Despite my eclectic interests and fairly regular change of place and resulting circumstance, research on the effects of high altitude on the cardiovascular system has remained an enduring allure in my career. Initially, I was drawn to this field as a consequence of the India-China war. In 1962, the Chinese army launched an attack in different sectors of the Himalayan regions. The Government of India, caught off guard, reacted in panic by hastily dispatching several thousand soldiers to elevations of 3,500 to 5,000 meters above sea level. Woefully ill equipped, ill trained and unacclimatized for mountain warfare, these soldiers succumbed in massive numbers; still larger numbers developed a mysterious illness within a few days of arriving at these altitudes, coughing up blood and unable to breath. It was only about a year later that it became clear that these men were developing high altitude pulmonary edema (HAPE), a condition that had hitherto been described only in case reports. As a doctor in training, I was curious about this little known condition. When the mountainering institute at Manali was established as part of a strategy to prepare for any possible future confrontation in the mountains, I immediately joined to train as a mountaineer.

At the institute I developed an abiding fascination for the yak. I was intrigued with their dexterity on the slopes and indifference to the rarified air. All expeditions to Mount Everest had used the yak to haul heavy loads to camps over 6,000 meters. I learned that the yak belonged to the bovine family, and was in fact the first cousin...
of the cow. Nonetheless, cattle cannot survive at high altitude, developing brisket disease with severe pulmonary hypertension and right heart failure. I could not find an explanation as to why the yak is so totally adjusted to high altitude, while the cow has an adverse reaction. My intuition prompted me to believe that perhaps the vital clue for comprehending mountain sickness lay in this distinction.

A scholarship to Oxford necessitated a change of focus, and I obtained a D.Phil in physiology and acquired experience in cardiology. By now I had developed a keen interest in heart failure. In 1984, I returned to England to study the role of neurohormonal activation in heart failure with Peter Harris. Although dormant, my desire to understand the differences in response to altitude between the yak and the cow still held my attention. I was aware that Peter and his dear friend, the eminent pathologist, Donald Heath, had found that the cameldids, like the llama in the Andes, do not respond to hypobaric hypoxia by vasoconstriction. I asked Peter if it was possible that the yak may have similar mechanisms of adaptation. Peter suggested we undertake to examine the conjecture. The initial studies at the Whipsnade Zoo, near London, confirmed that the pulmonary artery pressure of the yak was indeed low.

The effects of high altitude on the cardiovascular system have remained an enduring allure in my career

Encouraged by the preliminary results, a team comprising Peter Harris, Donald Heath, David Williams, Roberto Ferrari, and myself was organized to go to the Himalayas to investigate the hemo-dynamics of the yak at 3,500 to 5,000 meters. We were able to substantiate that the yak at those altitudes did not show an increase in pulmonary arterial pressure and that the lack of hypoxic vasoconstriction was due to the absence of vascular smooth muscle in the pulmonary arterioles. Over the next ten years, we made several expeditions to the region. The monks of the Tak Tok monastery, in the village Shakti (4,500 m) where most of our research was done, slowly warmed to our presence. They brought to our attention that the yak was not only an immediate relative of the cow but also mated with the cow, the dzo being the progeny. Like most crossbreeds the male dzo is sterile, while the female dzo-mo is very fertile; she mates with the yak bull to produce the stoll, and with the cow bull to produce the gar. Soon we were able to show that the lack of pulmonary vascular smooth muscle, and consequently the hypoxic vasoconstrictive response, in these crossbreeds is inherited as an autosomal dominant trait. After work on a number of animal species in different parts of the world and over a number of years, it became obvious that animals indigenous to high altitude were adapted to hypobaric hypoxia by developing biochemical, physiological and anatomical features that were genetic in nature, allowing the species to explore the environment to its best advantage.

It was now only natural to question if humans indigenous to high altitude had also adapted to hypoxia. In 1928, Carlos Monge described chronic mountain sickness (CMS) in Peru, a condition in which severe polycythemia develops in some individuals after a prolonged stay at high altitude. Generally attributed to a breakdown in acclimatization, we wondered whether it was no more than a few months of arrival from the plains of China to Lhasa (3,500 m). Upon examination and reexamination of the autopsy material, we realized to our amazement that we were observing a syndrome that, to our knowledge, had never been seen or reported. The condition affected mostly infants of Han origin who presented with congestive heart failure within 2 months of being brought from low altitudes to live in Lhasa. The most striking feature was right ventricular hypertrophy, and a dilated pulmonary trunk. The ratio of the right to left ventricular weight was 3 times that of age matched Tibetan controls. The pulmonary arterioles showed muscularization with development of a thick muscular media. In contrast, the Tibetan age matched controls had thin walled pulmonary arteries and a single elastic lamina in the pulmonary arterioles. Clearly, unlike the Han infants, the Tibetan infants appeared to be genetically adapted to hypoxia. We called this condition subacute infantile mountain sickness.

The news bulletin of the international society for heart research

In this issue of Heart News and Views, we begin a new series of autobiographical articles entitled, “The Heart of the Matter: A Career in Cardiovascular Research”. We are privileged to begin our series with the contribution of Dr Inder S. Anand, Professor of Medicine at the University of Minnesota Medical School and Director of the Heart Failure Program at the VA Medical Center in Minneapolis, MN.

Dr Anand was charged to provide an autobiographical account that must include specific advice to young scientists interested in pursuing a similar career path, and he has graciously responded with a fascinating account of his research experiences in the field of high altitude physiology and medicine. We are greatly indebted to Dr Anand for setting such a high standard with this initial installment in the series, and hope that you enjoy sharing the wisdom of your colleagues as they recount their stories of a life lived in the pursuit of cardiovascular research.

Leslie Anderson Lobaugh, Ph.D.
Regardless of my responsibilities as a cardiologist in Chandigarh, the question of whether humans indigenous to high altitude were adapted never receded from my mind. In 1988, chance gave me yet another opportunity to find the answer. A call from the army headquarters informed me that they were unable to comprehend a strange epidemic that was bedeviling their personnel at the Siachin Glacier (5,000 to 6,500 m). The next six weeks were spent investigating these patients, who evidenced a remarkable pathophysiology of heart failure. Healthy soldiers, after ten to twenty weeks of being stationed at altitudes of 5,000 to 6,500 meters, started to develop a syndrome: insidious in onset, with gradual increase in shortness of breath and edema leading to gross anasarca. ECG and echocardiography showed right ventricular hypertrophy and dilatation but normal LV structure and function. Hemodynamics confirmed moderate pulmonary hypertension. All the abnormalities reverted to normal within 12-16 weeks of transfer to sea level. I decided to call this syndrome "adult subacute mountain sickness," because it bore all the characteristics of "subacute infantile mountain sickness." Both the infantile and adult variety could be considered the human counterpart to brisket disease in cattle.

To continue our work on mountain sickness, we established a permanent laboratory in Leh (3,500 m). Here we found that the pulmonary arterioles of native Ladakhi highlanders, who had died accidentally, lacked vascular smooth muscle, much like those of the yak. Taken together, the rarity of the syndrome in Tibetan infants and Ladakhi adults, and the absence of vascular muscle in native Ladakhis, strongly suggested that humans indigenous to high altitude have also adapted to hypobaric hypoxia.

At the end of my venture across the terrain of high altitude medicine, there is a deep sense of gratitude in knowing that, by making the classification of mountain sickness more comprehensive, we have made a contribution to the understanding of the impact of altitude on humans and animals. However, over and above all, there is the feeling of immense fulfillment and the lessons learned. I offer these in the hope that young scientists may find something therein to assist them on their journey. As Einstein said, "your generation must put a critical aspect of our research would have been lost.

- A hypothesis, while needed to direct observation and design experimentation, should never impede the ability to remain open to all probability. An attitude to discover rather than to prove engenders humility to be sensitive to alternate opinion. If we had not listened to Dr Sui, the monks were, after all, the source of the crucial information on the crossbreeds.

In the final analysis, scientific research is not only for stimulating the reasoned mind to scrutinize and explain phenomena, it is as much a dedication of spirit to unravel the truth in the service of wisdom and beauty. The resolve to defy the many challenges this entails can only be sustained by an unwavering passion for the calling. Science is a jealous lover craving absolute commitment of time and ardor.

Inder S. Anand, M.D.
Minneapolis, MN
Dear Reader,

As you know, August is holiday time in Italy. Being 100% Italian, I began my holiday two weeks early and I am writing this letter while relaxing in Boca di Magra by the sea. My thoughts have turned to an earlier visit by Tom Ruigrok and his family. We went out on my boat, and I still have the memory of a Dutch professor basking in the shade of the caves on a fantastic stretch of Ligurian coast called Le Rocce Rosse, while every Italian was fighting to get more sunshine! Time goes by so fast, and I only hope that we will be able to repeat this experience.

Because I am on holiday, instead of giving you an official report of ISHR business I thought that I would tell you a little about the history of Bologna, and thus tempt you to attend the 2007 World Congress which will be held there.

The University of Bologna was founded in 1088. It is the oldest university in the western world, and has influenced the entire history of European Universities. Throughout history, Bologna has been an important centre for learning in many fields of study: methodology, logic, judicial and theological sciences during the Middle Ages, and medicine, physics and mathematics during and after the Renaissance. Bologna produced major contributors to these areas of knowledge, including such scholars as Aldrovandi, Malpighi, Marsili, and Galvani. The University has also had teachers of the highest caliber, among them Carducci, who received the Nobel Prize for Literature in 1906. Thanks to Carducci, the University of Bologna virtually symbolised the new Italian culture.

Bologna is the chief town in Emilia Romagna. You must pass through it when going from the north to the south of Italy. The town began as an Etruscan centre, then Gallic, and finally it became a Roman colony. The wonders of Bologna are the arches and towers throughout the city. Bologna is considered La Mecca for food, and it is best know for Tortellini, Parmesan Cheese and an enormous variety of sausages – Mortadella being the most famous. However, for the Italians it is the city of homemade fresh pasta, and if you visit Bologna you will be able to meet the families that still produce it. Lambrusco is the local wine. Bologna has a population of around 500,000, and a famous international airport that connects it to cities worldwide.

I hope that you will forgive me for such an informal letter, but I promise that I will be back again soon with more “official” ISHR business. Tom, please be assured that Le Rocce Rosse are still there, and that my new boat has a roof to provide shade.

Roberto Ferrari
The key to Köhler and Milstein’s success lies in the fusion of cells; they fused mouse myeloma tumor cells to spleen cells derived from a mouse which previously had been immunized, using Sendai Virus to accomplish fusion. Later polyethylene glycol or addition of a strong electric field were used. Köhler and Milstein were lucky because they chose normal antibody producing cells from a mouse that had already been activated by immunization. Activated cells fuse 100 times better than non-activated cells. In addition, their hybrid cells were stable and continued to produce antibodies. Hybridomas were selected out in a tissue culture medium which did not support growth of the parental cell types. By successive dilution or by plating out, single clones could be established in which all the antibodies produced were identical, had the same Ig class and allotype, and the same structure, affinity and specificity for a given epitope.

While Milstein and Köhler’s hybridomas were fusions from mouse cells, human clonal antibodies can now be constructed by using recombinant DNA technology; this can entail chimeric constructs in which the mouse domains are spliced onto human genes, resulting in antibodies which are less immunogenic in humans.

Who were Georges Jean Franz Köhler and César Milstein? Köhler was born in 1946 in Munich and died in 1995 of myocardial failure when he was only 48 years old. He studied biology at the University of Freiburg and in 1971 went to the Basel Institute of Immunology where he obtained his Ph.D. In 1974 Köhler moved to England for a 2-year postdoctoral fellowship to work with Milstein in the medical research council laboratory in Cambridge. César Milstein was born in Argentina in 1927, the son of European immigrants. He obtained his Ph.D. in Argentina, as he writes “with no economic support.” He then moved to the department of biochemistry in Cambridge, then returned to Argentina, and then moved permanently to Cambridge to work with Fred Sanger, the head of the division of protein chemistry. Upon the advice of Sanger, he switched from biochemistry to immunology and began to work on the fusion of two myeloma cell cultures. It was then that Köhler joined him and this cooperation led to the production of hybridomas. Instead of hybridizing two myelomas, they hybridized a myeloma and an antibody producing cell. The two men had met first at a lecture Milstein gave in Basel, and their cooperation led to work which changed immunology. As Milstein wrote in his obituary of Köhler: “We both concluded that it was this combination of knowing how to fuse cells and wanting specific myelomas that led to the substitution of one fusion partner for spleen cells of an immunized animal.”

It was, so Milstein reported, the speed at which everything happened which was so remarkable. The paper in Nature was submitted when Köhler had been in the laboratory for just one year. But the journal agreed to publish the report only on condition that it be condensed to an abbreviated letter format. This misjudgment of great advances by editorial reviewers is all too common. How difficult it is to cross the barrier from obscurity to recognition! Government agencies also failed to appreciate the potential of the discovery and no patents were applied for. After finishing his second year as a postdoctoral fellow in Cambridge, Köhler returned in 1976 to the Institute in Basel and hence to Freiburg as Director of a Max Planck Institute. He left it to others to commercially exploit monoclonal antibodies, which became a billion dollar business.

Köhler’s tenacity to learn and his approach to difficult situations was shown in Cambridge when he found himself living in a house with a piano and decided to learn to play. He refused to take piano lessons, so he bought the (continued on page 9)
Tess became quite frightened and discoloration of his lips, fingers, and toes. She then aroused James, who had a bluish disposition at birth; James, on the other hand, now seemed listless and his lips discolored and his strength restored. The following day, James was released, no longer cyanotic, could be due to a hole in James’ heart,” he reported. “Congenital heart disease, it’s called. We must obtain a chest x-ray.” The x-ray revealed James’ heart to be of normal size and there was no suggestion of cardiovascular malformation. Nonetheless, Dr Hutchins met with the Pearsons and recommended James be admitted for observation. Later that day, Dr Hutchins looked in on James in the nursery. He was receiving \( O_2 \), but his cyanosis persisted. The following day, James was released, no longer discolored and his strength restored. After only a few days at home, James again turned blue. He was readmitted to the hospital, where he once again recovered.

At 1 month of age, John was growing nicely despite his seemingly weak disposition at birth; James, on the other hand, now seemed listless and his lips were gray. Perhaps it was the dim light in the nursery that lit the house. The next morning was bright and sunny. Tess rose early to change John’s diaper and fed him. She then aroused James, who had a bluish discoloration of his lips, fingers, and toes. Tess became quite frightened and immediately awoke Fester. “Fetch my father!” she exclaimed. “We need to go to the doctor. James is ill.” Within the hour they had driven to the hospital in Vita.

Practitioner Dr Hutchins learned that James had been the larger and healthier of the two at birth. He inquired as to whether either boy had recently been sick. Diarrhea, perhaps? Except for the present spell and perhaps some listlessness, James had not been ill. John was well. “This bluish discoloration, or cyanosis, could be due to a hole in James’ heart,” he reported. “Congenital heart disease, it’s called. We must obtain a chest x-ray.” The x-ray revealed James’ heart to be of normal size and there was no suggestion of cardiovascular malformation. Nonetheless, Dr Hutchins met with the Pearsons and recommended James be admitted for observation. Later that day, Dr Hutchins looked in on James in the nursery. He was receiving \( O_2 \), but his cyanosis persisted. The following day, James was released, no longer discolored and his strength restored. After only a few days at home, James again turned blue. He was readmitted to the hospital, where he once again recovered.

Rain finally appeared on Tuesday, July 1. Evenings were now warmer and the wood-burning stove no longer needed. The next morning Tess woke James to find him with a third episode of cyanosis. However, on this occasion the cyanosis was so generalized and intense that when Dr Hutchins saw James he referred the Pearsons back to Dr Medwick. That evening, Tess made provisions to take the train to Winnipeg. John would remain behind and stay with Tess’ mother, Mary, who would feed him with milk from Bessie, Tess’ cow.

Upon their arrival in Winnipeg on Thursday, James’ cyanosis was barely perceptible. Additionally, Dr Medwick could find no evidence of heart or lung disease. He was perplexed as to the cause of James’ intermittent cyanosis. “I suggest the next time James has an episode you call me straightaway. I will catch the first train to Vita and examine James.” A frustrated Fester and worried Tess returned home that day to find Tess’ father, George, pacing the pavement, his anxiety evident. “John has turned blue, and your mother is fit to be tied,” he reported. Upon their arrival at the Barnett farm, Tess found John to be cyanotic. “Fester, telephone Dr Medwick in Winnipeg and ask him to come to Vita. Tell him James appears to be fine while John is now the one who is blue.” Medwick arrived on Saturday morning.

What is your diagnosis?

Since the Pearson’s visit to clinic and during the train ride to Vita on July 4th, Dr Medwick considered various possibilities. He was prepared to address them. Parents and grandparents had not taken ill while James had become cyanotic on several occasions and now so had John.

Cyanosis, the presence of 5 g/dL or more of reduced hemoglobin in capillaries, may be subdivided into central and
Peripheral types. In the central type, there is either arterial blood desaturation or an abnormal hemoglobin derivative. Mucous membranes and skin are both affected and discolored. Peripheral cyanosis is due to either a slowing of blood flow or an abnormally large degree of $O_2$ extraction from normally saturated arterial blood. Causes of peripheral cyanosis include generalized vasoconstriction, low cardiac output associated with blood loss, cardiogenic shock, or venous occlusion.

James did not have any of these. Two other things argued against peripheral cyanosis: it was generalized; and did not improve on supplemental $O_2$.

Medwick found John to be drowsy and his skin, lips, hands, and toes had a bluish discoloration but there was no evidence of cardiopulmonary disease. From his doctor’s bag Medwick pulled out a test tube and syringe. He withdrew blood from John. It was chocolate-colored! Even when he swirled the tube and its blood, mixing it with ambient oxygen, the chocolate color remained. This further ruled out reduced hemoglobin due to hypoxemia and entities such as congenital heart disease with a right-to-left shunt or intermittent respiratory airway obstruction. He then removed a vial from his bag; it contained a solution of methylene blue. He administered 0.5 cc intravenously and within the hour John’s cyanosis disappeared. The diagnosis was now made: methemoglobinemia.

The question that now challenged Medwick was why the twins had developed methemoglobinemia (metHb). The fact that James recovered in the hospital and on the train to Winnipeg could also be explained by his removal from an offending agent in the environment. Aniline dyes from blankets or wax crayons? Aniline derivatives, such as phenacetin? None seemed likely. Nitrates? But from where? Well water? He would have to search the premises.

The well at the Barnett’s had been drilled last year and was well constructed. Nevertheless, Medwick took a sample for subsequent examination. Over at the Pearson’s decrepit farmhouse Medwick requested “show me where you obtain your drinking water.” Tess took him out back to the rain barrel, which was filled with water after the recent rain. “When the barrel is empty, Fester fetches water from our well,” she remarked. “With the drought we had these past weeks, James’ formula was prepared with well water.” The well was old, in poor repair, and situated in a gully downstream from the Pearson’s house, Bessie’s small barn, and the outhouse. Medwick could only imagine the high nitrogenous concentration that had accumulated underground from human and animal waste. This must be the source of nitrates as he took a sample. “Let’s see Bessie,” he asked Tess. They found the old cow drinking water from her trough. “Where does this water come from?” he asked. “Rain water mostly. But with the drought, Fester filled it with well water.” Medwick noted “ruminants, like cows and sheep, have a rumen filled with bacteria that can convert nitrates to more toxic nitrites. Monogastric animals, like pigs and chickens, have no rumen and the nitrate they consume is eliminated in urine. So the milk John received from Bessie must have been high in nitrites and nitrates. Newborn infants have little acid in their stomach and the bacteria present converts nitrates to nitrites. Let me have a sample of Bessie’s milk for analysis.”

“This is all so fascinating,” said Tess. “Maybe Bessie has nitrate poisoning and that’s why she seems so run down and why her milk production is so scanty. Should we check her for cyanosis?” Medwick beamed at the idea. Sure enough, when he lifted Bessie’s upper lip to examine her mucous membranes, there was the telltale bluish discoloration.

That night, Tess called over to her husband dozing in a chair. “Fester, I’ve been thinkin’. I’m going back to high school this fall to get my diploma. Maybe I could go on to the university and become a veterinarian.” She paused a moment. “By the way, Fester, go fetch rain water for James’ formula. And take your time. I have a headache.”


Karl T. Weber, M.D.
HAVING arrived in the culturally rich city of New Orleans to attend the 27th annual meeting of the American Section of the International Society for Heart Research we were greeted by an abundance of both sunshine and heat (two entities not associated with Scottish summers!). Although the good weather was a more than adequate reward for the long trek across the Atlantic the best was yet to come as we were treated to a fantastic meeting full of numerous educational and social highlights.

A stimulating forum, compiled by the exceptional conference committee, commenced with an inspirational lecture from Prof. Louis J. Ignarro, a Nobel laureate from the University of California, Los Angeles. Prof. Ignarro’s talk described nitric oxide (NO) as a unique signalling molecule and illustrated how exercise can enhance the beneficial effect of NO on the cardiovascular system, a belief he appears to hold steadfast as evidenced by an entertaining collection of slides documenting his triumphs as a marathon participant. His seminar also described his successful path to achieving the coveted Nobel Prize and served to dispel some of the mystique surrounding the award ceremony, which proved to be of great interest to both senior and junior investigators alike.

The high quality of Prof. Ignarro’s lecture proved not to be an isolated event, as it was followed by a series of excellent educational speakers all of whom are highly accomplished in the field of cardiovascular research. Amongst them were Dr Edward Frohlich, who delivered the excellent ‘Janice Pfeffer Distinguished Lecture’ on left ventricular hypertrophy, and Dr Eric Olson (2005 Outstanding Investigator) who gave an elegant speech on transcriptional control of heart development and disease.

The 16 symposia, which were presented throughout the course of the meeting, encompassed a wide spectrum of cardiovascular research and were well received by a diverse audience. The conference also showcased 150 posters of an extremely high standard in the form of two evening poster sessions, the abstracts of which were published in the JMCC 38(5), 2005.

The extravagant Grand Ballroom of the Fairmont Hotel was the setting for the banquet dinner on the penultimate day of the conference. A winning combination of mouth-watering cuisine, an abundance of wine, and live entertainment provided by one of the finest local jazz bands proved to be the makings of a highly enjoyable night. The informality of the evening allowed the participants to indulge in an ‘uninhibited’ environment, which extended to the buzzing Bourbon St. after dinner, and the acquisition of an assortment of traditional Mardi Gras beads. The evening was also highlighted by the announcement of the winner of the prestigious young investigator award, Dr Asa B. Gustafsson from the Scripps Research Institute, for her talk on the contribution of Bnip3 to myocardial ischaemia/reperfusion injury and autophagy.

As research students we thoroughly enjoyed the meeting and revelled in the opportunity to converse with so many distinguished and experienced scientists. In our opinion, meetings such as these are ideal opportunities to amalgamate senior and junior scientists, facilitating the exchange of both ideas and experience, and thus more post-graduate students should be encouraged by their supervisory teams to attend. Finally, the meeting was both an educational and social triumph, which was thoroughly enjoyed by all participants, and all in all an event...
New Orleans August 2005

I was asked to write a short note about hurricane Katrina. Thankfully, Mobile received only wind damage from Katrina’s fury as the city is mostly on high ground. Since the storm came ashore 100 miles from Mobile, we felt that we would escape; in fact, we experienced a strong hurricane with many trees down and power outages all over the city. My electricity came back on 4 days after the storm and most Mobilians had some property damage. For those that were closer to the sea the storm was equivalent to a tsunami as a 10-meter wall of water surged over the beach along 100 miles of the Gulf Coast. All structures near the water were ravaged from Mobile Bay to New Orleans. The civil defense here is good; all low-lying areas were evacuated prior to the storm so the loss of life was minimal. Even so, some people either failed to obey the evacuation or were unable to leave and several hundred people died. That number surely will rise as they drain New Orleans.

My university in Mobile was only lightly damaged and we were back in operation within a week of the storm. Those in New Orleans were not so lucky. People evacuated the city and then were unable to return due to the flooding. Many people were scattered, and employers, friends, and even their families have had trouble locating them. The American Physiological Society has maintained a bulletin board to exchange messages between displaced physiologists. Tulane and LSU physiology faculty have turned up in California, Mississippi, Georgia, Arkansas, Texas, and even Guatemala. They will not be able to return to their homes any time soon. LSU plans to hold medical school classes at a Baton Rouge campus while Tulane students will probably resume their classes in Houston, Texas.

Mobile is flooded with evacuees who have no homes to return to. Those that are fortunate are renting apartments and hotel rooms while those of less means still live in shelters. The government has been simply overwhelmed and a clear plan has not yet emerged from them. Private charities have led the way for much of the recovery, foremost among them the American Red Cross. Donations to them will go directly to Katrina’s victims. http://www.redcross.org/

Most of our members have been to a New Orleans meeting as it is one of the greatest venues in the world. Fortunately, the French Quarter was not flooded and most of the city that we knew remains intact. New Orleans will recover and be better than ever. Rest assured you will be able to enjoy many more meetings in the Big Easy.

Jim Downey, Ph.D.

Köhler, Milstein, and Monoclonal Antibodies

(continued from page 5)

score of a piece by Chopin and began to teach himself to play. Apparently he was able step by step to play this work. Köhler’s death was a terrible loss for humanity, for science, and for his family. As Milstein wrote, “Once I told him that we were being blamed for our alleged failure to take a patent. His comment was ‘we are not business men, we are scientists.’” How times have changed!

References


Richard J. Bing, M.D.
It was a great honor to receive the 2005 Young Investigator Award at the XXVII Annual Meeting of the American Section in New Orleans for my work on the mitochondrial pro-apoptotic protein Bnip3 in ischemia/reperfusion injury. I am originally from Stockholm, Sweden, and came to the United States to study at the University of California, San Diego, where I completed my Ph.D. in the Department of Pharmacology in 2001. I moved to the Scripps Research Institute to do my postdoctoral training with Dr Roberta A. Gottlieb, where I recently made the transition to faculty. Our laboratory is interested in the process by which cells self-destruct, and we are currently most interested in the mitochondrial alterations that develop during ischemia/reperfusion (I/R). My research has focused on elucidating the role of Bnip3 in I/R injury.

**Cell Death in Ischemia/Reperfusion**

Cardiovascular disease is the leading cause of death in North America and is predicted to become more prevalent as our population ages. Cardiovascular disease can be initiated by multiple factors, but in recent years it has become apparent that a major contributing factor is the loss of myocardial cells. Cell death can occur in a destructive, uncontrolled manner via necrosis or by the highly regulated process of apoptosis. Until recently, the loss of myocytes was attributed to necrosis; however, it is now clear that apoptosis may play an important role in the pathogenesis of a variety of cardiovascular diseases. Importantly, cardiac myocytes are terminally differentiated; once destroyed they are not replaced. Consequently, with fewer myocytes, the ability of the myocardium to sustain contractile function is reduced.

**Mitochondria and Bcl-2 Family of Proteins**

The mitochondria play a key role in apoptosis. They provide the energy necessary for the completion of apoptosis and release important pro-apoptotic factors such as cytochrome c into the cytosol. The Bcl-2 family proteins are important regulators of the mitochondrial pathway of apoptosis in the cardiovascular system. This family consists of both pro- and anti-apoptotic members. Bnip3 is a pro-apoptotic member of the Bcl-2 family and is primarily localized to the mitochondria. Although Bnip3 mRNA can be detected in multiple organs, its physiological function is unknown. We have found high levels of basal expression of Bnip3 in the adult myocardium, indicating that Bnip3 is maintained in an inactive state by an unknown mechanism in the absence of death stimuli.

**Contribution of Bnip3 to I/R Injury**

We have established a technique of TAT protein transduction into isolated perfused hearts where linkage of the 11-amino acid transduction domain of HIV TAT to a protein allows it to be readily transduced into cells in the heart (Reference). To investigate whether Bnip3 plays a role in mediating I/R injury in the rat heart, we generated a TAT-fusion

![Figure 1](image-url). **Effect of TAT-Bnip3ΔTM transduction on I/R injury.** Rat hearts were perfused with TAT-proteins for 15 min and then subjected to 30 min of global ischemia followed by reperfusion for 15 min (for superoxide production) or 120 min (for infarct size). **A.** Creatine kinase activity in the coronary effluent was measured for 15 min before ischemia and for the first 15 min of reperfusion (n=8, *p<0.05). **B.** Infarct size was determined by TTC staining (n=4, *p<0.05). **C.** Superoxide levels were assessed by measuring dihydroethidium (DHE) conversion to ethidium (n=4, *p<0.05 vs. control, **p<0.05 vs. I/R+TAT-β-gal).
protein encoding the carboxyl terminal transmembrane deletion mutant of Bnip3 (TAT-Bnip3ΔTM) which has been shown to act as a dominant negative to block Bnip3-induced cell death. Perfusion with TAT-Bnip3ΔTM significantly reduced both creatine kinase release and infarct size in hearts subjected to 30 min of global ischemia and 120 min of reperfusion compared to TAT-β-gal (Figure 1). I/R injury is associated with increased production of reactive oxygen species (ROS), such as superoxide anion, hydrogen peroxide, and hydroxyl radical. To assess whether the cardioprotective effects of TAT-Bnip3ΔTM could be attributed to a reduction in the production of ROS, superoxide generation was measured in heart slices obtained after 30 min of ischemia and 15 min of reperfusion. Using dihydroethidium staining to detect superoxide production, we found that TAT-Bnip3ΔTM attenuated superoxide production after I/R, suggesting that increased ROS production is partly due to activated Bnip3 in I/R hearts (Figure 1). Perfusion with TAT-Bnip3ΔTM before ischemia also improved functional recovery after I/R compared with TAT-β-gal perfused hearts. Moreover, we found that hearts perfused with TAT-Bnip3ΔTM exhibited reduced cytochrome c and AIF release after I/R, and that addition of recombinant Bnip3 to mitochondria isolated from the rat heart resulted in the release of cytochrome c and AIF.

Ischemia/Reperfusion Results in Bnip3-Mediated Induction of Autophagy

Autophagy plays an important role in cellular homeostasis and is the process by which cells recycle cytoplasm and dispose of excess or damaged organelles. Since several studies have linked dysfunctional mitochondria with up-regulation of autophagy, we speculated that Bnip3-induced mitochondrial damage in I/R might lead to induction of autophagy in cardiac myocytes. A characteristic of autophagy is the recruitment of the microtubule-associated protein light chain 3 (LC3) to autophagic vesicles, which can be detected as punctate patterns of LC3-GFP. We found that control cells transiently transfected with LC3-GFP showed predominantly a diffuse distribution of green fluorescence, whereas I/R resulted in an increased punctate pattern (Figure 2). Moreover, I/R-induced autophagy was significantly reduced by overexpression of Bnip3ΔTM, whereas overexpression of Bnip3 led to increased induction of autophagy in the absence of I/R (Figure 2).

Conclusions and Future Direction

Taken together, these findings implicate Bnip3 as a major contributor to myocardial injury by causing mitochondrial dysfunction. Moreover, I/R leads to Bnip3-mediated upregulation of autophagy. We are currently investigating the molecular mechanism(s) of Bnip3 activation which leads to mitochondrial dysfunction and upregulation of autophagy in cardiac myocytes.

Reference


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Figure 2. A. HL-1 cells were transfected with LC3-GFP and vector or Bnip3ΔTM prior to simulated I/R (sI/R). After 2 h of ischemia and 90 min of reperfusion, the extent of autophagy was assessed by analyzing staining patterns of LC3-GFP. The percentage of diffuse vs. punctate LC3-GFP-positive cells per condition is shown as the mean ± S.E.M. of three independent experiments. Simulated I/R caused upregulation of autophagy, which was significantly reduced by overexpression of Bnip3ΔTM (p<0.05). B. HL-1 cells were transfected with LC3-GFP and pcDNA3.1, Bnip3, or Bnip3ΔTM. After 48 h, the extent of autophagy was assessed by analyzing staining patterns of LC3-GFP. Overexpression of Bnip3 in cardiac myocytes significantly induced autophagy (*p<0.05 vs. control). The percentage of diffuse vs. punctate LC3-GFP-positive cells per condition is shown as the mean ± S.E.M. of three independent experiments.
While the European Union debates how far to expand its reaches to the east, the European Section of the ISHR made the bold and daring move to expand its meeting venues to the far north. As an American, I was excited to hear that this year’s meeting would be closer to the state of Alaska than to Rome. My only previous European Section meeting was the outstanding 2002 congress in Szeged, Hungary, and I was very interested in attending another. For years I have followed the research of my northern Norwegians colleagues, and I have enjoyed hearing stories of fjords, white nights, and seal physiology from Terje Larsen and others when they venture out from Tromsø to attend meetings closer to the equator. So when I saw that the 2005 meeting was in Tromsø, I simply had to go.

Tromsø is a small city (population 62,000) on an island situated between fiords and mountain peaks at almost 70 degree latitude, 400 km north of the Arctic Circle. From here the great Norwegian’s arctic explorers Fridtjof Nansen and Roald Amundsen took off on their daring expeditions to the North over 100 years ago. It is home of the University of Tromsø, which is the northern-most university in the world. (Tromsø is also home to the northern-most beer brewery and cathedral!) It has an excellent medical school, and an active program in “artic research” involving the physiology and behavior of the local wildlife, particularly reindeer and seals.

When making our travel plans, we saw that there were several flights a day between Oslo and Tromsø, however, with the encouragement of Terje, we decided to travel by boat. We flew to Bodø, 450 km south of Tromsø, and took the local “steamer” Hurtigruten up through the snow capped fiords. The trip took 24 hours and made several stops at isolated villages to drop off goods and exchange passengers. It was the longest day of the year, with beautiful sun-lit views all night long and a side trip to Troll’s Fjord full of trolls.

The local organizers must be commended for putting together an outstanding scientific and social program, and for attracting a first-rate collection of submitted abstracts. The meeting was held in a beautiful facility at the University. There were two parallel sessions, with posters stationed between the two lecture halls, which forced lots of positive interaction among participants at the breaks. The social program started with an opening reception at an architecturally intriguing refurbished old warehouse on the water front. We stayed at the reception quite late. I have attended many evening social events at scientific meetings, and they all have a natural ending when it is dark and one feels the natural desire to sleep. This was not the case in the white and almost sunny nights in Tromsø: I felt like I could have socialized all night long.

On a subsequent evening there was a barbeque dinner, which was a real adventure. About 400 of us were loaded onto a fast boat that took the scenic route out to the beautiful island of Sommarøy, where we dined on reindeer meat and wonderful fish. From the island one really had the feeling of being north: to the west was Greenland, Iceland was to the southwest, and Alaska was only a few thousand kilometers across the North Pole.
The Gala dinner was full of surprises, from a concert performance by the virtuoso flutist and editor Tom Ruigrok, to the lecture on the benefits of cod liver oil. We were all fortunate to receive volumes of complimentary samples of fish oil capsules from the sponsor of the meeting, Fri Flyt, and we had a very thorough and hilarious lecture on the benefits of cod liver oil from a local expert. I know that the economy of Norway is booming due to the export of high priced crude oil, however what I really envy is their production of cod liver oil. Since the meeting I have been taking Norwegian fish oil supplements, and I have greatly improved bodily functions that I did not previously know I even possessed!

All together, it was an outstanding scientific meeting and a great cultural experience. We left Tromsø with the strong desire to return. Perhaps it is time for the European Section of the ISHR to make another bold move, and hold a “Skiing Under the Aurora Borealis” meeting in Tromsø in January, with 24 hour darkness?

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Margaret Chwascinska-Sharel
Cleveland, USA

Report on the Nordic-Baltic Meeting on Cellular Bioenergetics: Mitochondria in Myocytes - Methodological Aspects and Dysfunction in Cardiovascular Disease
(June 15-21, 2005; Tartu, Estonia)

The meeting, organised by the Network of the research institutions of Nordic and Baltic countries, and supported by the European Section of the ISHR and the Research Council of Norway, was held as a postgraduate summer school in the Centre of Molecular and Clinical Medicine, Faculty of Medicine, University of Tartu.

(continued on page 15)
Greetings from Perth, Australia:
XXIX Annual Scientific Meeting of the Australasian Section
(August 5-8, 2005)

The XXIX Annual Scientific Meeting of the Australasian Section was recently held in conjunction with The Cardiac Society of Australia & New Zealand (CSANZ) in Perth, Western Australia, a beautiful city lavished with Mediterranean-like climate and unique landscape. CSANZ is a clinical society composed of cardiologists, cardiac surgeons, trainees, nurses and other allied professionals. More than 1800 participants, including overseas CSANZ and ISHR members from Germany, France, UK, USA, China, Japan, Taiwan, Malaysia, Indonesia, Singapore and New Zealand made the conference a busy and exciting occasion for the ISHR to contribute significantly to the design of the combined scientific program.

Dr Elizabeth Nabel (Maryland, USA) presented the CSANZ’s 2005 R.T. Hall Lecture on “Genetic Medicine and Cardiovascular Disease”. Based on her experience and clinical success, Dr Nabel emphasised in her excellent presentation that clinical breakthroughs can only arise with close interplay between original clinical observations, laboratory science, and subsequent clinical trials, often involving numerous iterations back and forth with the laboratory. This set a perfect scene and provided justification for ISHR’s joint meeting with CSANZ.

Symposia highlights included: Molecular Biology of Cardiac Failure; Signalling Mechanisms in Cardioprotection; Cardiac Myocyte Signalling in Adaptive Growth, Hypertrophy & Failure; Sex, Steroids & Angiotensin Peptides - Trophic Effects; Atherosclerosis and Inflammation; Cardiac Oxygen Signalling in Stress & Survival; Metabolic Syndrome, Diabetes & the Heart; and At the Heart of Function & Dysfunction. In addition, ISHR ran two “Leading Edge Research Techniques & Resource Workshops”: Proteomics & Genomics-Practice and Possibilities, and Assessing Cardiac Function - Meeting the In Vivo Challenge.

As in previous meetings, there was also a strong focus on research students. Outstanding oral presentations were made by the four finalists in the ISHR Student Prize Symposium: Edna Lekgabe (Melbourne, Australia), Sharon Tsang (Hong Kong, China), Iwan Alban Williams (Sydney, Australia), and the winner, Enzo Porrello (Melbourne, Australia), whose talk was entitled “Evidence suggestive of an angiotensin II-dependent cardiomyocyte cell in neonatal hypertrophic heart rat”. The popular Poster, Wine and Cheese session provided a relaxed atmosphere for animated discussions and contemplation on the work presented. The Best Student Poster Prize was awarded to Freya Sheeran (Melbourne, Australia) for her presentation of “Respiratory chain functional defects underlying mitochondrial complex-1 activity in human heart failure”, and the High Commendation Poster Prize went to Lu (Karen) Fang (Melbourne, Australia) who presented: “Gender affects remodeling and healing post acute myocardial infarct in mice”.

On behalf of all the delegates I would like to acknowledge and sincerely thank Dr Salvatore Pepe (Australasian Section President), A/Prof. Lea Delbridge (Secretary) and Dr Xiao-Jun Du (Treasurer) for all the hard work they put into organising a great program. Sincere appreciation also goes to Drs Leonard Arnolda, Peter Thompson, Richmond Jeremy, Ken Hossack, and the CSANZ Organising Committees for enabling a successful joint meeting. Indeed this success sets the precedence for the next joint meeting with CSANZ (4-6 August, 2006) to be held in Canberra, Australia’s capital. For those who love wine (we all know the cardiovascular benefits!), the Canberra regions cool climate wines are now receiving international recognition. With 140 vineyards with more that 30 cellar doors, most of which are only 30 minutes from the city, be sure to allow extra days to explore! The Australasian Section invites you to join us!

Helen Kiriazis, Ph.D.
Melbourne, Australia
A number of senior scientists from Australia, Czech Republic, Denmark, Estonia, Finland, France, Germany, Italy, Lithuania, Norway, Slovak Republic and UK, and PhD students from Nordic and Baltic countries exchanged their research results and scientific ideas. A wide range of topics was addressed, including Mitochondrial respiratory chain, its regulation and pathological alterations, Compartmentalization of the energy metabolism, Mathematic modeling of bioenergetic processes, Role of mitochondria in intracellular signalling, Alterations in skeletal muscles in conditions of heart disease, Role of mitochondria in the cell death process, and Methods of studying the normal and altered functions of the mitochondria. A significant part of the meeting was devoted to discussing the newest methodological approaches in the field of cellular bioenergetics, with practical instructions in different laboratories.

The format, scientific content and technological merits of the meeting were highly appreciated by the PhD students and lecturers. The participants also enjoyed the informal meetings and sightseeing tours in Tartu and its surroundings that helped us to recover after rather demanding and intensive workdays. It was a general understanding that the meeting was a great success in favor of establishing the academic and research Network between the Nordic and Baltic countries and of its integration into the European research area. The meeting was accepted as a part of the current academic PhD study programs in the participating Institutions.

After finishing their work in Tartu, many PhD students and lecturers attended the XXV European Section Meeting in Tromsø, Norway. Those attending both meetings were pleased to discover that the scientific program of Tartu’s meeting fairly complemented the content of the session *The Multifaceted Mitochondria: from Protection to Aging* held in Tromsø.

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