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Featured on the cover: Watercolor portrait of the late Dr. Peter Safar by Milly Scheffer from Buffalo, NY. This painting was created from a photograph taken of Dr. Safar on the occasion of his 79th Birthday—which occurred during the 2003/2004 academic year. The painting was presented to Mrs. Eva Safar by Dr. Larry Jenkins, Associate Director of the Safar Center for Resuscitation Research.

MISSION STATEMENT

The global mission of the Safar Center for Resuscitation Research is to improve understanding of the mechanism of secondary injury after trauma and cardiopulmonary arrest, from whatever cause, and to contribute to the development and implementation of novel therapies. The treatment and prevention of secondary injury after these life-threatening catastrophic events is a major goal in each venue of investigation.

A letter from the Safar Center's Director

Patrick M. Kochanek, MD Director, Safar Center for Resuscitation Research

The 2003/2004 academic year was another outstanding one for the Safar Center. Research. Efforts into five major areas of research and research training—including research in cardiopulmonary arrest and resuscitation, traumatic brain injury (TBI), training in pediatric neurointensive care and resuscitation research, hemorrhagic shock and suspended animation, and CNS rehabilitation research.

The most important findings generated by Safar Center investigators this academic year came from the laboratory of Dr. Robert Clark, working on a project within the cardiopulmonary arrest program, studying experimental pediatric cardiac arrest. Bob's group carried out an important study demonstrating innate gender-based effects on necrotic and apoptotic cell death pathways using a unique neuronal culture system that separately evaluated the response of XX and XY neurons to nitrosative stress, excitotoxicity, and staurosporine. They found that XY neurons were particularly sensitive to nitrosative stress and excitotoxicity, while XX neurons were most sensitive to the apoptosis inducer staurosporine. This suggests that male neurons are more apt to develop necrosis while female neurons are more inclined to develop apoptosis. They further demonstrated that this difference was, at least in part, associated with the incapacity of male neurons to maintain intracellular levels of the antioxidant reduced form of glutathione. These findings were extended to an *in vivo* model of asphyxial cardiopulmonary arrest in developing rats—further supporting the observation. That unique work was reported in the *Journal of Biological Chemistry* in a manuscript authored by Dr. Lina Du. The findings were featured as a press release on the *Nature* website. The importance of this work relates to the fact that this suggests the possibility that the clinical approach to cerebral resuscitation for any form of brain injury could be very different for females and males—i.e., a different drug cocktail or therapeutic regimen may ultimately be needed. These findings also suggest that gender differences in CNS injury are important even before puberty. This work, initially supported by funds from Pediatrics Department Chair Dr. David Perlmutter at Children's Hospital of Pittsburgh recently led to the successful acquisition of an RO-1 award by Dr. Clark—expanding his portfolio to include RO-1 funded studies in both TBI and cardiac arrest. I want to personally congratulate Dr. Clark and his talented group for this important high-impact work. In addition, Dr. Clifton Callaway, director of the cardiopulmonary arrest program of the Safar Center, and investigator in the Center for Emergency Medicine successfully competed for one of the funded sites within the NIH sponsored Resuscitation Outcomes Consortium (ROC) that emerged out of the Pulse Initiative. Special congratulations to Drs. Callaway as Principal Investigator (PI) and to Sam Tisherman as Co-PI on this important national initiative. Finally, Safar Center Scientist Dr. Robert Hickey served this year as the Vice Chairperson of the Emergency Cardiovascular Care Committee, American Heart Association. Dr. Hickey has served on that committee for a number of years, and has considerable experience, particularly in the area of pediatric resuscitation

guidelines. Congratulations to Bob. I look forward to great things from the cardiac arrest program on both the pediatric and adult fronts.

Our TBI program continues to be funded by a program project from the National Institute of Neurological Disorders and Stroke (NINDS), 9 RO-1 awards, an R-21, 2 KO-I, and 1 K23 award, participation by two of our faculty as PIs in the CDC-funded University of Pittsburgh Center for Injury Control and Research (CIRCL) grant (directed by Dr. Hank Weiss) and a variety of other grants. Our work in TBI spans a number of areas of study—including evaluation of novel resuscitative therapies targeting neuronal death, unraveling the mechanisms of secondary injury in both experimental models and in brain injured patients, the development of novel tools to facilitate detection of potentially missed cases of child abuse, and the testing of new strategies in brain injury rehabilitation. One of the most exciting developments in the area of TBI at the Safar Center this year was the successful acquisition of an RO-1 award by Dr. Anthony Kline, a scientist in our center in the Department of Physical Medicine and Rehabilitation. Anthony's work is in the area of serotonin pathways in experimental TBI. His recent studies in this area, published in the *Journal of Neurotrauma* suggest powerful beneficial effects through pharmacological manipulation of this transmitter system—specifically via 5HT-1A receptor agonists. I look forward to new discoveries by Dr. Kline, now one of the Associate Directors of our Center, and am pleased by the link between Rehabilitation and Resuscitation that has developed at the Safar Center. Our investigators published several other notable papers in experimental and clinical TBI. Dr. Kimberley Statler, working on our T-32 grant from the National Institute of Child Health and Human Development (NICHD) (see below) published a manuscript which explored one of the mechanisms underlying the potent neuroprotective effects of isoflurane—showing that this anesthetic markedly attenuated the posttraumatic hyperglycolysis believed to occur early after injury in response to excitotoxicity. She demonstrated that the commonly used analgesic in the ICU, fentanyl—failed to protect in this paradigm. Her work garnered the cover of two issues of the journal *Brain Research*. A series of two papers by Drs. Hülya Bayır and Amy Wagner demonstrated enhanced oxidative stress in adult males versus females after severe TBI. The effect of gender was more powerful than the use of therapeutic moderate hypothermia. Dr. Bayır continues to develop as a young investigator under the superb guidance of Dr. Valerian Kagan, Professor in the Department of Environmental and Occupational Health. Dr. Wagner is now an Associate Director in our Center. Both studies were published in the *Journal of Neurotrauma* and have been cited by a number of laboratories as clinical evidence for endogenous protection against oxidative injury conferred by female gender in experimental models of CNS injury. A third paper of note was generated by Dr. Paul Shore, also supported by our T-32. He carried out a unique study that compared the effect of method of CSF drainage in infants and children with severe TBI on CSF levels of a number of biochemical markers of brain injury and other endpoints, such as intracranial pressure (ICP). Remarkably, levels of mediators and ICP were lower in the children treated with continuous versus intermittent drainage. Finally, Dr. David Adelson just completed the first phase of his randomized controlled trial of therapeutic hypothermia in pediatric patients with severe TBI—an assessment of safety and feasibility of 48 hours of cooling. We look forward to analysis of those findings. I am pleased to say that both the work of

Drs. Paul Shore and Adelson represent a response to the plea in the recently published Guidelines for the Management of Severe Traumatic Brain Injury in Infants, Children, and Adolescents for more clinical studies in pediatric TBI. I look forward to future work by both of these investigators.

Research training continues to be a key priority in our Center –including the development of both postdoctoral fellows (MD and/or PhD) and junior faculty. This also represents the most important and enjoyable part of my own efforts. Postdoctoral clinician-scientist development in our Center has been greatly facilitated by our T-32 grant from the NICHD entitled "Training in Pediatric Neurointensive Care and Resuscitation Research." Graduates of our program in 2003/2004 included Drs. Paul Shore and Mary Hartman. Dr. Shore (see above for a description of some of his work) obtained a Masters degree in Clinical Research via the clinical scholars program at the University of Pittsburgh during his training on the T-32 and was highly sought after as a young clinical investigator in pediatric neurointensive care by a number of programs. He accepted a faculty position at the University of Texas Southwestern, Children's Hospital of Dallas. Dr. Hartman performed her T-32 work prior to completing her clinical training to facilitate the acquisition of an MPH degree. She has been working with Dr. Derek Angus of the Clinical Research, Investigation, and Systems Modeling of Acute Illness (CRISMA) laboratory studying the variability in the rates of triage of critically injured children with severe TBI to tertiary pediatric centers using a unique seven state database. She is reporting a remarkably high level of failure in transfer of these children in the specific population of patients who go on to die beyond 24 hours after admission. These concerning findings could have great public health impact and Dr. Hartman has presented this work to interested audiences at several national meetings. We look forward to seeing the full manuscript in press. One of our current T-32 trainees, Dr. Mandeep Chadha, also deserves congratulations for being selected as one of the top three presenters at the Annual NCMRR/NIH Training Workshop. That conference is held annually by NCMRR/NICHD and organized by Dr. Ralph Nitkin at NCMRR. It is a fantastic conference that is highly valuable to trainees. Congratulations Mandeep! Our trainees are thriving in a number of centers nationwide, including Drs. Michael Whalen, Michael Bell, and Courtney Robertson all funded by K08 awards at Massachusetts General Hospital, DC Children's Hospital, and the University of Maryland, respectively. Drs. Statler and Nguyen—recent graduates of our T32 program have applied for K awards at the University of Utah and Baylor College of Medicine, respectively. Finally, I wish to thank Drs. Ralph Nitkin, Michael Weinrich, and Carol Nicholson at NICHD for their valuable insight and support of this exciting program. In addition to clinician-scientists training on our T-32, Dr. Margaret Wilson has been working as a postdoctoral fellow in the laboratory of Dr. C. Edward Dixon, a highly respected experimental TBI scientist at our center. Margaret is carrying out studies examining striatal injury in the controlled cortical impact model and has been characterizing the Fluro-jade B method in that model.

Research productivity by the trainees continues to be spectacular, including a total of 10 fellow first-author peer-reviewed publications and 23 abstract presentations this academic year. In honor of Dr. Nancy Caroline, one of Dr. Safar's early trainees who went on to become the mother of CPR in Israel, and later, the head of the Israeli Red

Cross, we established in 2002 the Nancy Caroline Fellow Award at the Safar Center. This award is given annually to the fellow working with a Safar Center Scientist who has made the greatest contribution to the field of resuscitation medicine. Dr. Xianren Wu received this award at the 2003 Safar Symposium (see photo). Dr. Wu is a prolific young investigator working in the Suspended Animation (SA) program. His work is discussed later in this report.

Xianren Wu, MD, 2nd recipient of the Nancy Caroline Fellow Award

Many students have performed admirable research over the years at the Safar Center, and during the 2003-2004 academic year, a number of students were involved in studies at the Safar Center. Most notably, Kathleen Sachse carried out an interesting study in collaboration with both Drs. Edwin Jackson in the University of Pittsburgh Center for Pharmacology and Dr. Kochanek. Kathleen found extremely high levels of caffeine and its metabolites in the CSF of adults with severe TBI. Importantly, increased caffeine levels in the initial 24 hours after injury were associated with favorable long-term outcome. This work suggests a possible beneficial effect of caffeine via its well known up-regulation of adenosine A1 receptor number or function—making adenosine a more potent endogenous neuroprotectant. Our laboratory had previously demonstrated marked increases in adenosine in brain early after severe TBI in humans—making this a logical mechanistic possibility. Kathleen presented this work at the 2004 American Society of Anesthesiology meeting where the report received attention by the press. Dr. Kochanek was interviewed by CBS News about this work. Finally, Kathleen also presented this work at the 2003 meeting of the National Neurotrauma Society, where she was selected as one of the poster finalists. We are pleased to report that Kathleen will be joining the Anesthesiology Residency program here at Pitt—she will be a terrific addition to the program and we look forward to working with her in the future. We thank Dr. Jackson for his continued support of our work related to adenosine in CNS injury. His input is nothing short of genius.

Junior faculty development continues to be another vital component of our work at the Safar Center. Drs. Robert Hickey in the Division of Pediatric Emergency Medicine (mentored by Dr. Steven Graham) and Amy Wagner in the Department of Physical Medicine and Rehabilitation (PM&R) (mentored by Dr. Dixon), continue to be supported by KO-8 awards, and Dr. Rachel Berger in the Department of Pediatrics (mentored by Dr. Kochanek) is supported by a K-23 from NICHD. Finally, collaborator Dr. Sam Poloyac in the School of Pharmacy was funded by a grant from the American Heart Association—Pennsylvania-Delaware Affiliate for work on cytochrome-P450 metabolism in brain ischemia. Dr. Poloyac is a promising young investigator in this area. I continue to be particularly proud of our successes in fellow, resident, student, and faculty development, which I feel is the most important facet of our work.

The hemorrhagic shock and suspended animation program thrived in 2003/2004 guided by Drs. Samuel Tisherman and Patrick Kochanek. This program, which is focused on novel approaches to resuscitation of traumatic hemorrhagic shock and exsanguination

cardiac arrest, continues to be supported through a congressional appropriation funded through the United States Army. Dr. Peter Safar was the Principal Investigator of this project until his passing on August 3, 2003. Dr. Patrick Kochanek assumed the role of Principal Investigator with the approval of the United States Army. The program is focused on new approaches to the use of hypothermia and other pharmacologic strategies for protection and preservation of the entire organism during circulatory arrest. A number of studies were carried out in the 2003/2004 academic year. Most notable is the provocative study of Dr. Xianren Wu. Prior work in our dog model of suspended animation induced by aortic flush with ~20 liters of ice cold saline demonstrated that dogs could be preserved for at least 2 hours at ~7˚C after rapid exsanguination over 5 minutes to cardiac arrest. These animals could be successfully resuscitated to normal outcome using cardiopulmonary bypass to carry out a delayed resuscitation. Despite these impressive findings we were challenged by the US Army to address another important scenario—particularly relevant to combat casualty care—namely to determine if suspended animation could still be successful if a prolonged period of hemorrhagic shock preceded the arrest. Dr. Wu carried out a series of studies demonstrating unequivocally, that Suspended Animation—with normal long-term outcome—could be successful even if a hemorrhagic shock interval of 2 hours preceded the arrest. This work opens the door to the potential use of this novel modality, even in the setting where a casualty was pinned down for hours after being wounded. The potential relevance of the findings to civilian trauma is also obvious. Consultative and administrative support from Dr. Lyn Yaffe, former director of the United States Naval Medical Research Institute continues to be instrumental to the program. Dr. Yaffe is working with industrial partners to develop a novel approach to catheterization and is a vital resource and a special friend to our Center. We cannot thank him enough for this support. In addition, Mr. Dave McMurry and his team at Ardiem Medical have supported cooling device development for our studies. Dr. Miro Klain has also been working on field catheterization-related aspects of this work. We are also very thankful to COL Dean Calcagni and Mr. Robert Read of the United States Army for their continued encouragement and support at the Telemedicine and Advanced Technology Research Center (TATRC) of the United States Army Medical Research and Materiel Command. Finally, Dr. Tomas Drabek, a cardiac anesthesiologist from Prague, Czechoslovakia has just begun to work as a fellow on the suspended animation project. He is developing a rat model of SA to study mechanisms with Dr. Larry Jenkins, using proteomic methods and screen novel therapies. We welcome Dr. Drabek to our team.

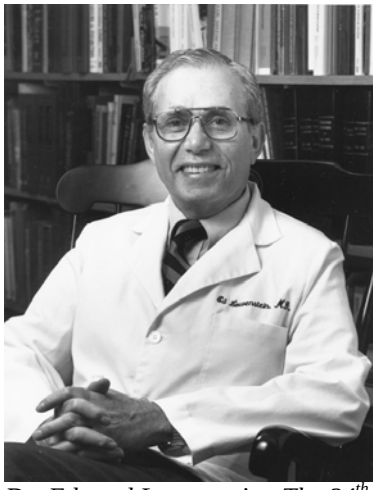
Pictured from left to right are United States Congressman John Murtha and Dr. Kochanek discussing emergency hypothermia at the ARMtech Exposition.

We would like to thank Congressman John Murtha for his support of this project in his role on defense appropriations. As the 2004/2005 academic year began, Dr. Kochanek presented our work on this project to Congressman Murtha

at the ARMtech exposition (see photo). We are indebted to Congressman Murtha for his support of our unique program.

Investigators in the Center published 29 peer-reviewed papers, 25 chapters and editorials, and 63 abstracts in 2003/2004. Included among these reports were publications in the *Journal of Neurotrauma, Journal of Cerebral Blood Flow and Metabolism, Journal of Neurochemistry, Journal of Biological Chemistry, Critical Care Medicine, Neurosurgery, Journal of Trauma,* and *Pediatric Critical Care Medicine*.

On October 30, 2003, we hosted the second Safar Symposium at the University of Pittsburgh School of Medicine. It featured sessions on *Breakthroughs in Resuscitation Research* and on the *Role of Human Simulation in Medical Education and Research.* The symposium was linked to a memorial service held in the late Dr. Safar's honor at the Heinz Chapel on the University of Pittsburgh Campus.



Dr. Edward Lowenstein. The 24th Peter and Eva Safar Lecturer

One hundred and sixty-five clinicians, scientists, and allied faculty, fellows, paramedics, and students attended the symposium. Invited speakers included Drs. John Hallenbeck, John Povlishock, Lance Becker, Donald Marion, Lyn Yaffe, Doris Ostergaard, and Mr. Tore Laerdal.

During the Symposium, we also held the 24th Peter and Eva Safar Annual Lectureship in the Medical Sciences and the Humanities on October 30, 2003. Dr. Edward Lowenstein addressed the topic of Ethics, physicians, and the relief of intolerable suffering: Lessons learned from the Oregon Death with Dignity Act. The issue of end of life care was important to Dr. Safar throughout his career, making this topic germane for the Safar Lectureship.

Pictured from left to right are Mr. Robert Read, Mrs. Eva Safar and COL Dean Calcagni during an award presentation from the US Army in Dr. Safar's honor

Immediately before the lecture, COL Dean Calcagni and Mr. Robert Read of TATRC presented Mrs. Eva Safar with a special award from the US Army, honoring Dr. Safar's special contributions to combat casualty care.

Professor David Hovda

Our Visiting Professor in 2003/2004 to the Safar Center for Resuscitation Research was Dr. David Hovda of the Department of Neurological Surgery at the UCLA School of Medicine. Dr. Hovda addressed the important

topic of optimal fuels for the brain early after injury. His group has pioneered work on a variety of alternative substrates such as ketones in experimental TBI. He is one of the top investigators linking bench to bedside via PET and microdialysis in TBI and a friend to all of us at the Safar Center. Our faculty and trainees thank him for his comments on their work.

Once again, I would like to thank everyone working at the Safar Center for a terrific job this year. I am indebted to Linda Amick, Marci Provins, Fran Mistrick, Val Sabo, and Julian Smith for their administrative and secretarial excellence. Linda and Marci are extremely dedicated to the Safar Center and its success. Linda continues to take on an increasingly greater administrative role on the business end of the Center while Marci continues to serve as our key secretarial resource for the academic programs in our Center –along with her dedicated work as my local editorial assistant for the journal *Pediatric Critical Care Medicine*. Fran Mistrick serves as the coordinator for the annual Safar Symposium, and is doing a superb job on this project, along with a number of other roles related to the archives and legacy of Dr. Safar. Fran also did a special job on the memorial service to Dr. Safar. What pleases me the most is all of the help that Linda, Marci, Fran, Val, and Julian have given to the many investigators working at the Safar Center. I would also like to personally thank Henry Alexander, John Melick, Keri Janesko, Vincent Vagni, Xiecheng Ma, Dr. Lina Du, Paula Nathaniel, Ray Griffith, Jackie Pantazes, Grant Peters, and Research Assistant Professor S. William Stezoski, who are senior administrative and technical staff members during the 2002/2003 academic year for their spectacular contributions to the individual missions of the Center. Bill Stezoski oversees the work of technicians Jeremy Henchir, Sherman Culver, and Jason Stezoski who work on our novel SA project and help it reach new levels of success. I continue to be amazed by the work ethic of all of the technical and secretarial staff at our Center.

I would like to thank Dr. Mitchell Fink for his support as the Chairman of the Department of Critical Care Medicine and Ms. Susan Stokes, departmental administrator. I am grateful to them for their support and particularly grateful to them for helping to launch the renovation that is about to begin in our center. Dr. Fink has been a steadfast leader of our center. I would also like to personally thank Drs. Clark, Dixon, Jenkins, and Tisherman for their incredible help at the Safar Center. These four faculty members have made very special contributions to the center to make my job easier and greatly enhance our success. Their close camaraderie and guidance has been incredible. I would also like to convey special thanks to Drs. Adelson, Wagner, and Kline. Their efforts in pediatric TBI and clinical and experimental rehabilitation, respectively, have made special contributions to these important niches for our center. Much thanks is also due to Drs. Bayır, Callaway, Thompson, Lunsford, Zafonte, Zhang, Yan, Klain, Graham, DeKosky, and Hickey for their efforts in the continued development of the Safar Center, its trainees, and its programs. They have been instrumental in its success. I am especially thankful to Drs. Lunsford and Zafonte for their contributions to our renovation, and to Dr. Ann Thompson who is the director of the PICU at Children's Hospital of Pittsburgh for her continued support and guidance. I also thank Dean Arthur Levine for facilitating our facility renovation and expansion. I also thank Frank Adams, Doug Schlach, Kelly

Brown, and Dick Aradine, the architects and administrators involved in this project, for their dedication to our new facility. Finally, I would also like to thank Dr. John Williams, Chairman of the Department of Anesthesiology, for supporting the Safar Symposium and the Peter and Eva Safar Lecture.

Special thanks are also due to Dr. Edwin Jackson in the Center for Clinical Pharmacology, Dr. Valerian Kagan in the Department of Environmental and Occupational Health, Drs. Chen Ho and Kevin Hitchens and Lesley Foley at the Pittsburgh NMR Center for Biomedical Research, Dr. Stephen Wisniewski in the Department of Epidemiology, Dr. Robert Garman of Consultants in Veterinary Pathology, Inc, Dr. Timothy Carlos in the Department of Medicine, Dr. Simon Watkins in the Department of Cell Biology and Physiology, Dr. Timothy Billiar in the Department of Surgery, Dr. Paul Paris in the Department of Emergency Medicine, Dr. David Perlmutter in the Department of Pediatrics, and Dr. Samuel Poloyac in the School of Pharmacy for outstanding collaborative expertise that raises the level of the research at the Safar Center. These collaborators have been tremendous resources for our faculty and trainees, and have contributed importantly to our funding successes. I cannot thank them enough.

I also owe a debt of gratitude to Mr. Tore Laerdal of Laerdal Medical and to Mr. Hans Dahl of the Laerdal Foundation. Their generous support of our young investigators through the Laerdal Foundation has been special throughout many years. Dr. Ake Grenvik has also served as an important liaison in this regard for our Center and we thank him for his many efforts.

Finally, with the help of Chancellor Nordenberg, we continue fundraising efforts for three funds, including a "Safar Legacy Fund," to provide a core budget for the Center, along with funds to support the "Nancy Caroline Fellowship Award" and, of course, the "Safar Symposium." We have enclosed a pledge card describing those funds in this year's report and thank you in advance for your support. I would also like to personally thank each of you who have already donated to these efforts. Our total goal for these three programs is an endowment of two million dollars toward Dr. Safar's goal of the resuscitation of "brains and hearts too good to die."

I once again look forward to success in 2004/2005 in our investigative efforts to develop new therapies in the field of resuscitation medicine, and thank you for your continued support of our work.

Respectfully submitted,

Patrick M. Kochanek, MD

Patrick M. Kochanek, MD, Director, Safar Center for Resuscitation Research
Director, Traumatic Brain Injury

Clifton Callaway, MD, PhD
Associate Director, Cardiopulmonary Arrest

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Associate Director, Molecular Biology

C. Edward Dixon, PhD
Associate Director, Functional Outcome

Larry W. Jenkins, PhD
Associate Director, Molecular Biology

Anthony E. Kline, PhD
Associate Director, Rehabilitation Research

Peter J. Safar, MD, Distinguished Professor*
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Amy K. Wagner, MD
Associate Director, Rehabilitation Research

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Nicholas Bircher, MD
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S. William Stezoski
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Zachary Repanshek
Kathleen Sachse
Ryan Santos
Josh Sokoloski
Chris Stangey
Matthew Tormenti
Mike Wenger
Lauren Willard

*Founding Director
Deceased – August 3, 2003

Funding

During the 2003/2004 academic year, Safar Center investigators had a total of 47 active grants. Forty-two of these grants were extramural. The direct and indirect costs for the full award period of these grants totaled **$19,262,156** and this is plotted for the current and preceding eight academic years on the following page. The specific sources of this grant support are shown on the subsequent page. Remarkably, the Safar Center is continuing to grow and maintain a high level of extramural support. This continues to require a monumental effort by our faculty since our support is almost completely derived from extramural grants. Congratulations to the faculty for their funding successes.

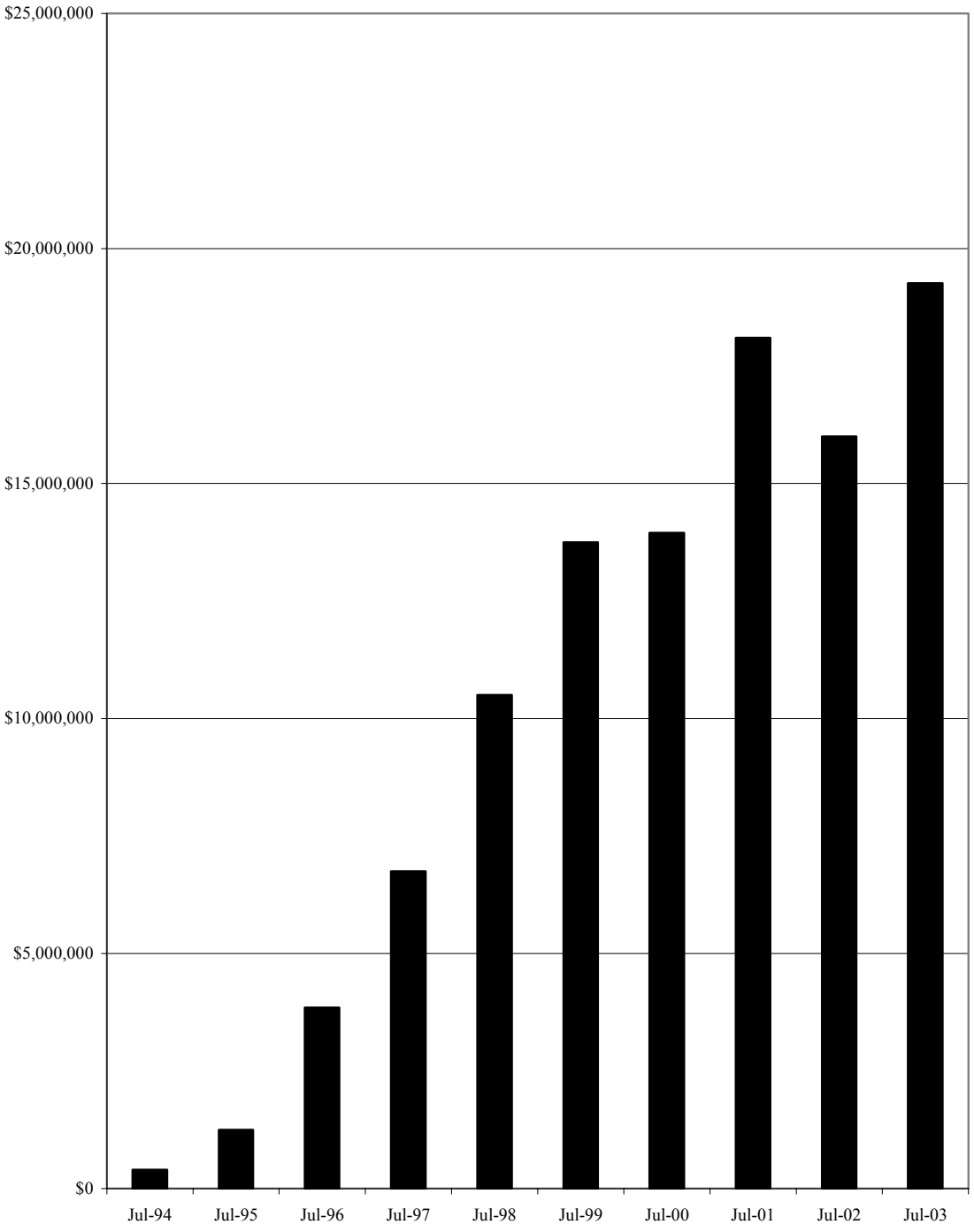
The portion of the budget for use in each academic year (July 1 through June 30) is also plotted for the current and preceding four academic years on the pages following. This represents direct and indirect costs and is shown for total, extramural, and intramural grant support.

Extramural funding sources included the National Institutes of Health, the United States Congress via the US Army**,** the Centers for Disease Control and Prevention, the Laerdal Foundation, and a variety of other sources, including contributions made to the Safar Center in memory of Eric Bundy.

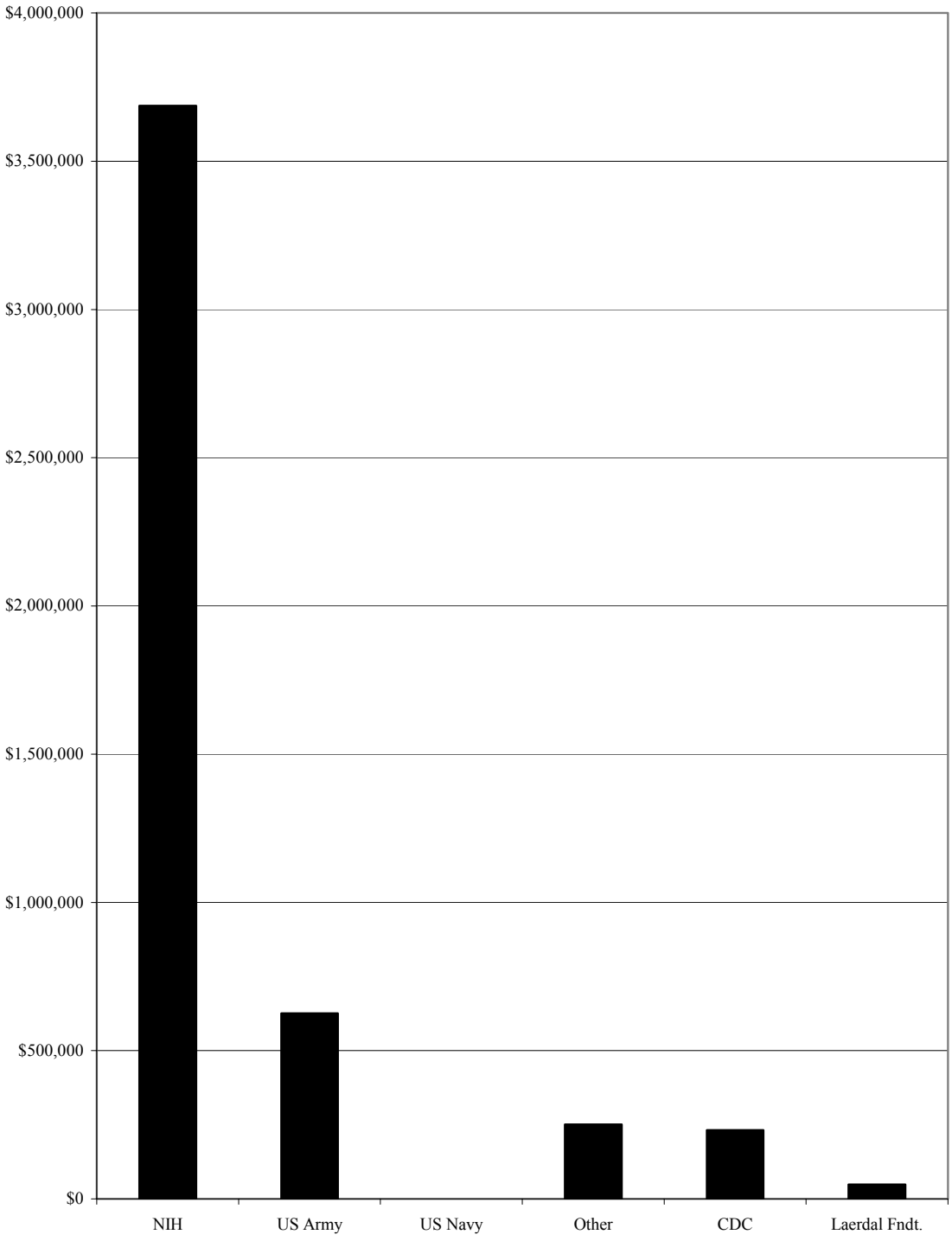
Intramural funding was provided by the Departments of Critical Care Medicine, and Anesthesiology, and the Children's Hospital of Pittsburgh.

We are deeply grateful for the prior and current support from all of these granting agencies and donors.

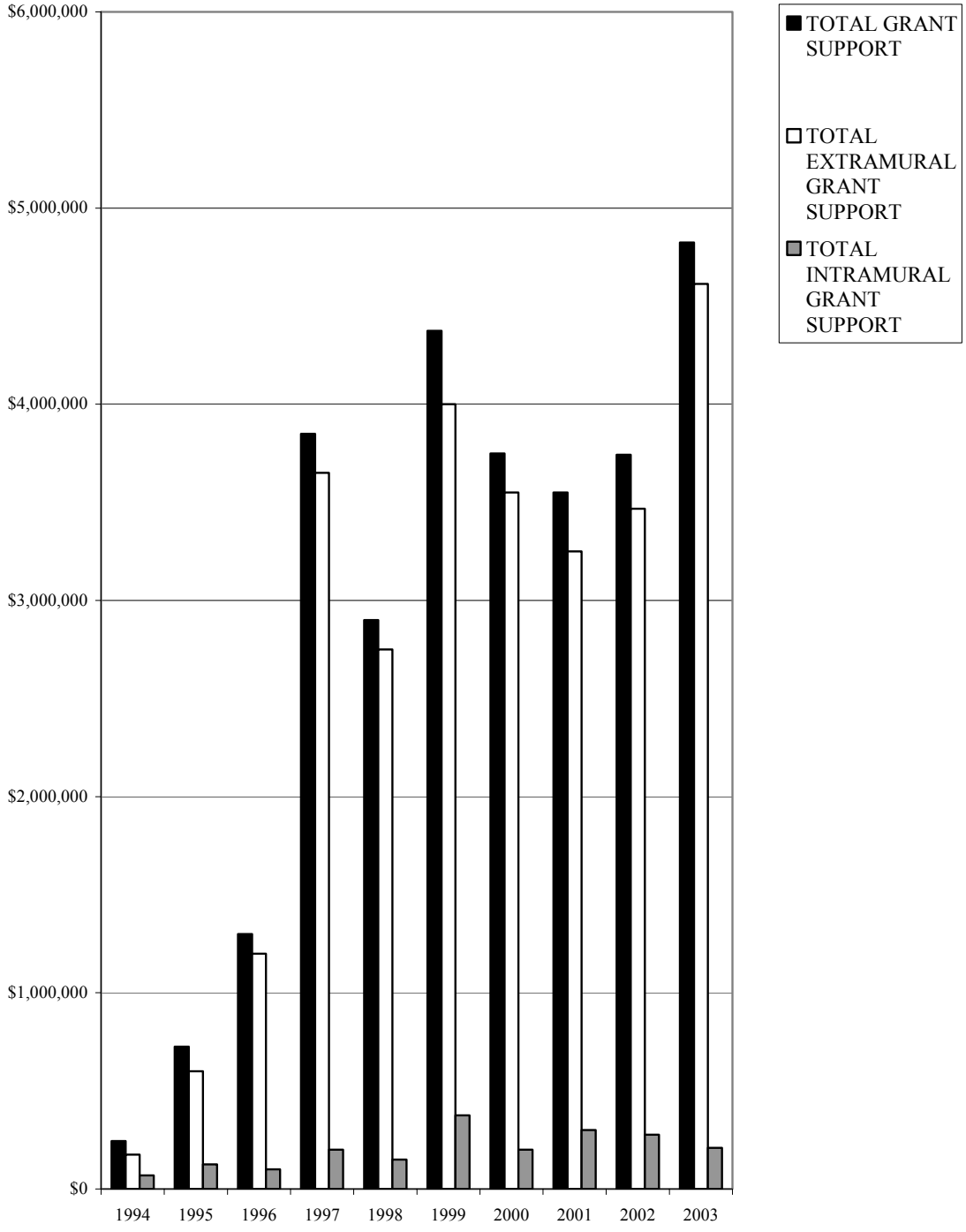
Direct and Indirect Costs for the Full Award Period of SCRR Grants



Specific Sources of Grant Support



Safar Center Grant Support thru 2003/2004
use in each academic year



TRAUMATIC BRAIN INJURY (TBI) PROGRAM

Traumatic brain injury (TBI) affects 1.5 to 2 million people in the United States each year, making it one of the more prevalent and debilitating of all neurological disorders. Approximately 300,000 of the cases are severe enough to warrant hospitalization. Of the 250,000 survivors of severe TBI, 100,000 endure long-term disabilities that require rigorous, lengthy, and costly medical and rehabilitative care. In addition to the medical expenses associated with TBI, societal costs are also significant in terms of lost wages due to the inability to resume employment. While the true cost of TBI is incalculable, it is estimated at $100,000 annually per patient or about $48.3 billion per year. TBI is a serious and survivable medical problem with no acknowledged treatment. Therefore, investigation of therapeutic strategies at the Safar Center that may facilitate the recovery process after TBI are essential. Equally important are studies identifying mechanisms involved in the evolution of secondary damage after TBI and determining if pharmacological agents are detrimental to the recovery process.

TBI Investigation by Safar Center Director and Associate Directors

1. Studies directed by Patrick M. Kochanek, MD

Patrick M Kochanek, MD, Director, Safar Center for Resuscitation Research, Professor and Vice Chairman, Department of Critical Care Medicine, University of Pittsburgh School of Medicine, and Professor of Anesthesiology and Pediatrics.

Dr. Kochanek's Research at the Safar Center is accomplished through a collaborative effort between a number of investigators, fellows, students and staff located principally in the Department of Critical Care Medicine (CCM), Neurosurgery, PM&R, and Neurology at the University of Pittsburgh School of Medicine. A number of collaborations are also ongoing with investigators in other University of Pittsburgh Departments including the Center for Clinical Pharmacology, Environmental and Occupational Health Medicine, Pediatrics, Epidemiology, Anesthesiology, and Surgery. A long-standing collaboration is also in place with the Pittsburgh NMR Center for Biomedical Research at Carnegie Mellon University. We have also had, this year, several important extramural collaborators, including Dr. Neal Thomas at Penn State Children's Hospital, Dr. Jiang-Fan Chen at Boston University, and Dr. Jurgen Schnermann at the National Institutes of Diabetes and Digestive and Kidney Diseases. These collaborations have allowed us to investigate a spectrum of mechanisms that may be important to the evolution of secondary damage after TBI. Our studies of mechanism of secondary damage and repair in human materials (CSF, brain tissue samples from resected contusions, and microdialysis samples) have generated new insight into the biochemistry and molecular biology of human head injury. Based on this mechanistic work, we are currently testing novel therapies in our experimental models. Our goal is to develop new therapies that can be translated to clinical application. Our clinical research taking the bench to the bedside has been featured many times in the lay press. As outlined in the opening letter, this year, our work on caffeine levels in CSF after severe TBI in adults was featured in the national news media.

A. Biochemical Assessment of Secondary Mechanisms of Injury and/or Repair after Severe TBI in Infants and Children: The Role of Child Abuse

This continues to be an important area of research for our group and, as indicated above, continues to generate considerable publicity. We are using samples of CSF and blood collected from infants and children suffering severe TBI to study a variety of biochemical mediators of secondary damage and/or repair. These samples are collected by Dr. Rachel Berger in the Department of Pediatrics, and members of our critical care team including Drs. Clark, Bayır, Shore, Lai, Chadha, and Fink, and Dr. Adelson in the division of Neurosurgery at Children's Hospital of Pittsburgh. Dr. Kochanek is funded by the CDC (University of Pittsburgh Center for Injury Control and Research [CIRCL]) to generate this CSF repository. We have over 1,000 samples from over 100 infants and children who have suffered a severe TBI—including victims of inflicted TBI (shaken baby syndrome). We continue to collaborate with Dr. Neal Thomas at the Penn State Children's Hospital, Hershey, PA, who is also collecting samples.

Studies using the pediatric CSF bank at the Safar Center

Pediatric and adult CSF studies have produced several interesting findings in the area of TBI at the Safar Center in the 2003-2004 academic year. Work continues in a number of areas as outlined below, and in the report on Dr. Rachel Berger's investigation.

Oxidative stress in TBI

Oxidative injury is believed to be a fundamental pathway in mediating secondary damage after both ischemic and TBI. We believe that it is a key target for future development of new therapies in CNS injury. This area of study is overseen by Safar Center Scientist Dr. Hülya Bayır, a former fellow who has now joined our PICU faculty. Dr. Bayır was mentored in this area by Dr. Valerian Kagan, an international authority in free radical biology and has worked closely with him to carry out a number of studies. This year, building on a strong investigative base in this area, Dr. Bayır demonstrated a powerful endogenous antioxidant protective effect of female gender after severe TBI. A marked increase in the lipid peroxidation marker F2-isoprostane was seen exclusively in adult males after severe TBI. The gender difference was dramatic enough that it dwarfed the attenuating effect of therapeutic hypothermia on lipid peroxidation. These findings, along with the work of Dr. Wagner (see later in this report) support the possible need for gender specific therapy in TBI in adults, and specifically suggest that novel therapies targeting this mechanism are likely to be more efficacious in males. We hope to see Dr. Bayır apply novel lipidomics methods to this area in the near future in a continued collaboration with Dr. Kagan.

Adenosine in TBI

Dr. Kochanek's group is beginning experiments funded by an RO-1 from NINDS that was just successfully renewed in the area of adenosine and TBI. Translational work continues to be an important part of this effort and the CSF bank represents a key resource. Last year, we reported that T-32 fellow Dr. Paul Shore reported at the 31st Congress of the SCCM increased levels of vascular endothelial growth factor (VEGF) in CSF after severe TBI in children. The elaboration of VEGF is stimulated in part via adenosine receptor activation and local tissue hypoxia. This year, Dr. Shore published that work as a full paper in the journal Neurosurgery. Dr. Shore's work thus builds on our studies of the adenosine pathway and provides additional support for an early regenerative phase in clinical TBI. A second clinical project in this area that has created a great deal of excitement is the work of medical student Kathleen Sachse who has been collaborating with Dr. Edwin Jackson to assess the levels of caffeine and its metabolites in the CSF of adults with severe TBI. Kathleen observed remarkably high levels of caffeine in CSF of many adults with severe TBI and surprisingly, an association between CSF caffeine concentration and favorable long-term outcome. Kathleen presented this work at the 2004 meeting of the ASA where, as previously discussed, it garnered some media coverage. This potential beneficial effect of caffeine could be related to up-regulation of A1 receptor number or function—making endogenous adenosine a better neruoprotectant after injury. A beneficial effect of caffeine has recently been reported in Parkinson's disease that may be mediated via caffeine's inhibition of the A2a receptor. Further investigation is now underway in our laboratory.

CSF markers of neuronal death in TBI

For a discussion of these findings, please see the report of Dr. Robert Clark's group later in this annual report.

Effects of therapeutic hypothermia on the response to severe pediatric TBI

As Dr. David Adelson's clinical trials of therapeutic moderate hypothermia in pediatric TBI progress, we have been taking advantage of this important clinical investigation by studying CSF samples drained from the subgroup of patients at Children's Hospital of Pittsburgh. Last year, Dr. Bayır demonstrated that hypothermia attenuated oxidative injury after TBI in children. This year, she surprisingly showed no effect of hypothermia on nitrotyrosine levels in CSF in children after severe TBI. This suggests a differential effect of hypothermia on oxidative vs nitrative stress in patients. That work was presented at the 33rd SCCM Congress. In addition, Dr. Paul shore reported that therapeutic hypothermia (versus normothermic treatment) failed to attenuate increases in adenosine and purine-related metabolites, the cytokines IL-6 and IL-8, and several growth factors. That work, presented at the 2003 National Neurotrauma Society Meeting, further supports the concept of a differential effect of hypothermia on selected mechanisms of secondary damage and repair after severe TBI—most notably, oxidative stress, excitotoxicity and selected aspects of inflammation.

Effect of modes of CSF drainage

Dr. Paul Shore, in collaboration with Dr. Neal Thomas at Penn State Children's Hospital and Dr. David Adelson at Children's Hospital of Pittsburgh, completed and published an interesting descriptive study assessing the effect of continuous versus intermittent CSF drainage on mediator levels and pathophysiology after severe TBI in infants and children. These two approaches to CSF drainage in the treatment of intracranial hypertension have not been compared, and are used at the discretion of the treating physician/institution. Dr. Shore, reported that CSF levels of essentially all mediators tested were substantially lower in patients treated with continuous versus intermittent drainage. In addition, the amount of CSF drained in the continuous group was considerably greater than in the intermittent group and ICP was lower in the children treated with continuous drainage. This study was published by Dr. Shore in the *Journal of Neurotrauma* and suggests the need for a prospective clinical trial to compare these two approaches to treatment since dramatic differences were seen in this preliminary clinical trial.

Our pediatric CSF repository continues to represent a key research tool of our trainees to help bring the bench to bedside in the study of secondary injury mechanism in clinical TBI research.

Support: Improving the Diagnosis and Prognosis of Inflicted Head Trauma in Infants R49/CCR310285-03, (9/1/03-8/31/08), $139,163 (DC $95,265 and IDC $45,898), P Kochanek, PI, M Heyes, PhD, [Curagen Corporation], R Berger, S Wisniewski, PhD, and P David Adelson, MD, Co-investigators); collaborators. CDC, CIRCL (H Weiss, PhD, PI); Adenosine and TBI, NS38087, $311,420 (DC $213,749 and IDC $97,671), P Kochanek, PI; iNOS and TBI, NS30318 (P Kochanek, PI), Project 3 in the University of Pittsburgh Brain Trauma Research Center (BTRC), CE Dixon, PI. Protocol #3480500 (3/1/03-2/29/04), $179,434 (DC $122,525 and IDC $39,047), R Berger, PI, CHP GCRC. Oxidative Stress after Severe Head Injury in Infants and Children: Effect of Therapeutic Hypothermia, Laerdal Foundation, (01/01/03-12/31/03), $11,975, H Bayır, PI.

B. Adenosine and TBI

Adenosine is produced during the breakdown of adenosine triphosphate (ATP) after TBI. Its powerful vasodilator, anti-excitotoxic, and anti-inflammatory effects may represent an important endogenous defense mechanism in injured brain. The role of adenosine after TBI is being pursued both in the rat TBI model and in patients after TBI. This program includes both bench and bedside investigation, as discussed above with the clinical CSF studies. In the laboratory, we are completing studies examining the effects of adenosine agonists and antagonists on CBF as assessed by MRI. Dramatic and sustained increases in CBF were observed. This is discussed further in our section on MRI and TBI. We have also begun to carry out studies in two important knockout (ko) mice – namely the A2a receptor ko provided by Dr. Jiang-Fan Chen at Boston University, and the A1-receptor ko mouse supplied by Dr. Jurgen Schnermann at NIDDK. This project continues to be the most active area of research in Dr. Kochanek's laboratory this year and is being carried out in collaboration with Dr. Edwin Jackson in the Center for Clinical Pharmacology

Support: NIH RO-1, Adenosine and TBI, NS 38087-05, (8/1/03-7/31/04), $311,420 (DC $213,749 and IDC $97,671), P Kochanek, MD, PI, National Institute of Neurological Disorders and Stroke (NINDS) and Dr. Shore is supported by NIH T32, Pediatric Neurointensive Care and Resuscitation Research, T32-HD40686, (5/1/03-4/30/04), $249,304 (DC $232,195 and IDC $17,109), P Kochanek, PI, National Institute of Child Health and Development (NICHD).

C. Role of Inducible Nitric Oxide Synthase (iNOS) in the Inflammatory Response after TBI

iNOS is induced by cytokines and NF-κB is suggested to play an important role in the pathophysiology of sepsis outside of the central nervous system. Both beneficial and detrimental actions of iNOS have been reported. Using both inhibitors of iNOS and knockout mice, Dr. Elizabeth Sinz (1996-97 Charles Schertz Fellow) reported a powerful endogenous neuroprotectant effect of iNOS in experimental TBI. New faculty member Dr. Hülya Bayır, in collaboration with Drs. Kagan and Timothy Billiar, has been carrying out studies to define some of the endogenous neuroprotectant effects of iNOS. Her preliminary work suggests an important endogenous antioxidant effect of iNOS-derived NO *in vivo* and was presented at the 2003 meeting of the National Neurotrauma Society. Dr. Bayır is also examining the contribution of iNOS to nitration and nitrosylation in brain after experimental TBI using iNOS ko mice. She is also investigating the potential roles for iNOS in post-translational modification of proteins, and immunomodulation. This area of study is carried out as part of our funded project within the University of Pittsburgh Brain Trauma Research Center (BTRC) Program Project.

Support: NIH 2P50 NS30318, iNOS and TBI, $179,434 (DC $122,525 and IDC $39,047), P Kochanek, MD, PI, Key Collaborators: H Bayır, MD, RSB Clark, MD, CE Dixon, PhD, T Billiar, MD, V Kagan, PhD, L Jenkins, PhD, X Zhang, PhD, and T Carlos.

D. Emergency Interventions after TBI: Effect on Secondary Damage

Last year, work was completed on this project that was focused on the assessment of the effects of the administration of various anesthetics and sedatives early after experimental TBI in rats. We are now publishing the findings. Previously we reported powerful neuroprotectant effects of isoflurane versus fentanyl in rats anesthetized with these agents at the time of trauma. We also carried out a study comparing 7 different sedative/analgesic regimens—applied early after injury and again showed that isoflurane was the most beneficial. This year Dr. Kimberly Statler published a study demonstrating that isoflurane powerfully attenuated the marked early increase in cerebral glucose utilization that accompanies excitotoxicity early after TBI. In contrast, increased glucose utilization was elevated after TBI in rats anesthetized with fentanyl. Figures from Dr. Statler's work were featured on the cover of two issues of *Brain Research*. Dr. Statler (one of our T-32 fellows) has been the leading investigator on this work and is joining the

faculty of the University of Utah, where she plans to continue her research. These studies suggest that isoflurane may limit excitotoxicity in experimental TBI, making it more difficult to demonstrate beneficial effects of therapies. In addition, clinically used narcotics such as fentanyl and morphine fail to provide any anti-excitotoxic protection.

Support: This work was previously funded by the US Army, the Laerdal Foundation, and Dr. Statler was supported by T32-HD40686, from the National Center for Medical Rehabilitation Research (NCMRR), National Institute of Child Health and Development (NICHD), P Kochanek, PI .

E. Magnetic Resonance Imaging (MRI) Assessment of Experimental TBI

Contemporary and novel MRI methods are being used to characterize our injury model and facilitate the testing of novel therapies in experimental TBI in rats. The goal of this work is to use non-invasive NMR methods to access acute physiologic derangements early after injury and to couple these to assessment of functional outcome at more delayed times after TBI. MRI methods were used to augment investigation in our study of both adenosine (see above) and anesthetics in experimental TBI. Our work examining the effects of adenosine agonists on CBF in normal rats and in rats after experimental TBI is in preparation for publication. We have begun to use MRI to assess CBF in our mouse CCI model with the help of Kevin Hitchens and Lesley Foley. Our initial studies show feasibility of this method in mice and we look forward to the first full study of this application. Dr. Ho's outstanding multidisciplinary NMR Center for Biomedical Research continues to be a key collaboration for our work in experimental TBI.

Support: NIH-NINDS 2P50 NS3031809 A1, Rat/Surgery/Imaging Core C, $192,628 (IDC $131,534 and DC $61,094 - $655,696 over 5 years, P Kochanek, MD, PI, C Ho, PhD, Co-PI, Kevin Hitchens, Lesley Foley, and Edwin Jackson, Co-investigators). NIH Grants RR-03631 and RR-10962, (C Ho, PI) support the Multidisciplinary Pittsburgh NMR Center at Carnegie Mellon University. NIH PAR00-031, In-Vivo MR Microscopy Instrumentation at 11.7 Tesla ($500,000, C Ho, PhD).

Miscellaneous

Dr. Kochanek served as one of the four editors of the new edition of the Shoemaker Textbook of Critical Care Medicine--a huge project that has just gone to press. He also continues to serve as the Editor in Chief of the journal *Pediatric Critical Care Medicine.* Finally, he authored or oversaw the preparation of a number of chapters in books in the area of experimental and clinical TBI and pediatric neurointensive care, including a comprehensive chapter on the topic of hypothermia in experimental and clinical TBI that will be published in a new book co-edited by Drs. Samuel Tisherman and Fritz Sterz on Therapeutic Hypothermia. Other chapters are included in the bibliography of the TBI program.

2. Studies directed by C. Edward Dixon, PhD

C. Edward Dixon, PhD, Professor of Neurological Surgery, Anesthesiology, Neurobiology, and PM&R, University of Pittsburgh School of Medicine. Director, University of Pittsburgh Brain Trauma Research Center

Research Interests

Research in Dr. Dixon's laboratory is directed towards understanding the molecular mechanisms of cognitive deficits following TBI. Current studies are evaluating the effects of brain injury on dopaminergic and cholinergic systems and the relationship between these changes and the induction and recovery cognitive deficits. Experimental neurotherapeutic studies are ongoing to evaluate the effects of neurotrophic growth factors and neurotransmitter receptor activation on recovery of function. Clinical studies include measuring CSF and extracellular levels of catecholamines and markers of oxidative injury in humans acutely after brain trauma. Dr. Dixon also collaborates closely with many of the Safar Center investigators as the director of the functional outcome core facility for experimental TBI research.

A. Dopaminergic/Cholinergic Mechanisms of TBI

Recovery of cognitive function after TBI is a dynamic process in which alterations in neurotransmitter systems do not likely occur in isolation. Prior work in our laboratory demonstrated that substantial cholinergic neurotransmission deficits occur without a chronic (4-wk post injury) loss of cholinergic cell bodies and that that TBI causes chronic changes in key dopaminergic proteins that occur concomitantly with these cholinergic changes. Numerous studies have shown that the dopaminergic innervation of medial septum and diagonal band of broca (medial septal area [MSA]) regions that are dense with cholinergic neurons, can affect hippocampal acetylcholine (ACh) release, especially via D1 receptor agonists. Furthermore, our data suggest that dopaminergic innervation of cholinergic nuclei is reduced after TBI. In this project, our hypothesis is that cognitive deficits after TBI may be, at least partially, attributable to decreased dopamine (DA) modulation of septohippocampal cholinergic function. A systematic series of studies are testing this hypothesis. Our focus is on DA modulation of the selectively vulnerable septohippocampal cholinergic system. To better grade an effect of TBI on these systems, we will compare in the MSA the effects of TBI to an established model of DA deafferentation effects; 6-hydroxydopamine (6-OHDA)-induced DA denervation. We will examine the effects of TBI and 6-OHDA lesions on DA modulated ACh release in the hippocampus and DA release in the medial septum. We will also determine whether changes in hippocampal ACh release are associated with altered D1 receptors in the MSA. Dr Dixon's group will determine the effect of exogeneous administration of neurotrophic factors on DA biochemical markers, cognitive deficits, as well as hippocampal ACh release and MSA DA release after TBI. Lastly, we will determine the effects of clinically relevant DA agonist therapies on cognitive deficits, as well as hippocampal ACh release and MSA DA release after TBI. Our long-term goal is to develop new therapies to accelerate cognitive recovery following TBI.

During this year, we reported at the National Neurotrauma Society meeting that DA transporter expression was reduced in striatum after TBI in rats. Similarly, in collaboration with Dr. Amy Wagner, we demonstrated effects of environmental enrichment on frontal cortex DA transporter and BDNF. Those results are discussed in greater detail in Dr. Wagner's section. Finally, in related studies, postdoctoral fellow Margaret Wilson has been working on striatal injury in the CCI model, under the direction of Dr. Dixon, and gave presentations this year related to this work to both the Society for Neuroscience and National Neurotrauma Society. Dr. Wilson has been using Fluro-Jade B staining to study neurodegeneration in the rat CCI model and reported on both the time course of injury and the lack of effects of 8-OH-DPAT therapy on neurodegeneration after injury.

Support: NIH-NINDS, Chronic Changes in Neurotransmission Following TBI, R01 NS-33150-06 ($1,000,000/$484,819 over 5 years, 4/1/00-3/31/05, CE Dixon, PhD, PI).

B. Functional Outcome Core

The Functional Outcome Laboratory Core Facility provides a centralized site and highly standardized procedural control for all animal experiments employing functional outcome as an endpoint following TBI to rats. The Functional Outcome Laboratory Core gives the investigators of the University of Pittsburgh BTRC the capability to assess the effects of physiological manipulations and therapeutic interventions of recovery of function after experimental brain injury.

During this year, the Functional Outcome Core has evaluated post-injury function in several hundred rats and mice for seven different Principal Investigators associated with the Safar Center. This included important contributions to publications from the labs of Drs. DeKosky, Kline, and Wagner, along with a number of additional preliminary abstract reports.

Support: NIH, BTRC Supplement—Functional Core to P50 NS-30318-041A ($274,583 over 4 years, 4/1/96-3/31/00, CE Dixon, PhD, PI).

C. Examination of the Cellular Mechanisms of Mesocortical Dopaminergic Deficits after TBI in a Rodent Model Using Biochemical Indices of DA Autoxidation and Biochemical, Molecular Biological and Immunohistochemical Indices of DA Metabolism and Neurotransmission.

The goal of this project is to examine the cellular mechanisms of mesocortical dopaminergic deficits after TBI in a rodent model using biochemical indices of DA autoxidation and biochemical, molecular biological and immunohistochemical indices of DA metabolism and neurotransmission. Neurochemical and immunohistochemical markers of DA neurotransmission in the dopaminergic ventral tegmental/forebrain systems, as well as functional deficits, will be assessed after injury. The effects of therapies that either reduce oxidative damage of DA terminals and/or chronically stimulate DA activity on neurochemical and immunohistologic markers, and on

functional performance will be assessed following TBI. Lastly, the relationship between early biochemical markers of DA activity to neuropsychological outcome measures specific to frontal lobe function will be evaluated in severe TBI patients. This project represents the first systematic examination of the mechanisms of induction and recovery of catecholaminergic cognitive deficits after TBI. Our long-term goal is to develop new therapies to attenuate the induction and enhance the recovery of DA-mediated neurobehavioral deficits after TBI. Some of this work has been carried out in collaboration with Dr. Amy Wagner, and details of that work are reported later in this annual report.

Support: NIH-NINDS, Mechanisms of Prefrontal Dysfunction Following Brain Trauma, R01 NS-40125-01 ($800,000/$376,775 over 4 years, 3/1/00-3/31/04, CE Dixon, PhD, PI).

D. Transcriptomic Analysis of Therapeutics in Brain Trauma

Recovery of cognitive function after TBI is a dynamic process that likely involves multiple neural systems. Several studies by our laboratory and others indicate that cognitive recovery can be enhanced by post injury activation of dopaminergic systems or exposure to an enriched environment. The effectors of such therapeutic activation are likely to involve simultaneous gene expression changes in numerous neural systems. The recent development of DNA microarrays has allowed scientists for the first time the ability to observe thousands of gene expression changes in parallel. While there are limitations, DNA microarrays provide a new systemic view to study brain injury and the treatments that stimulate and enhance recovery of function. We have evaluated a number of DA agonists that are clinically used "off label" for their ability to enhance recovery of cognitive function in our experimental model of TBI and found three to be beneficial: amantadine hydrochloride, bromocriptine, and methylphenidate. While all are putative DA agonists, they have varying degrees of specificity. We have also observed that bromocriptine treatment, when initiated 24 h after TBI, can attenuate hippocampal cell death and lipid peroxidation. This suggests that DA agonists may have mechanisms of action beyond just being DA replacement therapies (e.g. cell survival effects). Supporting this concept, our preliminary microarry data suggest that relative to a vehicle treatment, the DA agonist methylphenidate can enhance the gene expression of DA receptors and alter injury-induced inflammatory responses. DNA microarrays are well suited to investigate the effects of DA agonists on multiple pathways. The overall goal of the project is to determine common genes that are changed by these therapies and whether these gene expression changes can be further enhanced by the addition of enriched environment therapy. This project will obtain the information needed for a larger-scale R0l study to increase the number of cases, refine and increase the number of genes analyzed, and to comprehensively study those genes whose expression are related to recovery of function after TBI. These studies of message also dovetail with the proteomic work being carried out in the lab of Dr. Larry Jenkins in the Safar Center.

Support: NIH-NINDS, R21 NS47919, Transcriptomic Analysis of Therapeutics in Brain Trauma. 07/01/03–06/30/06. $95,000-annual direct costs. CE Dixon, PhD, PI.

E. Miscellaneous

Several other areas are being investigated in the Dixon laboratory including novel studies on the effect of acupuncture treatment on functional recovery in the rat CCI model. Those studies are being carried out by Dr. Hong Yan. Similarly, preliminary work has begun in two areas, one focused on the interaction between the adenosine and dopamine systems in experimental TBI and one on the role of calcineurin signaling in histopathological and functional outcome after experimental TBI. More on these new projects will follow in next year's report.

Finally, Dr. Dixon was involved in aiding Dr. Geoff Manley in developing a pig model of CCI. That work was presented at the 2003 meeting of the National Neurotrauma Society.

Support NIH, R21 NS47919, Transcriptomic Analysis of Therapeutics in Brain Trauma. CE Dixon PI. 07/01/03–06/30/06. $285,000 total direct costs; NIH, R01 NS40125, Mechanisms of Prefrontal Dysfunction Following Brain Trauma. CE Dixon PI. 03/01/00-03/31/04. $1,000,000 total direct costs; NIH, R01 NS33150, Chronic Changes in Neurotransmission Following TBI. CE Dixon PI. 04/01/00-03/31/05. $1,645,223 total direct costs; CDC, R49 CCR312296, CIRCL: Acute Care Core Project 1–Effects of Amantadine Hydrochloride on Functional Outcome After TBI: a Randomized, Multi-Center, Placebo-Controlled Clinical Trial; and Acute Care Core Project 2-Relationship Between Amantadine Hydrochloride Efficacy and Brain Function Using PET Imaging, CE Dixon PI. 09/01/98-08/31/02. $2,709,778 total direct costs; USAMRMC, 00-451-4360, Novel Resuscitation from Lethal Hemorrhage. P Safar PI, CE Dixon Co-I. 09/15/02-09/14/03. $712,336 annual direct costs; NIH, R21 NS40049, Protein Synthesis, Memory and Pediatric Brain Injury. LW Jenkins PI, CE Dixon Co-PI. 04/01/00-03/31/03. $375,000 total direct costs; NIH, R01 NS38087, Adenosine and TBI. P Kochanek PI; CE Dixon Co-PI. 08/02/99-07/31/03. $747,440 total direct costs; NIH, R03 HD41399, Gender Differences in DA Function after TBI. AK Wagner PI, CE Dixon Co-PI. 02/06/02 – 01/31/04. $100,000 total direct costs; NIH, K08 HD40833, DA Function in TBI and Effects of Therapeutic Intervention. AK Wagner PI, CE Dixon Primary Sponsor. 09/01/01-09/30/06. $576,165 total direct costs; NIH, R03 HD043851, Interaction of Serotonin and Cholinergic Systems after TBI. AE Kline PI, CE Dixon Co-I. 04/01/03–03/31/05. $100,000 total direct costs.

3. Studies by Robert S.B. Clark, MD

Robert S.B. Clark, MD, Associate Professor of Critical Care Medicine and Pediatrics, University of Pittsburgh School of Medicine, Fellowship Director, Pediatric Critical Care Medicine Program, Children's Hospital of Pittsburgh.

A. Endogenous Neuroprotectant Gene Expression after TBI

This research focuses on the genetic regulation and execution of delayed neuronal death in selectively vulnerable neurons after TBI. We have now characterized the expression of several potential cell death-suppressor genes and their translated proteins including bcl-2 gene family members and heat shock protein 72 (endogenous neuroprotectants), as well as potential cell death-effector genes including the pro-apoptotic bcl-2 gene family member bax. These genes appear to be up-regulated and/or activated after TBI in both our experimental model (CCI injury with secondary hypoxemic insult followed by resuscitation in rats) and in humans. Studies documenting that bcl-2 family genes may be important in both adult and pediatric patients after TBI were reported previously in the *FASEB Journal* and the *Journal of Pediatrics*, respectively.

A role for heat shock proteins after human head injury is also being investigated. Regulation of some of these proteins is via post-translational modification, including the bcl-2 family members bad and bag-1. Bag-1 regulates the chaperone function of heat shock proteins, pointing to a direct interaction between these two classes of endogenous neuroprotectants. This interaction was demonstrated in human brain after injury by Dr. Neal Seidberg, a PCCM fellow, and others in the laboratory. This work was published in the *Journal of Neurochemistry*.

This year, PCCM fellow Dr. Yichen Lai carried out a study examining the stress response to TBI in infants and children and reported that the stress protein HSP-70 was markedly increased in CSF after injury, particularly (over 3-fold) in victims of inflicted TBI (child abuse). This represents another mechanistic pathway demonstrating a unique profile in the abuse victims. In this case, chronic injury or stress, among other factors, could play an important role. Dr. Lai's paper on this work was published in the *Journal of Neurotrauma*. Also, summer student, Christopher Stange studied the endogenous neuroprotectant protein HSP-60 and demonstrated increases in CSF after severe TBI in infants and children. Chris gave an outstanding presentation of that work at the 2003 Congress of the Society of Critical Care Medicine (SCCM). Similarly, summer student J'Mir Cousar, working in the University of Pittsburgh School of Medicine Summer Enrichment Program, authored an abstract on increases in heme oxygenase-1 in CSF after pediatric TBI. The Heme oxygenase pathway is well known to have important neuroprotective effects in preconditioning and related models. That work was presented at the 2003 Congress of the SCCM. Congratulations to Christopher and J'Mir for a job well done.

B. Divergent Pathways of Cell Death after Brain Injury

Increasing evidence suggests that activation of caspases regulate and execute programmed cell death after TBI in experimental models and in humans. Accordingly, the objective of this research is to develop pharmacological and molecular treatment strategies that reduce caspase-mediated programmed-cell death after TBI. We previously described potential roles for caspase-1 and –3 after severe TBI in humans in a paper

published in the *FASEB Journal*. Studies examining other more potent caspase inhibitors, and combination treatment strategies targeting multiple points in the programmed cell death cascade are ongoing.

This year, to continue to bridge bench and bedside in this research area, Dr. Lai expanded upon the work of prior T-32 Dr. Margaret Satchell and reported marked increases in CSF levels of cytochrome-c in additional pediatric TBI patients. That expanded study was presented at the SCCM meeting this year and a full manuscript will follow. The release of cytochrome-c was again associated with inflicted TBI—further supporting unique facets of this injury mechanism related to secondary neuronal death—presumably by apoptotic pathways. This suggests potential unique therapeutic targets for secondary brain injury in child abuse victims. I am pleased to say that this year Dr. Lai will join our T-32 program.

It is clear that both apoptotic and necrotic cell death contribute to neuronal cell loss after acute brain injury; however, recent data suggest that this is in fact over simplistic, and that multiple, interrelated pathways exist. A key regulator in this regard is the mitochondrial protein AIF. Work by Dr. Xiaopeng Zhang under the direction of Dr. Clark has shown that AIF-mediated cell death occurs after experimental TBI. That work was published in the *Journal of Neurochemistry*. Last year Drs. Zhang and Clark demonstrated an important role for an additional pathway of delayed neuronal death after experimental and clinical TBI—namely—the Fas/Fas ligand pathway. They reported, in the *FASEB Journal*, caspase-8 expression and proteolysis in human brain after severe TBI—suggesting the need for additional experimental and clinical investigation of this pathway in TBI, and the possibility of novel avenues for therapy. Ongoing studies are determining the contribution of these divergent pathways of cell death to secondary damage in TBI using multiple strategies in collaboration with Drs. Jun Chen, Steven Graham, Patrick Kochanek, Csaba Szabo (Inotek Corp., Beverly, MA), Simon Watkins, Hector Wong (Cincinnati Children's Medical Center), and Ian Reynolds.

This year Dr. Zhang presented two papers germane to this area of work. First, he presented, at the 2003 meeting of the Society for Neuroscience, a proteomic analysis of proteins released from rat brain mitochondria after depolarization. Second, he presented at the National Neurotrauma Society meeting evidence for activation of the protein kinase B signaling pathway after experimental TBI. He is studying this important cell death-regulating pathway in brain samples from both experimental and clinical TBI in work that is being carried out in collaboration with Dr. Larry Jenkins (see Dr. Jenkins's section later in this report).

C. PARP Activation after TBI

The study of PARP in experimental TBI is an expanding area of investigation at our center. PARP is an abundant nuclear enzyme with a role in DNA repair pathways. However, in the setting of energy failure, it is suggested that excessive ADP-ribosylation of proteins resulting from activation of PARP leads to marked nicotine adenine dinucleotide (NAD) depletion and exacerbation of energy failure. Drs. Whalen, Clark, and Kochanek collaborated with Dr. Csaba Szabo (an expert in the area of PARP and

sepsis at the Inotek Corporation) to study the PARP ko mouse in our model of experimental TBI. We previously reported highly significant levels of protection against functional deficits after TBI in PARP ko vs wild-type mice, and a role for PARP inhibitors in improving outcome in experimental TBI in mice. However, we also noted deleterious effects of PARP inhibitors on memory acquisition in normal mice—supporting a role for PARP in memory acquisition. Last year, we published a report showing that intra-mitochondrial PARP activation contributes to NAD depletion and cell death, both in neuronal culture and in experimental TBI—which provided novel and valuable insight into the cascade of cell death in the setting of PARP activation—a mechanism that is believed to contribute importantly to a number of important diseases in critical care medicine including CNS injury, stroke, cardiac arrest, sepsis, shock and MOF. In addition, this work further establishes the presence of PARP in mitochondria.

This year, recently graduated T-32 fellow Dr. Margaret Satchell published a full manuscript on the in vivo work evaluating PARP inhibitors in our TBI mouse model in the *Journal of Neurochemistry*. Those studies suggest interesting direct effects of PARP on learning and memory and have stimulated the evaluation of targets of mitochondrial poly-ADP ribosylation by the Clark laboratory. Initial work reporting the discovery of mitochondrial poly-ADP-ribosylation as an important post-translational modification were published in the *Journal of Biological Chemistry*. Additional studies with PARP inhibitors are underway.

Finally, Dr. Clark's laboratory contributed a number of important chapters and reviews on cell death pathways, including a chapter in the upcoming Shoemaker Textbook of Critical Care Medicine and a review article in the journal *Critical Care.*

Support: RO1-NS38620-04, Caspase-Mediated Neuronal Death after Head Injury ($584,022 total direct costs over 4 years beginning 2/1/99, R Clark, MD, PI); RO1-NS38620 competitively renewed under the new title Divergent Pathways of Cell Death after Brain Injury ($1,187,500 total direct costs over 5 years beginning 2/1/03). P01-NS30318, PARP Activation After TBI, Project 4 of the BTRC Program Project ($595,000 total direct costs over 5 years beginning 6/1/00, R Clark, MD, PI). R44 NS37985, Ultrapotent PARS Inhibitor for CNS Trauma, NIH SBRI Subcontract Csaba Szabo ($120,000, total direct costs over 1 year beginning 9/1/02).

4. Studies directed by Larry W. Jenkins, PhD

Larry Jenkins, PhD, Associate Professor of Neurological Surgery, University of Pittsburgh School of Medicine

A. Protein Kinase B and C in Head Injury

The PKB and PKC enzyme families participate in many cellular functions including protein synthesis. Hippocampal protein synthesis after TBI is critical for neuronal

survival, learning and memory, and synaptic plasticity. TBI alters hippocampal protein synthesis and while improved protein synthesis enhances recovery after cerebral ischemia, this has not been examined after TBI. Pathological changes in protein synthesis mediated by dysfunction of eIF2 and eIF4 pathways after TBI may impair the initiation and fidelity of protein synthesis and injury related restorative and growth responses. Pathological changes in the phosphoinositide 3-kinase-protein kinase B (PI3K-PKB), PKC, GSK-3, mitogen activated protein kinase (MAPK) and mTOR pathways may all be involved in abnormal protein synthesis after TBI. Protein synthesis can be modified by cap-dependent (eIF4E), cap-independent (internal ribosome entry segment [IRES]), and 5'TOP-5' oligopyrimidine tract (mTOR) protein synthesis initiation. This project tests the hypothesis that improved functional recovery following TBI can occur by therapeutically activating beneficial stress related IRES protein synthesis after injury causing stress induced tolerance to secondary injury processes. Hypothermia has been shown to be one therapeutic mechanism by which protein synthesis can be manipulated and will be examined. This year we examined a number of kinase systems with and without hypothermia treatment and have documented a number of important and surprising changes. In addition to the role of protein kinases in translation control, phosphorylation also regulates gene expression via epigenetic mechanisms via post-translation modification of histones and transcription factors. Gene expression changes exert proximal control over the types of mRNA to be translated and will also be examined in this project.

Thus, the aims of this proposal are to determine fundamental kinase and chaperone protein pathways that regulate protein synthesis in relation to hypothermia treatment after TBI by examining the control of three major initiation pathways, namely, cap-dependent, cap-independent (IRES) and 5’ terminal oligopyrimidine tract (5’TOP) translation. We will further examine the expression of key protein products representative of these pathways involved in recovery from injury. Protein synthesis regulation is fundamental to most cellular processes. Recent advances in understanding the complexities of protein synthesis regulation contribute to the potential for therapeutic manipulation of protein synthesis. However, the manipulation of signals controlling protein synthesis after TBI may not only affect regional injury and restorative responses, but the normal function of relatively uninjured brain regions after TBI.

Control of protein synthesis primarily occurs at the rate-limiting step of initiation. Pathological changes in protein synthesis mediated by dysfunction of eIF2 (eIF2 - rate of translation - quantitative) and eIF4 (eIF4-mRNA selection-qualitative) pathways after TBI may impair the rate and fidelity of protein synthesis and injury repair. Protein kinases and phosphatases modulate many critical control steps in the initiation and fidelity of protein synthesis, especially the initiation steps mediated by eIF2 and eIF4 protein pathways and thus the activity of these kinase and eIF pathways can be determined in part by their phosphorylation status. Using a reproducible and clinically relevant model of controlled cortical impact (CCI) in the rat, (resulting in spatial memory dysfunction as occurs in humans, we have identified a number of important hippocampal signaling changes that affect protein synthesis initiation. Time dependent changes in PKB, PKC isoforms, PKA, GSK-3B, 4E-BP, mTOR, p70S6K, eIF4E, and eIF2a

phosphorylation after TBI have been documented and will be explored further in this project. In addition, changes in regulation of the histone code and epigenetic signaling have been documented and will be further examined.

This year we presented three abstracts in this area of research. First, Dr. Jenkins presented a report at the 2003 meeting of the National Neurotrauma Society showing that inhibitory phosphorylation of glycogen synthase 3 beta was decreased after CCI in rat pups, which may play a role in the reduction of protein synthesis after TBI. Second, Dr. Mandeep Chadha, in his first year as a T-32 fellow, presented a paper at the 2004 meeting of the Society for Pediatric Research that represented the initial proteomic assessment of a delayed time point after experimental TBI in the developing rat—specifically, PND 17. The initial results suggest feasibility of this approach since 2-D gel analysis revealed that some proteins such as GFAP showed marked increases vs controls at this delayed time point. Further analysis of these samples using both 2-D gel and power blot are underway. Finally, Dr. Weimin Gao reported our initial findings in the area of epigenetic signaling in experimental TBI. Using the CCI model in PND 17 rats, he reported that histone H3 acetylation was decreased after injury—providing the first evidence of a role for this important pathway regulating transcription. That paper was presented at the 2003 meeting of the National Neurotrauma Society, and a manuscript of that work is in preparation by Dr. Gao.

Support: NIH-NINDS, PKB and PKC in Head Injury, R01 NS42648, $ 231,250 annual direct cost, 02/15/04-01/31/08, LW Jenkins, PhD, PI).

5. Studies directed by Anthony E. Kline, PhD

Anthony E. Kline, PhD, Assistant Professor, Department of Physical Medicine and Rehabilitation (PM&R), University of Pittsburgh School of Medicine

A. Protective Effects of Serotonin$_{1A}$ (5-HT$_{1A}$) Receptor Agonists Against TBI-Induced Cognitive Deficits and Histopathology

5-HT$_{1A}$ receptors (5-HT$_{1A}$R) are abundant in brain regions, such as the cortex and hippocampus, that play key roles in learning and memory and that are susceptible to neuronal damage by TBI. During the past few years, our laboratory has been investigating the effects of 5-HT$_{1A}$ receptor agonists on neurobehavioral, cognitive, and histological outcome. We first evaluated the high affinity 5-HT$_{1A}$R agonist Repinotan HCL (BAY x 3702), which was given (iv) as a 4-h continuous infusion commencing 5-min after TBI or sham injury. The data revealed that repinotan significantly attenuated spatial learning deficits as demonstrated by decreased latencies to locate a submerged (hidden) platform in a water maze task compared to the injured vehicle-treated group. Repinotan also attenuated histopathology as evidenced by more hippocampal CA$_1$/CA$_3$ neurons and smaller cortical lesion volumes vs. the vehicle group. This study, which was published in the journal *Neuroscience* in 2001, was the first to investigate a 5-HT$_{1A}$R agonist intervention in any model of TBI. Next, we investigated whether the widely used 5-HT$_{1A}$R agonist 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) would produce

similar beneficial effects. Using our standard injury paradigm, we found that 8-OH-DPAT-treated rats exhibited significantly reduced latencies in locating the hidden platform vs. the vehicle-treated group over time, which is indicative of improved learning and memory. Significantly more CA_3 surviving neurons were observed in the group treated with 8-OH-DPAT vs. vehicle. This study was published in *Neuroscience Letters* in 2002. Because 5-HT_{1A}R agonists produce mild hypothermia, which may have contributed to the benefits observed, we recently completed a study designed to clarify this issue. Briefly, the experiment was similar to the previous, but included an 8-OH-DPAT group that was maintained at a normothermic temperature (37 ± 0.5°C). Both the Injury+DPAT+Normothermic and Injury+DPAT+Hypothermic groups exhibited enhanced cognitive performance (spatial acquisition and retention) and reduced histopathology (CA_3 cell loss and cortical lesion volume) vs. the Injury+Vehicle group (P<0.05), but did not differ from one another despite a rapid (15 min), mild (34.4 – 34.9°C), and transient (~1 hr) hypothermic effect in the latter. These data, which were recently published in the *Journal of Neurotrauma*, confirm that a single systemic administration of 8-OH-DPAT confers neurological protection after TBI, and demonstrate that the beneficial effect is not mediated by concomitant hypothermia. We are currently evaluating the potential efficacy of a delayed and chronic 5-HT_{1A}R agonist treatment paradigm, as well as the added benefit conferred by environmental enrichment. Our published and ongoing studies lend credence for continued investigation of this therapeutic strategy and the recent acquisition of an R01 application combined with an ongoing R03 grant provide the support necessary to fully explore this exciting avenue. Collaborators include Drs. C. Edward Dixon, Amy Wagner, and Ross Zafonte from the Departments of Neurological Surgery, and PM&R.

B. Role of Environmental Enrichment (EE) after TBI

Enriched housing, which provides a complex, stimulatory, and social environment, and may be considered a rodent correlate of physiotherapeutic intervention, has been extensively studied in numerous experimental conditions. EE has been reported to increase brain weight, dendritic arborization, synaptogenesis, and to decrease apoptosis of neuronal precursor cells in the hippocampal dentate gyrus. Rats housed in EE for 30 days exhibit significantly higher levels of nerve growth factor mRNA in the rat visual cortex and hippocampus than rats housed in standard conditions. EE has also been shown to increase the expression of brain-derived neurotrophic factor mRNA in the rodent hippocampus. Furthermore, EE has been shown to improve spatial memory and reduce contusion lesion volume. EE has also been demonstrated to improve motor performance on a beam walk task or sensory neglect after cortical lesions. In our laboratory we are comparing the effect of 28 days of EE with standard living conditions on functional and histological outcome after TBI. The data suggest that EE is superior to standard housing in facilitating functional recovery and suggests that this interventional strategy may be useful in a rehabilitative setting by augmenting pharmacotherapies. On-going studies in our laboratory are examining the role of EE coupled with the 5-HT_{1A} receptor agonists 8-OH-DPAT and buspirone on neurobehavioral and histological outcome after TBI. Recently acquired NIH funding for an R01 grant entitled "Novel Rehabilitative Approaches for Recovery from TBI" will provide further opportunity to examine the

relationship between EE and 5-HT_{1A} receptor agonists on the recovery process after TBI. Collaborators include Drs. A Wagner and R Zafonte from the Department of PM&R, R Gibbs from the Department of Pharmaceutical Sciences, and CE Dixon from the Department of Neurological Surgery.

C. Effects of Atypical Antipsychotics on Functional Outcome after TBI

Over 1 million survivors of TBI receive maintenance pharmacotherapy, of which a substantial number receive antipsychotic agents for the treatment of psychoses, agitation and aggression, and other maladaptive behaviors. The incidence of agitation after severe TBI varies from 11% to 50%. In spite of the common clinical use of antipsychotics, the motor and cognitive risks vs. benefits are unclear. Seminal studies by Feeney and colleagues have shown that treatment with antipsychotics (e.g., haloperidol) after TBI retard functional recovery. Moreover, the administration of such agents reinstates deficits in subjects appearing to be "recovered." More recent work has shown similar detrimental effects on motor function with haloperidol and clozapine after ablation-induced brain injury. Our laboratory is currently evaluating the effects of single (24 h after TBI or sham injury) and/or chronic (24 h–28 d) administrations of the atypical antipsychotic risperidone on motor (beam-balance and beam-walk) and cognitive (spatial learning and memory) functioning in rats. Additionally, risperidone is being compared to the classical antipsychotic, haloperidol. The results from these studies should provide a clearer understanding of the effects of antipsychotic treatments in the recovering brain. An NIH grant is being prepared to further explore this important avenue. These studies are being conducted in collaboration with Drs. R Zafonte and CE Dixon.

Support: NIH-NICHD R03 HD043851-01, Interaction of serotonin and cholinergic systems after TBI, $144,233 for two years (04/01/03 – 11/30/05). AE Kline, PhD, PI. The Pittsburgh Foundation, Evaluation of the serotonergic$_{1A}$ receptor agonist, 8-OH-DPAT, on biochemical, functional, and histological outcome following TBI in rats, $19,096 for one year (2001-2002). AE Kline, PhD, PI. The Pittsburgh Foundation.

6. Studies Conducted by Amy K. Wagner, MD

Amy K Wagner, MD, Assistant Professor, Department of PM&R, University of Pittsburgh School of Medicine

A. Clinical Gender Differences in TBI Pathophysiology

There is conflicting evidence as to whether there are gender differences with TBI pathophysiology and outcomes. Some studies have reported that with brain injuries of equal magnitude, women sometimes fair worse. Previous work by Dr. Wagner shows that one year after hospitalization with TBI, women have more disability. Yet several

animal studies show that female hormones are neuroprotective in attenuating aspects of secondary injury such as excitotoxicity, ischemia, and oxidative stress. We completed a retrospective clinical study using the NIH funded BTRC database to identify if there are gender differences in CSF markers of TBI and if hypothermia affects these markers in a gender specific manner. Multivariate regression modeling techniques were used to show that there are gender differences with the production and time-course of a cerebrospinal fluid marker of excitotoxic injury and a marker of ischemia early after injury. Females appear to have some neuroprotection against excitotoxic and ischemic injury. However, based on this study, hypothermia appeared to reduce excitotoxic injury primarily in males. This finding may be due to an apparent "floor effect" with hypothermia in reducing excitotoxic injury in females. Ischemic injury and excitotoxicity were also linked to a marker of oxidative stress. Again there were gender differences in the relationship of ischemia/oxidative stress and excitotoxicity/oxidative stress. Females have much lower oxidative stress loads than males for a given excitotoxic or ischemic insult. These findings indicate that there may be acute clinical correlates to the early neuroprotection previously reported in studies on experimental brain trauma. A manuscript reporting this work is currently submitted for review. Another manuscript has recently been published in the *Journal of Neurotrauma*. Dr. Wagner was just funded for a project in the successful competitive renewal of the CDC/CIRCL center grant. This grant is focusing on the role of sex hormones in mediating gender differences in CSF markers of TBI and evaluating the role of acute and chronic hormone levels on neuropsychological and functional outcome, and quality of life. Collaborators include the NIH funded Brain Trauma Research Center CSF Bank [(CE Dixon (Neurosurgery), Mary Kerr (Nursing), Ava Puccio (Neurosurgery)], Anthony Fabio (CIRCL), Ross Zafonte (PM&R, Hülya Bayır (CCM), and Sarah Berga (OB/GYN Emory University).

B. Gender Specific Effects of Environmental Enrichment on Dopamine (DA) Markers and Neurotrophin Production after Experimental TBI

Environmental enrichment has been shown in a variety of animal models to improve behavioral performance and impact neural substrates affecting plasticity such as angiogenesis, neurotrophin production, gliogenesis, and dendritic sprouting. Enrichment of the housing environment also improves spatial memory after experimental TBI in male rat models. Recently we reported that 3 weeks of environmental enrichment after experimental TBI improved cognitive recovery in male but not female rats. We then investigated the effects of gender and an enriched environment on dopaminergic markers and neurotrophin production after TBI. Using Western Blot techniques, we evaluated dopamine transporter (DAT) levels in the striatum and frontal cortex. Results showed injury related reductions in DAT protein levels both in frontal cortex and striatum for males. Females generally did not have injury related reductions. However, enriched housing post-injury did result in reductions in regional reductions for injured females.

In the second experiment, we used western blot to evaluate brain derived neurotrophic factor (BDNF) levels in the frontal cortex and hippocampus after TBI and housing in an enriched environment. In males, no enrichment or injury effects were observed with hippocampal BDNF expression, but there was a significant post-injury increase in frontal

cortex BDNF expression that was not augmented by EE. Neither injury nor EE altered frontal cortical BDNF expression in females, but there was a trend for decreased BDNF expression in the hippocampus of injured females vs. sham. In contrast, there were robust increases in hippocampal BDNF expression for EE injured females compared to both sham and injured animals placed in standard housing. These results reveal significant, region-specific gender differences in chronic BDNF expression with both injury and EE that may impact enrichment-mediated improvements in cognitive recovery and responses to therapeutic interventions. Portions of the work were funded through Dr. Wagner's NIH K08 award. Some of this work will be submitted to the journal *Neuroscience*. Future work will focus on the role of sex hormones on these findings as well as continuing to explore relevant neurotransmitter systems affecting a dimorphic response to environmental enrichment with cognitive recovery. This work was presented at the 2003 National Neurotrauma Society. Collaborators include Xiangbai Chen (PM&R), CE Dixon (Neurosurgery), A Kline (PM&R), and R Zafonte (PM&R).

C. DA Kinetics and TBI

Altered DA neurotransmission is hypothesized to play a role in neurobehavioral deficits after traumatic brain injury. DA enhancing agents (DA agonists) have been shown clinically to improve aspects of mental functioning after TBI, and have been shown in multiple animal studies to improve behavioral performance. This laboratory has reported reductions in striatal dopamine transporter (DAT) protein and increases in tyrosine hydroxylase (TH) chronically after TBI. These proteins play a critical role in DA release and reuptake. However, the effects of DAT reduction and TH increases on DA neurotransmission are unknown. Fast scan cyclic voltammetry (FSCV) permits real time in vivo evaluation of DAergic kinetics. The goal of this project was to assess striatal DA neurotransmission by evaluating presynaptic striatal DA kinetics in conjunction with neuroprotein and neurobehavioral correlates after experimental TBI. We evaluated electrically evoked DA release and DA clearance kinetics 2 weeks after injury. Striatal dopamine release during bilateral electrical stimulation of the medial forebrain bundle was monitored in anesthetized rats by FSCV in conjunction with Nafion-coated carbon fiber microelectrodes. Prior to FSCV, we also evaluated rotational behavior. After FSCV, we evaluated a variety of striatal DA markers, including DAT, TH, Dopamine type 2 receptors (DRD2), and Vesicular Monoamine Transporter (VMAT). Striatal evoked overflow of DA was lower in injured rats, versus naïve. We also showed differences in zero and first order DA clearance for injured rats as well as an increase in DAT efficiency (function) after TBI. Decreases in DAT expression were noted post-injury, despite no changes in VMAT, indicating a regulatory change in DAT concentration. Behavioral data suggested a low incidence of rotational behavior in this injury model and correlated well with bilateral changes in presynaptic kinetics and DA marker expression. Increases in DAT efficiency post-TBI provide one explanation for the potential efficacy of DAT inhibitors (DA agonists) with improving cognitive recovery. A manuscript for this work was submitted. We plan to investigate regional and post-injury time course differences in DA kinetics as well as response to acute and chronic pharmacotherapies. This work is being conducted in conjunction with Dr. A Michael in the Dept. of Chemistry, whose research focuses on electrochemical techniques

and the measurement of neurotransmitters using microsensors. Other collaborators and students include CE Dixon (Neurosurgery), R Zafonte (PM&R), Joshua Sokoloski, (PM&R/Chemistry) and Zachary Repanshek (PM&R/Chemistry). This and other pilot work (see genetics section) were used to submit an NIH R01 application evaluating the role of DAT genotype in striatal neurotransmission and responsiveness to treatment with methylphenidate in a clinical population with TBI

D. The Impact of Gender & Hormonal Status after Experimental TBI

Some studies have shown that sex hormones have neuroprotective qualities in the setting of acute traumatic brain injury. However, less is known about endogenously circulating sex hormones or if particular hormone levels at the time of injury effect behavioral recovery. Recently, we reported that females appear to have a neuroprotective advantage with behavioral recovery on motor tasks performed early after injury. However, no gender differences were noted with spatial learning later after injury. A manuscript on this work was recently published in *Brain Research*. Currently, we are beginning to evaluate the role of physiological hormone replacement in female rats on behavioral recovery after TBI. Additional work will focus on how hormone manipulations affect histochemical markers of injury. Students and collaborators include Xiangbai Chen (PM&R), Michael Wenger (PM&R/Neuroscience), Lauren Willard (PM&R/ Neuroscience), CE Dixon (Neurosurgery), A Kline (PM&R) and R Zafonte (PM&R).

E. Associations between DAT Genotype, Outcome, & CSF DA Levels after Severe TBI: A Follow-up Analysis

DA pathways have been implicated in cognitive deficits after TBI. While not associated with alterations in protein structure, the DAT genotype is associated with differences in DAT protein density and development of DA mediated pathophysiological conditions. For instance, the DAT 10/10 genotype is associated with higher DAT protein levels and is implicated in the development of attention deficit disorder. Differential DAT expression presumably also affects both pre-synaptic DA release, via reverse transport, and DA reuptake. DAT regulation may have a role in DA mediated neurotoxicity acutely after TBI and play a compensatory role with DA neurotransmission chronically after TBI. Catecholamines, including DA and its metabolites, are subject to auto-oxidation, resulting in the formation of reactive oxygen species that can contribute to oxidative stress associated with secondary injury. Prior work from this laboratory has shown reductions in DAT protein after experimental TBI. The role of DAT genotype on injury and outcome has not been studied. We hypothesized that genetic & gender related differences in DAT density would affect CSF DA production & metabolism post-TBI, through reverse transport of DA via DAT. We genotyped & collected CSF for DA & metabolite (DOPAC & HVA) analysis via HPLC for 73 patients with acute severe TBI. Mixed effects multivariate regression analyses showed an impact of DAT genotype and a trend for female gender to increase CSF DA levels. Gender impacted CSF DOPAC & HVA production without affecting DA turnover, while DAT genotype impacted DA turnover**.** Further, preliminary analyses suggest acute CSF DA levels are linked to functional recovery curves. Data from this project was used to submit an NIH R01

application evaluating the role of DAT genotype in striatal neurotransmission and responsiveness to treatment with methylphenidate in clinical TBI. This work is being done in collaboration with the University of Pittsburgh BTRC, Dianxu Ren (Public Health), CE Dixon (Neurosurgery), Yvette Conley (Health Promotion and Development), Robert Ferrell (Human Genetics), Sue Beers (Psychiatry), R Zafonte (PM&R), and Mary Kerr (Nursing).

Support: NIH K08HD40833, AK Wagner, MD PI, *DA Function and the Effects of Therapeutic Intervention* $622,258 beginning 2001 for 5 years (Sponsors: CE Dixon, PhD, AC Michael PhD, and RD Zafonte, DO); NIH R03HD41399, AK Wagner PI *Gender Differences in DA Function after TBI* $145,535 beginning 2002; CDC R49/CCR323155-01-1---CIRCL, AK Wagner, MD Project PI (H Weiss PhD PI Center Grant), *Evaluating the Impact of Neuroendocrine Hormones on Pathophysiology and Outcomes after TBI* $772,948; CDC CCR310285-07---CIRCL, Small Grants Program AK Wagner, MD Project PI (H Weiss PhD PI Center Grant) $10,000 beginning 2002 for *Characterization of Alterations in the Female Rat Estrous Cycle after Experimental TBI*; NIH P50NS30318 Clinical Core--*University of Pittsburgh BTRC*, CE Dixon PI; NIH Loan Repayment Program; Department PM&R, University of Pittsburgh.

TBI Investigation by Safar Center Scientists and Visiting Scientists

7. Studies by P. David Adelson, MD

A. Severe TBI in Immature Rats

Dr. Adelson's laboratory is focused on the effect of hypothermia following TBI acutely and long term on recovery. They have been examining the role of hypothermia and its effect on excitotoxicity, cell death, and synaptic recovery following experimental TBI in developing rat, studying both postnatal day (PND) 7 and PND 17 rats and demonstrated important age-related differences in the different ages at injury. His lab has been able to demonstrate the efficacy of therapeutic hypothermia on outcome as it relates to age at injury in these same developmental paradigms. Dr. Adelson and his investigative team presented work in both of these areas at the annual meeting of the National Neurotrauma Society. He has focused his recent efforts on the CCI model of TBI. His lab is also beginning to carry out exciting studies in collaboration with Dr. Patrick Card examining reorganization of the developing brain after experimental TBI.

Support: NIH Grant No. 1 R01 NS42298, Efficacy of Hypothermia in Pediatric TBI

B. Hypothermia for Severe TBI in Children

The major goal of this project was to test the safety and efficacy of therapeutic hypothermia in children after severe head injury. This program has been funded at an R01 level by the NIH/NINDS and investigated hypothermia as a treatment of TBI in children, with a special emphasis on the development of novel methods for the initial and outcome assessment. Dr. Adelson is the principal investigator of this important multi-

center study that includes 7 centers. Dr. Harvey Levin, at the Baylor College of Medicine, is a co-investigator on that study, along with Drs. Sue Beers and Tom Campbell, at the University of Pittsburgh, that is assessing long-term functional outcomes including language and speech acquisition, long-term effects of mild to moderate head injury, and a number of other collaborative and related efforts. This phase II study is now underway.

Collaborative studies of the effect of therapeutic hypothermia on a variety of biochemical and molecular mediators of secondary injury and repair are ongoing (please see prior discussion of this area in Dr. Kochanek's report) from CSF samples obtained from patients enrolled at the Children's Hospital of Pittsburgh.

Support: NIH Grant No. 1 R21 NS043293, Hypothermia for Severe TBI in Children (planning grant) and NIH Grant No. 1 R01 NS38448, Hypothermia for Severe TBI in Children.

C. Pediatric Neurotrauma Center

The Pediatric Neurotrauma Center (PNTC) was developed through the generous support of the Federation of Independent School Alumnae and serves as a web based database and is the central data core for clinical trials locally, nationally and internationally. The many projects that have been supported by the Data Center of the PNTC include the biochemistry analyses of children following TBI and following treatment with hypothermia, speech and language outcomes, mild and moderate TBI, cerebral blood flow and metabolism following TBI, to name but a few. The PNTC has also been instrumental in its support of international clinical trials serving as the data center for a multicenter TBI project in Latin America based out of Argentina and supported by the Latin American Brain Injury Consortium (LABIC).

8. Studies by Rachel P. Berger, MD, MPH

Rachel P. Berger, MD, MPH. Assistant Professor of Pediatrics, University of Pittsburgh School of Medicine and Children's Hospital of Pittsburgh

A. Use of Serum Biomarkers in the Detection of Silent Inflicted Childhood Neurotrauma

Infants who are victims of inflicted TBI are often injured on multiple occasions or brought to care many hours to days after their injury. In addition, their injury is often not recognized since caretakers rarely provide a history of trauma and the infants often do not have any external signs of trauma. In the past year, Dr. Rachel Berger, a general pediatrician working in the area of child abuse at Children's Hospital of Pittsburgh, has broadened the potential relevance of this project by studying the potential use of serum

markers of brain injury with the hope of detecting otherwise unidentified brain injury in possible victims of child abuse. Rachel first showed that CSF levels of markers of neuronal (neuron specific enolase [NSE]) and glial (S-100B) death were massively increased versus control after severe TBI in infants and children—including child abuse victims. That work was published last year in the journal *Pediatrics*. Dr. Berger also published a report in the *Journal of Neurotrauma* showing that these markers of brain injury are increased in the serum in over one-third of infants and children with mild TBI—children that are often sent home from the emergency department. This study has set the stage for an assessment of the use of these biomarkers in a target population of infants in diagnostic categories that occasionally represent missed cases of inflicted TBI-such as vomiting without diarrhea, a seizure without fever, unexplained bruising, etc. A positive serum test for such biomarkers would not confirm trauma as etiology of the increase, rather it would "point to the head" and suggest to the health care provider, the need to either obtain additional history, perform a careful fundoscopic examination, or perform a cranial imaging study. That important prospective study is the centerpiece of Dr. Berger's K-23 award—from NICHD, and the project of Drs. Berger and Kochanek that is funded by the CDC-University of Pittsburgh CIRCL.

This year work continued in this important area of research. The macrophage marker quinolinic acid (QUIN) was considered as a potential marker that could provide clues to the timing of injury—i.e.—serving as a biological clock of chronic vs acute inflammation. Despite the lack of a history of trauma in 82% of children with iTBI, 100% had a peak QUIN concentration of >100 nM. There was an increase in the CSF concentrations of QUIN after severe nTBI and iTBI in children. Dr. Berger recently published that paper in the *Journal of Neurotrauma*. Higher initial and peak QUIN concentrations after iTBI may be due to severity of injury, young age, and/or delay in seeking medical care, which allows for increased secondary injury—thus, delay in presentation may not be the only reason for the marked increase in CSF QUIN in abuse victims. Another limitation of using a CSF marker such as QUIN is that in many cases of missed child abuse, CSF is not available, and the injury is mild. In contrast, a blood test showing brain injury could potentially be valuable in this setting—and this approach is the backdrop upon which the current NIH and CDC-funded work of Dr. Rachel Berger is progressing.

Dr. Berger also presented an abstract describing biomarker levels in serum in an expanded sample of children with known TBI. That work was presented at the 2003 meeting of the National Neurotrauma Society and a full report is in preparation for the submission to the *Journal of Neurosurgery*. This study adds further support to the potential utility of biomarkers across the spectrum of pediatric TBI. Dr. Berger also presented the first preliminary report on the potential use of serum biomarker to identify brain injury in infants presenting to an emergency department who are at increased risk for inflicted TBI. That presentation was made at the 2004 meeting of the Society for Pediatric Research (SPR). Those results are the initial data demonstrating feasibility of this approach in the target population. The clinical trial of this approach in infants with nonspecific symptoms such as vomiting without diarrhea—symptoms typical of the well-described cases of missed child abuse—is underway.

In addition, Dr. Berger was the lead author of an invited review on biomarkers in inflicted TBI that was published in the journal *Child Abuse and Neglect*. This supports the considerable interest that this approach has garnered among the brain injury and child abuse communities for TBI detection.

Finally, it is important to have information on other potentially confounding neurological diagnoses in children. To this end, Tina Dulani, a study coordinator working with Dr. Berger, reported, in another abstract at the 2004 meeting of the SPR, data on biomarkers in pediatric meningitis and seizures. Biomarker levels after hypoxic-ischemic encephalopathy are also currently under study in Dr. Berger's laboratory.

Support: 1K23HD43843-01 "Using Biochemical Markers to Detect Abusive Head Trauma," R. Berger, MD, MPH, PI. General Clinical Research Center (GCRC) M01RR00084 "Using Biochemical Markers to Detect Silent Brain Injury," R49/CCR323155-01, R Berger, MD, MPH, PI. University of Pittsburgh Center for Injury Research and Control (CIRCL), and "Can We Detect Brain Injury by Looking in the Blood?" P Kochanek, MD, PI. Children's Hospital of Pittsburgh of the UPMC Health System–Faculty Start-up Grant – "The Use of Biochemical Markers to Assess Accidental and Abusive Head Trauma in Infants and Young Children." R Berger, MD, MPH, PI

Collaborators: P. Kochanek, Critical Care Medicine; P. David Adelson, Neurosurgery; Mary Clyde Pierce, Emergency Medicine, John Leventhal, Department of Pediatrics, Yale University.

9. Studies by Steven T. DeKosky, MD

Steven T. DeKosky, MD. Professor and Chairman of the Department of Neurology and Director of the Alzheimer's Disease Research Center, University of Pittsburgh School of Medicine

A. Antioxidant and Neurotrophic Response after TBI

Dr. DeKosky's laboratory studies the role of neural cells and their products in the brain's attempt at repair following TBI. The laboratory is particularly interested in the cytokine and antioxidant cascades that occur over the course of days to weeks after injury (secondary injury processes), and their relationship with the upregulation of neuroprotective proteins such as neurotrophins. The goal is to elucidate the brain's injury response and provide insight into possible therapeutic interventions that could be used in clinical settings to treat human TBI patients.

Dr. DeKosky's group has examined the timecourse of changes in antioxidant activities (catalase, glutathione and superoxide dismutase) and neurotrophins (such as NGF) expression after experimental TBI. Close temporal relationships were observed between the upregulation of NGF protein and complex changes in antioxidant enzyme activities. To further investigate the relationship between NGF and the antioxidant enzyme

response, Dr. DeKosky's group examined the effect of hypothermia on the post-injury level of NGF and on antioxidant enzyme activity, and showed that in rats subjected to post-traumatic hypothermia, both NGF protein levels and catalase and glutathione peroxidase activity levels are suppressed. In an attempt to restore post-injury antioxidant enzyme activities in hypothermia-treated animals, NGF protein was infused immediately after injury, and during the course of hypothermia treatment. The study showed that NGF infusion was ineffective in restoring enzymes' activities to post-injury levels. These results suggest several possibilities. First, based on work by several laboratories, including studies by Drs. Bayır and Wagner previously discussed in this report, hypothermia may reduce oxidative stress, spare small molecule antioxidants such as ascorbate, and thus blunt the induction of antioxidant proteins such as catalase and glutathione peroxidase. Alternatively, since infusion of exogenous NGF failed to restore normal antioxidant enzyme activity after injury—other pharmacological antioxidants may be required to maximize the beneficial effects of therapeutic hypothermia in TBI. The results of these two studies were published in the *Journal of Neurotrauma* and the *Journal of Neurochemistry*, respectively.

B. Effects of TBI on Amyloid Precursor Protein (APP) Metabolism

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by neuronal loss in discrete brain regions and by formation of neurofibrillary tangles and beta-amyloid associated neuritic plaques. A major component of these plaques is the 42-43 amino acid amyloid beta (Aβ) peptide that is cleaved from the transmembrane region of amyloid precursor protein (APP).

One of the known risk factors for AD is TBI. Therefore, alterations in APP processing may play an important role in the pathogenesis of both TBI and AD. To better understand the relationship between TBI and AD, Dr. DeKosky's laboratory is conducting experiments using both mouse models and surgically excised tissue and CSF samples from patients with severe head injury. Collectively, these studies center on the cytokine-related molecular cascades involved in pathological alterations in APP and Aβ production and metabolism after TBI, and the effect of therapies designed to interrupt these cascades.

The humanized Aβ mouse model of TBI

In collaboration with Cephalon Inc., Dr. DeKosky's lab has developed a colony of mice that produce detectable levels of human Aβ (the "hAβ mouse"). This mouse represents a significant advance of previous transgenic mouse models of AD in that the APP gene is under its endogenous promoter, and APP itself is produced at normal levels. This mouse is therefore particularly important for the studies of Aβ changes after TBI because 1) unlike in rats or wildtype mice that produce rodent Aβ, we are able to employ well characterized biochemical assays to detect *human* Aβ and 2) the continuous over-expression of APP as seen in transgenic mice is avoided, which is particularly important in our injury and intervention paradigms. Dr. DeKosky's laboratory is currently

examining post-injury changes in APP and Aβ proteins, as well as components of a molecular cascade involving interleukin-1β, nuclear factor κB, and caspase-3 that are involved in post-injury upregulation and amyloidogenic processing of APP. Ultimately, the goals are twofold; 1) to define the pathways causing, and pathological effects of, Aβ overproduction after TBI, and 2) to assess the effect of therapies designed to prevent Aβ overproduction after TBI in mouse models, that could potentially be translated into therapeutic strategies to treat TBI patients.

This year, studies demonstrating increased expression of cholesterol transporter for ABCA1 and APP in experimental TBI were presented at the annual meeting of the Society for Neuroscience.

Studies in TBI patients

To better understand the relationship between TBI and AD, Dr. DeKosky's laboratory is examining the distribution and levels of APP and Aβ protein in surgically resected temporal cortical tissue and serial CSF samples obtained from head-injured patients. This study is the first to demonstrate AD-like Aβ plaques in freshly resected brain tissue after severe TBI. Furthermore, within hours after TBI, human temporal cortex reacts to injury with a robust up-regulation of APP in pyramidal neurons, which likely represent the main source of Aβ. This process is paralleled by increased neuronal accumulation of amyloidogenic APP fragments, as well as a marked up-regulation of apolipoprotein E in both neurons and glial cells. These observations are important for our understanding of TBI as a potential risk factor for later development of AD, suggesting a pathological cascade that involves neuronal overproduction of APP and Aβ, and glial upregulation of apoE, the latter of which has been known to facilitate Aβ deposition in AD brains. Of additional importance, the development of acute Aβ pathology after TBI is not paralleled by formation of neurofibrillary tangles (another pathological hallmark of AD), indicating that intracellular neurofibrillary changes and progression to dementia of AD can occur only after extended survival periods (i.e., months to years). This suggests a large window of opportunity for therapeutic interventions after TBI before the onset of cellular pathology that could lead to AD dementia. Collectively, these studies convincingly show that increases in Aβ after injury result in acute AD-like pathological alterations that could be an important target for therapies that are being developed in our humanized Aβ mouse model. These clinical studies are being carried out in collaboration with the clinical TBI core within the BTRC. A full manuscript is being submitted to *Experimental Neurology*. Dr. Milos Ikonomovic is a key co-investigator on this project.

Support: Core C of 2 P50 NS30318-04A21, Project #3 in the University of Pittsburgh Head Injury Research Center (S DeKosky, MD, PI).

10. Studies by Steven Graham, MD, PhD

Steven Graham, MD, PhD, Professor and Vice-chairman, Department of Neurology, University of Pittsburgh School of Medicine; Associate Chief of Staff for Research and

Director, Geriatric Research Educational and Clinical Center, V.A. Pittsburgh Healthcare System.

A. Bcl-2 Family genes in TBI

Dr. Graham's laboratory studies the molecular and cellular mechanisms of neuronal cell death. In collaboration with the Safar Center, Dr. Graham's laboratory investigates neuronal death in TBI. This work is part of the University of Pittsburgh BTRC funded by NINDS. The recent emphasis of the laboratory has been the genetic mechanisms that regulate neuronal cell death. In particular, the role of genes that regulate programmed cell death, the bcl-2 and the cysteine protease family of genes, is being investigated in trauma. Recent studies in Dr. Graham's laboratory focus on the role of the Fas cell death receptor and caspase 8 in traumatic brain injury.

Support: Core C of 2 P50 NS30318-04A21, Project #1 in the University of Pittsburgh BTRC (Steven Graham, MD, PhD, PI). Technician: Marie Rose

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CARDIOPULMONARY ARREST PROGRAM

A. Clifton Callaway and the Department of Emergency Medicine

Clifton W. Callaway, MD, PhD, Assistant Professor, Department of Emergency Medicine, University of Pittsburgh School of Medicine

We continue to emphasize the fact that resuscitation from cardiac arrest must attend to both heart and brain. About one-third of attempted resuscitations result in restoration of spontaneous circulation. Therefore, improved approaches to cardiac resuscitation are needed. Only about one-quarter of patients with restoration of circulation will regain consciousness. Therefore, therapies to improve neurological recovery are also required. Without attention to both of these organ systems, overall survival from cardiac arrest is unlikely to increase.

Work this year continued to focus on the molecular mechanisms of neurological recovery after cardiac arrest using our rat model. We committed more effort to clinical cardiac arrest research. Because induced hypothermia after cardiac arrest appears to be an effective therapy for improving brain recovery, we have advocated its acceptance as therapy rather than as research. Several presentations at Grand Rounds and departmental meetings within UPMC and Mercy Health Systems were conducted for this purpose.

1. Altered Cellular Signaling in Brain after Resuscitation

Two mitogen-activated protein kinases (MAPKs) increase in hippocampus over the 24-hour period after resuscitation from cardiac arrest: the p42/p44 MAPK (extracellular-signal regulated kinase, ERK) and the Jun-N-terminal kinase (JNK). Likewise, levels of an extracellular signaling molecule, brain-derived neurotrophic factor (BDNF), increases in hippocampus at 24 hours after resuscitation from cardiac arrest. Induction of mild hypothermia (33°C) between 1 and 23 hrs after reperfusion, further increases activity of ERK and BDNF relative to normothermic (37ºC) controls. We have speculated that the beneficial effects of induced hypothermia are related to these increases in ERK and BDNF activation.

In order to study the role of BDNF expression during hypothermia, antisense oligonucleotides (AO) were infused into the lateral ventricles of rats in an effort to reduce total BDNF. A sequence for the AO against BDNF was obtained from previously published studies in rats. Rats (n=6-7 per group) received intracerebroventricular infusions (0.1 nmol/hr) of AO against BDNF or of a missense control AO. After 72 hours, the hippocampus was collected, total protein extracted, and BDNF levels measured using immunoblots. We were not able to detect any change in BDNF protein level after AO. In the event that poor penetration of the AO into cells or into parenchyma was the basis for this failure, we repeated this experiment including lipofectin in the AO vehicle, and also with direct infusion into the hippocampus. Still, there was no evidence of decreased BDNF levels. In order to confirm that AO was being taken up into neurons, fluorescein-labeled AOs were injected into separate rats. At 72 hours after injections, we

were able to visualize fluorescence in hippocampal CA1 neurons. Taken together, these data indicate that this AO can be delivered to hippocampal neurons, but produces no decrease in total BDNF protein levels.

We also have examined the effects of BDNF infusions on ERK activation. BDNF was infused into the lateral ventricles of rats at a rate of 0.025 mcg/hr using osmotic minipumps. Rats (n=3-4 per group) were sacrificed after 12, 24, 48 or 72 hours. The levels of BDNF and active ERK in hippocampus were measured using immunoblotting. This type of infusion increased tissue BDNF levels somewhat at 12 hours and robustly by 24 hours. Active ERK levels change only slightly in these brains. These data suggest that prolonged delivery of BDNF has only modest effects on ERK signaling in brain.

Last year, we determined that injection of 100 μg of U0126 into the lateral ventricle specifically reduced ERK activation in bilateral hippocampi for 12 hours. ERK activity had returned to near normal levels by 24 hours. In addition, we examined the influence of U0126 injections on several transcription factors. Rats subjected to cardiac arrest, received injections of vehicle or U0126 at 30 minutes after reperfusion (n=3-4 per group). These rats were maintained at normal temperature (37°C) or hypothermia (33°C) between 1 and 24 hours after reperfusion. Hippocampal levels of the phosphorylated forms of p90Rsk, ATF2 and CREB were examined with immunoblotting. These data indicate that hypothermia increases activation of ATF2 and CREB, and that this activation is blocked by U0126. Thus, hypothermia-induced signaling through these transcription factors is ERK-dependent.

2. Effects of Resuscitation on Brain Gene Transcription

We have prepared total RNA from rats treated with cardiac arrest followed by 12 or 24 hours of normal temperature or hypothermia (33ºC). Sham rats received all surgery but no cardiac arrest. This RNA was processed by the genomics core facility for hybridization to an Affymetrix U34 Rat DNA array. Results of this gene array study should become available in summer of 2004.

In parallel with this analysis, we have set up PCR-based measurements of gene transcription. Relative changes in hsp70, hsp60, hsp27, c-fos, bdnf, GAPDH and cyclophilin were measured in reverse transcribed cDNA. A scheme for quantification has been adopted using serial dilutions of cDNA and relative intensity of the PCR products on ethidium-bromide gels. Using this technique, we have been able to measure changes in bdnf transcripts. The transcript for BDNF includes four alternative exons (called exon 1-4) that are under the control of different promoters. The PCR technique was able to determine that cardiac arrest increases expression of exon 1 and exon 3. Hypothermia specifically increases expression of exon 3. Exons 2 and 4 were not altered significantly and appeared to decrease after hypothermia. These results demonstrate that the temperature regimen after resuscitation can produce very specific changes in gene expression that are probably related to effects on specific transcription factors. These results were presented at the Society for Neuroscience Meeting and are submitted for publication.

3. Effects of Manipulation of Cellular Signaling on Brain Injury after Cardiac Arrest

Previously, we confirmed that induced hypothermia improved neurological recovery after cardiac arrest and resuscitation. However, there were no significant differences in functional outcome between U0126-treated and vehicle-treated rats. We have subsequently completed histological analysis of these rats, and determined that hypothermia markedly reduces loss of hippocampal CA1 neurons after 14 days, and that U0126-treatment does not affect this loss. These data suggest that hypothermia-induced improvements in outcomes are not dependent upon forebrain ERK activation.

A study is in progress to examine the influence of exogenous BDNF administration on functional and histological outcome after cardiac arrest. Osmotic minipumps deliver BDNF or vehicle to both lateral ventricles at a rate of 0.025 mcg /hr for 72 hours after cardiac arrest. All rats are maintained at normal temperature. To date, BDNF (n=7) and vehicle (n=10) groups do not differ in terms of 14-day survival (71% vs 70%), weight loss, or neurobehavioral scores. Brains have not yet been examined histologically. These data suggest that BDNF infusion alone is not sufficient to reverse the severe brain injury observed after cardiac arrest.

4. Vasopressin in Cardiac Arrest

We initiated a study with the City of Pittsburgh Bureau of Emergency Medical Services to study the effect of adding vasopressin (40 IU) or placebo to standard care for out-of-hospital cardiac arrest. This study employs an Exception from the Requirement from Informed Consent for Emergency Research. The first subjects were enrolled in May 2003. Approximately 150 subjects of a planned 324 were enrolled during this year. The study remains blinded. However, overall survival of these subjects does not differ from historical patients treated in Pittsburgh who would have met the inclusion criteria. Therefore, we suspect that there is no large difference attributable to vasopressin administration.

The process of notification of subjects about enrollment in this trial has provided insight into research conducted under an Exception from Informed Consent. The rate of family member completion of informed consent documents is poor. This fact appears to be related to competing activities related to end-of-life decision making rather than lack of opportunity or active resistance. Preliminary observations about this process were presented in abstract form to the National Association of EMS Physicians.

5. Out-of-Hospital Cardiac Arrest in Pittsburgh

In preparation and during the vasopressin study, we have examined the electronic recordings collected by automated external defibrillators (AED) used by firefighter first-responders. Analysis of these recordings reveals several systematic problems with the conduct of resuscitation that may be amenable to improvement. First, there is a low incidence of ventricular fibrillation that is converted to a perfusing rhythm by AED shocks. Second, rhythm analysis and repeated rescue shocks consume a lot of time that is not devoted to chest compressions. Finally, the ratio of 15 compressions to 2 ventilations

results in less than 60% of the time devoted to chest compressions during resuscitation. Based on these observational data, we plan to retrain the first-responder system in June-July 2004 to use 30 compressions: 2 ventilations. At the same time, AEDs will be reprogrammed to allow more hands-on time during chest compressions. This activity will be quality improvement, although we plan to observe and collect data as part of our ongoing research database.

Support**:** Hypothermia and Gene Expression after Cardiac Arrest, (#R01 NS046073) National Institute of Neurological Disorders and Stroke (07/99 – 06/04) total award $848,032 ($166,250 direct costs + $80,631 indirect costs per year) Clifton W. Callaway, MD, PhD, PI. Vasopressin in Cardiac Arrest, Pittsburgh Emergency Medicine Foundation, total award $1525, Clifton W. Callaway, MD, PhD, PI.

B. Pediatric Cardiopulmonary Resuscitation

There is an expanding pediatric cardiac arrest program at the Safar Center that now has both bench and clinical components. Dr. Robert Clark (see prior report in TBI), Associate Professor in the Department of Critical Care Medicine, Associate Director of the Safar Center and Pediatric Critical Care Medicine specialist at Children's Hospital, has received funding from Children's Hospital of Pittsburgh to initiate laboratory studies in a new model of asphyxial cardiopulmonary arrest in rats. This research is off to a spectacular start. In addition, Dr. Robert Hickey in the Department of Pediatrics, Division of Emergency Medicine at Children's Hospital of Pittsburgh has ongoing mechanistic studies in the area of developmental brain injury and has played a key role in the national guidelines committees in resuscitation. Finally, Dr. Howard Ferimer of the Mercy Hospital Department of Pediatrics completed some studies in asphyxial cardiopulmonary arrest in collaboration with Dr. Edwin Jackson.

1. Laboratory Research in Pediatric Resuscitation

A. Asphyxial Cardiopulmonary Arrest in the Developing Rat (Robert Clark, MD)

In 1995, Drs. Larry Katz and Peter Safar published a seminal paper in the *Journal of Cerebral Blood Flow and Metabolism* describing a clinically relevant model of asphyxial cardiopulmonary arrest in adult rats. Based on that work, and with special talents of senior laboratory technician, Henry Alexander, Dr. Robert Clark developed an important pediatric analog of that asphyxial cardiopulmonary arrest model using post-natal-day (PND) 17 rats. This is an important development in that the PND 17 rat models a toddler or young child—the population most commonly afflicted by cardiopulmonary arrests resulting from asphyxiation (i.e., near drowning, trauma, child abuse, choking, SIDS). Although there are established models of perinatal ischemia, there are no small animal models mimicking cardiopulmonary arrest. Equally important is the fact that this is a clinically relevant model that includes a global insult to the entire organism and all of the standard clinical components of resuscitation as guided by contemporary pediatric advanced life support (i.e., mechanical ventilation, chest compressions, epinephrine).

This year, T32 fellow Dr. Ericka Fink published the description of the model as a full manuscript in the journal *Pediatric Critical Care Medicine*. In addition, Dr. Fink presented three abstracts of her work in this exciting new model. She presented abstracts, at both the Society for Neuroscience and the Fellows' Research Day Conferences held by the Pennsylvania/Delaware Affiliate of the American Heart Association, reporting beneficial effects of post-resuscitation mild hypothermia on histopathological and functional outcome in this model. She also presented studies demonstrating a gender-dependent effect on behavioral outcomes and rate of neuronal death in this model. These findings lend further support to the notion that male neurons are more apt to die of necrosis while female neurons tend to die in a more delayed fashion—presumably via apoptotic mechanisms. That paper was presented at the 2004 meeting of the Society for Pediatric Research. Dr. Fink has been extremely productive and has taken great advantage of this new model. She is preparing a manuscript on the hypothermia work and is beginning studies of novel antioxidants as therapeutics in this model. Finally, Dr. Clark has submitted an RO-1 award on this model, and we are pleased to say that funding of this new program is anticipated.

Support: Children's Hospital of Pittsburgh. Robert SB Clark, MD, PI. The Laerdal Foundation for Acute Medicine. Ericka Fink, MD, PI

B. Developmental Aspects of COX-2-mediated Brain Injury (Robert Hickey, MD)

Dr. Robert Hickey continued work on his KO-8 award from NICHD to study developmental aspects of the role of COX-2 in brain injury. This research is being carried out under the mentorship of Dr. Steven Graham in the department of Neurology at the VA Hospital. Dr. Kochanek is a co-sponsor of the grant. COX-2 plays an important role in secondary injury in models of stroke, trauma, and cardiac arrest in adult investigation. Its role in pediatric brain injury remains to be defined. Studies to evaluate the effects of COX-2 inhibitors in the developing rat subjected to asphyxial cardiopulmonary arrest are in progress. Dr. Hickey also began work in neuronal culture to study potential mechanisms whereby COX-2 can contribute to ischemic injury.

Support: COX-2 and Injury in the Immature Brain, KO-8 (#HD40848) National Institute of Health, National Institute of Child Health and Development, (7/01-7/06), total award $623,430 ($115,450 direct + $9,236 indirect per year), Robert W. Hickey, MD, PI, Steven Graham, MD, Patrick Kochanek, MD, Co-Investigators; Robert Clark, MD, C. Edward Dixon, PhD, Peter Safar, MD, Consultants. COX-2 and Excitotoxicity in Developing Rat Brain, Competitive Medical Research Fund (CMRF), University of Pittsburgh, (7/1/03-6/30/05), total award $25,000. Robert W. Hickey MD, PI: Steven H Graham MD, PhD, Co-investigator.

Public Education and National Guidelines Committee

Dr. Robert Hickey is the current Vice-Chair of the American Heart Association Emergency Cardiovascular Care Committee (ECC) and the immediate past-chair of the American Heart Association subcommittee on Pediatric Resuscitation. The ECC is

responsible for overseeing the American Heart Association's pediatric advanced life support (PALS), advanced cardiovascular life support (ACLS) and basic life support (BLS) courses. The AHA has approximately 250,000 instructors that train over 7 million people annually. Dr Hickey developed a new teaching module for the PALS course entitled "Catastrophic Illnesses in Children Presenting with Common Chief Complaints". In his capacity as Vice-Chair of the ECC, Dr. Hickey also serves as a representative to the International Liaison Committee on Resuscitation (ILCOR) and has recently participated in meetings in Australia, Italy, Brazil, and Budapest to develop international consensus on new developments in resuscitation science. Dr. Hickey is the Co-Chair of the ILCOR Pediatric Taskforce responsible for developing the evidence-based Pediatric Guidelines scheduled for release in 2005. Dr. Hickey also serves on the Science Advisory and Coordinating Committee (SACC) of the AHA. SACC advises the AHA on scientific issues and helps to develop the strategic goals and research initiatives of the AHA.

Pediatric Cardiopulmonary Arrest: Clinical Studies

Dr. Hickey has initiated the assembly of a multidisciplinary team to evaluate children resuscitated from cardiac arrest. The team has representatives from the entire continuum of care including pre-hospital, emergency medicine, critical care, neurology, neuroimaging, behavioral pediatrics, and rehabilitation medicine. The team will, 1) characterize early molecular markers of hypoxic ischemia brain injury, 2) evaluate strategies for prognosis of neurologic recovery, 3) identify patterns of functional deficits in long-term survivors, and 4) develop targeted strategies for rehabilitation of patients with hypoxic ischemia brain injuries. This information will facilitate comprehensive evaluation and treatment for individuals suffering from hypoxic ischemia brain injury and also develop a profile of the natural history of injury and recovery that can be used for evaluation of anticipated neuroprotective therapies. The study has been approved by the IRB and enrollment has begun.

Peer-Reviewed Manuscripts: Cardiopulmonary Arrest Program

1. Du L, Bayır H, Lai Y, Zhang Z, Kochanek PM, Watkins SC, Graham SH, Clark RSB: Innate gender-based proclivity in response to cytotoxicity and programmed cell death pathway. J Biol Chem (in press).

2. Fink EL, Alexander H, Marco CD, Dixon CE, Kochanek PM, Jenkins LW, Lai YC, Donovan HA, Hickey RW, Clark RSB: Experimental model of pediatric asphyxial cardiopulmonary arrest in rats. Pediatr Crit Care Med 5:139-144, 2004.

3. Hazinski MF, Markenson D, Neish S, Gerardi M, Hootman J, Nichol G, Taras H, Hickey R, et al. Response to cardiac arrest and selected life-threatening medical emergencies: The medical emergency response plan for schools. Ann Emerg Med 43:83-99, 2004. Simultaneously published in Circulation 109:278-91, 2004 and Pediatrics 113:155-168, 2004.

4. Lai CS, Hostler D, D'Cruz BJ, Callaway CW: Prevalence of troponin-T elevation during out-of-hospital cardiac arrest. Am J Cardiol 93:754-756, 2004.

5. Sherman LD, Flagg A, Callaway CW, Menegazzi JJ, Hsieh M: Angular velocity: a new method to improve prediction of ventricular fibrillation duration. Resuscitation 60:79-90, 2004.

6. Menegazzi JJ, Callaway CW, Sherman LD, Hostler DP, Wang HE, Fertig KC, Logue ES: Ventricular fibrillation scaling exponent can guide timing of defibrillation and other therapies. Circulation 109:926-931, 2004.

7. Menegazzi JJ, Wang HE, Lightfoot CB, Fertig KC, Chengelis NL, Sherman LD, Callaway CW: Immediate defibrillation versus interventions first in a swine model of prolonged ventricular fibrillation. Resuscitation 59:261-270, 2003.

8. Lightfoot CB, Callaway CW, Hsieh M, Fertig KC, Sherman LD, Menegazzi JJ: Dynamic nature of electrocardiographic waveform predicts rescue shock outcome in porcine ventricular fibrillation. Ann Emerg Med 42:230-241, 2003.

Chapters, Editorials and Invited Papers: Cardiopulmonary Arrest Program

1. Clark RS, Lai Y, Hickey RW: Hypoxic-Ischemic Encephalopathy: Pathobiology and Therapy of the Post-Resuscitation Syndrome in Children. In: Pediatric Critical Care 3rd edition, Fuhrman B, Zimmerman J (eds.), Elsevier Science, St. Louis (in press).

2. Hickey RW, Callaway CW: Therapeutic hypothermia. In: Molecular and Cellular Biology of Critical Care Medicine. Tisherman, S, Sterz F (eds.), Kluwer Academic Publishers, New York (in press).

3. Hickey RW, Graham SH: Eicosanoids: Roles in the pathophysiology of cerebral ischemia. In: Prostaglandins and Eicosanoids, Curtis-Prior, P (ed.), John Wiley & Sons, London, 44:481-486, 2004.

4. Illievich UM, Kalkman CJ, Katz LM, Knape J, Kochanek PM, Nellgard B, Safar P, Sakabe T, Warner DS: Brain resuscitation in the Drowning Victim In: Handbook on Drowning-Prevention, Rescue, Treatment, Bierens J (ed.), Springer-Verlag, Heidelberg (in press).

5. Kochanek PM: World Congress on Drowning, 2002: Task- Force on "Brain Protection" Pediatric Considerations (in press).

6. Kochanek PM, Hickey RW, Bayır H, Fink EL, Ruppel RA, Clark RSB: Pediatric neurointensive care. In: Textbook of Critical Care 5th Edition, Fink MP,

Abraham E, Kochanek PM, Vincent JL (eds.), WB Saunders, Philadelphia, Chapter 60 (in press).

7. DeFranco DB, Ho L, Falke E, Callaway CW: Small molecule activators of the heat shock response and neuroprotection from stroke. Current Atherosclerosis Reports 6:295-300, 2004.

Abstracts: Cardiopulmonary Arrest Program

1. Fink E, Marco CD, Donovan HA, Alexander H, Dixon CE, Kochanek PM, Jenkins LW, Clark RS: Brief induced hypothermia improves outcome in a pediatric model of asphyxial cardiopulmonary arrest in rats. Crit Care Med 31:89, 2003.

2. Fink EL, Alexander H, Donovan H, Marco C, Dixon CE, Kochanek PM, Clark RSB: Therapeutic hypothermia improves neurologic recovery in a model of pediatric asphyxial cardiopulmonary arrest in rats. Society for Neuroscience 2003 (electronic).

3. Fink EL, Marco CD, Donovan HA, Alexander H, Dixon CE, Kochanek PM, Jenkins LW, Clark RS: Brief induced hypothermia improves outcome in a pediatric model of asphyxial cardiopulmonary arrest in rats. American Heart Association; Pennsylvania/Delaware Affiliate Fellows' Research Day, Hilton Hotel, Pittsburgh, PA, February 13, 2004.

4. Fink EL, Marco CD, Donovan HA, Alexander H, Kline AE, Dixon CE, Bao L, Kochanek PM, Clark RSB: Gender-dependent differences in behavioral outcome and rate of neuronal cell death after asphyxial cardiac arrest in post-natal day 17 rats. Pediatric Research 55 (Suppl):25A, 2004.

5. Lubin JS, Hostler DP, Doshi AA, Callaway CW: Implementation of a waiver of informed consent in out-of-hospital cardiac arrest research. Prehosp Emerg Care 8: 92, 2004.

6. Millin MG, Doshi A, Hughes M, Callaway C, Hostler DP, Roth R, Lo B, Quinn S, Wasielewski W, Rodgers M: Amiodarone vs. lidocaine in out-of-hospital cardiac arrest-combined data from two EMS systems. Prehosp Emerg Care 8:90, 2004.

7. Menegazzi J, Callaway C: Intravenous infusion of ice-cold normal saline rapidly induces hypothermia after resuscitation from cardiac arrest. Prehosp Emerg Care 8:81, 2004.

8. Ramos R, Menegazzi J, Wang H, Callaway C: Post-resuscitation hemodynamics and relationship to duration of ventricular fibrillation. Prehosp Emerg Care 8:81, 2004.

9. Min A, Wang H, Hostler D, Lo B, Quinn S, Callaway C: Clinical factors have time-dependent influences on death rate after cardiac arrest. Acad Emerg Med 11:563, 2004.

10. Wang HE, Callaway CW, Peitzman AB: Admission hypothermia is associated with adverse outcomes after trauma. Acad Emerg Med 11:513-514, 2004.

SHOCK AND SUSPENDED ANIMATION PROGRAM

The hemorrhagic shock (HS) and suspended animation (SA) program consists of project I on HS in rats and pigs (PI, Dr. Tisherman; Co-P.I., Dr. Safar); and project II on suspended animation (SA) in dogs (PIs., Dr. Safar [7/03] Dr. Kochanek [8/03-6/04]; Co-PI, Dr. Tisherman). The funding since 1997 was made possible through special "plus-up" funds from Congress initiated by former Navy Commander Lyn Yaffe, MD during 2001/2003. The HS studies were funded separately by the Office of Naval Research. During the 2003/2004 academic year, separate funding was completed on the rat HS program, and both of these programs were supported by our re-tooled and expanded single program entitled "Emergency Hypothermia" again funded by Congress through Telemedicine and Advanced Technology Research Center (TATRC). We received total funds of approximately $956,949 during 2003/2004.

Our research ICU for large animals, initiated in the 1970s, is still considered a unique resource for the documentation of novel cardiopulmonary cerebral resuscitation methods. It must be maintained continuously to be cost-effective, with at least three technicians, two full-time MD research fellows with CCM experience, and about 40 long-term large animal experiments per year. Maintaining this ICU program alone requires over $0.5M per year. In 2003/2004, the research fellows were Dr. Ala Nozari (in his third year) and Dr. Xianren Wu (in his fifth year); Mr. William Stezoski has continued as lab coordinator. This was the late Dr. Safar's final year as PI of this important project. Subsequent to his passing, Dr. Kochanek assumed the role of PI of this project with Dr. Tisherman as Co-PI. Drs. Kochanek and Tisherman will work together on this important project. Specifically, Dr. Kochanek will serve as overall PI and PI for the laboratory work, with Dr. Tisherman as Co-PI. While, for the planned clinical feasibility trial of SA in the future, Dr. Tisherman will serve as PI. Co-investigators or consultants included Drs. Yaffe, Klain, Jackson, Dixon, Clark, Kagan, Jenkins, and Radovsky, and most recently Robert Wagner (DVM), Joseph Carcillo, and Robert Garman.

The objective of the HS-SA program has been to help maximize the reversibility of presently lethal traumatic hemorrhage resulting in exsanguination CA (ExCA). The HS studies in rats and pigs were designed to extend the golden hour of HS tolerance; HS (low blood flow), with viscera as the most vulnerable organs, is the prevalent cause of death in soldiers "dying of wounds" (DOW). Exsanguination cardiac arrest (ExCA) (no blood flow), with the brain as the most vulnerable organ, is the prevalent cause of death in soldiers "killed in action" (KIA). SA is a totally new approach for presently unresuscitable conditions. While SA has been considered science fiction, colleagues are now increasingly using this term seriously, as representing rapidly induced preservation of the organism for delayed resuscitation. This idea was initiated in the 1980s by Drs. Safar and COL Ronald Bellamy. For HS and SA we have explored mainly hypothermic strategies – specifically mild hypothermia (33-36°C) for HS and profound hypothermia (5-15°C) for SA. In the laboratory, we plan to extend the duration of SA to address the practicalities of prolonged transport. In addition, we plan to develop a SA model in rats to take advantage of the many molecular tools available for use in rats that are not

available for use in dogs or pigs. Finally, Dr. Tisherman, as discussed above, is planning clinical feasibility studies for both in selected trauma centers. Devices needed for such studies are being developed concurrently with additional laboratory studies, including collaboration with our industrial partners (Ardiem Medical for cooling devices, and Dr. Yaffe's group working on smart catheters and other aspects of trauma bay and field application of this exciting technology.

The HS models in rats and SA models in dogs used in 2002/2003 had been initiated and further developed over the years by our group. They have several unique features, the most important being clinical relevance in terms of insult, resuscitation strategy, ICU management, and outcome.

1. HS Studies

Work in the HS program during academic year 2003/2004 included publication of studies that were completed last year from work funded by the Office of Naval Research (PI: SA Tisherman, MD; Co-PI: P Safar, MD), and studies supported by the "Emergency Hypothermia" congressional appropriation. Fellow Xianren Wu, MD supervised all studies. The rat studies were completed by technician Jason Stezoski. The pig studies utilized the ICU team led by S. William Stezoski, with technicians Jeremy Henchir, Sherman Culver, Alan Abraham, Jason Stezoski, Scott Kostelnik, and Murugan Subramanian. Fellow Ala Nozari, MD, also assisted with the pig studies.

Mild Hypothermia and Prolonged HS

As described in last year's report, we showed, using models of uncontrolled HS or pressure-controlled HS, that a mean arterial pressure (MAP) of 50 mmHg was insufficient to allow long-term survival after very prolonged (6 h) HS. Even a MAP of 60 did not consistently allow survival. We also showed that mild hypothermia (34^{o}C) improved survival after prolonged HS. A full manuscript is in preparation.

Mild Hypothermia and HS: Mechanisms of Benefit

This year, Dr. Wu published two full manuscripts in this area from work previously completed. First, he studied a variety of mechanistic endpoints to probe the potential mechanisms underlying the beneficial effects of mild hypothermia in HS. Mild hypothermia conferred protection to the liver but did not attenuate increases in either cytokines or markers of oxidative stress. Early increases in blood glucose, and reductions in both serum potassium and transaminases were seen with hypothermia. That paper was published in the *Journal of Trauma*. In a second paper, Dr. Wu demonstrated that after spontaneous hypothermia during HS, continued mild hypothermia did not improve long term outcome, but favorably influenced survival time, particularly with severe HS. Both of these studies suggest that the beneficial effect of mild hypothermia in HS is early, and attenuation of late secondary injury mechanisms by hypothermia is not readily seen. This paper was also published in the *Journal of Trauma*.

Solutions and HS

Recent fellow graduate Dr. Rainer Kenter carried out several studies (described in prior reports) examining the optimal fluid for hypotensive (limited) resuscitation during HS.

This includes work evaluating hypertonic and hyperoncotic solutions and related combinations. Full manuscripts of that work are being prepared by Dr. Kentner.

Hypothermia and Poikilothermia

In last year's report, we discussed the preliminary work of Dr. Wu testing the new neurotensin analog (NT-69L), which former fellow Dr. Larry Katz found to induce rapid and sustained mild hypothermia in rats after asphyxial CA. As discussed, a poikilothermic state could be extremely essential to the benefits of mild hypothermia, and a pharmacological agent that induces hypothermia is an appealing concept. However, in our volume-controlled HS model, NT had no further beneficial effect on survival than did our standard application of hypothermia via surface cooling. This highlights the fact that one must carefully determine the optimal application conditions for hypothermia (target temperature, duration, re-warming rate, sedation/analgesia, and associated pharmacological agents) in each disorder that it used. This work is in preparation by Dr. Wu as a full manuscript.

Large Animal Outcome with Mild Hypothermia

Previous studies of mild hypothermia during HS have been performed in rats. Clinically, there is great concern that hypothermia is associated with worse outcomes in trauma patients. Prior to making final plans for clinical trials of mild hypothermia during HS in trauma patients, we felt that a large animal study using a clinically-relevant model of HS plus trauma, with prolonged life support, was needed. Also, we wanted to test the safety and efficacy of ice-cold fluid infusion for induction of hypothermia during HS. Studies have suggested benefit in patients after CA. Thus, we developed a pig HS model with controlled continuous bleeding (75 ml/kg over 3 h) and trauma induced by laparotomy and splenic transection (delayed splenectomy). At HS 40 min (simulating arrival of paramedics) pigs were randomized into 3 groups: Group-1, normothermia (38°C) with warmed saline, Group-2, hypothermia (34°C) induced with 2°C i.v. saline and surface cooling, and Group-3, hypothermia (34°C) with 24°C i.v. saline and surface cooling. Resuscitation fluids were given when MAP was <30 mmHg until HS 3 h. Remarkably, rapid cooling with ice-cold saline was not as effective as slower i.v. cooling using room temperature resuscitation fluids. Use of ice-cold fluid increased blood pressure and lactate. It may be that rapid cooling using ice-cold fluid during HS produces peripheral vasoconstriction, leading to underestimate of the fluid needs. Further studies are needed to prove that hypothesis. This year, Dr. Wu presented those interesting results at the Fellows' Research Day hosted by the American Heart Association, Pennsylvania/Delaware Affiliation.

In this area of research, Dr. Tisherman also published a review in the *Journal of Trauma* entitled Trauma Fluid Resuscitation 2010, and several related chapters in the Saunders Manual of Critical Care.

2. SA Program

Studies in dogs

A. Successful SA after 2 hours of ExCA

In prior reports, we described studies by former fellow Wilhelm Behringer in our group demonstrating that an SA of 2 hours in duration could be achieved with intact outcome in some animals. Intact outcome for SA of 90 minutes in duration was remarkably consistent using a target tympanic temperature of 10^{o}C. These landmark studies were published this year as a full paper in the journal *Critical Care Medicine*.

B. Addition of tissue trauma to the SA model and the effects of plasma exchange

In prior SA experiments, we reported that induction of profound cerebral hypothermia (a tympanic membrane temperature of ~10^{o}C) can allow intact survival after a 90 min ExCA. The potential benefits of drugs or specialized solutions have been disappointing. The anti-oxidant Tempol and a specially developed fluid (by Michael Taylor, PhD) for organ preservation with hypothermia (Unisol) seem promising. In last year's report, our studies suggested that the addition of tissue trauma (thoracic incision, laparotomy, splenectomy) caused extracerebral organ system dysfunction, although brain histopathology is normal after 60 min SA. This year, Safar Center Fellow Dr. Ala Nozari published the results of that study in the *Journal of Trauma*.

In children with multiple organ dysfunction and thrombotic microangiopathic anemia, use of plasma exchange has resulted in significant clinical improvement. Thus, we hypothesized that plasma exchange might help alleviate some of the extracerebral complications seen after trauma and SA. After 120 min SA in dogs, plasma exchange decreased the coagulopathy and improved overall performance, without affecting neurologic deficits and brain histopathology. These studies support the potential use of 2 h of SA even in the setting of ExCA that is accompanied by considerable tissue trauma. This is an important study toward the potential clinical use of SA. Plasma exchange may be needed as a clinical adjunct. However, it must be recognized that our studies in dogs have been carried out without the resources of a canine blood bank (i.e., we are limited by lack of therapies such as platelets, cryoprecipitate, and fresh-frozen plasma). The results of this interesting study were presented at the 2003 meeting of the Society of Critical Care Medicine. These studies were carried out in collaboration with Ann Hale of Midwest Laboratories who provided blood typing for the plasma exchange. We are grateful to Ann for her help with these complex studies.

C. *Small volume induction of SA using veno-arterial re-circulation*

SA in its current form could be applied in a trauma bay or operating room in the setting of civilian or military trauma. However, one of the greatest potential limitations to the application SA—in its current form—in the field is the need for large (20 liters or more) quantities of iced flush solution. Pharmacologic adjuncts to the flush solution are one possibility. In the laboratory, another approach that can be used is to re-circulate the flush solution. This year, Dr. Nozai successfully tested this approach in our dog model using a 90 min SA protocol. This approach, using a femoral route for delivery, proved to be highly efficacious. These findings were presented at the 2003 congress of the Society of Critical Care Medicine. Recirculation of the flush is an interesting potential approach

for lab use—specifically in models without tissue trauma. However, wounds with vascular disruption—and resultant loss of the flush solution rather than re-circulation—would prevent the use of this approach for clinical ExCA. Re-circulation, however, could be used for normovolemic CA—in situations where SA might be considered, such as refractory CA.

D. Mild hypothermia during CPCR rather than after resuscitation

Recent clinical trials have demonstrated beneficial effects of mild hypothermia in adults when induced by surface cooling after restoration of spontaneous circulation (ROSC). This year, Dr. Ala Nozari was the team leader on an important study at our center that tested the hypothesis that benefits of mild hypothermia would be even greater if it were begun during CPCR. The most likely scenario for benefit of this approach would be the prolonged resuscitation of a patient refractor to ACLS. To test this hypothesis, we used extracorporeal veno-venous cooling during prolonged (a total 40 min insult including 3 minutes of no-flow, 7 minutes of BLS, and 30 min of ACLS) CPCR to achieve target temperatures of either normothermia mild (34°C) or moderate (27°C) hypothermia. With this approach mild or moderate hypothermia during CPCR dramatically improved outcome at 96 hours after resuscitation. This work was published this year as a full paper, published by Dr. Nozari in *Critical Care Medicine* and strongly suggests the need to carry out clinical trials of intra-arrest cooling. This manuscript was the subject of a very favorable editorial.

We are beginning several new projects using the dog SA model, including pilot work to study the impact of prolonged HS prior to SA and preliminary investigation to test approaches to break the two-hour barrier of SA. New additional facets of our work that we also hope will further our goals will be the addition of neuropathologist Dr. Robert Garman to our group and expanding our collaboration with Dr. Carleton Hsia and his talented group at Synzyme. More to follow on these important new approaches and collaborations in next year's report.

Studies in rats

A. Proteomic studies in a rat model of complete global cerebral ischemia without reperfusion

Two years ago, Drs. Larry Jenkins and Peter Safar initiated a project linked to the SA program that seeks to probe into the mechanisms of cellular (neuronal) degradation at prolonged global cerebral ischemia during profound hypothermia—at levels of cooling that are successfully used in our SA experiments. An intriguing question is–during prolonged (1-2 h or more) complete global cerebral ischemia (at profound hypothermia, 10°C), what cellular derangements occur. Is cellular degradation during prolonged periods of hypothermia occurring, does it set the stage for damage during reperfusion, or is reperfusion and re-warming the key? Although mechanisms involving lipid degradation, or DNA or RNA damage, may be important, a key initial focus of our work in this area has been on proteolytic damage. Dr. Jenkins has used proteomics to study protein degradation and post-translational modification in TBI and previously published a manuscript on this approach. In last year's annual report, the initial work on this project was described including work by Dr. Jenkins, and PICU T-32 fellow Dr. Mandeep

Chadha, who used 2-D gel electrophoresis to examine the effect of 30 min of complete global brain ischemia (at either normothermia or profound hypothermia, 10°C) on the proteome of the isolated rat hippocampus. Their initial work suggested that 30 min of complete global brain ischemia produced only modest changes in the proteome of the rat hippocampus. Obviously, the limitations of the sensitivity of 2-D gel electrophoresis for low copy proteins and protein fragments must be taken into consideration. Nevertheless, these initial studies are provocative. This work was presented by Dr. Chadha at both the NCMRR/NIH Training Workshop and at the American Heart Association Pennsylvania/Delaware Affiliate Fellows' Research Day. Dr. Chadha was selected as having one of the three top presentations at the NCMRR/NIH conference—congratulations. Studies of the impact of longer ischemia durations and the effect of reperfusion are underway using the rat SA model descried below.

B. A rat model of SA

In the middle of this academic year, Dr. Tomas Drabek—a cardiac anesthesiologist from Prague—joined our group. His specific goal is to develop a rat model of SA that can be used both for molecular studies and to screen therapies. Drs. Drabek, Kochanek and technician Jason Stezoski visited the laboratories of Drs. David Warner and Hillary Grocott to acquire the method of cardiopulmonary bypass in rats which is critical to the resuscitation phase of SA—and thus to the development of a rat SA model. We wish to thank them for their remarkable help and generosity and Dr. Drabek and Jason Stezoski have begun to carry out the initial studies in this important and novel direction for our laboratory.

C. Device Development

During the 2003/2004 academic year, Safar Center investigators (Drs. Safar, Tisherman, and Kochanek, and Mr. William Stezoski) working on the SA project continued to provide consultation to Dr. Lyn Yaffe and his "Smart Catheter" group working on the development of novel catheters for the clinical and experimental implementation of SA. We continue to evaluate catheter prototypes for aortic insertion. In addition, this same group of Safar Center investigators provided consultation to the Ardiem Medical Company in the development of cooling devices for use in induction of hypothermia, both in SA and HS paradigms. We continue to meet with the development team of Ardiem Medical at the Safar Center and are currently using their initial devices for experimental cooling in our SA and mild hypothermia projects in large animals.

D. Emergency Hypothermia, Clinical planning

Dr. Tisherman is beginning to organize an initial meeting of a clinical consortium for SA—including important and interested trauma centers across the USA. As we look to the future, our new congressional application will be entitled "Applied Emergency Hypothermia," to highlight the fact that we feel that we are approaching the possibility of a clinical feasibility trial. This will build on the initial groundwork laid by Dr. Tisherman with investigators in the American Association for the Surgery of Trauma (see last year's

report for details). Future meetings of this consortium group of potential investigators are planned.

Finally, several review articles on SA were published this year by Drs. Tisherman, Yaffe and/or Kochanek. Most notable are the outstanding review articles on SA and "Smart Catheter" approaches, written by Drs. Tisherman and Yaffe, respectively that were published in the Festschrift to Dr. Safar published in the journal *Critical Care Medicine*. Dr. Kochanek was the lead author of a chapter on SA in the new textbook on hypothermia published this year by Dr. Hayashi.

Support: Novel Resuscitation from Lethal Hemorrhage. Suspended Animation for Delayed Resuscitation, US Army-Combat Casualty Care, DAMD 17-0102-0038 (9/15/03-9/14/04), $956,949, Patrick M. Kochanek, MD, PI and Samuel Tisherman, MD, Co-PI

Peer-reviewed Manuscripts: Shock and Suspended Animation Program

1. Behringer W, Safar P, Wu X, Kentner R, Radovsky A, Kochanek PM, Dixon CE, Tisherman SA: Survival without brain damage after clinical death of 60-120 min in dogs using suspended animation by profound hypothermia. Crit Care Med 31:1523-1531, 2003.

2. Nozari A, Safar P, Stezoski SW, Wu X, Henchir J, Radovsky A, Hanson K, Klein E, Kochanek PM, Tisherman S: Mild hypothermia during prolonged cardiopulmonary-cerebral resuscitation increases conscious survival in dogs. Crit Care Med (in press).

3. Nozari A, Safar P, Wu X, Stezoski WS, Henchir J, Kochanek PM, Klain M, Radovsky A, Tisherman: Suspended animation can allow survival without brain damage after traumatic exsanguination cardiac arrest of 60 min in dogs. J Trauma (In press).

4. Tisherman SA, Barie P, Bokhari F, Bonadies J, Daley B, Diebel L, Eachempati SR, Kurek S, Luchette F, Puyana JC, Schreiber M, Simon R: Clinical practice guideline: Endpoints of resuscitation. J Trauma (in press).

5. Wu X, Stezoski J, Safar P, Bauer A, Tuerler A, Schwarz, N, Kentner R, Behringer W, Kochanek PM, Tisherman SA: Mild hypothermia during hemorrhagic shock in rats improves survival without significant effects on inflammatory responses. Crit Care Med 31:195-202, 2003.

6. Wu X, Stezoski J, Safar P, Nozari A, Tisherman SA: After spontaneous hypothermia during hemorrhagic shock (HS), continuing mild hypothermia (34°C) improves early, but not late, survival in rats. J Trauma 55:308-316, 2003.

Chapters, Monographs, and Editorials: Shock and Suspended Animation Program

1. Kochanek P, Tisherman S, Stezoski SW, Nozari A, Wu X, Safar P: Novel potentials for emergency hypothermia: suspended animation with delayed resuscitation from exsanguinations cardiac arrest. In: Brain Hypothermia 2004, Hayashi (ed.), Springer-Verlag Publishers (in press).

2. Tisherman SA: Trauma fluid resuscitation 2010. J Trauma 54:231-234, 2003.

3. Tisherman SA: Suspended animation for resuscitation from exsanguinating hemorrhage. Crit Care Med 32:S46-50, 2004.

4. Tisherman SA: Myocardial contusion. In: Saunders Manual of Critical Care. Kruse JA, Fink MP, Carlson RW (eds.), Saunders, Philadelphia, 2003, pp 73-74.

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Founding Director – Peter J. Safar, MD
1979 - 1994

Dr. Peter J. Safar received his MD degree in 1948 from the University of Vienna. He came to the United States permanently in 1954 along with his wife, Eva Kyzivat Safar. Over the course of his career Dr. Safar earned many awards and honors. He is generally considered the father of "CPR" and pioneered the development and implementation of this vital life-saving technique used worldwide. Dr. Safar also had important roles in the development of a number of other areas in medicine, such as the fields of intensive care and emergency medicine. He trained hundreds of anesthesiology, critical care medicine and emergency medicine specialists working around the globe. You can read about his many accomplishments and view his extensive publication list on the Safar Center website. Dr. Peter Safar was also highly revered by his colleagues, peers, and trainees.

In 1979, Dr. Safar founded the International Resuscitation Research Center (IRRC) after a highly successful tenure as the founding Chairman of the Department of Anesthesiology and Critical Care Medicine, 1961-1979. In July 1994, Dr. Safar turned over the directorship of the IRRC to Dr. Patrick M. Kochanek. One of Dr. Kochanek's first directives was the renaming of the IRRC to "Safar Center for Resuscitation Research."

On August 3, 2003, after a 15-month fight against cancer, the Safar Center for Resuscitation Research lost its Founding Director, a great colleague, and a loyal friend – Peter J. Safar, MD. Now that we can no longer walk into his office and ask for his advice, we must accept the challenge to continue his work and preserve his legacy for future generations of researchers and scientists. Not only was Dr. Safar our leader, mentor, and colleague for many years, but he was also a personal friend to many of us. Though he is greatly missed, the way he lived life to its fullest continues to inspire us to seek breakthroughs and make the world a better place for all.