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Featured on the cover are photographs from the March 31, 2002 Sunday Magazine section of the Pittsburgh Post-Gazette. The article featured in that issue, entitled “The Beat Goes On,” provided insight into the life’s work of Peter and Eva Safar. *Top:* Dr. Peter Safar posing in front of an abstract painting of the girl who’s death mask inspired the face of the Resusci-Anne manikin that is used worldwide to teach CPR. *Middle:* Dr. Safar amidst his files. *Bottom:* Peter and Eva Safar at the 2002 University of Pittsburgh Honor’s Convocation.



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.



*Patrick M. Kochanek, M.D.
Director, Safar Center for
Resuscitation Research*

A letter from the Safar Center's Director

It is, once again, an honor to present the annual report of the Safar Center for Resuscitation Research. Our center has continued to flourish during the 2001-2002 academic year—its 22nd year of operation. The Safar Center is now formally a division of the new Department of Critical Care Medicine of the University of Pittsburgh School of Medicine. That department, chaired by Dr. Mitchell Fink is the first medical school based Department of Critical Care Medicine in the United States. That Critical Care Medicine has achieved departmental status in Pittsburgh is logical both on the basis of the unprecedented academic strength of the department and its historical status as the site of the nation's first multidisciplinary ICU and training program—pioneered by Dr. Safar in the late 1960s. I am pleased that the Safar Center is part of this new department.

The multidisciplinary nature of the Safar Center produces a unique and exciting environment for both trainees and faculty and the productivity and successes of the investigators and trainees continues to amaze me.

Three major areas of research and research training are in full swing and well funded—including research in traumatic brain injury, hemorrhagic shock and suspended animation, and training in neurointensive care, resuscitation, and rehabilitation research. Our traumatic brain injury program is funded by a program project grant from the National Institute of Neurological Disorders and Stroke, five RO-1s, one R-21 and KO-8, and a variety of other grants. It spans a number of areas of investigation—such as the study of novel resuscitative therapies targeting neuronal death, unraveling the mechanisms of secondary injury in both experimental models and in brain injured patients, development of novel tools to facilitate detection of occult cases of child abuse, and testing of new strategies in brain injury rehabilitation. We were all sad to see Dr. Donald Marion leave Pittsburgh. Don was a tremendous collaborator and integral member of the Safar Center family and he will be sorely missed. We wish him well in his new position as chairman of the Department of Neurosurgery at the Boston University School of Medicine. However, I am thrilled that Dr. C. Edward Dixon will assume the position of principal investigator of the program project. Ed is the perfect choice to continue to foster the outstanding collaboration that has developed between the Department of Neurological Surgery and the Safar Center. His strong links to the Department of Physical Medicine and Rehabilitation (PMR) will also help further unite



*C. Edward Dixon, Ph.D. assumed the
directorship of the University of
Pittsburgh Brain Trauma Research
Center.*

these important components of the continuum of care in traumatic brain injury with the strong resuscitation and Critical Care medicine faculty within the Safar Center. The success of the link between PMR and our Center is further reflected by the fact that two PMR junior faculty members, Drs. Anthony Kline and Amy Wagner, now have sections within this report within the area of traumatic brain injury.

The hemorrhagic shock and suspended animation program—guided by Drs. Peter Safar and Samuel Tisherman also continues to break important new ground in the area of trauma resuscitation. This program continues to be well supported through congressional plus-up funding via the United States Army and a grant from the United States Navy. Experiments in this program have routinely achieved intact survival after exsanguination cardiac arrests of 90 minutes in duration, and in some cases, after two hours. This work is breaking new frontiers in cerebral preservation and resuscitation research. It is an honor to be able to watch the genius of Peter Safar as he carefully crafts this important project into the masterpiece that it has become. Consultative and administrative support from Dr. Lyn Yaffe, former director of the United States Naval Medical Research Institute has been instrumental to the success of this program, as has been the enthusiastic support of Col. Dean Calcagni and Robert Read of the United States Army.

Research training continues to be a key priority in our center –both postdoctoral fellow (MD and/or PhD) and junior faculty development. This represents the most important part of my own efforts. Postdoctoral clinician-scientist developments in the field of pediatric critical care medicine is greatly facilitated by our T-32 grant from the National Institute of Child Health and Development entitled “Training in Pediatric Neurointensive Care and Resuscitation Research.” I wish to thank Drs. Ralph Nitkin, Michael Weinrich, Carol Nicholson, and Beth Ansel at NICHD for their valuable insight and support of this important program. We have also received funding from the Charles Schertz Grant from



Rachel Berger, M.D. is carrying out an important study at Children's Hospital of Pittsburgh using serum biomarkers in an attempt to identify infants who are unrecognized victims of inflicted childhood neurotrauma (shaken baby syndrome).

our Department of Anesthesiology. I cannot say how pleased I am to work with Dr. John Williams, Chairman of the Department of Anesthesiology, to ensure that the multi-departmental mission of the Safar Center continues to flourish. Finally, some postdoctoral fellowship positions are supported by individual faculty grants. Junior faculty development is supported by a number of grants, including KO8 awards to Drs. Robert Hickey in the division of pediatric emergency medicine (mentored by Dr. Steven Graham in the Department of Neurology) and Amy Wagner (mentored by Dr. C. Edward Dixon) in the Department of PMR. Also, Dr. Rachel Berger, in the Department of Pediatrics has submitted a K-23 award entitled “Using Biochemical Markers to Detect Abusive Head Trauma”

(mentored by Dr. Kochanek) and we are optimistic that Rachel's important work will also be funded. Productivity by the trainees continues to be spectacular, including a total of 25 fellow first-author peer-reviewed publications this academic year. Several fellows received awards. Dr. Hülya Bayır received the Scientific Award from the Society of Critical Care Medicine during the 2001 Critical Care Congress for her work showing a marked gender effect of lipid peroxidation after traumatic brain injury in adult patients. Also, Dr. Berger received the Ambulatory Pediatric Association Fellows Award from the Ambulatory Pediatric Association for her work on the astrocyte marker S100B in pediatric head injury. The successful development of academic faculty in intensive care, resuscitation, and rehabilitation-relevant fields is our most important mission. I am proud of our successes on this front. Seven of our recent trainees have successfully competed for K awards from NIH and three of these individuals have gone on to achieve support as principal investigators at the RO-1 level.

Investigators in the Center published 59 peer-reviewed papers, 23 chapters, 48 abstracts, and 5 editorials during the 2001-2002 academic year. Included among these reports were publications in the *Journal of Cerebral Blood Flow and Metabolism*, *Pediatrics*, *Critical Care Medicine*, the *Journal of Neurotrauma*, *Shock*, *Brain Research*, *Pediatric Research*, *Neuroscience Letters*, *NeuroReport*, *Contemporary Neurosurgery*, *Journal of Trauma*, *Neurosurgery Clinics of North America*, *New England Journal of Medicine*, *Neuroscience*, *Journal of Neurochemistry*, *Resuscitation*, *Pediatric Neurosurgery*, *Academic Emergency Medicine*, *Pediatric Critical Care Medicine*, *Current Opinion in Anesthesiology*, and *Critical Care Medicine*. Particularly noteworthy publications included invited reviews authored by two of our T-32 fellows--Dr. Kimberley Statler in the *Journal of Neurotrauma* entitled "The Simple Model versus the Super Model," and Dr. Trung Nguyen in *Pediatric Critical Care Medicine* entitled "Microvascular Thrombosis in Pediatric Multiple Organ Failure—Is it a Therapeutic Target?" Dr. Kochanek and co-authors published an invited review on "Cerebral Resuscitation after Traumatic Brain Injury and Cardiopulmonary Arrest in Infants and Children in the New Millennium" in the journal *Pediatric Clinics of North America*. Two medical students published a first author manuscript in *Pediatric Critical Care Medicine*--Jonathan Amick and Kristen Yandora, and a high school summer student, Sumeeta Varma, now at



Michal Schwartz, Ph.D. was the 2000 Peter and Eva Safar lecturer and discussed her pioneering work in the area of protective autoimmunity after CNS injury.

Stanford University--gave an impressive presentation of her paper entitled "Lipid Peroxidation after Severe Traumatic Brain Injury in Infants and Children: Assessment of F₂-isoprostane" at the annual meeting of the Society of Critical Care Medicine. Fellow, Dr. Wilhelm Behringer, under the mentorship of Drs. Safar and Tisherman, authored an important report on the suspended animation project--describing survival of 30 minutes of cardiac arrest with cooling by aortic flush in dogs--in the journal *Anesthesiology*. Dr. Safar's paper of 1958 in *JAMA* was selected as the first "classic paper" in a new series in *Anesthesiology*.

The 2001 Peter and Eva Safar Lecturer was Michal Schwartz, Ph.D., who gave a provocative talk on "Protective

Autoimmunity after CNS Trauma and in Chronic Neurodegenerative Disorders: A Paradigm Shift.” Professor Schwartz was born in Tel Aviv, Israel. She received a B.Sc. in chemistry from The Hebrew University of Jerusalem in 1971 and a Ph.D. in chemical immunology from the Weizmann Institute of Science, Rehovot, Israel, in 1977. In 2000, Professor Schwartz was named Career Woman of the Year in Israel. She is the first woman to be invited to deliver the Peter and Eva Safar Lecture at the University of Pittsburgh School of Medicine.

Visiting Professor to the Division of Pediatric Critical Care Medicine was Jacques R. Lacroix, M.D., Associate Professor of Pediatrics and Director of the Pediatric Critical Care Medicine Program at the University of Montreal, Sainte-Justine Hospital gave a lecture on “Red Blood Cell Transfusion: The Good, the Bad and the Ugly.” Similarly, our critical care medicine and Safar Center fellows presented their research to him on the second day of his visit, and his suggestions to them were outstanding.



From left to right Safar Center research fellows, Ala Nozari, M.D., Kimberly Statler, M.D., Margaret Satchell, M.D., Pak Chan, Ph.D. (annual Safar Center visiting professor), Xianren Wu, M.D., Hülya Bayır, M.D., Trung Nguyen, M.D., and Margaret Wilson, Ph.D., after their scientific presentations to Dr. Chan during his visit. Dr. Safar’s watchful eye is appropriately in the background.

Our annual visiting professor to the Safar Center was Dr. Pak Chan from Stanford University School of Medicine, Dept. of Neurology and Neurological Sciences. Dr. Chan lectured on “Oxidative Signaling as Molecular Switch for Cell Death or Survival in CNS Injury.” Each of our fellows also presented their work to him for critique on the second day of his visit. His presentation and comments to our

young investigators was extremely helpful and greatly appreciated.

Beginning on July 1, 2002, Dr. Clifton Callaway, a faculty member in the University of Pittsburgh Center for Emergency Medicine will take over from Dr. Nicholas Bircher as the director of the cardiopulmonary arrest program at the Safar Center. Clif is a talented young investigator, and I am optimistic that we can work together to cultivate an important interaction between the Emergency Medicine Department (chaired by Dr. Paul Paris) and the Safar Center. I would like to thank Nick for his longstanding dedication to cardiopulmonary resuscitation research at the Safar Center. I have revamped some of the programs and named several new directors—to reflect the current composition of our Center. Dr. C. Edward Dixon has taken over as the director of the new Functional

Outcome Core, while Drs. Robert Clark and Larry Jenkins have taken over as co-directors of the Molecular Biology Core of the center. These titles are long overdue for Ed, Bob, and Larry, who are both talented scientists and irreplaceable colleagues and friends.

Once again, I would like to thank everyone working at the Safar Center for a terrific job this year. I am personally indebted to Linda Amick and Marci Provins for their administrative and secretarial excellence, respectively. 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 serves as our key secretarial resource for the academic programs in our center –along with her incredible work as my local editorial assistant for the journal *Pediatric Critical Care Medicine*. I would also like to personally thank Henry Alexander, John Melick, Keri Janesko, Xiecheng Ma, Fran Mistrick, Ray Griffith, Jackie Pantazes, Grant Peters, and S. William Stezoski, who were senior administrative and technical staff members during the 2001-2002 academic year for their spectacular contributions to the individual missions of the Center. I continue to be amazed by the dedication and work ethic of these individuals and all of the technical and secretarial staff at our Center.

I would like to thank Dr. Mitchell Fink for his support as the new Chairman of the Department of Critical Care Medicine and I look forward to working with Susan Stokes, the new departmental administrator. I would like to thank Drs. Robert Clark, C. Edward Dixon, Larry Jenkins, Donald Marion, Ross Zafonte, Clifton Callaway, Nicholas Bircher, P. David Adelson, Xiaopeng Zhang, Anthony Kline, Amy Wagner, Hong Qu Yan, and of course Peter Safar for their camaraderie and guidance with the continued development of the Safar Center and its programs. I would also like to thank Dr. John Williams, Chairman of the Department of Anesthesiology, for his interest in our Center.

Special thanks are also due to Dr. Chien Ho and Kristy Hendrich at the Pittsburgh NMR Center for Biomedical Research, Dr. Edwin Jackson in the Center for Clinical Pharmacology, Dr. Valerian Kagan in the Department of Environmental and Occupational Health, Dr. Stephen Wisniewski in the Department of Epidemiology, Dr. Rachel Berger in the Department of Pediatrics, 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. Melvyn Heyes at the Curagen Corporation for outstanding collaborative expertise that raises the level of the research at the Safar Center.

I once again look forward to success in 2002-2003 in our investigative efforts to develop new therapies in the field of resuscitation medicine.

Respectfully submitted,

Patrick M. Kochanek, M.D.



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

Director, Traumatic Brain Injury

Peter J. Safar, M.D., Distinguished Professor

Director, Shock and Suspended Animation

Samuel A. Tisherman, M.D.

Associate Director, Shock and Suspended Animation

Robert S.B. Clark, M.D.

Associate Director, Molecular Biology

Larry W. Jenkins, Ph.D.

Associate Director, Molecular Biology

C. Edward Dixon, Ph.D.

Associate Director, Functional Outcome

Clifton Callaway, Ph.D.

Associate Director, Cardiopulmonary Arrest

Scientists

P. David Adelson, M.D.
 Nicholas Bircher, M.D.
 Miroslav Klain, M.D., Ph.D.
 Anthony Kline, Ph.D.
 Donald Marion, M.D.
 Ernesto A. Pretto, M.D.
 S. William Stezoski
 Amy Wagner, M.D.
 Xiaopeng Zhang, M.D.

Fellows

Hülya Bayır, M.D.
 Mandeep Chadha, M.D.
 Yong Y. Han, M.D.
 Trung Nguyen, M.D.
 Ala Nozari, M.D.
 Margaret Satchell, M.D.
 Paul Shore, M.D.
 Kimberly Statler, M.D.
 Margaret Wilson, Ph.D.
 Xianren Wu, M.D.

Technicians

Alan Abraham
 Henry Alexander
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 Dwight Davis
 Weimin Gao
 Raymond Griffith
 Yaoqiong Hao
 Jeremy Henchir
 Keri Janesko
 Scott Kostelnik
 Youming Li
 Xiecheng Ma, M.D.
 John Melick
 Paula Nathaniel
 Grant Peters
 Dan Santone
 Jason Stezoski
 Murugan Subramanian
 Vince Vagni

Guest Scientists

Rachel Berger, M.D.
 Steven DeKosky, M.D.
 Howard Ferimer, M.D.
 Robert Garman, D.V.M.
 Steven Graham, M.D., Ph.D.
 Kristy Hendrich, B.S.
 Robert Hickey, M.D.
 Sam Poloyac, Ph.D.
 James V. Snyder, M.D.
 Hong Qu Yan, M.D.

Support Staff

Linda Amick
 Janice Hasch
 Fran Mistrick
 Jackie Pantazes
 Karen Perkins
 Marci Provins
 Valerie Sabo
 Julian Smith

Students

Aaron Gordon
 Laura Griffith
 Danielle Kausler
 Jaime Massucci
 Nicole Williams

Visiting Scientists

Ann Radovsky, D.V.M.
 Lyn Yaffe, M.D.

Funding

During the 2001-2002 academic year, Safar Center investigators had a total of 48 active grants. 43 of these grants were extramural. The direct and indirect costs for the full award period of these grants totaled \$18,908,318 and this is plotted for the current and preceding four 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.

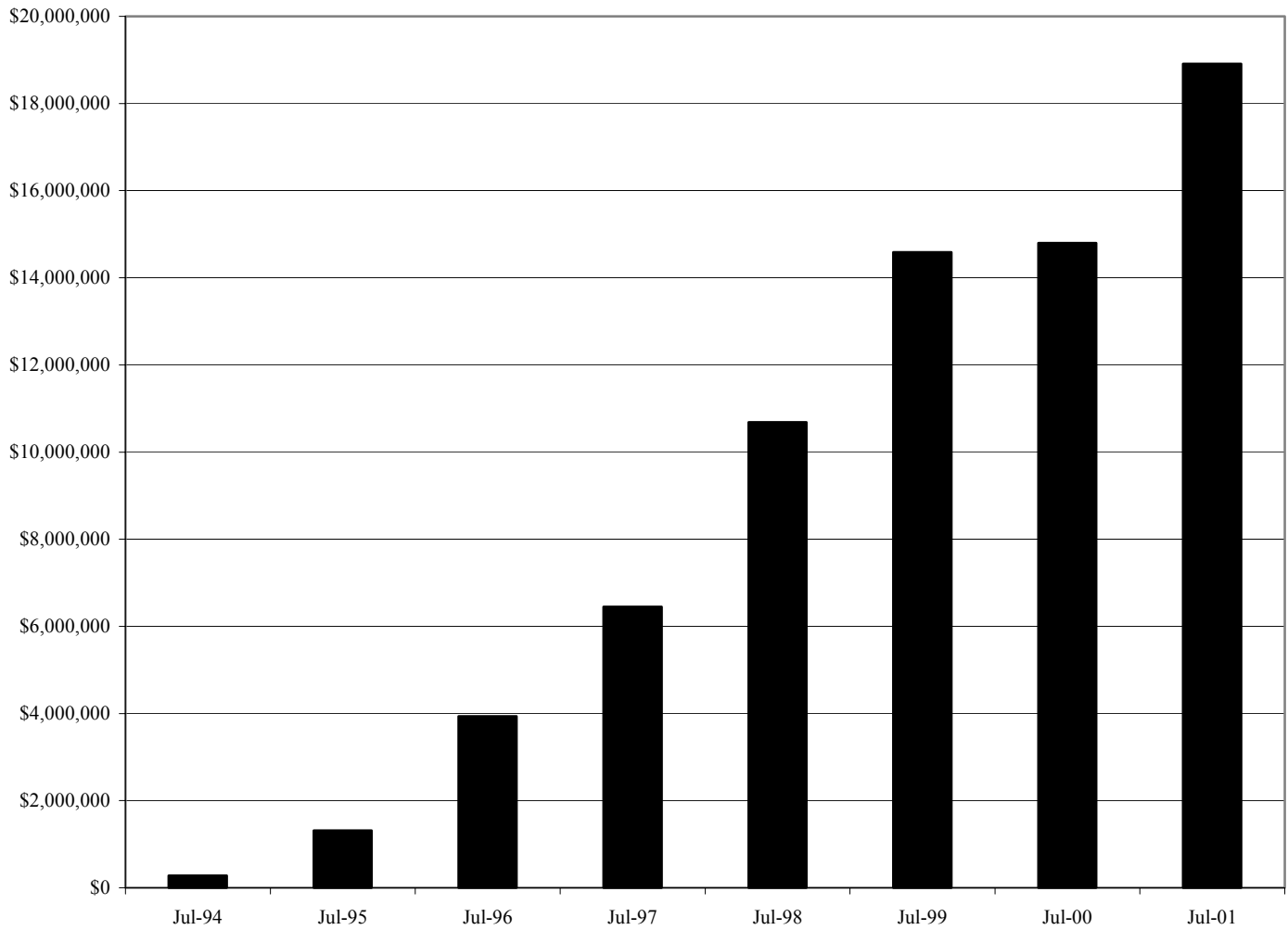
The portion of the budget for use in each academic year (July 1 through June 30) is also plotted for the current and preceding four academic years on the pages following. This represents direct and indirect costs and is shown for total, extramural, and intramural grant support.

Extramural funding sources included the National Institutes of Health, the United States Army, the United States Navy, the Centers for Disease Control and Prevention, the Laerdal Foundation, and a variety of other sources including The Pittsburgh Foundation. Contributions were made to the Safar Center in memory of Eric Bundy.

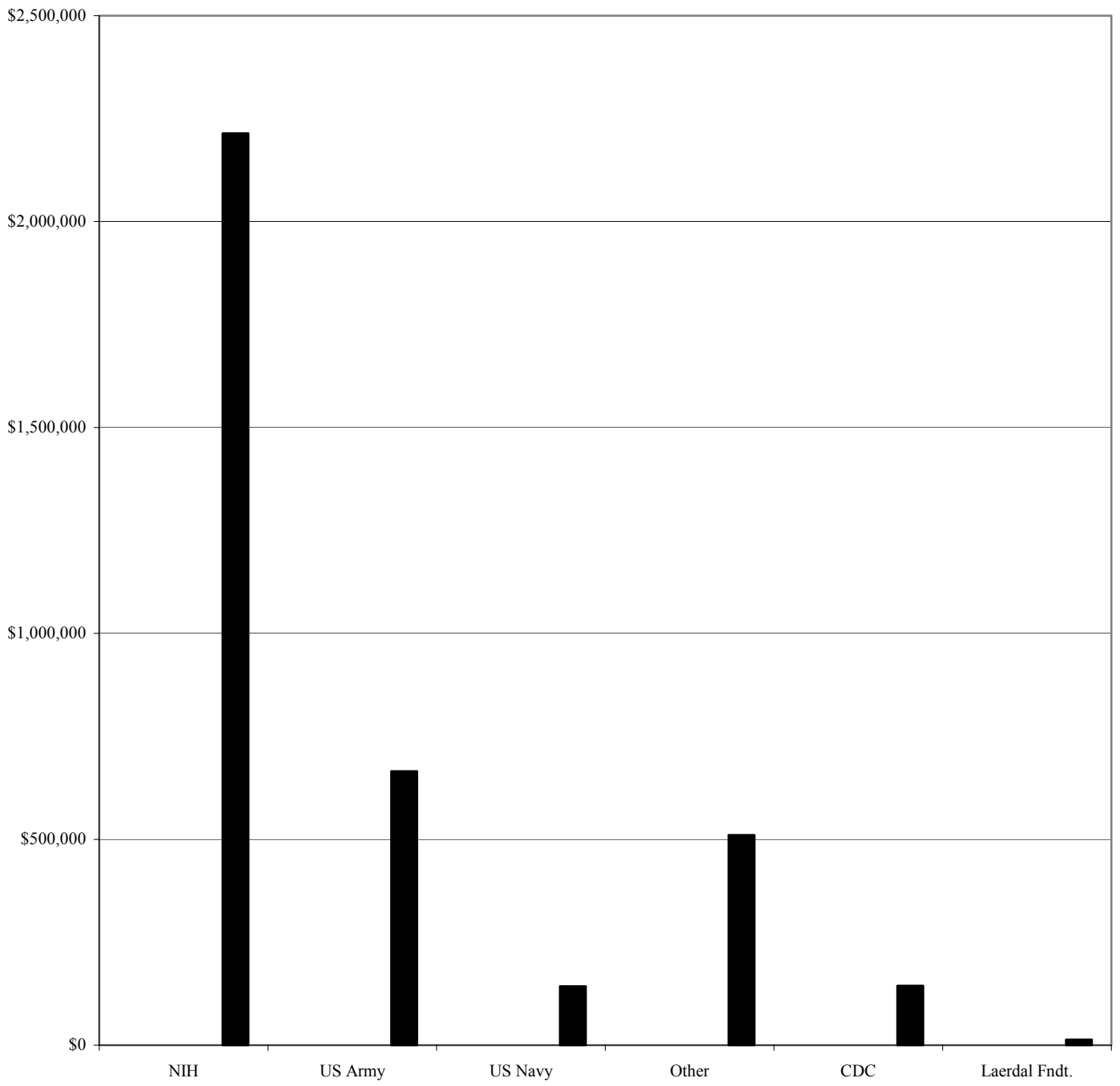
Intramural funding was provided by the Departments of Anesthesiology, Critical Care Medicine, Children's Hospital of Pittsburgh, and the Pittsburgh Mercy Foundation, Mercy Hospital of Pittsburgh.

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

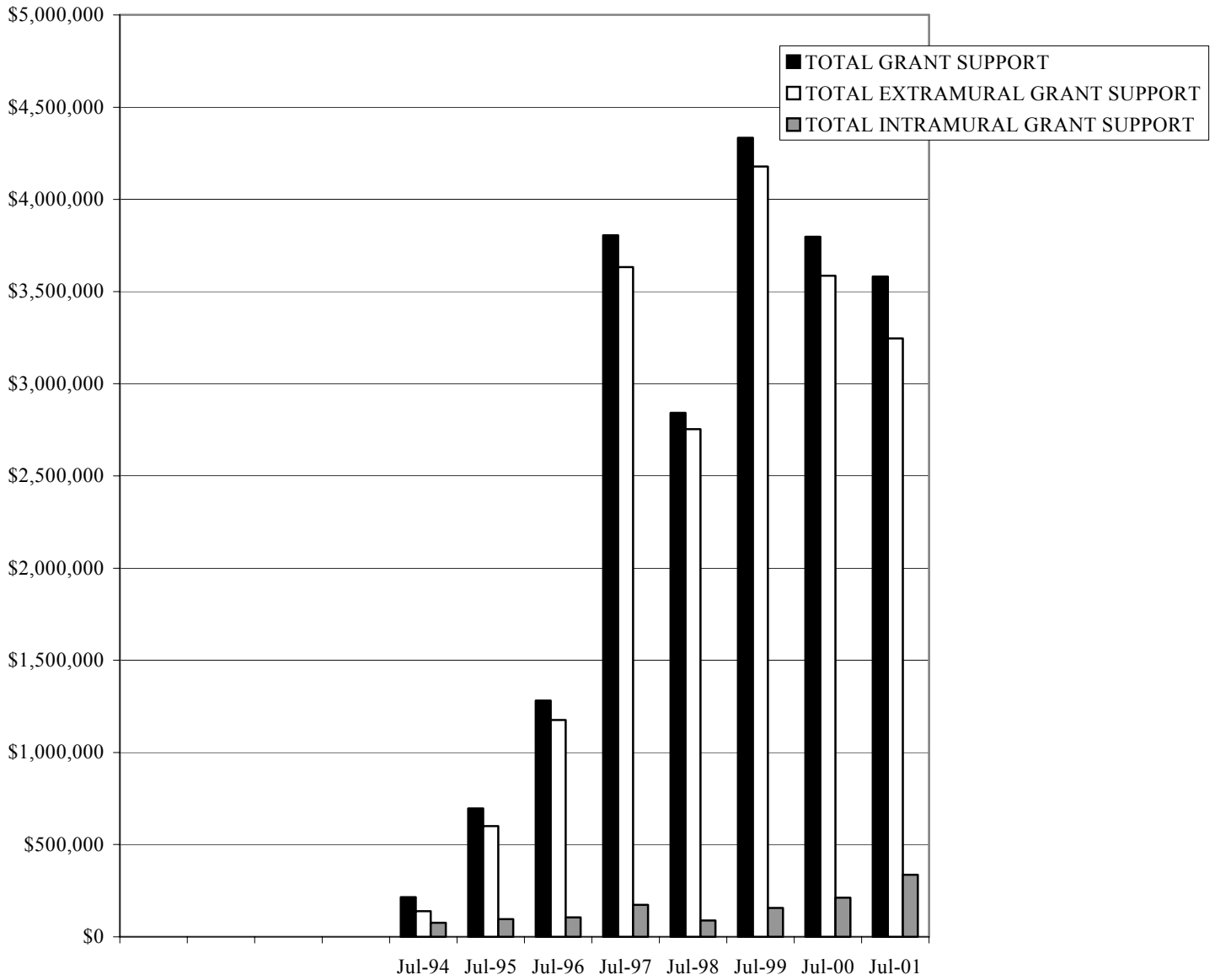
Direct and Indirect Costs for the Full Award Period of SCRR Grants



Specific Sources of Grant Support



**Safar Center Grant Support thru 2001/2002
use in each academic year**



TRAUMATIC BRAIN INJURY (TBI) PROGRAM

Research in TBI 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), including the Clinical Research, Investigation, and Systems Modeling of Acute Illness (CRISMA), Neurosurgery, and Neurology at the University of Pittsburgh School of Medicine. A large 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, Neurological Surgery, Pediatrics, Neurology, Epidemiology, Anesthesiology, and Surgery. In addition, a long-standing collaboration is in place with the Pittsburgh NMR Center for Biomedical Research at Carnegie Mellon University. We have also had a number of important extramural collaborators, Dr. Melvyn Heyes at the Curagen Corporation, Dr. Naoto Minamino at the National Cardiovascular Center Research Institute in Osaka, Japan, Dr. Jiang-Fan Chen at the Harvard Medical School, Dr. Jurgen Schnermann at the NIH, and Dr. Ann-Christine Duhaime at Dartmouth University. Taken together, these collaborations have allowed us to investigate a broad spectrum of mechanisms that may be important to the evolution of secondary damage after TBI. Our most important work continues to be in the area of defining the mechanisms important to secondary brain injury both after experimental TBI and in the human condition. Our studies of mechanism of secondary damage and repair in human materials (cerebrospinal fluid [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 successfully translated to clinical application. Our clinical research of taking the bench to the bedside—particularly as it relates to child abuse—has been featured many times in the lay press.

1. Studies directed by Patrick M. Kochanek, M.D.

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 member of our critical care team including Drs. Robert Clark, Hülya Bayır, Paul Shore, Randall Ruppel, Yi-Chen Lai, Mandeep Chadha, and Erica Fink in the division of Critical Care Medicine, Dr. Rachael Berger, in the Department of Pediatrics, and Dr. David Adelson in the division of Neurosurgery at Children's Hospital of Pittsburgh. To generate a CSF bank for this purpose, Dr. Kochanek is funded by the CDC (University of Pittsburgh Center for Injury Control and Research/CIRCL). We have now over 1000 samples from nearly 100 infants and children who have suffered a severe TBI—including over 20 victims of inflicted TBI

(shaken baby syndrome). In addition, we continue to collaborate with Dr. Neal Thomas at the Hershey Medical Center, Hershey, PA, who is also collecting samples.

Studies using the pediatric CSF bank at the Safar Center

The pediatric CSF bank and related clinical projects have produced some of the most interesting findings in the area of TBI at the Safar Center in the 2001-2002 academic year. Work has progressed in seven major areas including 1) oxidative stress, 2) detection of occult inflicted childhood neurotrauma, 3) adenosine and related metabolites in TBI, 4) markers of neuronal death study, 5) growth factors and markers of regeneration and repair, 6) studies of the effect of hypothermia on markers of secondary damage after TBI, and 7) assessment of the effect of the mode of CSF drainage in pediatric TBI.

Oxidative stress in TBI

This is an exciting area of research spearheaded by Dr. Hülya Bayır, a senior PICU fellow and the 2002 Charles Schertz Fellow in the Department of Anesthesiology. Dr. Bayır's work entitled "Assessment of antioxidant reserve and oxidative stress in CSF after severe TBI in infants and children" was published as a full paper in *Pediatric Research*. That work, done in collaboration with Dr. Valerian Kagan, provided substantial evidence for oxidative stress in brain after severe TBI in infants and children. Dr. Bayır followed up on that study an equally important report entitled "Effect of hypothermia on oxidative stress after TBI in humans: a preliminary report" that she presented at the 2001 meeting of the National Neurotrauma Society and will present at the 2002 meeting of the SCCM. She has begun to use the battery of markers of oxidative stress and damage that she has developed with Dr. Kagan, to evaluate the effect of therapies—including moderate hypothermia in adults. Current studies by Dr. Bayır of the effect of hypothermia on oxidative stress in pediatric TBI are also underway—done in conjunction with the RCT being carried out by Dr. Adelson at Children's Hospital of Pittsburgh. Dr. Bayır has also worked under the direction of Dr. Kagan to study a novel marker of nitrosative stress in pediatric TBI, namely, S-nitrosylation. Their work in this novel area was presented at the 2001 meeting of the Society for Neuroscience and a full manuscript is *in press* in the *Journal of Cerebral Blood Flow and Metabolism*.

Detection of occult inflicted childhood Neurotrauma

This important area of research originated from a small grant awarded to Dr. Kochanek within the University of Pittsburgh Center for Injury Control and Research (CIRCL) focused on the use of inflammatory markers in CSF as a biological clock to provide insight into the timing of injury in infants who were victims of the shaken baby syndrome. Often these infants are either chronically injured, or there may be a delay in presentation. In the past year, Dr. Rachel Berger, a general pediatrician working in the area of child abuse at Children's Hospital of Pittsburgh, has done an exemplary job in broadening 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]), glial (S-100B), and axonal (myelin basic protein) were massively increased versus control after severe TBI in infants and

children—including child abuse victims. That work was published this year in the journal *Pediatrics*. She also published an important report in the *Journal of Neurotrauma* showing that these markers of brain injury are increased in 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 abusive head injury—such as vomiting without diarrhea, a seizure without fever, unexplained bruising, etc. That study is the centerpiece of Dr. Berger’s recently submitted K-23 award—that we are optimistic will be funded by NICHD this year. Rachel is quickly becoming a leading investigator in this area. Relevant to this area, Dr. David Adelson was the co-editor of an issue of *Neurosurgical Clinics of North America* that was devoted to child abuse and several Safar Center faculty and fellows were authors on review papers in that issue.

Adenosine in TBI

Dr. Kochanek is beginning year-4 on an RO-1 from NINDS focused on adenosine in TBI. Translational work is an important part of this effort and the CSF bank represents a key resource. Dr. Courtney Robertson’s article on CSF adenosine in pediatric TBI (see last year’s report) was published this year in the journal *Critical Care Medicine*. Investigation of the effect of hypothermia on adenosine and purine related markers of energy failure are ongoing in collaboration with Dr. Edwin Jackson. Similarly, Ava Puccio is evaluating the relationship between CSF adenosine and tissue oxygen levels in adults with severe TBI, in work done with Dr. Marion at Presbyterian Hospital.

CSF markers of neuronal death in TBI

As part of the impressive work of Dr. Robert Clark’s group on mechanism of neuronal death, including studies on caspases, apoptosis inducing factor (AIF), and poly ADP ribose polymerase (PARP), translational studies are similarly taking advantage of our CSF bank and brain tissue samples. Drs. Margaret Satchell and Xiaopeng Zhang have published a series of abstracts on PARP activation and protein kinase B signaling after TBI in humans. Some of Dr. Satchell’s work was discussed in last year’s annual report. This translational approach is providing important human data on contemporary and intensively investigated mechanisms of neuronal death in experimental TBI—and has the potential to help guide the development of novel therapies.

Growth factors and markers of regeneration and repair

Building on the prior work of both Dr. Steven DeKosky on nerve growth factor, and Edwin Jackson on the relationship between adenosine A₂β receptor activation and elaboration of vascular endothelial growth factor (VEGF), Dr. Paul Shore presented a paper at the 31st Congress of the SCCM reporting marked increases in VEGF after severe TBI in infants and children. At the same meeting, Dr. Erica Fink, a senior pediatric resident working with Dr. Clark reported increases in hepatocyte growth factor in CSF after injury. We have been struck by the robust and rapid regenerative response that occurs after TBI and by the fact that this is readily detected using CSF.

Effect of modes of CSF drainage

Dr. Paul Shore is carrying out a comparative study (in collaboration with Dr. Neal Thomas at Hershey Medical Center) assessing the effect of continuous versus intermittent CSF draining on mediator levels and pathophysiology after severe TBI in infants and children. We are pleased to collaborate with Dr. Thomas, a former fellow in our program, in this study that addresses a basic treatment approach (CSF drainage) that has been subjected to remarkably little investigation.

Our pediatric CSF bank 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: Quinolinic Acid in Cerebrospinal Fluid Early after Severe Head Injury in Victims of Child Abuse R49/CCR310285-03, (9/1/01-8/31/02), \$45,110, Patrick Kochanek, PI, Melvyn Heyes, Ph.D., [Curagen Corporation], Stephen Wisniewski, Ph.D., Donald Marion, M.D., and P. David Adelson, M.D., Co-investigators); collaborators. CDC, Grants for Injury Control Research (Donald Marion, M.D., PI); Adenosine and TBI, NS38037, (8/2/01-7/31/02) \$263,910, Patrick Kochanek, PI; iNOS and Traumatic Brain Injury, NS30318 (Patrick Kochanek, PI), Project 3 within the University of Pittsburgh Brain Trauma Research Center (BTRC), Donald Marion, PI. Protocol #3480500 (5/1/00-4/30/01), \$11,215, Rachel Berger, PI, CHP GCRC. Oxidative Stress after Severe Head Injury in Infants and Children: Effect of Therapeutic Hypothermia, Laerdal Foundation, Hülya 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 as an endogenous neuroprotectant molecule, particularly early after TBI, and its potential participation in delayed cerebral swelling are being pursued both in the rat TBI model and in patients after TBI. We are beginning the 4th year of this RO-1-funded project. This project continues to be the most active area of research in Dr. Kochanek's laboratory this year and has produced a number of reports of studies in both patients and experimental models of brain injury. This work is being carried out in collaboration with Dr. Edwin Jackson in the Center for Clinical Pharmacology. In laboratory aspects of the research on this project, we have continued to evaluate the effect of local injection of adenosine receptor agonist and antagonists on cerebral blood flow. That work is carried out in collaboration with Dr. Chien Ho and Kristy Hendrich at the Pittsburgh NMR Center. A recent study was presented at the 2001 meeting of the National Neurotrauma Society—and demonstrated that adenosine receptor agonist mediated cerebrovasodilatory effects are mediated by the A2a receptor and can increase cerebral blood flow in both the normal and traumatically injured rat brain. A number of outcome studies of adenosine agonists are ongoing in collaboration with Dr. C. Edward Dixon in our center using the controlled cortical impact (CCI) model. Manu Varma, an undergraduate from the University of Michigan who worked on that project in our laboratory again this summer, was just informed that his manuscript on this work is accepted for publication in the

journal *Brain Research*. Using our CCI model, we are currently studying the A2a-receptor knockout mouse, obtained from Dr. Jiang-Fan Chen at the Massachusetts General Hospital and the A1-receptor knockout mouse obtained from Dr. Jurgen Schnermann at the NIH to begin to unravel the role of specific adenosine receptors in the mechanisms of secondary damage and repair after experimental TBI. Key collaborators on the RO-1 are Drs. Edwin Jackson, C. Edward Dixon, Chien Ho, Steven Graham, Donald Marion, and Ms. Kristy Hendrich.

Support: NIH RO-1, Adenosine and Traumatic Brain Injury, (\$1,593,730, 08/02/99-07/31/03, Patrick M. Kochanek, M.D., PI).

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

iNOS is induced by cytokines and NF- κ B 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. 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. In collaboration with Drs. Kagan and Timothy Billiar, Hülya Bayır has been studying protein nitration and nitrosylation after experimental TBI using iNOS knockout mice. Nitrosothiols may represent a nitric oxide reservoir and could play important roles in signal transduction, immunomodulation, vascular regulation, and neurotransmission.

Support: NIH 2P50 NS30318, iNOS and Traumatic Brain Injury, (\$582,986), Patrick Kochanek, M.D., PI, Key Collaborators: Robert SB Clark, M.D., C. Edward Dixon, Ph.D., Timothy Billiar, M.D., Valerian Kagan, Ph.D., Larry Jenkins, Ph.D., Xiaopeng Zhang, Ph.D., Hong Qu Yan, M.D., and Timothy Carlos, M.D., collaborators.

D. Emergency Interventions after TBI: Effect on Secondary Damage

Studies in this area of investigation were funded, this year, by both the Laerdal Foundation and the Curagen Corporation. Dr. Kimberly Statler (one of our T32 fellows) has been the leading investigator on this work. Dr. Statler presented a surprising paper showing that moderate hypothermia, applied after experimental TBI, expands lesion volume at 72 hours after injury in rats anesthetized with the narcotic fentanyl. That work was presented at the National Neurotrauma Society meeting and is *in press* as a full manuscript in the journal *Critical Care Medicine*. In that study, Dr. Statler discovered that hypothermia after TBI produces an enhanced stress response—reflected by higher serum catecholamine levels—compared to the normothermic condition. These studies are in contrast to the remarkable neuroprotection that others and we have consistently observed with hypothermia in rats anesthetized with isoflurane. The importance of this work lies in the fact that patients are sedated with narcotics after TBI. It may be that to maximize the potential benefit of therapeutic hypothermia after TBI, sedation must be

optimized. To further understand the mechanism underlying the effect of hypothermia on experimental TBI, we are carrying out studies evaluating the effect of hypothermia on gene expression using our mouse model of controlled cortical impact. This work is being carried out in collaboration with Dr. Melvin Heyes at the Curagen Corporation, a leader in gene culling technology. In an initial report, two summer students, Becky Sullivan and Gilna Alce published an abstract of work in *Critical Care Medicine* showing a robust beneficial effect of the resuscitative application of transient, moderate hypothermia in this model. This, to our knowledge, is the first report of the beneficial effects of hypothermia in a mouse model—and sets the stage for studying the combined effects of hypothermia in genetically modified mice. Finally, Dr. Statler also published an invited review on this area of work in the *Journal of Neurotrauma* that was based on a plenary talk by Dr. Kochanek, entitled “The Simple Model Versus the Super Model: Translating Experimental TBI Research to the Bedside.” We hope to also soon apply proteomics approaches to the study of hypothermia in TBI in collaboration with Dr. Larry Jenkins in our Center.

Support: Laerdal Foundation, MRI Assessment of cerebral blood flow and calcium accumulation after TBI in rats: Effect of isoflurane versus Fentanyl, (\$7,500, 1/1/00 – 6/30/01, Kimberly Statler, PI). Training in Pediatric Neurointensive Care and Resuscitation Research, T32-HD40686, National Center for Rehabilitation Research, National Institute of Child Health and Development, Patrick Kochanek, PI 9/25/00-4/30/01.

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

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 and anesthetics in experimental TBI. We have just begun to expand this application this year to the study of our mouse model of experimental TBI with the help of Kevin Hutchins. Dr. Ho’s outstanding multidisciplinary NMR center for biomedical research continues to be a key collaboration for our work in experimental TBI and we hope to begin to collaborate with Dr. Eric Aherns in the area of microimaging applied to our mouse TBI model.

Support: NIH-NINDS 2P50 NS3031809 A1, Rat/Surgery/Imaging Core C, (\$470,095 over 5 years, Patrick Kochanek, M.D., PI, Chien Ho, Ph.D., Co-PI, Kristy Hendrich, Donald Williams, Ph.D., and Steven DeKosky, M.D., Co-investigators). NIH Grants RR-03631 and RR-10962, (Chien 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, Chien Ho, Ph.D., --submitted 3/13/00).

2. Studies directed by C. Edward Dixon, Ph.D.

Research Interests

Research in Dr. Dixon's laboratory is directed towards understanding the 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 an ongoing randomized clinical trial of amantadine hydrochloride on neuropsychological measures of frontal lobe function and measuring CSF and extracellular levels of catecholamines and markers of oxidative injury in humans acutely after brain trauma.

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. During the previously funded period we have observed that substantial cholinergic neurotransmission deficits can occur without a chronic (4-week post injury) loss of cholinergic cell bodies. We also have extensive data that TBI causes chronic changes in key dopaminergic proteins that occur concomitantly with these cholinergic changes. Numerous studies have demonstrated 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, we have compelling preliminary data that dopaminergic innervation of cholinergic nuclei is reduced after TBI. For this project, we propose to logically extend our previous findings to hypothesize that cognitive deficits following TBI may be, at least partially, attributable to decreased dopamine (DA) modulation of septohippocampal cholinergic function. A systematic series of studies are proposed to test this hypothesis. For this project, we will focus on DA modulation of the selectively vulnerable septohippocampal cholinergic system. This provides us with a prototypical system to examine the effects of TBI on interactive neurotransmitter systems. 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. Dr. Dixon will also determine whether changes in hippocampal ACh release is associated with altered D1 receptors in the MSA using quantitative autoradiography, and DA-fiber/cholinergic neuron interactions using a tyrosine hydroxylase/choline acetyltransferase double-immunolabeling method following TBI. Dr. Dixon's group will determine the effect of exogenous administration of neurotrophic factors that promote DA neuronal survival on DA biochemical markers, cognitive deficits, as well as hippocampal ACh release and MSA DA release following TBI. Lastly, Dr. Dixon will determine the effects of clinically relevant DA agonist therapies on cognitive deficits, as

well as hippocampal ACh release and MSA DA release following TBI. Our long-term goal is to develop new therapies to accelerate cognitive recovery following TBI.

During this year, we have found that TBI can produce chronic changes in proteins necessary for DA neurotransmission. We have also found that TBI can produce a reduction in DA release in the medial septal region at 2-weeks postinjury and that the number of tyrosine hydroxylase (TH)-positive fibers with the medial septum and diagonal band are decreased after TBI. Immunohistochemical and Western blot studies have revealed a distributed upregulation of TH and downregulation of DAT protein levels. Western blot studies have found decreases in D2 receptor protein levels in the striatum at 4-wks postinjury. We have also demonstrated that DA agonists can enhance recovery of cognitive function after TBI. Overall, there is new evidence that ACh and DA systems are altered chronically after TBI. We also have preliminary data that markers of DA innervation of the septal region are chronically diminished after TBI.

Support: NIH-NINDS, Chronic Changes in Neurotransmission Following Traumatic Brain Injury, R01 NS-33150-06 (\$1,000,000 / \$484,819 over 5 years, 4/1/00-3/31/05, C. Edward Dixon, Ph.D., PI).

B. Functional Outcome Core

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.

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 brain injury to rats. The Functional Outcome Laboratory Core gives the investigators of the University of Pittsburgh Brain Trauma Center the capability to assess the effects of physiological manipulations and therapeutic interventions of recovery of function after experimental brain injury.

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

C. Oxidative Mechanisms of Severe TBI: Relationship to Chronic Frontal Lobe Cognitive Deficits.

The primary goal of this project is to test the hypothesis that TBI will produce an increase in DA which autoxidizes to generate hydroxyl and free radicals that produce oxidative damage to proteins in frontal lobe regions, and these changes are predictive of long-term performance on neuropsychological measures of frontal lobe function. We will test this hypothesis by utilizing in vivo microdialysis to determine whether extracellular levels of markers of catecholamine protein oxidation, nitration markers, and catecholamine levels predict 6-month neuropsychological outcome on measures of frontal lobe function in humans after severe TBI.

The University of Pittsburgh BTRC has a productive infrastructure to conduct mechanistic studies of available human extracellular fluid, and CSF specimens collected during the acute phase of TBI. The funds provided by this grant are being used to provide the additional support necessary for this existing multidisciplinary team to collect extracellular and CSF samples for analysis of DA and behavioral outcome in TBI patients. If a link is established between DA oxidation and frontal lobe behavioral deficits after severe TBI in humans, then our long-term goal will be to clinically evaluate the effects of therapies that reduce catecholamine-mediated oxidative brain injury on behavior outcome. Our ultimate objective is to reduce disability following TBI and consequently the cost of TBI to society.

Support: The Pittsburgh Foundation – The John F. and Nancy A. Emmerling Fund, Oxidative Mechanisms of Severe Traumatic Brain Injury: Relationship to Chronic Frontal Lobe Cognitive Deficits, reference #: M1999-0080, (\$95,958 over 3 years – no indirect costs, 07/01/99-06/30/02, C. Edward Dixon, PI).

D. 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 at specific time points following injury suggested by our preliminary data. 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.

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, C. Edward Dixon, Ph.D., PI).

E. Effects of Amantadine Hydrochloride on Functional Outcome after TBI: A Randomized, Multi-center, Placebo-Controlled, Clinical Trial.

This project will study one hundred patients with TBI. These will include patients admitted to participating regional rehabilitation hospitals following the acute phase, post-injury, as well as chronic TBI patients recruited through the Neurobehavioral clinic of a major urban academic medical center. Efficacy of drug treatment will be assessed with a double-blind, cross-over design. At each study site, the patients will be randomized into an initial amantadine hydrochloride (AMH) or a placebo group, treated for 3 months, then crossed over to the other treatment for an additional 3 months. A set of measures of each patient's functional status (including the Neurobehavioral Rating Scale, the Glasgow Outcome Scale, and the Disability Rating Scale) will be administered at the time of their admission to the study (baseline), three months after initiation of the intervention, and 3 months after the opposing treatment. Additionally, neuropsychological tests specific to memory and frontal lobe function will also be administered at the same time points. These assessment data will provide the requisite dependent measures to evaluate the following specific hypotheses involving the AMH and placebo patient groups.

Support: CDC, CIRCL - Acute Care Project 1, R49 CCR-312296 (\$151,000 / \$45,000 year 2, 9/01/98–8/31/02, C. Edward Dixon, PhD., PI).

3. Studies by Robert S. B. Clark, M.D.

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 upregulated 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 head injury 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. More recent studies have suggested that 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. A report by pediatric CCM fellow, Neal Seidberg, entitled "Alterations in inducible 72-kDa heat shock protein and the chaperone cofactor BAG-1 in human brain after head injury" is in press in the *Journal of Neurochemistry*. Future studies aim to determine to what extent these genes and their translated proteins contribute to the regulation and execution of neuronal death by using novel molecular (antisense oligonucleotides and viral transgenes) and pharmacologic (fusion protein-coupled heat shock protein) therapies in our experimental models, to provide support for future clinical trials using similar strategies. Finally, in studies addressing another endogenous neuroprotectant pathway, Dr. Xiaopeng Zhang presented a paper at the

National Neurotrauma Society Meeting entitled “Increase in phosphorylated protein kinase substrates after human head injury.”

B. Caspase-Mediated Neuronal Death after Head 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. Last year, we described the expression and activity of caspase-3 in our experimental model of TBI in the *Journal of Neurochemistry*. Importantly, a caspase-3 inhibitor administered after trauma reduced cell death; although, no effects in behavioral outcome could be demonstrated. Potential roles for caspase-1 and -3 were also described in the above-mentioned 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.

C. Divergent Pathways of Cell Death after Brain Injury

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 has clearly demonstrated that AIF-mediated cell death occurs after experimental TBI. That work was presented by Dr. Zhang at the 2001 Meeting of the Society for Neuroscience. This process also appears operative in neurons in vitro as work by Dr. Zhang in concert with Dr. Lina Du has shown that peroxynitrite-induced injury in neurons is associated with nuclear translocation of AIF, large-scale DNA fragmentation, and cell death. This cell death is inhibited using a peroxynitrite decomposition catalyst or PARP inhibitors. These data were combined and published recently by Dr. Zhang et al., as a full manuscript entitled “Intranuclear localization of apoptosis-inducing factor and large scale DNA fragmentation after TBI in rats and in neuronal cultures exposed to peroxynitrite” in the *Journal of Neurochemistry*. Further work intends to tease out the contribution of these divergent pathways of cell death 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), Ian Reynolds, and Donald Marion.

D. 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 nicotinic 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 knockout mouse in our model of experimental TBI. We previously reported highly significant level of protection against functional deficits after TBI in PARP knockout vs wild-type mice. This effect was the greatest beneficial effect that we have observed with any agent in the CCI model of TBI. That work was published as a rapid communication in the *Journal of Cerebral Blood Flow and Metabolism*. Dr. Margaret Satchell, a 4th year pediatric CCM fellow who is working on our T-32 grant from NICHD, has aggressively pursued this promising mechanism in collaboration with Dr. Szabo using novel inhibitors of PARP activation in our TBI model. Abstracts of that work were presented last year as described in the 2000-2001 annual report. Dr. Satchell has pursued clinical studies of PARP activation and nitrosative stress after TBI in adults. She presented this work recently at the 31st SCCM Critical Care Congress and received an Annual Scientific Award from the Society of Critical Care Medicine. She previously received the Murray Goldstein Award from the Neurotrauma Society for her work. This exciting project, with Dr. Clark as PI, is funded within the University of Pittsburgh BTRC Program Project Grant application (Donald Marion, PI).

Support: RO1-NS38620-03, Caspase-Mediated Neuronal Death After Head Injury (\$584,022 total direct costs over 4 years beginning 2/1/99, Robert Clark, M.D., P.I.); KO8-NS01946-05, Role of Neuroprotective Genes After Traumatic Brain Injury (\$455,960 total direct costs over 5 years beginning 12/1/96, Robert Clark, M.D., PI; Steven Graham, M.D., Ph.D. and Patrick Kochanek, M.D., Sponsors); 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, Robert Clark, M.D., P.I.).

4. Studies by P. David Adelson, M.D.

A. Severe TBI in Immature Rats

Diffuse injury and cerebral swelling are important components of the clinical picture in children with severe TBI. Unfortunately, there has been only limited investigation of pediatric TBI in laboratory models, and specific treatments are lacking. The major goal of this program is to define the pathophysiologic response to severe TBI in the immature rat and to better understand the mechanisms involved with neural injury and recovery.

This year, support for this area of research has been obtained from the multiple sources including the NIH, Copeland Foundation, Betty Freiberg Fund, and the University of Pittsburgh. Studies being carried out in Dr. Adelson's laboratory include focal and diffuse experimental TBI. Characterization of the long-term functional and histopathological consequences of severe diffuse and focal TBI was performed, in collaboration with Drs. Dixon and Jenkins. This work has been presented at the annual meetings of the National Neurotrauma Society. Remarkably, sustained functional deficits were observed out to three months following diffuse injury. This indicates that the diffuse injury model, unlike fluid percussion or CCI, produces enduring functional deficits in the immature rat. Other, collaborations with Drs. Whalen, Jenkins, and

Kochanek, include the histopathologic response to TBI of both the CCI and the diffuse injury models in the immature rat. There were striking differences in the injuries incurred and in the expression of adhesion molecules and leukocyte influx in these models. Acute inflammatory response was observed only in the area of contusion produced by CCI. This, to our knowledge, is the first direct comparison of the inflammatory response in focal and diffuse injury models, and suggests that the deficits produced by diffuse injury are manifest despite a paucity of acute inflammation. These comparisons between focal and diffuse will be the mainstay of the laboratory over the next few years. In collaboration with Dr. Chien Ho, Kristy Hendrich and the talented group at the Pittsburgh NMR Center at Carnegie Mellon University, the time course of changes in blood flow and brain swelling are being identified using non-invasive MRI methods in the diffuse and focal injury models. Lastly, additional areas of study include the testing of novel therapies in the focal and diffuse injury models of TBI in the immature, including moderate hypothermia, excitotoxic blockade and cyclosporin A as well as others to be introduced after further defining mechanistic etiologies of damage.

Support: KO-8NS01809-01A1, Severe Diffuse Traumatic Brain Injury in Immature Rats (\$415,260 over 5 years beginning 12/96, P. David Adelson, M.D., PI, Patrick Kochanek, M.D.)

B. Hypothermia for Severe TBI in Children

The major goal of this project is to test the safety and efficacy of hypothermia in children after severe head injury. This program has been funded at an R01 level by the NIH and seeks to investigate hypothermia as a treatment of TBI in children, with a special emphasis on the development of novel methods for initial and outcome assessment. Dr. Adelson is the principal investigator of this multicenter study that includes 8 centers. Dr. Harvey Levin at the Baylor College of Medicine, is a key co-investigator on that important proposal. In addition, in collaboration with Drs. Kochanek, DeKosky, and Graham and others further ongoing research includes the effect of therapeutic hypothermia on both excitotoxic and inflammatory markers of brain injury in infants and children, the effect of severe TBI on language and speech acquisition and recovery, cerebral blood flow and metabolism after injury, long-term effects of mild-moderate head injury, and other collaborative and related efforts.

Support: KO-8 NS01809-01A1, Severe Diffuse Traumatic Brain Injury in Immature Rats (\$415,260 over 5 years beginning 12/96, P. David Adelson, M.D., PI, Patrick Kochanek, M.D., Sponsor); Pittsburgh Foundation, Functional and Cerebrovascular Response to Severe Diffuse Traumatic Brain Injury in Immature Rats (\$50,000 over 2 years beginning 7/96, P. David Adelson, M.D., PI); Competitive Medical Research Fund, Functional and Cerebrovascular Response to Diffuse Traumatic Brain Injury in Immature Rats (\$25,000 over 1 year beginning 8/97, P. David Adelson, M.D., PI).

5. Studies by Steven DeKosky, M.D.

A. Neurotrophic Response to TBI

Dr. DeKosky's laboratory studies assessing the role of neural cells and their products in the brain's attempt at repair following TBI. Our laboratory is particularly interested in the cytokine and anti-oxidant cascade following injury, and the role of these inflammatory proteins in the upregulation of neuroprotective proteins such as nerve growth factor (NGF). Our goal is to elucidate the brain's injury response and provide insight into possible therapeutic interventions.

We have examined several inflammatory and anti-oxidant molecules following injury. One important protective response to injury is the upregulation of antioxidant proteins such as catalase and glutathione peroxidase. Our data show that catalase (CAT) and glutathione peroxidase (GPx) increase in the lesioned cortex after TBI, peaking at 3 and 7 days, respectively. In the hippocampus, CAT and GPx increase at 1 day, peaking at 7 days, while superoxide dismutase (SOD) levels actually decrease by 6 hours post-trauma. Hypothermia treatment attenuates the rise in GPx, while increasing activity of SOD above sham levels. We previously had shown that reduction of IL-1 after TBI inhibits NGF expression and suppresses antioxidant upregulation. Subsequent experiments have examined whether p75 and trk A, the receptors for NGF, are elevated following TBI. Our analyses suggest that TBI elevates the mRNA and protein for both p75 and trk A in the cortex and hippocampus. These major studies are of interest to our laboratory because, in addition to furthering understanding of the biochemical changes following TBI, they may provide insight into human neurodegenerative diseases, including Alzheimer's disease.

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 (A β) 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. We are examining the factors involved in regulating the expression of APP after TBI in rats. Preliminary data demonstrate a trauma-induced increase in APP and A β levels. Determining the factors and conditions that regulate APP metabolism will provide information for the role of this compound in TBI and for the development of therapeutic strategies for AD. We are currently developing treatment strategies that will enable manipulation of APP processing after TBI, with an eye toward suppressing A β , which produces apoptosis and oxidative stress after injury and in AD. Information gained by these studies will establish the therapeutic importance of manipulating APP metabolism, and offer new opportunities for suppressing the pathological deposition of A β in AD.

To better understand the relationship between TBI and AD, we are investigating the expression of AD markers, including amyloid, in surgically resected tissue samples and CSF from head-injured patients. Our data demonstrate that amyloid-related pathological and biochemical changes after TBI are similar to those observed in AD. Shortly after head injury, there is increased production of APP. In addition, the two A β species (and particularly the “early” A β 42) are increasingly being deposited in the tissue, while they are depleted in the CSF. In contrast to these rapid changes in amyloid metabolism, neurofibrillary tangles, another major pathological hallmark of AD, are not found in these patients. Our present results suggest that A β (42) may play a critical role in plaque formation after brain injury, similar to findings in AD brains. Studies are underway to further characterize these and other changes that support common pathological mechanisms in TBI and AD. Because the normal biological function of APP is unknown, determining the factors and conditions that regulate APP metabolism will provide information for the development of therapeutic strategies for both TBI and AD.

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

6. Studies by Steven Graham, M.D., Ph.D.

A. Excitotoxicity and Programmed Cell Death

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. Ongoing studies concern the role of excitatory amino acids and free radicals in pathogenesis of brain injury. 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.

Cyclooxygenase-2 (COX2), the inducible isoform of the enzyme that catalyzes the formation of prostaglandins is also being investigated. Expression of COX2 is induced by neuronal excitation and COX2 activity produces free radicals, so COX2 may be an important mechanism whereby excitotoxicity is expressed.

Support: Core C of 2 P50 NS30318-04A21, Project #1 in the University of Pittsburgh Head Injury Research Center (Steven Graham, M.D., Ph.D., PI). Department of Veterans Affairs/Department of Defense Brain Trauma Initiative - Merit Review (Steven H. Graham, M.D., Ph.D., PI, 4/1/95 - 3/31/98, 20% of VA Time. Department of Veterans Affairs, The Role of Inducible Cyclooxygenase in Delayed Neuronal Death. Current Year Direct Costs: \$143,000. Technicians: Ann Stetler, Marie Rose, Post doc: Li Zhu, Ph.D.).

7. Studies directed by Larry W. Jenkins, Ph.D.

A. Protein Synthesis, Memory and Pediatric Brain Injury

We have further examined the potential role of impaired protein synthesis in memory deficits after experimental pediatric TBI. There is extensive data suggesting that protein synthesis is critical for the consolidation of hippocampal dependent learning and memory. Protein synthesis is involved in developmental synapse formation, long-term potentiation (LTP) and during memory consolidation. Our initial study employing 2-D gel electrophoresis to examine global protein expression during the consolidation of spatial memory acquisition has just been submitted for publication. Proteomic studies have significant potential to expand our understanding of neural injury and therapy but have yet to be applied to TBI. The purpose of the present study was to examine global hippocampal protein changes in 17 PND rats 24 hrs after moderate CCI. Analysis was limited to a wide pH range (nonlinear pH 3-10) for isoelectric focusing with immobilized pH gradients (IPG strips) and large format (22 x 22 mm) SDS slab gels. We evaluated only the most soluble cellular protein fraction using hippocampal tissue protein lysates from six paired sham and injured rats. Approximately 1500 proteins spots were found in each gel with 40% spot matching of proteins. Of these 600 matched proteins 50% showed either a 2 fold increase or decrease, 20%, a 5 fold increase or decrease, and 10%, a 10 fold decrease or increase. Limited spot matching with existing protein databases showed changes in some important cytoskeletal (actin and tubulin), and cell signaling (phosphatidylinositol transfer protein and superoxide dismutase) proteins suggesting that this approach is both feasible and informative in the study of protein changes after pediatric TBI.

Our long-term goals are to also characterize some of the most important changes in neuronal signaling known to influence cognitive dysfunction after injury and determine if these changes can be normalized by delayed treatment with trophic factors Protein synthesis may be altered after TBI by changes in the phosphorylation state of PKB. Phosphorylated PKB (p-PKB) activation alters protein synthesis by phosphorylating the target of rapamycin protein kinase (mTOR/FRAP) that in turn phosphorylates 4EBP (p-4EBP) the repressor binding protein (4EBP) of eukaryotic initiation factor 4E (eIF4E). p-PKB also activates eukaryotic initiation factor 2 α (eIF2 α) indirectly by phosphorylating glycogen synthase kinase 3 (GSK-3) reducing the phosphorylation of eIF2 α and activating p-eIF2 α . Thus, PKB phosphorylation modulates the selection of translated mRNA by eIF4E activity and the global rate of protein synthesis by increasing p-eIF2 α activity. We evaluated the level and distribution of brain p-PKB, p-4EBP, p-eIF4E, and p-eIF2 α activity in injured or sham 17 PND rats at 6, 24 or 72 hr after moderate CCI using immunohistochemistry (n=5/group). TBI increased the levels of all impacted hippocampal p-proteins at only 6 hr except p-eIF4E suggesting an early but unsustained upregulation of PKB linked protein synthesis activators after pediatric CCI. We are further expanding these studies.

Support: NIH-NINDS, Protein Synthesis, Memory and Pediatric Brain Injury, R21 NS-40049, (\$186,250/yr, 5/1/00-4/30/03, Larry W. Jenkins, Ph.D., PI).

B. Hypothermia and TBI

The overall goals of this project are to examine the effects of mild hypothermia treatment on secondary ischemic injury and neurotransmitter signal transduction after TBI. This project combines behavioral and structural morbidity studies with molecular pharmacology to examine some potential mechanisms of increased sensitivity of the posttraumatic brain to secondary cerebral ischemia and potential actions of hypothermic treatment on altered signal transduction after TBI.

Normally, secondary forebrain ischemia following mild TBI results in increased bilateral delayed CA1 neuronal death by 7 days after injury. During the last year we examined the use of a general serine/threonine protein kinase inhibitor staurosporine (10 ng) microinjected into the hippocampal CA1 sector before mild TBI and then examined CA1 neuronal death following mild TBI and combined secondary cerebral ischemia. Significant neuronal protection was found in the CA1 sector at 7 days of survival demonstrating that aberrant protein kinase activation may enhance posttraumatic ischemic sensitivity after even mild TBI. These studies are being prepared for publication submission.

We studied the effect of serum hyperglycemia on secondary ischemic brain injury after TBI. Serum hyperglycemia increases secondary ischemic brain damage after TBI; however, it is unknown if the traumatized brain is hypersensitive to the hyperglycemic exacerbation of secondary ischemic injury. We examined if posttraumatic serum hyperglycemia at levels of 400-mg% decreases the threshold for secondary ischemic injury after TBI. Normoglycemic rats receiving mild TBI and 6 min of forebrain ischemia also had CA1 neuronal death after 7-day survival. In contrast, hyperglycemic rats subjected to mild TBI and 6 min of forebrain ischemia developed seizures leading to status epilepticus within 18-24 hr after injury and had extensive neuropathological damage in many brain regions in proportion to seizure activity duration. These data suggest that mild TBI predisposes the traumatized brain to sustain more damage to hyperglycemia related exacerbation of ischemic injury compared to the non-traumatized brain by increasing parenchymal sensitivity to secondary ischemic damage in multiple regions. This manuscript is being prepared for publication.

We also published a comparison CA1 hippocampal CBF profiles before, during and after 6 min of forebrain ischemia in normal or mildly hypothermic rats using laser Doppler flowmetry for the first time. During the ischemic insult there were intergroup differences in the magnitude of CBF decreases in the CA1 region. In both normo- and hypothermic groups, CBF returned to preischemic values within one minute of reperfusion but hypothermic rats had more sustained hyperemia. Hypothermic rats also had a quicker EEG recovery and less delayed CA1 neuronal death. These data suggest ischemic blood flow to the CA1 sector was increased by intraischemic mild hypothermia, which may contribute to the greater benefit of intraischemic hypothermic neuroprotection as compared to immediate postischemic hypothermia treatment. This study was published by *Brain Research*.

These collective studies suggest that hypothermia may provide benefit to head injured patients by modifying signal transduction before posttraumatic secondary ischemia or delayed neuronal death after secondary ischemia. The suspected mechanism is by preserving more normal serine/threonine protein kinase function.

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8. Studies directed by Anthony E. Kline, Ph.D.

TBI affects 1.5 to 2 million people in the US each year, making it one of the more prevalent and debilitating of all neurological disorders. Approximately 300,000 of the TBI cases are severe enough to warrant hospitalization, where 50,000 die. Of the 250,000 survivors, 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 loss 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, empirical investigation of therapeutic strategies that may facilitate the recovery process after TBI, such as those directed by Dr. Kline and colleagues at the Safar Center and department of Physical Medicine & Rehabilitation (PMR) are essential. Equally important is the identification of pharmacological agents that may be detrimental to functional recovery after TBI.

A. Protective Effects of Serotonin_{1A} (5-HT_{1A}) Receptor Agonists Against TBI-Induced Cognitive Deficits and Histopathology

Serotonergic pathways originating in the raphe nuclei have extensive projections to brain areas involved in cognition and 5-HT receptor agonists and antagonists alter these processes. Of all the 5-HT receptors characterized thus far, the 5-HT_{1A} is the most widely studied. 5-HT_{1A} receptors are abundantly expressed 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. Several studies have reported decreased histopathology after focal or global cerebral ischemia in both rats and mice treated with 5-HT_{1A} receptor agonists. Because of the beneficial effects in these model systems, we examined them in our CCI model of TBI that produces many of the characteristics of human brain injury. The high affinity 5-HT_{1A} receptor agonist Repinotan HCL (BAY x 3702) was given (i.v.) as a 4-hr continuous infusion commencing 5-min after TBI or sham injury. Rats were evaluated for spatial learning in the MWM on post-operative days 14–20 and examined histologically at four weeks after TBI. 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 significantly more hippocampal CA₁/CA₃ neurons and smaller cortical lesion volumes vs. the vehicle group. This study was the

first to demonstrate beneficial effects with a 5-HT_{1A} receptor agonist in any model of TBI and is published in the journal *Neuroscience* (2001).

The positive demonstration of marked neuroprotection and cognitive improvement after acute Repinotan HCL treatment was the impetus for further investigation of 5-HT_{1A} receptor agonist treatments after TBI. In a follow-up study, we investigated whether 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) would produce similar beneficial effects. Using our standard injury and assessment paradigms, we found that 8-OH-DPAT exhibited significantly reduced latencies in locating the hidden platform vs. the vehicle group over time, which is indicative of improved learning and memory. Additionally, significantly more CA₃ surviving neurons were observed in the 8-OH-DPAT group relative to the vehicle group. This study is published in the journal *Neuroscience Letters* (2002). The beneficial effect on functional and histological outcome with the 5-HT_{1A} receptor agonist 8-OH-DPAT, coupled with our significant Repinotan HCL data, lend support for continued investigation of this novel therapeutic strategy. Collaborators include C. Edward Dixon, Ph.D., Jaime Massucci, B.S., and Jianyun Yu, M.D. from the Department of Neurological Surgery and Safar Center and Ross Zafonte from the Department of PMR.

B. The Effect of the Anti-Inflammatory Cytokine, Interleukin-10, Coupled with Moderate Hypothermia after TBI

TBI produces long-term disturbances in neurobehavioral and cognitive function. One mechanism for the detrimental effects may be the initiation of inflammatory processes such as the synthesis of proinflammatory cytokines that are implicated in secondary tissue damage. Experimental and clinical studies of TBI have shown robust inflammatory responses, including the early production of cytokines and the upregulation of (E)-selectin and intercellular adhesion molecule-1 (ICAM-1) on cerebrovascular endothelial cells. Previous studies using the CCI or fluid percussion (FP) injury models of TBI have shown that neutrophils accumulate in the brain as early as 4 hr after injury and reach peak levels by 24-72 hrs. Interleukin-10 (IL-10) is a potent anti-inflammatory cytokine that inhibits a variety of macrophage responses including the synthesis of cytokines, adhesion molecules, and chemokines. Moreover, hypothermia may also attenuate TBI-induced inflammatory responses. Hypothermia has already been shown to benefit outcome after TBI. Improved functional and/or histological outcomes have been demonstrated following FP, weight drop, and CCI injury. In humans, significant reductions in intracranial pressure, as well as improved outcome have been shown with moderate hypothermia. Thus, we sought to evaluate the effects of IL-10 coupled with hypothermia on functional and histological outcome after experimental TBI. Briefly, fifty isoflurane-anesthetized rats underwent a CCI or sham injury and then were randomly assigned to one of five conditions (TBI/Vehicle Normothermia (37°C), TBI/Vehicle Hypothermia (32°C for 3 hr), TBI/IL-10 Normothermia, TBI/IL-10 Hypothermia, and Sham/Vehicle Normothermia). Human IL-10 (5µg) or vehicle was administered (i.p.) 30 min after surgery. Function was assessed by established motor and cognitive tests on post-operative days 1-5 and 14-18, respectively. Cortical lesion volume and hippocampal CA₁/CA₃ cell survival were quantified at 4 weeks. Brain

sections from 15 additional rats were immunohistochemically assessed (MoAB RP-3) to determine neutrophil accumulation at 5 hrs after TBI. The administration of IL-10 after TBI produced an ~75% reduction in the number of RP-3-positive cells in both the normothermic and hypothermic groups vs. the normothermic vehicle-treated group ($P < 0.05$), but did not improve functional outcome. In contrast, hypothermia alone enhanced both motor and cognitive function and increased CA₃ neuronal survival after TBI. Contrary to our hypothesis, systemic administration of IL-10 combined with hypothermia did not provide synergistic neuroprotective effects after TBI. Rather, IL-10 administration suppressed the beneficial effects produced by hypothermia alone after TBI. Anti-inflammatory therapies can represent a dual-edged sword, exhibiting both beneficial and detrimental effects. Since hypothermia inhibits inflammatory responses – along with a variety of other mechanisms – after injury, combination with additional anti-inflammatory therapies may not be warranted. This study was published in the journal *Brain Research* (2002). Collaborators include Bryan Bolinger, B.S., C. Edward Dixon, Ph.D., Patrick Kochanek, M.D., Timothy Carlos, M.D., Hong Yan, M.D., Larry Jenkins, Ph.D., and Donald Marion, M.D.

C. 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. Collaborators include Amy Wagner, M.D., C. Edward Dixon, Ph.D., and Ross Zafonte, D.O. An RO1 grant entitled “Serotonergic and Environmental Therapies for TBI” has been submitted to further examine the relationship between EE and 5-HT_{1A} receptor agonists on the recovery process after TBI.

D. 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. Early experimental studies by Feeney and colleagues have shown that the administration of antipsychotics (e.g., haloperidol) retard functional recovery after TBI. Moreover, the administration of such agents reinstates deficits in subjects appearing to be “recovered.” Recently, Goldstein and colleagues have 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 hr after TBI or sham injury) and/or chronic (24 hr – 28 days) administrations of the atypical antipsychotic risperidone on motor (beam-balance and beam-walk) and cognitive (spatial learning and memory) functioning in rats after TBI. 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. These studies are being conducted in collaboration with Drs. Ross Zafonte and C. Edward Dixon.

Support: NIH-NICHD R03 HD043851-01, Interaction of serotonin and cholinergic systems after TBI, \$144,233 for two years (04/01/03 – 03/31/05). Anthony E. Kline, Ph.D., PI. The Pittsburgh Foundation, Evaluation of the serotonergic_{1A} receptor agonist, 8-OH-DPAT, on biochemical, functional, and histological outcome following traumatic brain injury in rats, \$19,096 for one year (2001-2002). Anthony E. Kline, Ph.D., PI. The Pittsburgh Foundation, Efficacy of interleukin-10 (IL-10) coupled with moderate hypothermia in neurobehavioral, cognitive, and histological outcome following traumatic brain injury in rats, \$24,261 for one year (2000-2001). Anthony E. Kline, Ph.D., PI.

Studies Conducted by Amy K. Wagner, M.D.

1. Clinical Gender Differences in TBI Pathophysiology

There is conflicting evidence as to whether there are gender differences with TBI pathophysiology and outcomes. Some clinical studies have reported that, even after adjusting for injury severity women often fare worse. Previous work by Dr. Wagner shows that one year after hospitalization with TBI, women have more disability. Yet several animal studies utilizing experimental TBI and stroke models show that female hormones are neuroprotective in attenuating aspects of secondary injury such as excitotoxicity, ischemia, and oxidative stress. The primary goal of this project was to characterize, in a clinical population, possible gender differences in the production of CSF markers of secondary TBI and gender specific responses to hypothermia in attenuating these markers of TBI. Multivariate regression modeling techniques were used to show that females appear to have some neuroprotection against excitotoxic and ischemic injury. However, hypothermia appeared to reduce excitotoxic injury primarily in males. Ischemic injury and excitotoxicity markers were also linked to a marker of oxidative stress. Again there were significant 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 hormonally

mediated neuroprotection previously reported in studies on experimental brain trauma. Portions of this work have been presented at the 2002 National and International Neurotrauma Society meeting. This work will be presented at the 2003 Association of Academic Physiatrists meeting. Manuscripts are currently in preparation and review. A large grant has been submitted as a part of the University of Pittsburgh's Center for Injury Research and Control's competitive renewal to the CDC based on this work. Collaborators include the NIH funded Brain Trauma Research Center CSF Bank (Ava Puccio MSN), Drs. Tony Fabio (Center for Injury Research and Control), Ross Zafonte and Hülya Bayır.

2. EE Promotes Cognitive Recovery in Male but not Female Rats after Experimental TBI

EE 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 has also been shown to improve spatial memory after experimental TBI in male rat models. However, the impact of gender on how EE affects behavioral performance after experimental TBI has not been studied. In this study, we used the CCI model of experimental TBI to determine the effects of EE on motor and cognitive behavioral performance for both male and female rats. The results of the study showed that early intervention with enrichment did not impair motor performance early after injury for either males or females. EE improved spatial memory performance for males only. This finding may be due to potential gender differences in enrichment-mediated neuroplasticity after injury or gender specific alterations in hormonal modulation of post-injury neuroplastic responses. The results of this work were presented by our undergraduate student, Joshua Sokoloski, in the 2002 National and International Neurotrauma Society student poster competition and recently published in *Neuroscience Letters*. This was the first enrichment study completed at the Safar Center, and it has served as valuable pilot data and treatment protocol for other investigators at the center interested in pursuing other grant funding involving enrichment based studies. We are currently characterizing how enrichment and gender impact dopaminergic markers post-TBI. Future work will focus on other neural and hormonal substrates that may impact this gender specific behavioral response. Collaborators include Drs. E. Edward Dixon, Anthony Kline, and Ross Zafonte.

3. DA Kinetics and TBI

Altered DA neurotransmission is hypothesized to play a role in neurobehavioral deficits after TBI. DA agonists have been shown clinically to improve aspects of mental functioning after TBI, and have been shown in multiple animal studies originating from Dr. Dixon's laboratory to improve behavioral performance. This laboratory has also demonstrated reductions in striatal DA transporter (DAT) protein and increases in 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 differences in striatal DA kinetics after experimental TBI using FSCV. In this study, we used the CCI model of experimental TBI to evaluate electrically evoked DA release as well as DA clearance 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. Striatal evoked release of DA was significantly lower on the injured side of the brain compared to the uninjured side two weeks after injury. First order, but not zero order, DA clearance kinetic profiles appear to be altered in the injured striatum. These findings provide a functional correlate to previously reported DA system protein changes. The relationship between behavioral performance, DAT concentrations, and DA kinetics will be studied. This work is being conducted in conjunction with Dr. Adrian Michael in the Department of Chemistry, whose research focuses on electrochemical techniques and the measurement of neurotransmitters using microsensor technology. A manuscript for this work is currently in preparation. We intend to investigate regional and post-injury time course differences in DA kinetics as well as response to acute and chronic pharmacotherapies. Collaborators include Drs. C. Edward Dixon, Adrian Michael, and Ross Zafonte.

4. The Impact of Gender and Hormonal Status after Experimental TBI

Some studies have shown that sex hormones have neuroprotective qualities in the setting of acute TBI. However, less is known about how endogenously circulating sex hormones or particular hormone levels at the time of injury effect behavioral performance. Here we used the CCI model of experimental TBI to evaluate how gender, pre-injury estrous cycle status, and pre-injury serum hormone status affect motor and cognitive behavioral performance after TBI. The results of this study showed that, for females, serum hormone levels and estrous cycle stage at the time of injury do not impact behavioral performance on any of the behavioral tasks. Females do significantly better than males on motor function tasks assessed early after injury. However, there were no significant gender differences in spatial memory performance later after injury, implying that female sex hormones confer early neuroprotection with behavioral performance, but endogenous hormones may not significantly impact later behavioral outcome. A manuscript for this work is currently in preparation. Portions of this work will be presented at the 2003 Association of Academic Psychiatrists. Future work will focus on how hormone manipulations affect behavioral performance and histochemical markers of injury. Collaborators include Drs. C. Edward Dixon, Anthony Kline and Ross Zafonte.

Gender Differences in Behavioral Performance with DA Enhancing Therapies

Several studies involving experimental TBI in male rats have shown that DA agonists improve cognitive recovery. Other literature shows that there are gender differences in how DA systems are modulated, and this modulation is largely affected by sex hormones. For example, striatal dopamine transporter concentrations are higher in females, and females are more behaviorally sensitive than males to dopamine agonists acting at the transporter. For this study, we used the CCI model of experimental TBI to evaluate the effectiveness of chronic treatment with the DA agonist, methylphenidate, on improving behavioral performance after TBI. The results show that daily treatment with 5mg/kg of

Methylphenidate improves beam balance performance for both males and females. However, the degree of improvement with this task was much larger in the males, compared to the females. Additionally, Methylphenidate improved spatial memory for males but not females. The difference in cognitive performance with methylphenidate may, in part, result from an increased sensitivity to side effects in females compared to males to the drug at this dose. A manuscript for this work is currently in preparation. Future work will focus on how hormone and drug dosing manipulations affect behavioral performance. Collaborators for this study include Drs. Dixon, Anthony Kline, and Ross Zafonte.

5. Associations between DAT Genotype, Outcome, and CSF DA Levels after Severe TBI: A Preliminary Analysis

DA pathways have been implicated in cognitive deficits after TBI. While not associated with alterations in protein structure, the DAT genotypes are associated with differences in DAT protein density and development of DA mediated pathophysiological conditions such as attention deficit disorder. Differential DAT expression presumably affects both presynaptic 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 in improving 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 brain injury. Previous work from this laboratory has demonstrated reductions in DAT protein after experimental TBI. The role of DAT genotype on injury and outcome has not been studied. For this study, we evaluated 30 patients with severe TBI. We determined their DAT genotype, measured post-injury CSF DA and DA metabolite levels, and evaluated six-month neuropsychological outcomes. The DAT 10/10 genotype was considered the risk genotype for the production of more DA and DA metabolites as well as for poorer outcome. Results showed no differences between genotype groups for post-injury CSF DA levels. However, there were significant increases in DA metabolite production for the DAT 10/10 genotype group compared to other genotypes. Results also showed that people with the DAT 10/10 genotype had worse six month disability scores and poorer performance with some neuropsychological tests involved with frontal lobe function. Future work will focus on repeating this analysis in a larger population. Additional work will focus on gender differences in DA CSF marker production and the relationship to DA CSF markers to outcome. This work was completed in collaboration with the University of Pittsburgh BTRC as well as Drs. C. Edward Dixon, Yvette Conley, Robert Ferrell, Sue Beers, Ross Zafonte, and Mary Kerr.

Support: NIH K08HD40833, Amy K. Wagner, M.D. PI, Dopamine Function and the Effects of Therapeutic Intervention \$622,258 beginning 2001 for 5 years (Sponsors: C. Edward Dixon, Ph.D., Adrian C. Michael Ph.D, and Ross D. Zafonte, D.O.); NIH R03HD41399, Amy K. Wagner, PI Gender Differences in Dopamine Function after TBI \$145,535 beginning 2002 for 2 years; CDC CCR310285-07---CIRCL Small Grants Program Amy K. Wagner, M.D. PI (Hank Weiss Ph.D. PI Center Grant) \$7,000 beginning 2001 for Gender Based Associations with Outcome after Severe TBI, \$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, C. Edward Dixon PI; Laerdal Foundation, Hülya Bayır PI, Oxidative Stress After Severe Head Injury; Department PMR, University of Pittsburgh.

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CARDIOPULMONARY ARREST PROGRAM

Research in cardiopulmonary arrest and resuscitation is carried out under the direction of Dr. Clifton Callaway at the University of Pittsburgh Center for Emergency Medicine. In addition, there are pediatric components to the cardiac arrest program that are being carried out by Dr. Robert Hickey of the Children's Hospital of Pittsburgh Division of Emergency Medicine and Dr. Howard Ferimer of the Mercy Hospital Department of Pediatrics.

A. Clifton Callaway and the Department of Emergency Medicine

Resuscitation from sudden cardiac arrest is dominated by two organs: heart and brain. Current clinical approaches for restoring the heart to a normal perfusing state are suboptimal. Only about one-third of resuscitations attempted by paramedics outside of the hospital result in restoration of circulation. This low success rate is in part because of inability to titrate drug and electrical therapy to the changing physiological state of the patient. Subsequent to restoration of circulation, brain injury contributes to the demise of many more patients. Brain injury involves many biochemical changes that develop over the subacute period after reperfusion, and can be modified by therapy delivered during the first few hours after return of circulation.

Our studies employ both large and small animal models to study these two different components of resuscitation from normovolemic, sudden circulatory arrest. Using a swine model of cardiac arrest and resuscitation developed in the Department of Emergency Medicine by Dr. James Menegazzi, we are studying the optimal timing and delivery of drug and electrical therapy for restoring spontaneous circulation. Using a rat model of asphyxial cardiac arrest developed at the Safar Center, we are studying the biochemical changes in brain that develop over the hours following resuscitation.

Finally, our department has a long relationship and commitment to emergency medical services (EMS). Because EMS is the initial point of care for most patients with sudden cardiac arrest, it is an essential clinical arena for any study of this disease. We continue to cultivate this resource for future translational research.

1. Altered Intracellular Signaling in Brain after Resuscitation

Intracellular signaling in rat brain after cardiac arrest has been studied courtesy of an NINDS Independent Scientist Award. We previously described changes in intracellular signaling in rat brain after resuscitation from eight minutes of normothermic asphyxia, resulting in five minutes of circulatory arrest. In particular, the activity of two mitogen-activated protein kinases (MAPKs) increase in hippocampus over the 24 hrs period after reperfusion: the p42/p44 MAPK (extracellular-signal regulated kinase, ERK) and the Jun-N-terminal kinase (JNK). Induction of mild hypothermia (33°C) between 1 and 23 hrs after reperfusion, selectively increases activity of ERK relative to normothermic (37°C) controls. This regimen of hypothermia also decreases histological and behavioral

signs of brain damage, prompting us to speculate that some of the beneficial effects of induced hypothermia are mediated via increased ERK activation.

Immunohistochemistry and immunoblotting reveal a similar pattern of MAPK activation after cardiac arrest and reperfusion in other brain regions. Interestingly, the influence of hypothermia on ERK activation during the first 24 hrs of reperfusion appears to be more pronounced in forebrain (cortex and hippocampus) than in hindbrain (cerebellum and brainstem). Because the forebrain structures are more sensitive to ischemic injury, this regional selectivity further supports a relationship between ERK activation and the neuroprotective effects of hypothermia.

Further studies sought to identify the upstream activators of ERK, as well as the downstream effectors of the biological activities of ERK. Potential upstream activators of ERK include several neurotrophic factors. In the hippocampus, immunoblotting revealed no change in levels of nerve growth factor or neurotrophin-3. However, brain derived neurotrophic factor (BDNF) appears to increase after ischemia, and this increase is potentiated by induced hypothermia. These findings were reported in the *Journal of Cerebral Blood Flow and Metabolism*.

The biological effects of ERK are mediated by activation of a variety of transcription factors that can be measured using immunoblotting. Induction of hypothermia increases activation of factors believed to be substrates of the extracellular signal regulated kinase (ERK), including ATF-2 and CREB. Activation of CREB may be related to an increase in activation of the p90 ribosomal S6 kinase, a kinase that is itself a substrate of ERK.

In order to determine whether these changes in transcription factor activation are reflected by altered gene transcription, we have spent the last few months developing tools for assessing global patterns of mRNA expression. In our first experiments, hippocampi were collected 24 hr after asphyxial cardiac arrest and reperfusion at 37°C (n=3). Sham rats (n=5) underwent anesthesia and operation without asphyxia. Total hippocampal RNA from each group of rats was reverse transcribed into radiolabeled cDNA. This cDNA probe derived from total hippocampal RNA was hybridized with a nylon membrane spotted with 1176 oligonucleotides complementary to known rat genes. Autoradiograms of this membrane revealed specific changes in expression of a subset of genes. Using a criterion of 50% change in expression on more than one array, we detected 15 genes with increased expression after ischemia/reperfusion. These genes included heat shock proteins, tyrosine kinase related proto-oncogenes, and genes related to lipid metabolism. Expression of 53 genes decreased after ischemia/reperfusion. These genes included many products related to neurotransmitter synthesis, neurotransmitter receptors, and intracellular receptor signaling. These data indicate that circulatory arrest and reperfusion triggers a specific pattern of gene expression in rat hippocampus.

Other experiments have been directed at developing a satisfactory pharmacological tool for inhibition of ERK activation during reperfusion after ischemia. The compound SL327 worked well after intravenous administration, but remains unavailable from the

manufacturer. Geldanamycin, an inhibitor of the MAPK kinase kinase, Raf, also did not penetrate the blood-brain barrier and proved to be toxic after intraventricular doses large enough to penetrate into the brain parenchyma. We conducted an intravenous dose-response study of the MAPK kinase inhibitor U0126 (0.2 – 2.0 mg/kg), which has been used by others in rodent studies of ischemia, and determined that this compound did not decrease ERK activation in the brain. It is likely that this compound does not cross the blood-brain barrier.

Our future directions will be to test for a causal link between increased ERK activation and the improved neurological outcome observed after induced resuscitative hypothermia. This test will require us to establish a reliable pharmacological tool for inhibiting ERK activation. Likewise, we will test whether the increased levels of BDNF are necessary and sufficient for the beneficial effects of hypothermia, by stimulating and antagonizing this pathway.

2. Use of ECG Waveform Analysis to Guide Resuscitation From Ventricular Fibrillation

The optimal timing of rescue shocks for reversal of ventricular fibrillation (VF) requires clarification. Certainly, rapid defibrillation is optimal for restoring circulation after brief periods of circulatory arrest. However, a period of reperfusion prior to rescue shocks may be a superior approach for prolonged periods of circulatory arrest. We have previously developed a metric for quantifying organization in the ECG during VF that is called the scaling exponent. The scaling exponent can discriminate between animals with brief periods of circulatory arrest (in which rescue shocks have a high likelihood of success) and animals with prolonged periods of circulatory arrest (in which rescue shocks have a low likelihood of success).

With support from Physio-Control Medtronic, Inc. to Dr. James Menegazzi, we have examined whether the scaling exponent calculated in real-time could be used to differentiate swine with VF that benefit from rescue-shock-first from those that benefit from reperfusion-first. Preliminary results of this study reveal that when the scaling exponent is low (after 1-4 minutes of circulatory arrest), beginning resuscitation with rescue shocks is effective for restoring circulation. In contrast, when the scaling exponent is higher (after 8-12 minutes of circulatory arrest), beginning resuscitation with countershocks only restored circulation for 50% of swine. The alternative strategy of beginning resuscitation with chest compressions and pressors for up to 5 minutes before the initial rescue shock restored circulation for 81% of swine. Moreover, spontaneous circulation was restored more quickly in these latter swine, despite the built-in delay prior to the first rescue shock. These data suggest that a real-time measurement of the VF waveform could be used to guide the timing of the initial rescue shock to be delivered during resuscitation from a VF cardiac arrest.

In order to determine whether this type of VF analysis will be useful during human cardiac arrest, we have also analyzed recordings of VF cardiac arrest obtained from

automated external defibrillators. We previously reported on an analysis of similar recordings from Pittsburgh. This year, we obtained a larger sample of recordings from a separate EMS system. Preliminary analyses of these data confirm that the scaling exponent can predict the likelihood of successful rescue shocks. When the VF waveform preceding a rescue shock has a low scaling exponent, the rescue shock is likely to result in defibrillation. However, when the scaling exponent is high, the rescue shock is likely to fail. Because repeated failed rescue shocks may be detrimental to cardiac function, this measure could provide a real-time monitor for rational application of electrical therapy during resuscitation. Anecdotal observations in these recording confirm that artificial reperfusion with chest compressions and pressors can restore VF with a high scaling exponent to VF with a low scaling exponent.

3. Altered Blood Coagulation after Circulatory Arrest.

Previous studies have shown that fibrinogenesis is elevated during and after cardiac arrest. Furthermore, this increase is not matched by a concomitant increase in fibrinolysis. It is not currently known if increases in fibrinogenesis occur during the period of no-flow, during low-flow during resuscitation, or during subsequent reperfusion. Dr. Hostler, an EMS fellow, examined thrombin-antithrombin III (TAT) levels, a marker of fibrinogenesis, during resuscitation and reperfusion in the swine model of cardiac arrest. There was a time-dependent increase in TAT levels over ten minutes of untreated cardiac arrest, and the increases were most pronounced after six minutes of cardiac arrest. TAT levels were elevated at the beginning of resuscitation (132% of baseline) and increased further at ROSC (523%) (5.2 ± 1.0 min later). This elevation persisted after 30 and 60 minutes of reperfusion. Thus, TAT levels rise in proportion to the duration of circulatory arrest beyond six minutes, and remain elevated during resuscitation and after reperfusion. These data were presented at the Society for Academic Emergency Medicine in St. Louis, MO.

Because the increases are persistent, even when sampled after reperfusion, TAT levels or other markers of thrombogenesis could be used to estimate the combined duration of no-flow and low-flow prior to resuscitation. In future clinical studies, this type of marker would be useful for stratifying samples into groups with brief versus prolonged cardiac arrest. Dr. Hostler has initiated protocols to confirm these observations in human subjects being treated by our local EMS service. Further work is required to establish the cause and pathophysiological significance of these changes.

4. Clinical Studies

The various issues surrounding research on emergency care of human subjects who are unable to provide consent have been well described. Criteria for waiving the requirement to obtain informed consent in emergency research have been set forth. In order to lay the groundwork for translation of our basic science investigations to patients, Dr. Margaret Hsieh studied 100 consecutive responses by City of Pittsburgh paramedics in order to better establish a case for one of these criteria in our target population. In this study, we determined that individuals who could serve as surrogate decision-makers for subjects

being treated for cardiac arrest were only available in about one-half of cases. Furthermore, these surrogate decision-makers could only be located after about 25 minutes of resuscitation, and were usually unable to answer simple questions because of their emotional state. Thus, we have established that the mechanism of waiver of informed consent, rather than reliance on a surrogate or proxy is appropriate for most research in out-of-hospital cardiac arrest, and absolutely required for studies of therapy with a therapeutic window of less than one-half hour after beginning resuscitation. These data were presented as a Brief Communication in *Academic Emergency Medicine*.

Dr. David Newman, an EMS fellow in our department, conducted a further study to test our capacity for collecting detailed data during resuscitation from out-of-hospital cardiac arrest. In this study, a cerebral oximeter (provided by Somanetics, Inc.) was placed on 16 patients as soon as possible during resuscitation attempts. This device provides a measure of arterial and venous blood oxygen saturation in the brain, and this measure correlates with jugular venous oxygen saturation. During chest compressions, the cerebral oximeter readings were below the lower limit of detection, suggesting that there is little if any oxygen delivery during typical resuscitations. Interestingly, the cerebral oximeter was a very sensitive detector of the return of spontaneous circulation, often reporting increasing values prior to detection of pulses by the treating team. After reperfusion, oximetry readings increased and decreased with blood pressure, suggesting that cerebral perfusion after resuscitation lacks autoregulation, and is supply-dependent. This pilot study suggests a potential role for cerebral oximetry in titration of hemodynamics after reperfusion.

5. Plans

Research efforts will continue to focus on the molecular aspects of brain injury after cardiac arrest and on the immediate clinical questions of acute cardiac resuscitation during cardiac arrest. The rodent research to this point has led us to the question of what role do neurotrophic factors, signaling kinases, and new gene expression play in the response to cardiac arrest? Furthermore, what is the specific interaction between these events and the beneficial effects of post-reperfusion hypothermia? Swine studies will continue to refine the optimum approach to restoring circulation after prolonged circulatory arrest. We hope to recruit additional clinicians interested in translation of our basic knowledge about the situation of sudden cardiac arrest to the practical care of patients.

B. Pediatric Cardiopulmonary Resuscitation

1. Public Education and National Guidelines Committee

Dr. Robert Hickey is the current chair of the American Heart Association subcommittee on Pediatric Resuscitation. The subcommittee is responsible for overseeing the American Heart Association's pediatric advanced life support (PALS) course. PALS is taken by approximately 150,000 healthcare providers per year. The latest revision to the course was distributed in December 2001 with 153,000 PALS textbooks sold in the first 8

months of release. In his capacity as chair of the Pediatric Subcommittee, Dr. Hickey also serves as a representative to the international liaison committee on resuscitation (ILCOR) and has recently participated in meetings in Melbourne, Australia and Florence, Italy to develop international consensus on new developments in resuscitation science.

2. Laboratory Research in Pediatric Resuscitation

Drs. Robert Hickey and Howard Ferimer are principal investigators on the rodent asphyxial arrest projects.

A. Developmental aspects of COX-2-mediated brain injury

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 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.

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, M.D., PI, Steven Graham, M.D., Patrick Kochanek, M.D., Co-Investigators; Robert Clark, M.D., C. Edward Dixon, Ph.D., Peter Safar, M.D., Consultants.

B. Role of Adenosine after Asphyxial Cardiac Arrest

Adenosine is produced by the breakdown of ATP during ischemia. It is neuroprotective through multiple mechanisms; reduction in free radical production, hypothermia, improved cerebral blood flow and reduction in cellular metabolism. Systemic administration of adenosine is limited by its short half-life, inability to cross the blood brain barrier and the adverse cardiovascular side effects of hypotension and bradycardia. The beneficial neurologic effects of augmenting adenosine levels locally in the brain have been documented in TBI and Stroke models of cerebral ischemia. Whether augmenting adenosine levels in the brain after asphyxial cardiac arrest is beneficial is not known. It is the focus of these investigations.

In collaboration with Dr. Edwin Jackson, Dr. Ferimer is continuing to study the effects of adenosine modulating drugs on interstitial levels of purines in the brain of animals subjected to asphyxia-induced cardiac arrest. We employed state-of-the-art microdialysis and analytical methods, including high-performance liquid chromatography, to conduct these detailed studies. Our investigations are addressing pharmacological strategies to increase brain adenosine levels in the acute phase after resuscitation.

Our future work will focus on employing these methods to improve neurological and histological outcome following resuscitation from asphyxia-induced cardiac arrest. Ultimately, our studies may improve therapy in this all-to-common problem in the pediatric population.

3. 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 HI 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 HI brain injuries. This information will facilitate comprehensive evaluation and treatment for individuals suffering from HI 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.

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SHOCK AND SUSPENDED ANIMATION PROGRAM

Drs. Peter Safar and Samuel Tisherman

“Novel resuscitation from lethal hemorrhage; increasing survival of combat casualties” is a Department of Defense (DOD) supported program which began in 1997 and was, during 2001/2002, in its fourth year. It consists of project I on hemorrhage shock (HS) in rats and pigs (P.I., Dr. Tisherman; Co-P.I., Dr. Safar); and project II on suspended animation (SA) in dogs (P.I., Dr. Safar; Co-P.I., Dr. Tisherman). For the overall program in 1997-2002, Dr. Safar has been P.I. and Dr. Tisherman Co-P.I. The funding since 1997 was made possible through special “plus-up” funds from congress initiated by former Navy Commander Lyn Yaffe, M.D. His successor during years 3-4 was Jeannine Majde-Cottrell, Ph.D. Each year requires a new proposal, which is peer-reviewed by a DOD committee. For the two programs combined, we received total funds (including 50% institutional “indirect costs”) of approximately \$2.4M for year 1; \$1.9M for year 2; \$1.2M for year 3; and \$1.1M for the current year 4. The most expensive part of the budget are team and animals for the research intensive care unit (ICU) studies in dogs.

Our research ICU for large animals, initiated in the 1970s, is still considered a unique resource for the documentation of novel CPR methods. It must be maintained continuously to be cost-effective, with at least four technicians, two full-time MD research fellows with CCM experience, and about 80 long-term dog experiments per year. Maintaining this ICU program alone requires over \$0.5M per year. In 2001/2002, the research fellows were Dr. Nozari (in his first year) and Dr. Wu (in his third year); Mr. William Stezoski has continued as lab coordinator. The co-investigators or consultants included Drs. Kochanek, Klain, Jackson, Dixon, Clark, Kagan, Jenkins, and Radovsky (pathologist). Outside consultants included Dr. Hsia of the Synzyme Corporation, Dr. Taylor of the Organ Recovery Systems, Inc., Mr. Samson of the Cardeon Corp. (cardiopulmonary bypass catheters and other devices), and others. In spring 2001, Dr. Safar divided the projects’ funding by having the HS projects continue with Dr. Tisherman as P.I. and Dr. Safar as Co-P.I., funded by an intramural grant from the Navy (ONR). The congressional plus-up money continued supporting the SA project, with Dr. Safar as P.I. and Dr. Tisherman as Co-P.I.

The objective of the HS-SA program has been to help maximize the reversibility of presently lethal traumatic hemorrhage. The HS studies in rats and pigs were 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 (CA) (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. Bellamy and Safar. For HS and SA we explored pharmacologic as well as hypothermic strategies – specifically mild hypothermia (33-36°C) for HS and profound hypothermia (5-15°C) for CA. Dr. Tisherman is planning,

for both, a clinical feasibility study in selected trauma hospitals to start in the near future. Devices will have to be developed by outside companies under the guidance of our team.

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 outcome.

1. Hemorrhagic Shock (HS) Studies (Tisherman)

The hemorrhagic shock studies of academic year 2001-2002 were completed under year 5 of funding by the Office of Naval Research (PI: Samuel A. Tisherman, MD; Co-PI: Peter Safar, MD). A total of 267 rats were used. The studies were completed by technician Jason Stezoski under direct supervision of fellow Xianren Wu, MD.

Spontaneous Cooling during HS

In previous studies of hypothermia during HS in animal models, normothermia was maintained until therapeutic hypothermia was induced. In trauma victims, however, spontaneous hypothermia is common. The clinical issue for resuscitative hypothermia may be more a question of whether or not to *rewarm*, than to actively *cool* since the patients will already be mildly hypothermic. We hypothesized that after spontaneous cooling during HS, continuing mild, therapeutic hypothermia during resuscitation is beneficial. We felt that this study was important before considering clinical trials of hypothermia. After moderately severe HS (pressure-controlled HS at mean arterial pressure (MAP) 40 mm Hg until 30% reuptake of blood is needed), only 3 of 8 rats in the normothermia group survived to 72 hr, whereas 2 of 8 hypothermic rats survived. Most deaths were after 24 hr. After more severe HS (to 50% reuptake), survival time was longer with continued hypothermia. It appears that mild hypothermia is more beneficial to prevent early deaths, presumably from cardiopulmonary dysfunction, than late deaths, presumably from the systemic inflammatory response and multiple organ failure. This study was presented as a poster at the American Association for the Surgery of Trauma meeting in Orlando, FL. The manuscript is in preparation for the *Journal of Trauma*.

Very prolonged HS

Thus far in year 5, we have focused on development of a model of very prolonged HS in rats. The goal is to develop a resuscitation strategy for trauma victims that require prolonged extrication and transport times as in the military and rural settings. In the first part of this study, using a model of uncontrolled HS via tail cut for 6 hr, we compared target MAP of 50 vs 60 vs 70 mmHg for limited fluid resuscitation with whole blood (shed plus donor). Mortality increased with lower MAP goal, but there was significant variability in MAP. To control MAP more precisely we are currently repeating the study using a more classic Wiggers-type pressure-controlled HS model including systemic heparinization. An abstract for this study has been submitted to the Society of Critical Care Medicine.

Dr. Wu presented 3 posters at the meeting of the Society of Critical Care Medicine, January 26-30, 2002, entitled "Mild hypothermia (34°C) does not increase initial

bleeding from the injured liver after HS in pigs, Resuscitation with Ringer's ethyl pyruvate solution (REPS) fails to improve long term survival compared to lactated Ringer's (LR) solution after severe volume-controlled HS in rats," and "Inhibition of sodium/hydrogen ion (Na⁺/H⁺) exchange with methyl isobutyl amiloride impairs tolerance to hemorrhagic shock in rats." At the same meeting, Dr. Tisherman presented a poster entitled "Critical care experiences during surgical residencies do not affect surgical practice." Dr. Rainer Kentner presented a paper at the American Society of Anesthesiologists meeting entitled "Doubling the "golden hour" of traumatic HS tolerance with mild hypothermia and an anti-oxidant." Dr. Tisherman participated in a debate (with Larry Gentilello, MD) regarding the pros and cons of resuscitative hypothermia at the Advanced Technology Applications for Combat Casualty Care meeting in Fort Walton Beach, FL, on September 10, 2001. He also gave the following invited talks: "SA Research" at the Association of Neurological Surgeons, Chicago, IL, 4/11/02; "Hypothermia for Resuscitation from Trauma: It's Cool to be Cool" at the Society of Air Force Clinical Surgeons, Las Vegas, NV, 4/19/02, and at the Washington DC Area Critical Care Society, Washington, DC, 6/13/02. "Development of an Evaluation Instrument for Surgical Crisis Resource Management" at the Association for Surgical Education, Surgical Education Research Fellowship Forum, Baltimore, MD, 4/5/02.

2. Suspended Animation (SA) in Dogs (Safar)

In 2001/2002 we continued to search in dogs for breakthrough effects among 14 different drugs and different temperatures, added at the start of CA to the saline flush into the aorta. Alone, without drugs, when we delivered saline at 0-4°C, we achieved considerable preservation and resuscitation. The drugs were selected according to one or more of six mechanistic strategies, documented by us and others in the past, and on the basis of published results in rodents and advice from co-investigators and consultants. Almost all drugs tested had not yet been evaluated for outcome after prolonged CA in large animals. We were seeking a breakthrough effect, namely overall performance category (OPC) = 1 at 72 hr, i.e., functionally normal dogs, in the majority of experiments of exploratory mini-series. OPC = 1 was achieved in very few experiments. With exsanguination CA 20 min no flow, only mild hypothermic aortic arch flush with the antioxidant *Tempol* resulted in OPC 1 or 2 (good outcome) in all 8 dogs. *Tempol* flush was also effective in other settings. Strangely, *Tempol* improved function but not histologic brain damage. An explanation for this is speculative. Some mechanism studies were added. Treatment with any of 11 other drugs resulted only in dogs achieving OPC 3 or 4, severe neurologic deficit and severe histologic damage. Thus, using 84 exploratory dog outcome experiments with the same model by the same team, only the water soluble antioxidant *Tempol* (which crosses the blood brain barrier) improved neurologic outcome.

We then used the same models for exploring *hypothermic strategies*, without drugs, merely using flush of isotonic saline, pushing the insult beyond that of the previous years. We extended the CA no flow period from 20 min and 30 min, to 60, 90, and 120 min. Some results were "firsts." Saline at 2-4°C, flushed with a 1 l/min flow rate into the

aorta, decreased Tty by 3°C/min. This is faster than any other cooling method, except for using cardiopulmonary bypass (CPB). With CA 20 min no-flow, aortic arch flush rapidly lowered tempenic temperature (Tty) to 34°C and achieved survival to 72 hr with functional normality (OPC = 1) and histologically minimal damage. A delay in flush during normothermic CA to 8 min no-flow before start of cold-flush negated the preservation achieved with flush starting at CA 2 min or 5 min. When we increased CA to 30 min no flow we found that the flush volume of saline at 4°C had to be increased to 100 ml/kg to achieve functionally normal brains. This in some dogs achieved even histologically normal brains. The aortic catheter had to be withdrawn into the abdominal aorta to also protect by cold flush the viscera and spinal cord against ischemic damage. Aortic flush to Tty 20°C, 15°C, or 10°C preserved the brain and organism long enough to achieve intact survival (OPC 1) after 60 min, 90 min, and in some dogs even 120 min no flow. We now know that with Tty 10°C (induced with saline 0-4°C flush starting at CA 2 min) one can count on full preservation of all organs' viability after up to 90 min no flow. All 6 dogs with CA 90 min and Tty 10°C were functionally normal. One dog after CA 60 min, one after CA 60 min, one after CA 120 min, and one normal dog received cognitive function tests months later; these were normal. Functional and histologic studies of extracerebral organs after 72 hr have been initiated. CA up to 60 min was survived with intact viscera; liver function values were abnormal only transiently.

In the last study, with CA 120 min, when flush was with theoretically optimized solutions instead of saline – namely Normosol, Unisol, and Tempol *combined* -- all 6 dogs achieved good outcome. Thus there was a small additional outcome benefit in using solutions other than saline for the cold flush, stasis, and reperfusion. Polynitroxylated albumin with Tempol (Synzyme Co.) gave slightly better neurologic outcome. Unisol (Dr. Michael Taylor) resulted in easier restoration of stable spontaneous circulation. We found that the antioxidant Tempol is more effective when given at the beginning of HS or CA than when given during reperfusion.

We are working in communication with Drs. Peter Rhee and Hasan Alam of the Uniformed Services University of Health Sciences Office of Naval Research (USUHS-ONR), who were inspired by our work. They researched SA for over 3 hr with asanguinous low flow (not CA), using CPB via thoracotomy in pigs.

In spite of Dr. Safar's three life-threatening operations in winter 2001/2002, we visited in summer 2002 the U.S. Army Medical Research and Materiel Command/Telemedicine and Advanced Technology Research Center (USAMRMC/TATRC) in Washington, D.C. A team led by Dr. Safar presented the SA project's present and future plans and had funding for years 2003 reviewed and approved. In April 2002, Drs. Safar and Nozari presented cerebral topics at the American Society of Neurology meeting in Denver.

The SA dog projects in 2001/2002, using 3-4 day long outcome experiments, conducted 80 experiments. These experiments were under the team leadership of Dr. Nozari, supported by technicians Jeremy Henchir, Sherman Culver, Scott Kostelnik, Alan Abramson, and Murugan Subramanian: 1) Special aortic SA flush solutions (Unisol; Tempol) gave better outcome results than saline. 2) Exsanguination CA of 60 min no

flow is more difficult to reverse to intact survival if the insult includes trauma; the latter causes coagulopathy. 3) SA with aortic flush using recirculated cooled, diluted venous blood gives better outcome than one-way cold saline flush. 4) We simulated “unresuscitable” hearts with prolonged CPB, in prolonged normovolemic ventricular fibrillation (VF)-CA, mild hypothermia during prolonged CPR steps A-B-C, using veno-venous cooling, gave significantly better outcome data after mild hypothermia, in one study with 40 min steps airway (A)-breathing (B)- circulation (C) and another with 60 min steps A-B-C.

3. Miscellaneous

We have coached development of devices and methods for rapid vessel access and portable pumping-cooling. The latter is being initiated by the Biocontrol Company of Western PA, guided by us. Dr. Miroslav Klain received a spinoff grant in the fall of 2001 for helping develop a “smart catheter” and vessel access -- \$0.1M from the Army via Illinois Institute of Technology Research Institute (IITRI). Additional funds for the catheter project are to Dr. Lynn Yaffe as PI; the Cardeon Co. to produce the catheter, and to IITRI to produce the sensors.

We guided devices developments needed to move SA into the out-of-hospital arena. Five years ago two patents had been obtained by Drs. Safar, Klain, and Mr. Stezoski for the University of Pittsburgh, one for a portable multi-modular cardiopulmonary bypass apparatus, and the other for single or double balloon aortic catheters, as licensed to the Cardeon Corp. of California. The company has used these patents so far for the development of a “Cobra Catheter” for heart surgery. They are now planning to go into CPB for resuscitation.

Drs. Safar and Klain and Mr. Stezoski have been urging the Biocontrol Co. of Western Pennsylvania to develop a portable device for rapid cooling and pumping of blood and fluids. In patients after CA and ROSC, with circulation, there is an urgent need for rapid lowering of brain temperature to mild hypothermic levels (from 38 to 34°C). Our team found in dogs with circulation, with 10% cardiac output pumped through an improvised cooler, to achieve mild hypothermia within 6 min. For patients with cardiac arrest (SA), we are coaching industry to develop a rapid delivery system for aortic cold flush.

Since 2001, we co-initiated and helped the NIH PULSE initiative to include in resuscitation research, traumatology. Our group led new initiatives at local, national, and international levels for boosting the weakest link in civilian EMS, life supporting first aid (LSFA) skill acquisition by the public. Among several efforts to advance resuscitation delivery, Dr. Safar moved LSFA training programs with self-training systems into Pennsylvania and perhaps soon beyond, by guiding the Save a Life Foundation (SALF) of Chicago (Carol Spizzirri, R.N.) in its move to Pittsburgh. Dr. Steven Orebaugh, anesthesiologist, emergency physician, and educator, assumed the chair of a community LSFA committee. That program will be housed in the WISER, which, chaired by Dr. John Schaefer, is formally affiliated with the Safar Center.

Dr. Tisherman is promoting better understanding of controlled mild hypothermia vs. uncontrolled accidental cooling in patients with traumatic hemorrhagic shock. Drs. Safar and Behringer, and SCRR visiting professor Dr. Bernd Boettiger of Heidelberg, Germany, jointly represented cerebral resuscitation at the Wolf Creek conference for resuscitation research in June 2001 in Palm Springs, CA. The highlights of the HS-SA projects of 2001 will be presented in seven posters/talks at the forthcoming SCCM meeting in January 2002 in San Diego.

An important extension of the fruits of past CA research by Dr. Safar, Dr. Tisherman, and other team members, has been the following: Our group's discovery and documentation in dogs of mild resuscitative hypothermia after prolonged normothermic CA in dogs (1987-1994), has led to randomized clinical outcome studies abroad. This was not possible in the USA where the needed waiving of prospective informed consent was outlawed. Our alumnus Sterz of Vienna and colleagues in Australia and Japan have obtained statistically positive outcome data with mild hypothermia after prolonged cardiac arrest in patients. The two key papers have been published in the *New England Journal of Medicine* in early 2002. In 2001, the journal invited Dr. Safar to write an editorial on this subject which will appear (by Safar and Kochanek) in the same issue with the two clinical trials reports. This will create a boost for resuscitative hypothermia for various indications.

On September 11, 2001, Drs. Safar and Tisherman were returning from a DOD meeting in Fort Walton Beach when the Attack on America occurred. Dr. Safar delegated to Dr. Doris Cope his lecture for the Fifth International Symposium on the History of Anesthesia in Santiago de Compostela, Spain, on September 20, 2001. His lecture was on "Development of Cardiopulmonary-Cerebral Resuscitation in the Twentieth Century." Dr. Safar presented and discussed resuscitative hypothermia at the Cleveland Clinic's Neurocritical Care 2001 Conference on September 29, 2001. At the American Society of Anesthesiologists Congress in October 2001, Dr. Safar discussed suspended animation at a panel meeting with major interest from the media. In April 2002, Drs. Safar and Nozari presented "CPCR, From Animal Models to Clinical Trials" at the American Academy of Neurology.

Dr. Safar continued guiding the SA laboratory activities in spite of major operations in October 2001, November 2001, and May 2002. In March 2002, Dr. Safar's work was featured in the Pittsburgh Post-Gazette. In April 2002, Dr. Safar was visiting professor in Vienna to help plan the world's second large animal research ICU. Finally, in the summer of 2002, Dr. Safar prepared a major slide series on the SA project for the *DOD*.

Peer-reviewed Manuscripts: Shock and Suspended Animation Program

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

1. Safar P, Behringer W: Cerebral resuscitation from cardiac arrest. In: A Textbook of NeuroIntensive Care. Layon AJ, Gabrielli A, Friedman WA (editors). WB Saunders Publ. Sept. 2001 (in press).
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