeology Museum (MAAM) in Salta. Llullaillaco Children were found in 1999 at 6739 m. over the sea level, on the summit of Llullaillaco volcano in Salta-Argentina.
Ways and Techniques: this complex has a cooler core of etinelglicol of -32ºC, and it is stimulated into the enclosure. This cooling system was adopted in order to diminish the cyclic variation, reaching -20ºC in a continuous and inalterable way. The system gives the possibility to modify the atmosphere inside the chamber, (pressure, nitrogen and oxygen concentration can be regulated.
It is a monitoring system in real time about main variables as: weight, relative humidity, temperature and pressure.
All this, plus the alarm system by gap of the set point values, the possibility of monitoring locally or remotely, make it a very secure and reliable system.
This work came up to different faces of the project: Preliminary studies Hexogen and endogen agents affecting the preservation. Others cases in the world. Preservation condition, the preservation chamber, containment laboratory, levels of security, operation and preservation.
Results: it is known that this type of project have not got comparable examples, because they are unique and isolated cases of special applications of cryobiology. The plan was to obtain temperature stability, which was obtained in a range of + / - 1ºC.
The versatility of the system for switching equipment and predictive maintenance system in place, along with monitoring of the system locally and remotely, on a permanent state that it allows us to meet the complex originally forecast. The system has given consideration upon histological analysis, adipocere and DNA by Dr. Angelique Corthals (Stony Brooks University NY) in December 2008, which expressed the magnificent stay of preservation of bodies in recent years.

S1-1.

FUNCTIONAL AND HISTOMORPHOLOGICAL SCOPE FROM *IN VIVO* BEHAVIOUR OF IMPLANTED CRYOPRESERVED DESFROSTED VASCULAR ALLOGRAFTS

Bia D[1], Zocalo Y[1], Armentano R[1], Laza S[1], Craiem D[1], Perez Campos H[2], Saldias MC[2], Alvarez I[2], Panuncio A[3], Fernandez JD[3], Mariño A[3].
[1]Departmento de Fisiología. School of Medicine. Republic University – URUGUAY. [2]Instituto Nacional de Donación y Trasplante de Células, Tejidos y Organos (INDT). Health Minister – School of Medicine. Republic University - URUGUAY. [3]Departmento de Anatomía Patológica. Clinics Hospital Dr. Manuel Quintela. School of Medicine Republic University - URUGUAY.

Introduction: Cryopreserved/defrosted arteries (CA), have been a reasonable alternative in those clinical pictures with circulatory failure, mainly in lower limb revascularization surgeries with not available autologous veins or contraindicated synthetic prosthesis (i.e. e PTFE in infectd surgical fields).
Native vessels-grafts biomechanical mismatch (BM) is related to graft failure. Previously we demonstrated that our cryopreservation methods, do not affect *in vitro* biomechanic beheaviour.
Aims: 1) To characterize the *in vivo* biomechanical CA properties from Multi Organs Donors (MOD) and to compare them with recipients´ native arteries and arteries from subjetcs with similar characteristics to those of the recipients and multiorgan donors whose arteries were cryopreserved. 2) To analize the histo morphological picture between CA implanted-explanted segmental 2nd surgery with its *in vivo* biomechanical paramethers behaviour obtained.
Material and methods: Arterial presure, diameter, and wall thickness were obteined to quantify local and regional biomechanical parameters and evaluate the arterial eventual remodeling: 1) Non invasive *in vivo* recipients biomechanical studies were performed in: a) 9 implanted segments of femoro popliteal CA arteries; b) 9 native recipients femoral arteries partaker in proximal anastomosis of CA. 2) Non invasive *in vivo* recipients-like arteries: 15 control subjetcts without peripheral arterial desease or surgery; 3) Non invasive *in vivo* MOD-like arteries: 15 control subjetcs with similar haemodynamic conditions of MOD procured. 4) 7 CA segments obtained in 2nd surgery were histopathological analyzed by Optical and Electron microscopy. 5) *in vitro* biomechanical e PTFE evaluation: 10 segments 6 cm length were performed. To evaluate the arterial local biomechanical an functional behavior, the Incremental Elastic Modulus (EINC), the Arterial Compliance (AC), the Arterial Distensibility (AD), and the Characteristic Impedance (ZC) were calculated. To evaluate the bimechanical mismatching between the arterial recipients parameters and other vascular substitute parameters (implanted CA, MOD-like arteries, recipient - like arteries, or e PTFE), the Matching Factor (Γ), was calculated.
Results: Implanted CA were remodeled with increased wall thickness, wall-to-lumen ratio, and wall cross-section area. Implanted CA were stiffer than MOD like arteries, but more compliant than recipents´arteries. The proximal-distal gradual transition in stiffness remained unchanged in all CA length. Remodeling process was confirmed by histological studies with the clear increased thick of the wall, mainly by intimal thickness. There was a inner elastic lamina preservation and lost of elastic fibers from the media with increased collagenic component. Scattered myofibroblast cells of CA media showed fibropositors and collagen fibers emerging from cytoplasm. PCR myofibroblast technology showed the genetic matching with recipients linfocite cells. CA coupling with recipient´s arteries was always superior to e PTFE one.
Conclusions: The *in vivo* implanted CA maintain functional circulatory viability of lower limbs in spite of structural and morphological remodeling of arterial wall with a better haemodinamical behaviour obtained with e PTFE protheses.
Key words: Cryopreserved/Defrosted Arteries, Vessel biomechanical mismatch, *In vivo* Vascular Remodeling, Intimal hyperplasia.

S1-2.

PERCUTANEOUS CRYOABLATION OF SEPTAL ARRHYTHMOGENIC SUBSTRATES

Lanzotti ME.
Instituto de Cardiología del Sanatorio Británico – Instituto Cardiovascular de Rosario (ICR).
E-mail: mlanzotti@yahoo.com

Radiofrequency is the most commonly energy source used for treatment of cardiac arrhythmias. Despite its high success rate, in some cases radiofrequency energy may present limitations, especially in case of anteroseptal atrioventricular accessory pathways. In these patients, inadvertent atrioventricular block may occur during or after the procedure and a high recurrence rate is observed. Since the late 1970s, cryosurgery has been an integral part of the management of cardiac arrhythmias, and recently, animal and clinical studies demonstrated the feasibility and safety of applying percutaneous catheter cryoablation technology. These studies also showed that reversible "cryomapping" can be performed in high-risk arrhythmogenic sites, before creating permanent lesions. In this preliminary report, we describe the successful use of percutaneous cryoablation to permanently interrupt conduction over anteroseptal accessory pathways.
Key words: Wolff-Parkinson-White syndrome, cardiac arrhythmias, percutaneous cryoablation, "cryomapping".

S1-3.
COLD CARDIOPLEGIA IN CARDIAC SURGERY

Baumgartner N[1], Sujatovich V[1], Echeverría C[2].
[1]Banco de Homoinjertos Valvulares y Vasculares, C.U.C.A.I.B.A., La Plata, Argentina.
[2]Servicio de Cardiología Hospital "San Juan de Dios", La Plata, Rep. Argentina.
E-mail: norberto_baum@hotmail.com

Cardioplegic arrest (CA) using cold cardioplegia is clinically used since 1960s. It is currently applied in cardiac surgery and remains the preferred method of myocardial protection for many cardiac surgeons. Cold blood cardioplegia (CBC) has been reported to reduce ischemia reperfusion injury via apoptosis. Is important to analyze cold agglutinins (Immunoglobulin M), are of unique relevance in cardiac surgery because of the cardiopulmonary bypass with CBC. Immunoglobulin M auto antibodies will be catastrophic hemagglutination, microvascular thrombosis, or hemolysis.

The use of cardioplegia is an effective and widespread method for myocardial protection in cardiac surgery. Myocardial ischemia commonly complicates vascular surgery.

Hypothermic crystalloid cardioplegia was first introduced in 1960s, and posterior blood cardioplegia was introduced in the 1970s. Blood cardioplegia provides superior myocardial protection compared with crystalloid cardioplegia.

In terms of perfusion, retrograde cardioplegia (through of the coronary sinus), may be delivered alone or most frequently in combination with antegrade perfusion (through coronary arteries).

A variety of methods are in use and subject to investigations in terms of delivery, pressure, and temperature of the cardioplegic solutions, warm or cold cardioplegia. Cold blood cardioplegia (CBC) has gained reputation in recent years and is currently used in cardiac surgery. CBC provides superior ischemic tolerance. Cardiac arrest during cardiac surgery is a tool for mitigating the iatrogenic ischemia-reperfusion injury. Excessive generation of oxygen free radicals during early phase of reperfusion causes macromeloceluar damage, lipid peroxidation and tissue damages. The alteration in membrane permeability, configuration and cellular proteins caused by oxygen free radicals have been suggested as the main cause for ischemia-reperfusion injury.

Postoperative haemodynamic stability, the course of cardiac enzymes, and the clinical outcome are considered to be the indicators reflecting the efficiency of myocardial protection.

Several studies observed that myocardial lactate concentrations, glycerol at the end of the clamping time and the cumulative CK-MB release was significantly higher in those hearts protected with warm blood cardioplegia. Others studies reported that the viability of the myocardium, as assessed by oxygen utilization and functional recuperation, were better conserved with simultaneous antergrade and retrograde cold blood cardioplegia administered continuously. CBC produce a permanently lower septal temperature and with a significant reduction of the pressure variations in the coronary sinus. Several studies suggest that oxidative stress and anaerobic metabolism were significantly higher in patients undergoing coronary artery by-pass grafting (CABG) using warm cardioplegia.

Were compared, different techniques of cardioplegia and reported that lactate production is greatest during cardioplegia and increases during aortic cross-clamp interval. The most important difference was at the cross-clamp removal. Lactate production could persist after 10 minutes of reperfusion in those patients receiving warm antegrade cardioplegia. Increased lactate production may be an expression of elevated glycolytic activity or fatty oxidation with anaerobic production of high-energy phosphates. The lower CK-MB values in patients treated with cold cardioplegia suggest less myocardial injury, and are associated to better ventricular performance. Hearts protected at cold temperatures may have a higher sensitivity to circulating catecholamines, with can improve left ventricular performance.

It was observed markedly lower concentrations of lactate, and hypoxanthine in the CBC at the time of cross-clamp removal. Troponin T and CK-MB mass are consistent markers of injury. They demonstrated that CBC was associated with better left ventricular performance, reduced myocardial ischemia and significantly reduced incidence of sever events, combination of hospital deaths and post cardiopulmonary by-pass intraaortic balloon pump implantation.

Myocardial dysfunction was associated in cardiac arrest to local processes that contribute to stress such ischemia, ischemia-reperfusion injury (IRI), and associated to myocardial stunning.

Apoptosis has been considered as one mechanism of myocardial cell loss during IRI. CBC is superior that crystalloid cardioplegia in inhibiting apoptosis during ischemic arrest. Recent studies showed that cardiac arrest with cold crystalloid cardioplegia causes activation of the caspase-cascade and myocardial apoptotic cell death in humans. The apoptosis is induced in cardiomyocytes, suggesting that it may play a key role in myocardial dysfunction in cardiac surgery.

Retrograde cardioplegia alone is often used in aortic valve and aortic root surgery. Due to the differences in venous anatomy between the right and the left side of the heart, retrograde cardioplegia is associated with incomplete protection of the right side. Increased apoptotic death of cardiomyocytes after retrograde cardioplegia as compared with the antegrade procedure implicates that retrograde cardioplegia alone provides inferior cardio protection against IRI both in the right and the left ventricle.

It was demonstrated that despite continuing advances in cardioplegic solutions and myocardial protection, myocardial tissue suffered significant activation of caspase-dependent and caspase-independient apoptosis mediators. Progress in cardioplegic solutions would therefore likely benefit from incorporation of anti-apoptotic strategies for myocardial protection and improved clinical outcomes. Myocardial protection is crucial, not only for early recovery but also late survival.

Key words: cardioplegia, heart surgery, apoptosis, cold agglutinins

S2-1.
THE WHITE GENOME PROJECT

Mac Cormack W.
Universidad de Buenos Aires and Instituto Antártico Argentino.
E-mail: wmac@ffyb.uba.ar

Introduction: Several industrial products or biotechnological processes are based on the metabolic properties of microorganisms. Among these microorganisms bacteria are the most relevant ones. At the present days, due to their unexplored and novel character as well as for the biotechnological potential of their autochthonous microorganism, the extreme environments are the main targets for the bioprospecting programs (Podar and Reysenbach, 2006). Environments permanently exposed to low temperature are the best sites for isolation of psycrophilic and psychrotolerant microorganisms and a number of industrial processes have been developed using these microorganisms or their products. Several enzymes, as proteases phosphatases and lipases are commercialized due to their high catalytic activities at low temperature (Collins *et al*., 2007) and other kind of compounds, as exopolysacharides and polyunsaturated fatty acids are also members of the current "cold molecules" market. Antarctic continent, due to its climate conditions are one of the most promised sites for the isolation of new cold-adapted microorganisms.

Based in the above mentioned background, the Instituto Antártico Argentino subscribed with Biosidus company the "White Genome" project which included the following objectives: i) Isolation and characterization of psychrophilic and psychrotolerant bacteria from Antarctic biotopes for further screenings of biological activities of industrial interest, ii) Isolation and description of a new species of psychrotolerant bacterium, iii) Whole genome sequencing of the newly described bacterium.

Isolation and characterization of Antarctic psychrotolerant bacteria:

During the last 5 years a systematic search of Antarctic bacteria was carried out in different terrestrial and marine habitats. A culture collection composed by more than 400 isolates, preserved at -80°C and freeze-dried, was constructed. Although the identification studies are yet in progress, a significant fraction of the marine isolates have been characterized using amplified rDNA restriction analysis (ARDRA) with the enzymes *Hpa*II and *Alu*I. Obtained phylotypes were grouped and members of each group were identified by sequencing the 16S rDNA. A clear dominance of members of *γ-Proteobacteria* was observed, *Pseudoalteromonas, Pseudomonas, Psychrobacter* and *Psychromonas* being the most abundant and ubiquitous genera.

Isolation and description of *Bizionia argentinensis* sp. Nov:

One Gram-negative non-motile rod (JUB59T) isolated from Antarctic surface sea-water of Potter Cove (62° 14´S, 68° 40´W), 25 de Mayo Island (King George Island) was deeply characterized. Cells produced yellowish-orange colonies on marine agar. Optimum growth temperature was 22-25ºC (range 2-28ºC) and no growth was observed at 30ºC. According to the phylogenetic analysis based on the comparison of the complete 16S rRNA gene sequence with sequences from closely related strains, JUB59T belonged to the family *Flavobacteriaceae* and represented a novel species of the genus *Bizionia.* Although it showed a high sequence similarity with *Bizionia myxarmorum* ADA-4T (97.4%) and *Bizionia algoritergicola* APA-1T (97.1%), the DNA-DNA relatedness of JUB59T with these two strains was low (15.9-17.3% and 19.3-22.1%, respectively). The dominant fatty acids of strain JUB59T as well as their main polar lipids agree with those found in other members of the genus. The DNA G+C content was 34 mol %. On the basis of these data, strain JUB59T was classified as a novel species in the genus *Bizionia* and the name *Bizionia argentinensis* was proposed and accepted (Bercovich *et al*., 2008).

Whole genome sequencing of the new species *B. argentinensis*:

A draft of the whole genome of *B. argentinensis* was obtained with the Genome Sequencer FLX (Roche). Large-size genomic DNA samples were randomly fragmented into small (300-800-bb) fragments by physical shearing. Fragments were treated to obtain a library consisting of 404,465 reads with a length average of 277.1 bp. Assembly of these reads generated 77 contigs from which a consensus sequence consisting of 3.29 Mb was generated.

Conclusion: This work yielded an interesting cold-adapted bacterial culture collection for the search of new biotechnological applications. One of the isolated strains was a novel species from the recently described (genus *Bizionia* and its whole genome was sequenced. At present, the analysis and edition of the genome are in progress. Information about this new genome could offer new data about the genetic adaptation of psychrotolerant bacteria to the cold environment some of which could represent the base for new biotechnological processes.

Key words: *Bizionia argentinensis*, Antarctic bacteria, psychrotolerant, bacteria, genome sequencing

S2-2.

ANTARCTIC BACTERIA AS BIOREMEDIATION TOOLS

Ruberto L[1,2,3], Vazquez S[1,2], Dias R[1], Mac Cormack WP[1,3].
[1]Cátedra de Microbiología Industrial y Biotecnología, FFyB, UBA.
[2]CONICET.
[3]Instituto Antártico Argentino.

Bioremediation of cold soils require the use of low-temperature adapted organisms (Aislabie *et al.*, 2006). The most adequate places to obtain cold-adapted microorganisms are those permanently exposed to low temperature. In this sense, Antarctic soils are considered as model habitats to searching for this kind of microorganisms. Cold-adapted organisms are generally assorted into two groups: psychrophiles and psychrotrophs (or psychrotolerant). Psychrophilic bacteria are able to growth optimally below 15°C but are also unable to grow above 20°C. Psychrotolerant bacteria are able to grow at low temperatures but are not restricted to such condition and have optimum growth levels above 20°C (Morita, 1975). In summer, temperature in some regions of the Antarctic Peninsula can reach 10°C during sunny days rising soil temperature to 15 -20°C. For this reason, psychrotolerant bacteria represent the most adequate option for using in any soil bioremediation processes under these conditions.

The aim of this work was the isolation and characterization of psychrotolerant bacterial strains and consortia and the evaluation of their hydrocarbon removal efficiency under field assays carried out in Antarctica.

Isolation of the bacterial tools

The bacterial strains and consortia were isolated by enrichment cultures with hydrocarbons as sole carbon source (Jobson *et al.*, 1972). In this sense, strain DM1-41 was obtained using crude oil. Strains MP2-4 and M10dp and consortia J13 and M10 were obtained using polycyclic aromatic hydrocarbons (PAHs). All the strains were psychrotolerant gram negative rods. The partial sequence of the 16S DNAr showed that DM1-41 is related with *Stenotrophomonas rhizophila* (T) (99,7%), MP2-4 with *Pseudomonas migulae* (T) (99,4%) and M10dp with *Sphingobium xenophagum* (T) (97,3%). All these genera are referred as common inhabitants of Antarctic hydrocarbon contaminated soils (Stallwood, 2005; Saul *et al.*, 2005).

In order to isolate the main components of M10, an analysis of the culturable components of the consortia was also performed. Having these M10-derived isolates it would be possible to reconstruct the consortia in a reproducible and defined way for their use as innoculum. With this objective in mind, two liquid cultures of M10 were carried out using either gasoil (GO) or phenanthrene (Phe) as sole carbon and energy source. After 24 and 192 h cultures were sampled and serial dilution were plated in agarized saline basal media (SBM) containing gasoil or a mix of GO and Phe as carbon source. Four sets of isolates were obtained. Using RISA (ribosomal intergenic spacer analysis) the isolates were grouped in clusters and representative members of each group were identified by partial sequencing of 16s DNAr. Three main groups were detected: *Pseudomonas, Stenothrophomonas* and *Pedobacter.*However, members *of* the *Pseudomonadaceae* family were by far the most abundant ones. When the growth capacity of M10 consortia at different temperatures was analyzed, the results suggested that it is a psychrotolerant consortium rather than a psycrophilic one. Using GO as substrate, apparent growth rate at 20, 15 and 10°C were 0,350 h^{-1}; 0,078 h^{-1} and 0,032 h^{-1} respectively.

On site soil bioremediation assay in Antarctica

A bioremediation field assay was made using chronically hydrocarbon contaminated soil from Marambio Station. The assay was carried out in microcosms performed in metal trays containing 2,5 kg of soil. The experiment includes: abiotic control (AC), community control (CC), biostimulation of the autochthonous microflora (BAM), and three systems where biostimulation was combined with bioaugmentation: i) mix of bacterial strains described previously (S+B); ii) M10 consortium (M10+B); J13 consortium (J13+B). During the assay, temperature range was -7.8°C-+7.3°C (mean: + 0.5°C) and included 26 days in which temperature dropped below 0°C. Counts of total heterotrophic aerobic bacteria (THAB) and hydrocarbon degrading bacteria (HDB) were analyzed by plate-counting using caseine-peptone-starch (CPS) agar and agarized MSB-GO (AGO) respectively. Total petroleum hydrocarbons (TPH) were quantified by FT-IR (EPA 418 method). Results showed a rise in biological activity evidenced as an increase in THAB and HDB counts. At the end of the assay (45 d) the number of HDB was significantly higher in all systems (BAM, M10+B, S+B and J13+B) compared with the control system. TPH decreased from the initial concentration (11.972 ppm) to 6.460 ppm in the abiotic control (AC) and 1.687 ppm in the M10+B system. However, the efficiency of hydrocarbon removal observed in M10+B was not significantly different from those obtained with the BAM.

Conclusions

On site bioremediation of contaminated cold environments, like Antarctic soils, is possible using the potential of psychrotolerant bacteria and the biostimulation strategy. Bioaugmentation combined with biostimulation seemed to be not advantageous compared with biostimulation only.

Key words: bioremediation, Antarctic contaminated soil, hydrocarbon, psychrotolerant bacteria

S2-3.
BIOLOGICAL POTENTIAL OF ANTARCTIC VIRUSES

López JL.
Cátedra de Virología, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires.

At a salinity level of 34 g/l sea water freezes at approximately -1.8°C and the growing ice crystal matrix excludes the salts when it is formed. When ice is originated from freshwater, the result is a hard brittle solid with the primary inclusions being gas bubbles. In contrast, when sea water freezes, the resultant ice is a semisolid matrix, permeated by a complex system of brine-filled channels and pores. Changes in the properties and characteristics of pores and channels in sea ice therefore are caused by physical and possibly also by biological factors. An enlargement of pores has the advantage of increasing diffusive and even turbulent transport of organic and inorganic matter. Brine channel walls constitute large surface areas that can be colonized by algae and bacteria representing sites for attachment, locomotion and grazing. The total surface area of internal brine channels ranged from 0.6 to 4.0 m^2/kg of ice. Krembs *et al*. (1998) gave first evidence about the space which is available for colonization by microorganisms and they estimate that between 6 and 41% of the brine network surface area at -2°C may be covered by them. This is a high value compared with soils, where less than 1% of the total surface area is covered by microorganisms.

Sea ice is one of the coldest habitats on the earth for marine life, with temperatures ranging from 0 to -35°C. Despite the obvious low temperatures, sea ice is characterized by highly changeable salinity, pH, dissolved inorganic nutrients and dissolved gas and light signatures. Heterotrophic bacteria and unicellular algae represent the two major groups within sea-ice assemblages that have been best studied at present.

Although viruses were largely ignored in the marine biodiversity studies until about a decade ago, they are the most numerous of biological entities in the ocean and it is now known as influencing many biogeochemical and ecological processes (Sandaa, 2008). Viruses are concentrated in sea ice, when compared with the underlying water column, by the same factors acting on bacterial cells. They also occurred in greatest abundance in those parts of the sea ice where bacteria were most active. A few years ago, large (>110–424 nm capsid diameter) viruses were reported in Antarctic sea ice (Gowing *et al*., 2002). Although these viruses have the size and form to adequate to infect a range of algae and protozoan, no diatoms and only a few microheterotrophs and algae were observed to be infected. These bacteriophages belong to the tailed, double-stranded DNA phage families Siphoviridae and Myoviridae, and all the phages investigated as part of this study were host-specific.

Microbial diversity is fundamental for the maintenance and conservation of global genetic resources. As extreme environments are explored, the richness of microbial diversity emerges as is increasingly evident. This fact emphasizes the relevance of the studies about Antarctic environmental microbiology which are largely scarce at the present days.

On the other hand, biodiversity is affected by environmental disturbances and measures must be taken to estimate, record, and conserve microbial diversity as a way to preserve the human health.

The dynamic nature of virioplankton abundance may be affected by seasonal or climatic changes as well as physico-chemical parameters (Wommack and Colwell, 2000). Through lysis, viruses directly limit productivity of the host community and serve to maintain clonal diversity in the host communities.

The objective of our study is to determine the dynamic structure of Antartic virioplankton and baterioplankton populations by employing RAPD fingerprinting as well as cloning, sequencing and molecular phylogeny of most abundant genetic elements.

We have described the virioplankton morphology using electronic microscopy and the nucleotide sequences from a freshwater ice sample. This genetic information might have a biological and biotechnological potential mainly if some anti-freeze protein could be cloned.

Key words: virioplankton, Antarctica, biological potential.

S2-4.

COLD-ADAPTATION OF BACTERIAL MEMBRANE LIPIDS

Altabe SG, de Mendoza D.
Instituto de Biología Molecular y Celular de Rosario. IBR-CONICET.
E-mail: altabe@ibr.gov.ar

Variability and adaptability are crucial characteristics of organisms possessing the ability to survive and prosper in a wide variety of environmental conditions. A universally conserved adaptation-response observed among bacteria and most, if not all, poikilothermic organisms is the adjustment of membrane lipid composition at low temperatures, referred to as homeoviscous adaptation. Temperature markedly affects membrane physical state, and changes in lipid composition occur to assure optimal membrane structure and function.
In bacteria, membrane fluidity can be optimized by modifying the types of fatty acids that are attached to glycerol backbones since the structure of these fatty acids determine the biophysical properties of the membrane bilayer. The major way by which bacteria, lacking cholesterol, maintain this functional membrane physical state, is by increasing the proportion of low melting point fatty acids in the membrane lipids as the growth temperature decreases**.** Membrane fluidity can be optimized by modifying its fatty acids composition, that is: changing the proportion of branched-chain fatty acids, the acyl chain length or introducing double bonds. *cis*-Unsaturated fatty acids (UFAs) introduce a pronounced kink that disrupt the order of the bilayer and results in lower transition temperature than of one composed of straight –chain saturated fatty acids. Also, anteiso-branched chain fatty acids promote more fluid membranes than the iso fatty acids.
Our group has focused on the study of the biosynthesis and the regulation of UFAs by growth temperature in *B. subtilis.* Bacilli cells respond to a decrease in ambient growth temperature by introducing double bonds into fatty acids. These double bonds are inserted by specific fatty acid desaturase enzymes. *B. subtilis* contains a unique desaturase, which is encoded by the *des* gene which is tightly regulated by temperature at the transcriptional level. Desaturases are present in all groups of organisms and play a key role in the maintenance of the proper structures and function of biological membranes. They introduce a double bond at specific positions in fatty acids of defined chain lengths and utilize molecular oxygen and reducing equivalents from an electron transport chain. The importance of the desaturase enzymes in UFAs biosynthesis and the unique chemistry of the reaction that they catalyze have focused our interest in understanding the biochemistry of the desaturation reaction. It is known that the reaction requires oxygen and utilizes NAD(P)H and flavoproteins as electron donors, however the electron transport system of the Δ5-acyl lipid desaturase from *B. subtilis* has not been yet characterized. The "*in silico*" analysis of the genome of *B. subtilis* reveals the existence of two flavodoxins YkuN and YkuP, and a *fer* gene and we propose that they could act as putative electron donors for the Δ5-desaturase. We have demonstrated that mutants in any of these genes are not impaired in UFAs synthesis. Interestingly we found that in *B. subtilis* the function of de Δ5-desaturase is impeded when both, ferredoxin and flavodoxins are absent indicating that both flavoproteins can act as electron donors for UFAs synthesis in these bacteria.
In a previous work we reported that the double bond of UFAs synthesized by *B. subtilis* is located exclusively at the Δ5 position, regardless of the growth temperature and the length chain of the fatty acids. However, the determinants of the enzymatic specificity of membrane desaturases are not understood. Genes with significant similarity to fatty acid desaturases have been found in the genomes of many bacteria. The existence of structurally related desaturases, with different substrate recognition and double bond-positioning properties offers the opportunity to compare the active site structures of members of this family of enzymes. *In silico* analysis on Bacillus genomes allowed us the identification of highly similar desaturases. We have characterized two desaturases from *B. licheniformis* and two from *B. cereus*. Site-directed mutagenesis and domain swapping techniques will help us to elucidate which residues or regions in these enzymes are important for the substrate recognition and double bond-positioning activities of desaturases. The dissection of this molecular mechanism in these bacteria will provide important insights into the basic question on the desaturation reaction. Information gained from this research could potentially lead to the design of desaturases capable of producing new industrially useful isomers of monounsaturated fatty acids or specific inhibitors potentially useful as antibiotics.

S2-5.

STRUCTURAL INSIGHTS INTO THE SIGNAL TRANSDUCTION MECHANISM OF THE COLD SENSOR DesK

Albanesi D[1,2], Martín M[2], Trajtenberg F[3], Mansilla MC[2], Alzari P[1], Buschiazzo A[1,3], de Mendoza D[2].
[1]Institut Pasteur, Unité de Biochimie Structurale & URA CNRS 2185, 25 rue du Dr. Roux, 75015, Paris, France.
[2]Department of Microbiology, IBR-CONICET, National University of Rosario, Suipacha 531, (2000) Rosario, Argentina.
[3]Laboratory of Structural Biology, Institut Pasteur of Montevideo, Mataojo 2020, Montevideo 11400, Uruguay.

Temperature sensing and adaptation are essential for the survival of living cells. Organisms respond to changes in environmental temperature either by regulating body temperature or through adaptive behavioral thermoregulation. Although great strides have been taken towards understanding the fundamental process of temperature detection in many organisms ranging from bacteria to man, the molecular mechanisms of thermosensing are yet to be discovered in most systems. The best documented early effect of environmental cold on cellular processes is a decrease in membrane fluidity. In bacteria, generally lacking cholesterol, membrane fluidity is largely determined by the proportion of unsaturated fatty acids in the phospholipids. A simplified way to visualize the problem is to consider the shape of the fatty acids. The straight chain saturated fatty acids are linear and pack efficiently to produce a bilayer that has a high phase transition and low permeability properties. The double bond of unsaturated fatty acids introduces a pronounced kink in the chain, which disrupts the order of the bilayer and results in lower transition temperatures and higher permeability. Accumulating evidence indicates that cold-induced membrane lipid rigidification is sensed by membrane proteins acting as cold sensor regulators. In bacteria, the most studied system leading to thermal adaptation is the Des pathway of *Bacillus subtilis*. This pathway is composed of the DesK/DesR two-component system (TCS) and Δ5-acyl desaturase (Δ5-Des), the unique desaturase in this organism encoded by the *des* gene. DesK is a prokaryotic histidine kinase that has an N-terminal sensor domain (~150 residues) composed of four or five transmembrane (TM) segments connected to a C-terminal cytoplasmic catalytic core (DesKC, ~220 residues). DesR is a cytoplasmic response regulator that specifically controls the expression of the *des* gene. DesK is the founding example of a membrane bound thermosensor suited to remodel membrane fluidity when the ambient temperature drops below ~30ºC. Induction of the Des pathway is brought about by the ability of DesK to assume different signaling states in response to variations in membrane fluidity. At cold temperatures, the increase in the proportion of ordered membrane lipids favors a kinase-dominant state of DesK, which undergoes autophosphorylation in a conserved histidine residue (H188). The phosphorylated kinase then transfers the phosphate to DesR. DesR-P tetramer binds to the *des* promoter, leading to recruitment of RNA polymerase and activation of *des* transcription. Activation of *des* results in the synthesis of Δ5-Des, which introduces double bonds in the acyl chains of membrane lipids. These newly synthesized unsaturated fatty acids decrease the phase transition temperature of the phospholipids, favoring the phosphatase activity of DesK on DesR-P and turning off of transcription. Genetic and biochemical evidence suggests that the balance of two antagonistic DesK activities determines the DesR phosphorylation state: a phosphate donor for DesR and a phosphatase activity for DesR-P. As the activity of the transcriptional activator DesR is modulated by its phosphorylation state, the output of the DesK/DesR signal transduction pathway is determined by switches between kinase-biased and phosphatase-biased DesK activities. The balance between these activities would be regulated by changes in growth temperature that, in turn, dictates the fluidity of membrane lipids. It has been shown that *in vitro*, DesKC catalyzes its autophosphorylation, the phosphorylation of DesR and the desphosphorylation of phospho-DesR. However, the switch of DesKC from a kinase-biased to a phosphatase-biased activity is not temperature-regulated *in vivo*, suggesting that DesK TM segments play a crucial role in thermosensing and thermoadaptation. To establish how fluctuations in ambient temperature affect the phosphorylation state of DesK, we solved the crystal structure of its kinase cytoplasmic domain in three signaling states and determined the functional properties of the full length sensor in pure lipids vesicles. We propose a model in which the kinase-on to kinase-off transition involves a complex rearrangement of the central coiled-coil four helix bundle domain controlled by the TM sensing domain, which in turn detects a thermal input stimulus through changes in the order of the lipid bilayer. This regulation mechanism could be operational in a wide range of sensor proteins detecting a variety of different stimuli.

Key words: cold sensing, lipids, desaturase, regulation, signal transduction

1.

MODULE METER FOR THE DETERMINATION OF THE AORTIC VALVES ELASTIC PROPERTIES

Melissari B, Deri E, Lantero JC.
Laboratorio de Biomecánica, Instituto de Ensayo de Materiales. Facultad de Ingeniería de la Universidad de la República Oriental del Uruguay.

In order to determine the elastic properties of aortic valves, a device named module meter was designed, which measures the volumetric deformation of a small circular membrane subject to a pressure difference. The pressure-volume curve obtained allows to calculate the elastic module and therefore to characterize the properties of the valves. To validate the device, well known materials were tested, comparing the results with those obtained with standard methods. Determination of elastic properties of aortic valves presents the proper difficulties of any material obtained in reduced dimensions. The module meter designed allows the measurement of a mean Young's modulus representative of its elastic properties. The module meter is composed of an electric air compressor, a pressure regulator valve and a sample support. The pressure is applied from the bottom on a circular area of 5mm diameter and is measured by a 0-300 mm Hg Bourdon manometer with 0.5 mm Hg appreciation. The volume is measured by a water column over the sample with an appreciation of 0.5 mm3. The sample is cut approximately 8 mm diameter and is glued using Cianoacrylate drops on an Acrylic washer.
Device equation: a) Circular plate fixed on the edge. b) Isotropic material. This reduces the elastic constants to Young's modulus E, ratio between unit axial stress and deformation in a tensile test, and Poisson's ratio ν, ratio between transversal and axial deformations.
f = deflection, E = Young's module, p = pressure, h = thickness, r = radius,ν = Poisson's ratio, D = bending rigidity

$$f = \frac{p.r^4}{64D} \quad D = \frac{E.h^3}{12(1/\nu^2)}$$

Theoretic and experimental studies indicate that in a broad pressure range the membrane acquires a spherical configuration.

For the conditions of the tests, the relation between V and f is the equation of a spherical cap: $V = \frac{\pi}{6} f(3r^2 + f^2)$

For the module meter designed, r = 2.5 mm and is assumed ν = 0.45
E = Young's module, MPa; f = maximal deformation, mm; p = pressure, mm Hg; h= thickness, mm; V = volume, mm^3 $E = \frac{0{,}08\,p}{h^3}\frac{}{f}$ and $V = 0{,}52\,f\,(18{,}7 + f^2)$
To validate the results, rubber and polyethylene films were tested in the module meter, obtaining similar results than in standard tests. Finally, a sample of aortic valve was tested. The module meter allows the determination of the Young's modulus of a small thin sample without the precision of an standard test, but sufficient to characterize the material and to establish quality criterions as it is for the case of aortic valves for which the test was designed.
Key words: Young's module, aortic valves.

2.

HIPOTHERMIC PRESERVATION OF HEPATIC MICROORGANS (HMOS) IN BES-GLUCONATE SOLUTION. PROTECTIVE EFFECTS OF POLYETHYLENEGLYCOL (PEG) ON THE TOTAL WATER CONTENT AND FUNCTIONAL VIABILITY

Mandolino C[1], Pizarro M[1], Rodríguez J[1,2], Mamprin M[1,2].
[1]Centro Binacional (Argentina-Italia) de Criobiología Aplicada. CAIC. Suipacha 531 (2000) Rosario.
[2]Unesco Chair in Cryobiology.

We have previously reported the development of an alternative solution to preserve hepatocytes that have three key components: gluconate, sucrose and BES. This novel preservation solution (BGS) was equally effective as UW to protect rat hepatocytes against cold preservation injury due to ischemia and reoxigenation (1). In order to extend the use of this solution to other organs as the liver, we evaluate the effect of the addition of PEG of different molecular weights (8, 20 and 35 kDa) (2) to BG Solution on the total water content and functional viability of HMOs. The HMOs were manually cut from wistar rat livers into slices of 432±36 µm thickness, n=50. They were preserved up to 48 hs in anoxia at 0 ºC in 5 different solutions: ViaSpan, BGS, BG8 (BG + 4% PEG 8), BG20 (BG + 4% PEG 20) and BG35 (BG + 4% PEG 35). Daily, we have evaluated the LDH Release (%) and the Total Water Content. After 48 hs, only the HMOs preserved on BGS showed a significant difference on LDH Release (50.3±1.2* vs 25.7±0.7 for BG8, 20.2±0.9 for BG20, 18.9±0.5 for BG35 and 21.2±1.1 for ViaSpan) and were unable to regulate the Total Water Content (anova, $p<0.05$, n=4). After the preservation period, all the preserved groups were reoxygenated (120 min, 37ºC, KHR) and were evaluated the LDH Release, Total Water Content, Oxygen Consumption (µmolO_2/min mgprot) and Glycogen Content (mgGly/g liver). Freshly cut HMOs were used as controls. After 120 min, there was no difference between groups on the LDH Release and the Total Water Content and only the HMOs preserved on BG35 showed values similar to those of the Controls for Oxygen Consumption (4.5±0.8 vs 5.2±0.4) and Glycogen Content (18.0±1.5 vs 12.8±4.9 (n=4). Given that intracellular acidosis developed as a result of cold ischemia has a detrimental effect on proteins function, a preservation solution must have a high buffering capacity (BC) and must be able to regulate the pH. We have studied the BC (meqH^+/pH/L) of the solutions and the Evolution of the Extracellular pH of the HMOs suspensions. ViaSpan has the lowest BC (11.3 vs 39 for BG8, 38 for BG20 and 35 for BG35) and, after 48 hs, the extracellular pH of HMOs suspensions preserved in this solution was statistically different from 7.40 ($p<0.05$, n=3). The results described above demonstrate that BG35 could be an alternative preservation solution that is easier to prepare, has a lower cost and has a better capacity to maintain the pH between physiological values than ViaSpan. Also, only HOMs preserved for 48 hs in BG35 showed a respiratory activity and glycogen content similar to Controls.
Key words: hepatic microorgans, BGS preservation solution, PEG.

3.

ULTRASTRUCTURE OF HUMAN VALVULAR FIBROBLASTS THAT HAVE SUFFERED AN INCREMENT OF THE CRYOSTORAGE TEMPERATURE

Bessone V[1], Fiorucci MP[2], Izaguirre MF[2], Baumgartner N[3], Rodríguez J[1,4], Biancardi M[1,5], Quintana A[1,5].
[1]Laboratorio de Investigaciones en Criobiología (LIC). [4]Farmacología y [5]Morfología, Dto. de Ciencias Fisiológicas. Facultad de Ciencias Bioquímica y Farmacéuticas, Universidad Nacional de Rosario.
[2]Laboratorio de Microscopía Aplicada a Estudios Moleculares y Celulares, Facultad de Ingeniería, Universidad Nacional de Entre Ríos.
[3]Banco de Homoinjertos valvulares y vasculares. CRAISur, C.U.C.A.I.B.A., Hospital San Juan de Dios, La Plata. Argentina.

Human heart valve allografts are widely used for clinical purposes. Furthermore, with the improvement of preservation techniques such as cryopreservation, the use of human heart valve allograft for transplant has become more widespread. Therefore, the maintaining of preservation conditions of the allograft and the evaluation of indicators of this preservation are essential. In the present study, we analyzed by Transmission Electron Microscopy the tisular organization of human valve fibroblasts from two experimental groups: human cardiac valves conventionally cryopreserved (HVA_{cryo}) and human cardiac valves that suffered a gradual increment of the cryostorage temperature during 72 hs from -147ºC up to -47ºC and then restored to -147ºC ($HVA_{\Delta T}$). Pieces of valves from both experimental groups were fixed in a solution of 3% gluteraldehyde, 3% formaldehyde and 1% of picric acid saturated solution in phosphate buffer 0.1M, pH 7.40. After 2 hs, specimens were rinsed, dehydrated in acetone series and included in araldite. Sagital and longitudinal sections of 70 nm thick were obtained by a Reichert Ultracut S ultramicrotome, stained with uranyl acetate and lead citrate and photographed with a Philips EM 201 microscopy. Valves from group HVA_{cryo} showed active fibroblasts with fine nuclear chromatin, abundant secretory vesicles, and surrounded by abundant collagen fibers randomly distributed. Some fibroblast had cytoplasmic filaments actin- and myosin-like, suggesting myofribroblasts. In contrast, valves from group $HVA_{\Delta T}$ showed clear signals of cryo-injury. Alterations in cellular and nuclear membranes, less electrondensity of mitochondria, disorganization in the nuclear chromatin and collagen network suggest osmotic injury. We concluded that temperature elevation during cryostorage period (100ºC) seriously affect fibroblast morphology suggesting the impairment of its biosynthetic and functional properties. Damages suffered by collagen fibers could affect valve biomechanical properties.
Key words: Transmission Electron Microscopy, cardiac valves, cardiac valve fibroblasts, cryopreservation

4.

DETERMINATION OF CRYOPRESERVED CARDIAC VALVE VIABILITY USING THE MTT COLORIMETRIC ASSAY

Coda Zabetta C[1], Bessone V[1], Baumgartner N[2], Biancardi M[1,3], Rodriguez J[1,4], Sujatovic V[2], Quintana A[1,3].
[1]Laboratorio de Investigaciones en Criobiología. [3]Morfología y [4]Farmacología, Dto. de Ciencias Fisiológicas. Facultad de Ciencias Bioquímica y Farmacéuticas. UNRosario.
[2]Banco de Homoinjertos valvulares y vasculares. CRAISur, C.U.C.A.I.B.A., Hospital San Juan de Dios, La Plata. Argentina.

The current standard method of determining valvular viability in homograft is the XTT assay. The tetrazolium salt reduction is a quantitative method developed to evaluate the activity of mitochondrial dehydrogenase enzymes within living cells. Our purpose was to develop a method to determine cryopreserved cardiac valves viability using MTT assay instead of XTT. In order to achieve this aim, we assay the fibroblast mitochondrial activity from two experimental groups: human cardiac valves conventionally cryopreserved (HVA_{cryo}) and human valves that have suffered a gradual increment of the cryostorage temperature ($HVA_{\Delta T}$), from -147ºC up to -47ºC during 72 hs and then restored to -147ºC. This temperature rise was due to a technical failure. Also, porcine cardiac valves (PCV) were used to set up the protocol to perform MTT assay in valvular tissues. Briefly, PCV or HVAs were cut into small pieces weighting about 20, 40, 60 and 80 mg and each piece was maintained during the procedure in Krebs-Henseleit (KH) solution, pH=7.40 on ice. Each piece of tissue was placed into a dish containing 2.4 mL of KH solution at 37ºC in a Dubnoff shaker incubator bath and 600 µL of MTT solution (5mg/mL, pH=7.40, MTT final concentration: 1 mg/mL) was added. Samples were incubated during 60 minutes under carbogen atmosphere and gently shaken. After that, each sample was put in a test tube containing 3 mL of extraction reactive (30 volumes of aqueous 10% SDS and 70 volumes of isopropanol/HCl 0.04 N) to dissolve and extract formazan. Test tubes were shaken with a vortex for 30 seconds and sonicated during 2 minutes for a complete formazan extraction. Finally, the test tubes with the samples were incubated during 30 minutes in darkness to complete the extraction of the formazan. The absorbance of the formazan extracted was measured at λ=570 nm. Reaction blanks at time 0 for each piece of tissue were obtained cutting the leaflets into pieces of similar weight as the samples processed above. The only difference was that immediately after the MTT was added, the tissue was transferred to a test tube with extracting solution and incubated for 30 minutes in darkness. Although group HVA_{cryo} showed little formazan production it was significant higher than group $HVA_{\Delta T}$ since fibroblast viability from this last group could be seriously affected by temperature rise altering its mitochondrial enzymatic activity. In conclusion, the MTT colorimetric assay designed to evaluate fibroblast mitochondrial activity proved to be useful.
Key words: cardiac valve fibroblast, MTT, fibroblast viability

5.

EXPERIMENTAL OBSERVATIONS ON THE HYPOTHERMIC PERFUSION OF THE RAT LIVERS. I- THE EFFECT OF TEMPERATURE ON OXYGEN EXTRACTION

Rodriguez J, Federico MB, Scandizzi AL, Mamprin M, Guibert E[1].
Centro Binacional (Argentina – Italia) de Criobiología Clínica y Aplicada (CAIC) y Farmacología, Depto. Cs. Fisiológicas, [1]Biología Molecular, Depto Cs. Biológicas, Fac. Cs. Bioq. y Farm., Universidad Nacional de Rosario, Suipacha 531, S2002LRK Rosario, Argentina.

Introduction: New preservation strategies such as hypothermic machine perfusion (HMP) could facilitate the use of so-called marginal livers for transplant. One key question that has remained is whether oxygenation of the preservation solution during HMP is a requirement for an effective preservation. We have set up an experimental device to investigate the oxygen delivery and consumption while the liver is still exposed to a perfusion-preservation medium at low temperatures (1).
Aims: In this study we investigated the liver oxygen extraction during hypothermic perfusion at 10 and 5ºC, The preservation solutions tested were: Custodiol® solution (Franz Kholer Chemie GMBH, Germany) and the BGS solution prepared in our laboratory (2). Each solution was bubbled with Medicinal Synthetic Air at the selected temperatures at a gas flow of 600 mL/min during 20 min before use.
Experimental protocol: After excision, the liver flushed with the preservation solution was weighed and then connected to the perfusion circuit. Each liver was perfused at constant pressure up to 4 hours. A Clark´s electrode was used for O_2 concentration measurements.
Calculations: Liver oxygen concentration was calculated as was described in (1). Liver oxygen extraction (E_{ox}) was calculated using the standard formulae: $E_{ox} = \{[O_2]_{inflow} - ([O_2]_{outflow} - [O_2]_{cannulae}\} \div [O_2]_{inflow}$
Results: First, we investigate the effect of the perfusion time on the E_{ox} of isolated rat livers perfused at 10ºC with air-equilibrated BGS or Custodiol. We found a decrease of E_{ox} from 30 to 120 min of cold perfusion, and then the E_{ox} remains stable (Custodiol: 30 min-0,83 ± 0,07; 90 min-0,70 ± 0,20; 120 min-0,49 ± 0,07*; 240 min-0,45 ± 0,03*, n=3 preparations, * different from T 0 to 90 min. $P<0.05$). Second, we determine the effect of temperature on the E_{ox} of isolated rat livers perfused at 5 or 10ºC with BGS or Custodiol solutions after 120 min of perfusion. The results shows a clear cut difference of E_{ox} at normothermic versus hypothermic perfusion temperatures (Controls -37ºC: 0,86 ± 0,03; Custodiol- 10ºC: 0,45 ± 0,03 and 5ºC: 0,36 ± 0,01*; BGS- 10ºC: 0,38 ± 0,01 and 5ºC: 0,22 ± 0,02*, n=3 prep. * different from 37 and 10ºC, $P<0.05$).
Conclusion: Cooling process affects the handling of gases as oxygen by the liver; this information could be useful to determine the appropriate metodology to supply essential levels of oxygen during cold preservation and in HMP.

6.

OXYGEN CONSUMPTION OF HUMAN CARDIAC VALVES THAT HAVE SUFFERED AN INCREMENT OF THE CRYOSTORAGE TEMPERATURE

Bessone V[1], Pizarro M[1], Rodriguez J[1], Baumgartner N[2], Biancardi M[1], Furno G[3], Quintana A[1].*
[1]Fac.de Cs. Bioquímica y Farmacéuticas, UNRosario – Centro Binacional de Criobiología Clínica y Aplicada.
[2]Banco de Homoinjerto valvulares y vasculares. CRAI Sur, CUCAIBA. H.I.E.A. y C. San Juan de Dios, La Plata.
[3]Area Estadística Fac. de Ciencias Bioquímicas y Farmacéuticas, UNRosario.
**E-mail: aquintan@fbioyf.unr.edu.ar*

In the Valvular and Vascular Heart Banking, San Juan de Dios Hospital, La Plata City, Argentina (CRAISur, C.U.C.A.I.B.A.) in 2003, several cryopreserved human heart valve allografts (aortic and pulmonary) suffered a gradual increment of the cryostorage temperature (from -147ºC up to -47ºC) during 72 Hs due to a technical failure (group $HVA_{\Delta T}$). The temperature was restored to -147ºC. The valves were discarded for clinical purposes since there were no reports about the possible damages they could have suffered. Since the Health Ministry of Buenos Aires has authorized the use of the valves for research purposes, we have been performing morphological and functional studies to assess if the valves from group $HVA_{\Delta T}$ had been properly discarded for transplant. A good functional test is the measurement of tissue endogenous respiration, which is an index of metabolic integrity and is depressed in pathological states. The objective of the present study was to assess and compare the oxygen consumption of conventionally cryopreserved human cardiac valves (HVA_{cryo}) and $HVA_{\Delta T}$. Valves from experimental groups HVA_{cryo} and $HVA_{\Delta T}$ were cut in pieces between 100-250 mg. Each piece was put into a thermostatized oxygen electrode chamber containing 9.4 mL of respiration medium (Krebs-Henseleit media), pH = 7.40, equilibrated with air. Oxygen uptake rate was measured using a Clark-type oxygen electrode and recorded using a Sekonic chart recorder. The respiration rate was assessed under two different conditions: a) Basal or endogenous respiration (Vend) and b) Succinate-stimulated respiration (Vsucc), after the addition of 120 µL of a 940 mM succinate solution to the chamber (12 mM final concentration). Porcine cardiac valves (PCV) were used to set up the protocol. The oxygen consumption (V_{O2}) was calculated as follows:

$$V_{O2} = \left[\frac{\mu moles\ O_2/mL\ .\ Chamber\ Volume\ .\ \Delta s/\Delta t}{total\ scale}\right] \div g\ tissue$$

Where: µmol O_2/mL is 0.524 µmoles/mL; Chamber Volume is 9.4 mL; $\Delta s/\Delta t$ is the scale diminution per minute determined for each piece of tissue; total scale is the recorder full scale obtained after sensor calibration.
There were no statistical differences between groups HVA_{cryo} and $HVA_{\Delta T}$ in Vend and Vsucc but significant differences were found in the relationship Vsucc/ Vend (index of cellular membrane damages) and was higher for group $HVA_{\Delta T}$. Based on this last result, we conclude that valves that have suffered an increment of the cryostorage temperature have an altered cytoplasmatic and mitochondrial membrane integrity.
Key words: cardiac valves; oxygen consumption; cardiac valve fibroblasts