Fluid Resuscitation: State of the Science for Treating Combat Casualties and Civilian Injuries

Committee on Fluid Resuscitation for Combat Casualties, Institute of Medicine

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Fluid Resuscitation

State of the Science for Treating Combat Casualties and Civilian Injuries

Andrew Pope, Geoffrey French, and David E. Longnecker, Editors

Committee on Fluid Resuscitation for Combat Casualties
Division of Health Sciences Policy
INSTITUTE OF MEDICINE

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The serpent has been a symbol of long life, healing, and knowledge among almost all cultures and religions since the beginning of recorded history. The image adopted as a logotype by the Institute of Medicine is based on a relief carving from ancient Greece, now held by the Staatliche Museen in Berlin.

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This report has been reviewed in draft form by individuals chosen for their diverse perspectives and technical expertise, in accordance with procedures approved by the National Research Council's Report Review Committee. The purpose of this independent review is to provide candid and critical comments that will assist the Institute of Medicine in making the published report as sound as possible and to ensure that the report meets institutional standards for objectivity, evidence, and responsiveness to the study charge. The review comments and draft manuscript remain confidential to protect the integrity of the deliberative process. The committee wishes to thank the following individuals for their participation in the review of this report:

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While the individuals listed above have provided constructive comments and suggestions, it must be emphasized that responsibility for the final content of this report rests entirely with the authoring committee and the Institute of Medicine.

Preface

The U.S. military has a long tradition of making the safety of its troops a top priority. This goal is evident in military strategy and tactics and in the development of sophisticated technology that decreases or avoids human risk. This commitment is evident in the fostering and support of biomedical research that is applicable to the military as well. The spectrum of supported research ranges from acute through chronic care of the soldier. The U.S. Department of Veterans Affairs, for example, invests in research on long-term rehabilitation from wounds or military-related disease, whereas the armed services often focus on the acute medical needs of the injured soldier. One of the most important goals involves immediate resuscitation of the wounded soldier in the field, to support life during transport to a field facility, where definitive treatment may be instituted by highly trained medical personnel. The Office of Naval Research asked our committee to focus on this immediate resuscitation to ensure that current care is optimal and that future research is focused in the most fertile areas for advancement.

The National Academy of Sciences (NAS) has a long history of assisting the military in the evaluation of methods of resuscitation and shock. NAS volunteered its expertise to President Woodrow Wilson in 1916, and he responded by asking NAS to organize scientific agencies for national defense. At the conclusion of World War I in 1918, the president, by executive order, asked the Academy to perpetuate the National Research Council (NRC) for the government to have a consulting body available for a variety of needs. NRC played an important role in the studies of shock and resuscitation prior to and during World War II and provided advice for research policy and treatment protocols.

The challenges associated with the immediate resuscitation of the wounded soldier are daunting and are often unappreciated by civilian medical personnel. Military engagements often take place in mud, rain, snow, heat, or cold, at sea or on beaches, and often at night. The field medics, who are responsible for ini

tial resuscitation and treatment, can carry only very limited medical gear, and they are often engaged in battle and under fire while attempting to treat the wounded. Thus, the need for simple, compact, and effective approaches for immediate resuscitation is apparent.

Research in this area is extraordinarily challenging. First, there are few, if any, ways in which all aspects of human hemorrhagic shock can be fully reproduced in the laboratory. Various animal models can mimic specific aspects of the shock process, but no single model represents the entire spectrum of human hemorrhagic shock; there remain fundamental differences that apparently cannot be narrowed. Yet the use of unproven treatments in the field is simply unacceptable.

The committee had two goals as it thought about research in this area. First, it wanted to indicate to the Office of Naval Research which technologies were best suited for use in the immediate future. Second, it wanted to give some direction for longer-term research by identifying promising areas that might lead to leaps in the knowledge about hemorrhagic shock or care for the combat casualty. As part of the committee's review of the state of the science, the committee held a 2-day conference and heard from more than 40 scientists, medical researchers, and clinicians in the field of resuscitation research. Subsequently, the committee solicited information from several additional scientists and from the scientific community at large. In the end, the committee felt comfortable with its grasp of current research and with its view of the opportunities for future investigation. These conclusions are presented in the text of this report.

Finally, the committee wanted its work to have some relevance to the civilian medical community. Although its first responsibility was to the military, the committee understood that there are both similarities and differences between civilian emergency trauma care and acute military medicine. With this in mind, the committee was explicit in describing the similarities and differences between the combat and civilian environments, and it offered suggestions for technologies or approaches that would apply to civilian care as well. The committee hopes that this report will help energize and focus research in both military and civilian emergency medical care and help to save the lives of citizens and soldiers alike.

On behalf of the committee, I wish to express our gratitude to all who contributed to the production of this report. First, we appreciate the opportunity that was presented by the Office of Naval Research, which initiated the questions and sponsored the project. Second, a review of the science would not be possible if it were not for the many scientists and experts whose findings formed the scientific basis of this report. Third, the arduous process of conducting the complex task was made easier by the talented staff of the Institute of Medicine, especially Andy Pope, Geoff French, and Glen Shapiro. Most importantly, I want to add my personal gratitude to the rest of this committee who volunteered countless hours of their time and expertise to produce this document.

If the work that we have done here serves to assist in resuscitating even one casualty that would have otherwise been lost, as we believe it will, then this will have been a successful and worthwhile endeavor.

DAVID E. LONGNECKER, M.D.
CHAIR

Photographs

Page xii: A bottle of blood plasma hangs on a "wounded" man's rifle during a training exercise in 1943. Photograph by Marjory Collins. Courtesy of the Library of Congress.

Page 8: Medics helping a wounded soldier in France, 1944. Courtesy of the National Archives and Records Administration.

Page 18: An American soldier receives blood plasma in Sicily, 1943. Courtesy of the National Archives and Records Administration.

Page 46: A blood transfusion underway aboard a DUKW during the fighting on Iwo Jima, 1945. Courtesy of the Bureau of Medicine Historical Archives.

Page 78: Water and plasma being given to a marine at Eniwetok Atoll. Courtesy of the Bureau of Medicine Historical Archives.

Page 96: Soldiers at a battalion aid station await evacuation while being transfused in Korea, 1952. Reprinted with permission of the American Red Cross. All rights reserved.

Page 108: Wounded soldiers are evacuated aboard a tank in Vietnam, 1968. Photograph by John Olson. Copyright 1968 by Time, Inc. All rights reserved.

Contents

Executive Summary

Historically, one fifth of all injured combatants die in battle. Although this number has varied significantly depending on the specific campaign, 20 percent has been the mean mortality rate among injured combatants in all U.S. conflicts since World War II combined. Furthermore, of every 10 combatants who die from battle injuries, 9 die on the battlefield (i.e., are killed in action) before evacuation to a field hospital (Bellamy, 1998). Precise data are not available to verify the percentage of casualties who are potentially salvageable (Bowen and Bellamy, 1998; Koehler et al., 1994), but authorities have estimated this number to be approximately 20 percent (Bellamy, 1984, 1987a,b, 1995). The single major cause of death in potentially salvageable battlefield casualties is hemorrhage, and the greatest opportunity for reducing mortality and morbidity of battlefield casualties involves fluid resuscitation and treatment of hypovolemia (abnormally decreased volume of circulating fluid [plasma] in the body).

Current therapeutic regimens usually specify the use of crystalloid solutions for the treatment of hypovolemia and subsequent shock. However, numerous questions and concerns regarding the concentration, rate, and quantity of resuscitation fluid persist. Additionally, battlefield conditions further constrain the type, quantity, and delivery options of resuscitation fluids that can be administered in far-forward areas. Because of these and other concerns, the Office of Naval Research asked the Institute of Medicine to provide an independent assessment of the current status of resuscitation fluids and resuscitation protocols for combat casualties and to develop a series of findings and recommendations for future research directed at the acute treatment of massive blood loss on the battlefield. The Institute of Medicine committee reviewed the published literature, conducted an international conference, and interviewed numerous authorities to formulate an evidence-based response.

The committee found that there are at least theoretical disadvantages to existing resuscitation fluids (although these fluids are rarely questioned in clinical practice) and that many have not been modified for several decades. Still, both laboratory and clinical information demonstrated that some new protocols may reduce the rate of mortality among injured combatants on the battlefield. The committee concluded that new protocols for the fluid resuscitation of battlefield casualties should be implemented immediately and makes the following re-commentation:

Recommendation: **The initial fluid resuscitation of the hemorrhaging battlefield casualty should be a 250 ml bolus of 7.5 percent hypertonic saline delivered by a rapid-infusion system.** ***(Recommendation 5.2)***

Systemic administration of hypertonic saline solution would take place via an intraosseous needle placed in the anterior tibia, even through the uniform if necessary. The fluid bag would be placed under manual low pressure or attached to a simple and durable pump, which could be mechanical or electric. The option for intravenous access should also be provided, but the committee felt that it would be easier to teach nonmedical combatants the intraosseous route. The committee also noted that fluid resuscitation is only one component of the immediate care of the battlefield casualty. Fluid resuscitation is predicated on the control of bleeding and improvements in fluid therapy will be most effective when they are accompanied by improvements in management of the airway and ventilation in the field, and by rapid evacuation of the injured individual to a site where definitive care can be initiated by trained clinicians.

Even though the administration of hypertonic saline would be an improvement over current protocols, new resuscitation fluids should be developed and tested. Such fluids should address the metabolic and cellular consequences of traumatic shock and the potential disadvantages of existing fluid formulations. Future research directed at acute treatment of massive blood loss on the battlefield should explore the development of an improved resuscitation fluid. Ideally, such a fluid would provide adequate control of pH, partial CO_2 pressure/bicarbonate ratio, the phosphorylate potential, the redox state, and osmotic pressure; adequate control of sodium chloride, calcium, and potassium levels; and adequate control of the lactate and pyruvate ratio. Although the large volume of lactated Ringer's solution that is required for resuscitation in the field is simply not currently compatible with the expected functions of the first responder, who is both combatant and medic in most situations, evidence from a variety of sources suggests that modifications to lactated Ringer's solution might be of value, and the committee proposes that these be explored.

Recommendation: **Research involving modifications of existing lactated Ringer's solutions could include**:

1. **elimination of D-lactate**,
2. **reduction of total L-lactate load**,
3. **addition of ketones as an energy source, and**
4. **addition of free-radical scavengers and antioxidants (vitamins E and C, glutathione, or iron chelators).** ***(Recommendation 3.1)***

At present, there is insufficient evidence that proposed fluids, such as parenteral saline containing normochloremic carbonate/carbon dioxide: (1) restore blood volume effectively; (2) produce the desired hemodynamic, hematologic, cellular, and immunologic-related advantages; or (3) do not cause adverse events. The ideal solution for fluid resuscitation—one that addresses all of the physiologic and cellular aspects of hemorrhagic shock and that is easy to carry and use in the battlefield—is not apparent at this time. To hasten the development of such a fluid, several changes in the conduct, focus, and direction of resuscitation fluid research will have to take place.

Proper clinical evaluation of resuscitation fluids and protocols is hampered by the inevitably uncontrolled conditions that accompany clinical trauma and hemorrhage. These include variations in the site and extent of injuries, the duration of hypotension and hypoperfusion, the extent of hypothermia, and the interval before access to definitive care. Much laboratory research has been done with animal models that lack either reproducibility or clear relevance to the clinical scenario, or both. Some fluid resuscitation research has been limited by protocols that did not adequately distinguish or address (1) the differences between pure hemorrhagic shock and traumatic shock associated with tissue injury, (2) the need to standardize the animal models with regard to anesthesia and general care, and (3) the need to observe the subjects for longer-term survival. Clinical research specifically has been hampered by the lack of an organized national approach to trauma research that takes advantage of the considerable clinical material and research expertise among the regional trauma centers. Furthermore, approaches to both current treatment and future research are hampered by inadequate methods for classification of the severity of clinical trauma; such classifications are essential to evaluations of the efficacies of new treatment protocols that involve modifications in fluid formulations or novel therapies. Current trauma indexing systems are inadequate for use in future trauma research.

Recommendation: **A national study group should be convened to develop and implement clinical research, including multicenter clinical trials on selected topics at existing regional trauma centers. Federal agencies, including the U.S. Department of Defense, the U.S. Department of Veterans Affairs, and the National Institutes of Health, and national professional organizations, should collaborate with each other and with the private sector in this activity.** ***(Recommendation 6.2)***

Recommendation: **A new system for categorizing injury in trauma care should be developed.** ***(Recommendation 6.3)***

For these reasons, among others, the considerable research on fluid resuscitation for hypovolemia has not led to the implementation of new clinical approaches that improve hemodynamics or significantly reduce the rate of mortality among injured combatants on the battlefield. Furthermore, the key to improved care may not involve hemodynamics alone but likely requires an understanding of the molecular and cellular responses that are triggered by massive blood loss and shock. The goals of volume replacement therapy therefore include not only hemodynamic stabilization but also amelioration of the chain of events that lead to irreversibility in severe prolonged shock. Major therapeutic advances are most likely to result from new approaches that address the metabolic and cellular consequences of shock, many of which are triggered by the impaired oxygen delivery to tissue that accompanies hemorrhage and ischemia. Resuscitation approaches that enhance oxygen delivery to tissue deserve further evaluation.

Recommendation: **Evaluate the applicability of small-volume, stable oxygen (O_2-carrying and O_2-facilitating agents that improve and sustain O_2 delivery in the wounded subject for 24 to 48 hours.)** ***(Recommendation 4.1)***

Finally, the complex events that result from the initial insult, tissue injury, and resuscitative attempts provide numerous potential therapeutic targets for novel interventions. Novel therapeutic strategies might be conveniently, if somewhat artificially, categorized as those that prevent the early complications of the shock syndrome (prevention), those that treat the complications of shock syndrome and reperfusion injury (intervention), and those that render the subject less vulnerable to hypoxia and its consequences (tolerance). The military should maintain a research interest, if not research support, in each of these areas, recognizing that some are approaching sufficient maturity for clinical trials, whereas others are still at an investigative, basic science stage. The committee found that much of the research on hemorrhagic shock has remained focused on hemodynamics or has been directed toward the correction of a single biochemical abnormality that accompanies hemorrhage. Such strategies are unlikely to be successful, because multiple pathways lead to the cell death that results from severe hemorrhagic shock. Rather, novel therapies should be aimed at the multiple metabolic and cellular derangements that accompany traumatic shock. These approaches should take advantage of advances in other related fields (such as ischemia-reperfusion research on specific organs) and should be approached in a systematic manner that involves prophylaxis, immediate intervention, or the development of tolerance to global ischemia.

The committee's recommendations are listed in their entirety in Box 1.

BOX 1. THE COMMITTEE'S RECOMMENDATIONS

Pathophysiology of Acute Hemorrhagic Shock

Recommendation 2.1 Develop and validate diagnostic assays for substances in serum that Indicate the specific mechanisms involved in the molecular processes of cellular injury and cell death induced by shock and resuscitation.

Recommendation 2.2 Expand the use of transgenic experimental animals to further evaluate the role of specific proteins and enzymes in cellular injury and death induced by shock and resuscitation.

Recommendation 2.3 Study and rigorously evaluate polypharmaceutical approaches directed against the multiple and independent mechanisms of cellular injury and death induced by shock and resuscitation.

Experience with and Complications of Fluid Resuscitation

Recommendation 3.1 Research involving modifications of existing lactated Ringer's solutions could include:

1. elimination of D-lactate;
2. reduction of total L-lactate load,
3. addition of ketones as an energy source, and
4. addition of free-radical scavengers and antioxidants (vitamins E and C, glutathione, or iron chelators).

Recommendation 3.2 Studies examining modifications of the existing lactated Ringer's solution formula must include examining the effects of the modified solution on:

1. immunologic-related function,
2. cellular apoptosis,
3. intravascular retention, and
4. specific end-organ function such as pulmonary, renal, and cardiac function (i.e., the presence or absence of arrhythmias)

Recommendation 3.3 Previous concerns regarding the detrimental effects of aggressive fluid resuscitation with large volumes of crystalloids suggested the need to examine both the immunologic as well as hemodynamic consequences of small-volume lactated Ringer's resuscitation. Studies examining reduced volume of lactated Ringer's solution should examine the effects of this volume on:

1. hemodynamic function,
2. immunologic-related function,
3. cellular apoptosis,
4. specific end-organ function such as pulmonary, renal, and cardiac function.

Novel Approaches to Treatment of Shock

Recommendation 4.1 Evaluate the applicability of small-volume, stable oxygen (O_2)-carrying and O_2-facilitating agents that improve and sustain O_2 delivery in the wounded subject for 24 to 48 hours.

Recommendation 4.2 Therapeutic agents that target the toxic effects of hypoxic injury (e.g., antioxidants, chelating agents, hormones, and nitric oxide inhibitors) should be studied with animal models and subsequently in clinical trials. Combinations of several therapeutic agents should also be investigated.

Recommendation 4.3 Mechanisms that may induce tolerance to ischemia and cellular hypoxia (e.g., hibernation, ischemic preconditioning, and hypothermia) should be explored with appropriate preclinical models.

Protocols of Care at the Site of Injury

Recommendation 5.1 The number of trained first responders in the combat environment should be increased through development of a Military Trauma Life Support course.

Recommendation 5.2 The initial fluid resuscitation of the hemorrhaging battlefield casualty should be a 250 ml bolus of 7.5 percent saline delivered by a rapid-infusion system.

Recommendation 5.3 Efforts should be made to ensure that the airway of a battlefield casualty is patent and that ventilation is adequate.

Recommendation 5.4 If accessible, all severely injured battlefield casualties should be evacuated to a front-line high-echelon care site in less than an hour.

Future Directions

Recommendation 6.1 Laboratory research should be reproducible and relevant to the clinical scenario. For fluid resuscitation research, the experimental design of animal research should be guided by the following principles.

- when feasible, the experimental model should include soft-tissue injury in addition to hemorrhage;
- controlled hemorrhage protocols are preferred over uncontrolled hemorrhage models;
- when feasible, protocols that do not require anesthesia are preferred. If anesthesia is required, the depth of anesthesia should be reproducible, and the anesthetic agent should be selected to minimize alterations in the physiologic responses to hemorrhage;

- experimental animal species should be selected on the basis of clinical relevance, and will vary depending on the research question;
- if survival is an endpoint, mortality should be measured for at least 5 days; and
- the experimental design should establish a reliable 50 percent lethal dose (LD_{50}) for the control group.

Any and all animal research should be conducted in accordance with the *Guide for the Care and Use of Laboratory Animals* (NRC, 1996).

Recommendation 6.2 A national study group should be convened to develop and implement clinical research, including multicenter clinical trials on selected topics at existing regional trauma centers. Federal agencies, including the U.S. Department of Defense, the U.S. Department of Veterans Affairs, and the National Institutes of Health, and national professional organizations, should collaborate with each other and with the private sector in this activity.

Recommendation 6.3 A new system for categorizing injury in trauma care should be developed.

1

Introduction

Historically, 20 percent of all injured combatants die in battle. Although there has been tremendous variation in this number since World War II (depending on the campaign), this has been the mean mortality rate for all U.S. conflicts combined. A more important statistic, however, is that of every 10 combatants who die from battle injuries, 9 die on the battlefield (i.e., are killed in action) and never make it to the field hospital (Bellamy, 1998). It is also important to understand that, unlike in the civilian setting, there is no "golden hour" in combat trauma (Bellamy, 1984, 1987a,b, 1995); that is, whether the casualty receives care within the first hour after the injury is incurred is not a predictor of survival. Of those combatants who die on the battlefield, 40 percent die immediately and are not salvageable, and 25 percent die within 5 minutes and are probably not salvageable. As a role, Bellamy estimates that approximately 3 to 5 percent of those who make it to a higher-echelon point of care will die at a later time (i.e., will die of their wounds) (Bellamy, 1984, 1987a,b, 1995). In addition, those who die at the hospital will die of injuries to the central nervous system, whereas of those who die in the field, the majority will die of exsanguination (see Figure 1-1).

As noted above, of the battlefield casualties who die, 65 percent do so within 5 minutes of their injury (see Figure 1-2) and are not salvageable. The remaining 35 percent are probably at least potentially salvageable, in that 15 percent die up to 30 minutes after their injuries and the remaining 20 percent die after 30 minutes (Bellamy, 1984, 1987a,b, 1995). On the basis of data from the Vietnam conflict (Maughon, 1970), analyzed by Bellamy, almost 50 percent of the battlefield casualties died of exsanguination. Twenty-five percent died of massive mutilating torso injuries, 10 percent died of torso injuries that with timely surgical intervention could have been salvageable, and another 9 percent had peripheral exsanguinating injuries that also could have been salvaged with

timely intervention. In addition, another 1 percent died of airway obstruction, which was potentially reversible, whereas 10 percent died of tension pneumothorax, which also was potentially reversible. Thirty-one percent died of severe brain injuries and 12 percent died later of their wounds.

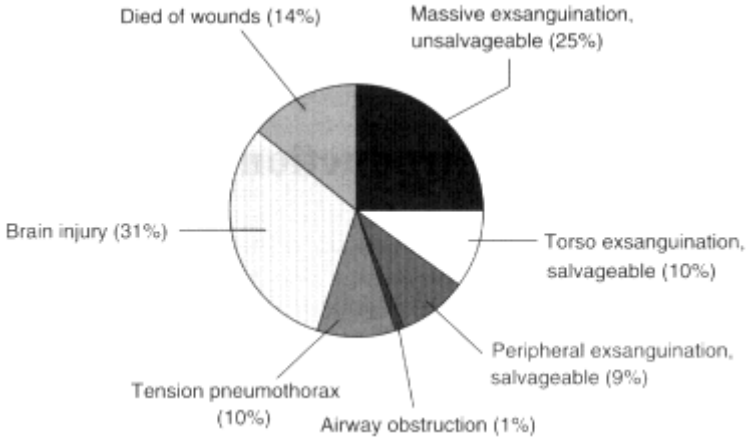


Figure 1-1
Distribution of fatal battlefield injuries in Vietnam.
Source: Adapted from Bellamy (1984, 1987a,b, 1995).

In all, Bellamy estimates that just under 20 percent of those who die on the battlefield are potentially salvageable (Bellamy, 1984, 1987a,b, 1995). Bruttig (1998) estimates that approximately 10 to 15 percent of battlefield casualties have potentially surgically correctable injuries. However, because of the delays to definitive surgery, these patients do not survive. Unfortunately, no good comprehensive wound data for the latter group are available, so it is impossible to identify clearly what percentage of these patients are truly potentially salvageable (Bowen and Bellamy, 1998; Koehler et al., 1994). It is clear, however, that the single major cause of death in the potentially salvageable battlefield casualty is hemorrhage.

It may thus be reasonable to expect that with immediate and appropriate care a significant number of these patients could be salvaged. This impression is bolstered by the experience of the Israeli Defense Forces, which have an aggressive system of field treatment by physicians who stabilize the wounded in the battlefield and then rapidly evacuate them to field hospitals. In addition, every other soldier in the Israeli Defense Forces is trained as a medic. It should be pointed out, however, that, in conflicts in which Israel has been involved, evacuation to definitive care required very short flight times (Krausz, 1998).

Still, the battlefield of the 21st century will be very different from that of the past. The new battlefield will be "asymmetric and non-linear" (Bruttig, 1998); the large-scale wars of the past are less likely. Wars will most likely be

fought in settings such as cities and towns, with vaguely identified combatants who will involve large numbers of civilians in their campaigns. The fighting of the future is also likely to involve terrorists and guerrilla interdictions as opposed to highly visible armies, and will be fought by small groups of combatants over shorter time periods with smaller numbers of casualties at any point in time. Because of the likely locations of these conflicts, evacuation by air may be difficult or impossible. As a result, immediate and even ongoing treatment of casualties may be significantly extended. As a consequence, lifesaving medical treatment may well come from a fellow combatant instead of a medic—both of whom are included in the term *first responders* as used in this report. Thus, the therapies used in the field may vary depending on the time frame from the injury to medical evacuation, the skills and resources of the first responder, and the field site of combatant injury. The availability of fluids of the appropriate volume and physiologic effect in the field may play a part in the provision of lifesaving treatments given the time frame from the injury to medical evacuation and the availability of care after initial resuscitation.

Thus, it is reasonable to conclude that there is a definite subset of battlefield combatants who now die of hemorrhagic shock but who are potentially salvageable with timely battlefield interventions (e.g., fluid resuscitation). As defined by the committee, *fluid resuscitation* is a treatment regimen involving fluid replacement that is intended to minimize the effects of hemorrhagic shock and to stabilize the hemodynamic response to trauma and hypovolemia. The report focuses on fluid resuscitation of the combat casualty, where a *casualty* is defined as a combatant who has been physically injured. The committee defines *shock* as a condition of inadequate tissue perfusion and inadequate removal of cellular waste products, leading to subsequent failure of oxidative metabolism. Shock may result from defects in (1) delivery, (2) transport, or (3) utilization of oxygen, or combinations of all three. Shock is described in greater detail in Chapter 2. To understand the issues involved in saving the lives of combat casualties it is useful to examine the historical developments in fluid resuscitation.

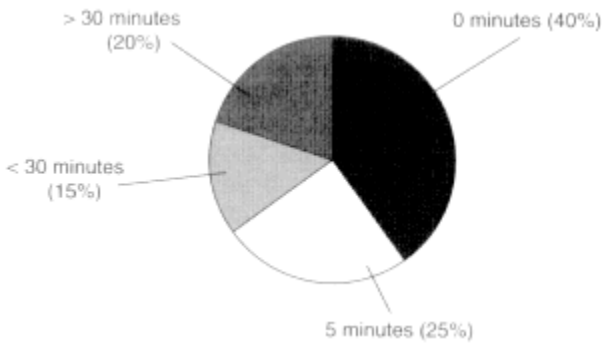


Figure 1-2
Time from injury to death of battlefield casualties.
Source: Adapted from Bellamy (1984, 1987a,b, 1995).

HISTORY OF FLUID RESUSCITATION

In recent conflicts, the majority of potentially salvageable fatalities in the battlefield setting involved exsanguinating hemorrhage (Bellamy, 1984, 1987b). Yet therapeutic approaches to the acute care of hemorrhagic shock have not substantially been altered in over 30 years. The committee found that there are at least theoretical disadvantages to existing resuscitation fluids (although these fluids are rarely questioned in clinical practice) and, as with therapeutic approaches, many have not been modified for several decades. In contrast, there have been significant advances in organ preservation resulting from new fluid formulations. Although lactated Ringer's solution has been the most widely used resuscitation fluid for the past 50 years, there remains substantial disagreement among respected researchers and practitioners in the field regarding the relative effectiveness of this and other resuscitation fluids. Part of the difficulty in achieving consensus is that trauma and hemorrhage do not produce standard clinical situations; and the variation in injuries, the duration of periods of hypotension, hypoperfusion, and hypothermia, and the time lapse before definitive care is rendered make it particularly difficult to evaluate treatment protocols. Furthermore, much of the past research has been done with animals whose responses do not wholly parallel those of humans and whose experimental injuries are dissimilar from those incurred in battle; that is, induced hemorrhage in healthy laboratory animals only partially resembles the hemorrhage with additional tissue damage typically observed in human trauma.

Scientists who try to understand the effects of blood loss and fluid replacement have investigated and documented the relative benefits of whole-blood transfusion, plasma, oxygen-carrying fluids, and colloid, crystalloid, and saline solutions at various volumes since the 17th century. To date, there remains considerable debate over the relative effectiveness of colloid versus crystalloid resuscitation fluids as well as hypertonic saline solutions. Even though studies of each approach have been conducted, the history of investigation has been primarily cumulative rather than comparative.

One of the first significant discoveries occurred in 1883, when Sidney Ringer found that hearts perfused with solutions made with tap water functioned longer than those perfused with distilled water solutions. He determined that the calcium in tap water was responsible. Over 40 years later, Krebs created a fluid that mimicked the composition of plasma, and in 1934, Hartmann, working in St. Louis, Missouri, found that children with infantile diarrhea were dying of hyperchloremic acidosis following a significant loss of sodium from the gastrointestinal tract that depleted the serum of sodium. The addition of sodium lactate to the intravenous solution allowed the sodium to bind with the excess chloride after the lactate was metabolized, and with additional modification, it became the lactated Ringer's solution now in widespread use. Nonetheless, occasional concerns have been voiced about the use of lactated Ringer's solution, and recent laboratory studies have suggested potential clinical complications from the administration of high volumes (this is discussed in more depth in Chapter 3).

For obvious reasons, military medicine has focused on hemorrhagic shock and fluid resuscitation for some time. During World War I, clinical observations of soldiers with mild to moderate blood loss were most often made because those suffering from severe hemorrhage usually died before they reached facilities capable of rendering definitive treatment. This experience seemed to support the prevailing concept of *secondary shock*, or multiple-organ failure following apparently successful resuscitation, observed after an initial period of stabilization. In his 1923 landmark work, Walter Cannon summarized the World War I experience and concluded that shock most often resulted from intravascular volume deficits and must be treated with restoration of blood volume to achieve homeostasis (Cannon, 1923).

Prior to and during World War I, work was also being done with whole blood and blood components, such as plasma, for fluid replacement. Karl Landsteiner described red blood cell isoagglutinins in 1900, thereby facilitating blood typing and cross matching. It was not until World War I that whole blood became available with the addition of citrate as an anticoagulant. Shortly after Landsteiner's discovery British researchers introduced gum acacia and gutta-percha for colloid replacement. In 1916, Bayliss reported the use of 6 percent gum acacia colloid for combat casualties during World War I, thus marking an important moment in what has been an ongoing debate on the use of colloids versus crystalloids. Subsequently, plasma replaced the 6 percent gum acacia as the colloid of choice, but plasma was itself not without drawbacks (see Chapter 3). The use of plasma products was restricted by limited supply, the availability of an adequate preservative solution, and access to refrigerated areas for proper storage (Imm and Carlson, 1993).

Alfred Blalock, Professor of Surgery at the Johns Hopkins University, extensively documented the importance of fluid volume and its effect on survival and was one of the first to note the importance of plasma in resuscitation. In more than 30 papers on shock published prior to World War II, he provided extensive documentation that hypovolemia (reduced blood volume in the body) was the most frequent cause of death and that relative hypovolemia from vasodilation (neurogenic shock) and mediator-caused vasodilation (vasogenic shock) were less frequent causes of death. In 1930, he demonstrated that tissue trauma resulted in the loss of extracellular fluid, which became unavailable to the intravascular compartment, and if the loss was sufficiently severe, the effectiveness of the systemic circulation was impaired (Blalock, 1940).

Blalock was an original member of the National Research Council Committee on Transfusions, which was chaired by Walter Cannon and which advised the armed forces. At its July 24, 1940, meeting the committee recommended the use of plasma, not blood, for resuscitation for four reasons:

1. Most instances of shock are associated with hemoconcentration (a decrease in the volume of plasma in relation to the number of red blood cells), and a given quantity of plasma is more effective than an equal quantity of whole blood in treatment.

2. Blood plasma is approximately as effective as whole blood in the treatment of hemorrhage.
3. The difficulties of preservability and transportability of plasma are considerably less than those of whole blood.
4. Matching and typing are not necessary when plasma is pooled (National Research Council, Minutes of Transfusion Committee).

Edward Churchill, in his memoir *Surgeon to Soldiers* (1972), disagreed with the first two reasons. The first statement about shock associated with hemoconcentration may be traced to a World War I observation by Cannon, who reported higher red blood cell concentrations in capillary blood than in venous blood. This became the centerpiece of the erroneous concept that shock was different from hemorrhage. Churchill further challenged the second reason, that plasma is as effective in treating hemorrhage as blood, because it ignored the advantage of the oxygen-carrying capability of whole blood. The last two reasons for recommending plasma are logistical and are valid to a large degree. Military physicians regarded desiccated plasma favorably because of its small volume, ease of transport, and oncotic effect, and they supported the decision.

Churchill, while Consulting Surgeon to the Eighth Army in Africa, was impressed with the results achieved by British surgeons who used blood for resuscitation. This was accomplished despite rudimentary collecting and storage systems (washed beer bottles). He applied unsuccessfully to the Pentagon for permission to use whole blood, and when he persisted was told to confine his communications to normal channels. Dissatisfied with this response, he contacted a reporter with the *New York Times*, telling him to report that plasma is not adequate for the treatment of wounded soldiers. The article was printed on August 23, 1943, and Churchill believed that it was critical to receiving authorization and equipment for the administration of whole blood in the Italian campaign.

Meanwhile, others also pursued methods to solve the problems related to the collection and transportation of whole blood. Paul Hawley, the European theater surgeon, pooled type-specific blood in gallon containers in England in 1943, and used this blood for transfusion in France in 1944. In 1944, the U.S. Navy successfully shipped blood by air from the United States to the South Pacific with stops at Pearl Harbor and Guam to change the ice in the containers. Several months later the U.S. Army sent its own shipments of blood from the United States to Europe.

In 1945, Coller and Moyer, of the University of Michigan, described fluid translocations produced by the administration of saline to postoperative patients (Jenkins et al., 1950), and later, G. Tom Shires in Dallas, Texas, documented the necessity of adding crystalloid to whole blood and plasma for successful resuscitation (Roberts et al., 1985; Shires et al., 1960a). This work has remained valid and central to present concepts of volume replacement. For the past 50 years, fluid balance in the treatment of shock and bums and understanding of the physiologic parameters of shock have been at the center of much scientific debate in the treatment of trauma.

On the basis of the work described above and related supporting work, the addition of crystalloids, either as saline or as a balanced salt solution, became the standard of care in the Vietnam conflict and resulted in a significant reduction in the rate of renal failure. Extremely high volumes were used in the care of severe casualties in a naval facility near Danang and highlighted the pulmonary problems in nonthoracic trauma which required prolonged respirator therapy and intensive care stays. The syndrome was popularly referred to as "Danang lung," "shock lung," or "traumatic wet lung" and was later labeled "acute respiratory distress syndrome." It had occasionally been noted in World War II and was described under the name of "congestive atelectasis" by Jenkins and colleagues (1950). Overhydration was the most frequently cited etiology. Although high-volume crystalloid infusion lowered the rate of posttraumatic renal failure, spared the use of blood or blood products from being the more exclusive resuscitation tools, and was successful in resuscitation, some adverse consequences were identified, pertaining to the complexity of distinguishing the process of resuscitation from the delayed effects of the shock situation. For example, high-volume fluid resuscitation in patients with shock can result in cell injury and fluid retention within the cell. Moreover, the capillary leak syndrome has been shown to have consequences from other types of resuscitation (e.g., crystalloid), including albumin, which will cross the cell membrane, and be retained within the cell, increasing wet-lung consequences.

Concern over the frequency and severity of this problem led to a conference conducted by the Committee on Trauma, Division of Medical Sciences, of the National Research Council on February 29, 1968. Numerous theories of the etiology of this problem were discussed, including fat embolism, oxygen toxicity, changes in surfactant, infection, overhydration, and vasoactive agents. A description of a final common pathway emerged: damage to the pulmonary capillaries permitted loss of proteinaceous material into the pulmonary parenchyma. Since then, observations on the etiology and treatment of acute respiratory distress syndrome have been helpful in reducing the rates of morbidity and mortality, but fluid volume remains a key consideration in the development of an effective resuscitation approach.

Although many researchers have compared casualties in the inner city to those that occur on the battlefield, those that occur on the battlefield are very different. The primary difference is that the vast majority of combat injuries are penetrating, whereas those in the civilian sector are blunt. Furthermore, in combat, penetrating wounds are caused not by bullets but by shrapnel from explosive munitions. This is compounded by the fact that evacuation in a combat setting, as opposed to the civilian sector, is not rapid and rarely transports a casualty to a setting where true definitive care can be administered. (It should also be recognized that the injuries in naval operations are still different again, in that the majority of these come from blasts, bums, and inhalation [Bellamy, 1984, 1987a,b, 1995; Ordog et al., 1984].)

ORIGIN, SCOPE, AND ORGANIZATION OF THE REPORT

In 1998 the Office of Naval Research requested that the Institute of Medicine appoint a committee that included individuals with expertise in cellular metabolism and biochemistry, emergency medicine, surgery, hematology, anesthesiology, and transfusion medicine to:

- review the state of the art of fluid resuscitation,
- identify targets for therapy, and
- make recommendations for future research directed at acute treatment of massive blood loss on the battlefield.

This report provides an independent assessment of the current status of resuscitation fluid design and resuscitation protocols for combat casualties and a series of findings and recommendations for future research directed at the acute treatment of massive blood loss on the battlefield. Additional attention is given to resuscitation strategies needed in the civilian sector.

The remainder of this report is organized into chapters that address the pathophysiology of acute hemorrhagic shock (Chapter 2), experience with and complications of fluid resuscitation (Chapter 3), novel approaches to the treatment of shock (Chapter 4), protocols of care at the site of injury (Chapter 5), and future directions (Chapter 6). Several appendixes (A to D) are included: Appendix A, acknowledgments; Appendix B, acronyms; Appendix C, the agenda from the committee's conference; and Appendix D, committee and staff biographies.

2

Pathophysiology of Acute Hemorrhagic Shock

DEFINITIONS OF HEMORRHAGIC SHOCK

A variety of definitions of hemorrhagic shock have arisen as more understanding of the mechanisms involved have been developed. Several definitions could be considered to be archaic but in general remain accurate (see Box 2-1). A modern definition of shock would acknowledge first that shock is inadequate tissue perfusion and inadequate removal of cellular waste products and second that shock is a failure of oxidative metabolism that can involve defects of oxygen (1) delivery, (2) transport, or (3) utilization, or combinations of all three. The diagnoses of clinical signs of shock are primarily related to organ failure, but organ failure is secondary to failure of the cells.

BOX 2-1 PAST DEFINITIONS OF SHOCK

Shock is used in reflections drawn from experiences with gunshot wounds.

— Le Dran

Shock is "a momentary pause in the act of death."

— John Warren I

"Shock is the manifestation of the rude unhinging of the machinery of life."

— Samuel V. Gross, 1872

Many authors have described the "vicious cycles" in shock (see Figure 2-1). They may cascade in a variety of ways such as decreased cardiac output, which leads to a decreased blood pressure, which in turn leads to decreased tissue per

fusion. Increased cardiac work may lead to failing myocardial function and decreased coronary perfusion. Decreased tissue perfusion at the cellular level leads to microcirculatory damage, cellular aggregation, and microcirculatory obstruction, followed by cell hypoxia, transfer of salts and fluid into the cells, and decreased venous return. These events lead to metabolic acidosis, which, if it becomes deep, can result in decreased myocardial contraction. There are time-honored classifications of shock, many of which were initiated by definitions described by Alfred Blalock in the late 1930s. They are:

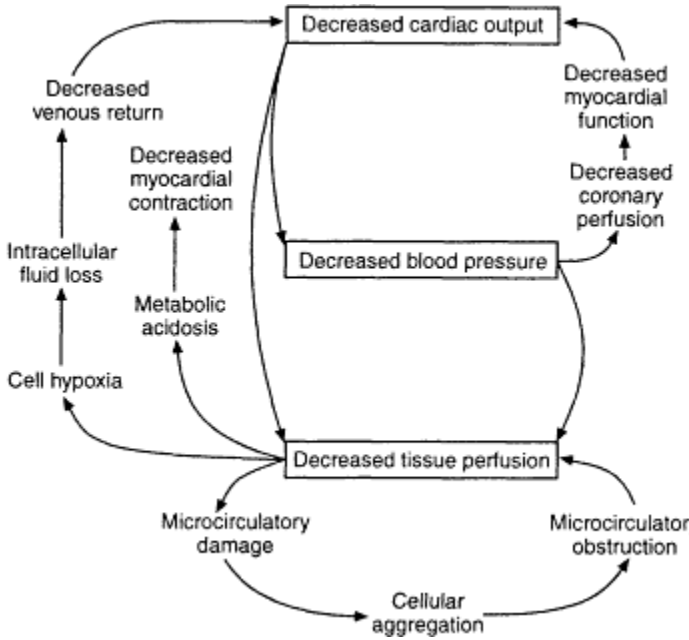


Figure 2-1
Vicious circles in shock. Initiation of shock can occur at any point, but the endpoint is often the same. Source: Reprinted, with permission, from Davis et al., (1995, p. 145). Copyright 1995 by Mosby-Year Book, Inc.

- *Hypovolemic shock*: shock secondary to inadequate circulating volume.
- *Traumatic shock*: shock secondary to inadequate circulatory volume plus soft-tissue injury.
- *Cardiogenic shock*: failure of the heart to provide circulation.
- *Neurogenic shock*: failure of the nervous system to provide peripheral vascular resistance.
- *Septic shock*: hemodynamic instability that may arise as a result of septicemia.

Organ Involvement in Shock

Shock can be evaluated from a number of standpoints (see Table 2-1) but is frequently described in terms of organ failure in the following systems:

- circulatory,
- endocrine,
- metabolic, and
- cellular.

Examples of such alterations are seen in the dynamics of human shock syndromes. Septic shock is manifested by high-output cardiac failure in which there is increased cardiac output but decreased systemic vascular resistance and decreased myocardial contractility. Low-output cardiac failure can also develop in sepsis and reflects a loss of response to catecholamines. Pulmonary changes in shock may include increased lung water levels, increased pulmonary vascular resistance, and increased alveolar-capillary permeability. Hyperpnea may compensate for metabolic acidosis, but the phenomena described above may also result in impaired gas exchange.

The next organ sequentially affected in the organ failure induced by shock is the kidney. Renal failure may ensue as a consequence of shock and, depending on the state of volume resuscitation and other factors, may have the following characteristics:

- initial high level of urine output,
- low pressure in the renal tubules producing sodium retention,
- renal parenchymal damage, and
- renal dysfunction and failure.

Renal failure as a consequence of shock has been a factor in most wars. In the 1973 Yom Kippur War in Israel, the mortality rate among patients who developed renal failure after injury was approximately 60 percent. In the Vietnam conflict in 1972, the mortality rate from renal failure after injury was approximately 70 percent, in the Korean War in 1955, it was about 60 percent, and in World War II, it was about 65 percent. Good civilian series reveal rates of morbidity from acute renal failure after injury that exceed 50 percent. In short, renal failure is a complication of severe shock and, historically in wars and more recently in civilian practice, is associated with a mortality rate of more than 50 percent. Vigorous fluid resuscitation has improved the situation by reducing the incidence of renal failure; early and adequate resuscitation can avoid this dreaded consequence of shock.

TABLE 2-1 Hemodynamic Responses to Different Types of Shock

	Type of Shock			
Indicator	Hypovolemic	Septic	Cardiogenic	Neurogenic
Cardiac index	↓	↑↑	↓↓	↑
Peripheral resistance	↑	↓	↑↑	↓
Venous capacitance	↓	↑	→	→
Blood volume	↓	→	→	→
Core temperature	↓→	↑	→	→
Metabolic effects	Effect	Cause	Effect	Effect
Cellular effects	Effect	Cause	Effect	Effect

NOTE: The hemodynamic response to different types of shock is indicated by arrows to show an increase (↑), severe increase (↑↑), decrease (↓), severe decrease (↓↓), or little effect (→). Hypovolemic shock is from blood loss and the cellular damage occurs as a consequence. By contrast, in septic shock the cellular injury is the initiated event and hemodynamic changes occur as a consequence of the cellular insult.

The gastrointestinal consequences of shock include increased acid production and increased permeability of the gastric mucosa. The increased permeability allows tissue penetration by acids, bacteria, and endotoxins. In the past, these complications resulted in the late morbidity from hemorrhagic gastritis, which has a high mortality rate. As understanding of the physiology of this problem has improved, the treatment of patients with shock with serotonin, H2 blockers, and ant-acids has resulted in a marked decrease in this complication's rate of occurrence.

The liver, like all other organs, responds to shock. The effect on the liver is not well delineated but does result in major changes in bilirubin, isoenzymes, protein synthesis, and, perhaps most importantly, the reticuloendothelial system. Decreased consciousness and changes in neural control mechanisms are the responses of the central nervous system to shock.

The metabolic effects of shock manifest as changes in homeostasis or hyperventilation, respiratory alkalosis, metabolic acidosis, and an excretion of nitrogen, phosphate, potassium, magnesium, zinc, and sulfate. Energy depletion of the cell in shock leads to failure of the sodium and potassium adenosin triphosphatase (ATPase) and a drop in resting membrane potential from ˜90mV to ˜60 mV associated with the loss of intracellular potassium and the translocation of sodium and water into the cytoplasm (Chiao et al., 1990; Fantini et al., 1987). Energy depletion is a consequence of hypoxia-induced failure of the Krebs cycle associated with increased lactate levels. Other biochemical effects of shock include hyperglycemia, gluconeogenesis, glycogenolysis, and the synthesis of triglycerides, free fatty acids, lipoprotein, and acute-phase proteins. The hormonal effects of shock result in glycogenolysis, lipolysis, gluconeogenesis, and insulin resistance. These are the results of an adrenergic stimulus and present hemodynamically as tachycardia and a hyperdynamic state. Immunologically

there is an impaired specific immunity at both the cellular and the hormonal levels; there is also an impaired nonspecific immunity of macrophages and opsonins. Hematologic changes include impaired hematopoiesis, altered hemostasis, and in some case, disseminated intervascular coagulation.

PHYSIOLOGIC RESPONSES TO HEMORRHAGE

Acute hemorrhage produces a decrease in arterial systolic, diastolic, and pulse pressures along with an increase in the pulse rate and a decrease in the cardiac stroke volume. The cutaneous veins are generally collapsed and fill slowly when compressed centrally. The skin is pale, moist, and slightly cyanotic. Usually, the respiration is rapid and shallow (Berne, 1983). However, the early stages of hemorrhage result in the initiation of a number of feedback mechanisms that tend to maintain arterial blood pressure in the presence of a decrease in circulating blood volume and a modest decrease in cardiac output. These regulatory mechanisms include (1) central circulatory reflexes originating from atrial stretch receptors and receptors located in the ventricles of the heart, (2) high-pressure baroreceptor reflexes from the carotid sinus and aortic arch, (3) chemoreceptor reflexes, (4) cerebral ischemia responses, (5) reabsorption of tissue fluids at the level of capillaries, (6) release of endogenous vasoconstrictor substances such as vasopressin, and (7) renal conservation of salt and water.

Early loss of less than 10 percent of the circulating blood volume may be associated with no change in arterial pressure because of compensatory increases in sympathetic nervous system activity and both arterial and venous constrictions initiated from low-pressure cardiac receptors (Chien, 1967). A further reduction in circulating blood volume results in decreased arterial pressure and diminished stimulation of aortic arch and carotid sinus baroreceptors and further decreased stimulation of intracardiac receptors. These alterations cause reduced vagal tone to the heart and increased sympathetic nervous system activity to the heart, arterial vessels, and venous capacitance vessels. The changes in peripheral resistance do not occur uniformly in all vascular beds. The cerebral and coronary circulations, which are not primarily regulated by direct sympathetic innervation, participate less in this response. Although the tachycardia and the increased sympathetic tone to arterioles have been studied extensively, the role of the carotid and aortic arch baroreceptors in regulating the sympathetic mediated tone of the regional venous beds has not been studied as extensively. Regulation of splanchnic capacitance vessels appears to involve conventional carotid and aortic arch baroreceptors and a significant contribution by cardiac receptors. Deep limb vessels are similarly regulated, but cutaneous vessels are less influenced by baroreceptors (Rothe, 1963). The activation of sympathetic innervation to splanchnic and deep limb veins provides a short-term autotransfusion of blood from venous reservoirs. Although more pronounced in some species such as the dog, this reflex mechanism also exists in the human (Guyton, 1986). In the early stages of moderate hemorrhage, the changes in total renal vascular resistance are slight because intrinsic autoregulatory mechanisms

within the kidney tend to maintain renal blood flow. The intense splanchnic and renal vasoconstriction may protect the heart and brain but can eventually lead to ischemic injury of the kidney and bowel resulting in kidney failure and further vascular injury and loss of fluids from the vascular compartment into the interstitial space.

When the arterial pressure falls below 60 millimeters of mercury (mm Hg), hypoxia of the peripheral chemoreceptors in the carotid body (because of decreased perfusion) results in activation of chemoreceptor reflexes. This augments peripheral sympathetic nervous system activity and also provides respiratory stimulation, resulting in increased breathing frequency and often an increase in minute ventilation. At very low levels of arterial pressure, below 40 mm Hg, inadequate cerebral blood flow produces an extremely strong activation of the sympathetic nervous system and intense vasoconstriction in response to cerebral ischemia.

A number of endogenous vasoconstrictors are released during hemorrhage. As a direct response to sympathetic nervous system activation, the release of epinephrine and norepinephrine from the adrenal medulla reinforces the actions of direct sympathetic nervous system innervation of the heart and peripheral circulation. Vasopressin, which is a potent vasoconstrictor, is actively secreted by the posterior pituitary gland in response to hemorrhage. Vasopressin release is activated by both the baroreflexes and receptors located in the left atrium. Diminished renal perfusion results in the secretion of renin from the juxtaglomerular apparatus and the subsequent conversion of angiotensinogen to angiotensin, which is also a powerful vasoconstrictor.

Shock Decompensation

Loss of the ability of the compensatory mechanisms described above to maintain arterial blood pressure and cardiac output in the presence of prolonged hemorrhage is usually the result of decreased cardiac function and failure to maintain sympathetically induced arterial and venous vasoconstriction. The decline in cardiac function and vasoconstriction may be the result of toxic peptides (Lefer, 1978, 1985) released from ischemic tissues in combination with metabolic acidosis. These changes are accompanied by alterations of immune function, aberrations in blood clotting, reticuloendothelial system dysfunction, and an inability to regenerate high-energy phosphate reserves at the cellular level. Tissue injury as a result of prolonged ischemia results in the loss of cell membrane integrity with respect to the maintenance of essential ionic gradients, widespread inhibition of metabolic activity because of mitochondrial dysfunction, and the activation of cellular hydrolases that further contribute to cellular injury.

The goal of early volume replacement in fact is to delay or prevent the chain of events that lead to irreversibility in severe prolonged shock, and restoration of blood volume, treatment of acidosis, and management of the metabolic

derangements can provide temporary restoration of blood pressure and cardiac output in severe prolonged shock. Delay in treatment usually results in death (Guyton, 1986). The question that has been posed repeatedly in shock research is what factor or factors lead to the eventual total deterioration of circulatory compensation. The answer to this question seems to be that beyond a certain point so much tissue injury has resulted in the release of toxic mediators, the destruction of metabolic machinery of the cell by lytic enzymes, and so much acidosis and failure of regulatory mechanisms aimed at preservation of homeostasis that even the most vigorous resuscitative measures are not capable of maintaining life (Hannon et al., 1990). The multifactorial nature of the processes leading to irreversibility in severe shock make it seem unlikely that simple therapeutic measures that occur late in shock will have any significant effect on outcome. It is more likely that early interventions that prevent the late extensive tissue injury phenomena are more likely to succeed in changing the outcome.

Cellular Responses to Shock

Reduced tissue perfusion and the pathologic events reviewed above initiate a syndrome characterized by alterations in:

1. energy metabolism, ion compartmentalization, lipid metabolism, and radical production and metabolism;
2. macrophage function;
3. transcription and translation that may lead to apoptosis; and
4. the secretion of and cellular responsiveness to growth factors.

Although during the last 30 years there has been considerable controversy regarding the ionic content and concentration of intravenous fluids used for resuscitation of patients in hemorrhagic shock, this controversy has not led to further consistent reductions in mortality. This suggests the possibility that the barrier to substantially improved care resides not in minutiae of volume resuscitation protocols but rather in an inadequate understanding of the molecular and cellular responses triggered by the shock syndrome. Therefore particular attention is paid to these phenomena and the causal mechanisms associated with them.

Altered Energy Metabolism, Ion Compartmentalization, Lipid Metabolism, and Radical Production and Metabolism

The physiologic cardiovascular and neurohumoral responses described above are able to provide considerable compensation for loss of up to 30 to 40 percent of the blood volume. However, when shock persists or volume loss continues to increase, hypoperfusion of first the bowel and then other vital organs impinges on the requirement for the delivery of oxygen (O_2) and metabolic

substrates to support adequate cellular levels of high-energy phosphate compounds such as adenosine triphosphate (ATP). The general consequences of this have been studied in a variety of organs, especially in the brain, which is extraordinarily sensitive to damage from ischemia and reperfusion.

ATP can be generated by anaerobic metabolism, which produces a net 2 moles of ATP for each mole of glucose metabolized. The end product of this pathway is pymvate, which is then normally transferred to further oxidative metabolism in the mitochondria. Mitochondrial aerobic metabolism is much more efficient than anaerobic metabolism in the production of ATP; in the aerobic system 36 moles of ATP are generated for each mole of glucose oxidized ultimately to carbon dioxide (CO_2) and water (H_2O). When aerobic metabolism fails because of inadequate O_2 delivery, persistent anaerobic metabolism and the conversion of accumulated pyruvate to lactate by pyruvate dehydrogenase lead to cellular accumulation of unoxidized reducing equivalents and decreased pH because of lactic acidosis.

The mitochondrial metabolism of the substrate generates electrons, which are added to O_2 one electron at a time. That is, the reduction of O_2 is stepwise, and the reaction can be written as follows:

$$O_2 + e^- \rightarrow {}^{\bullet}O_2^- + e^- \rightarrow H_2O_2 + e^- \rightarrow OH^- + {}^{\bullet}OH + e^- \rightarrow 2OH^-,$$

where e^- is an electron, ${}^{\bullet}O_2^-$ is superoxide, H_2O_2 is hydrogen peroxide, ${}^{\bullet}OH$ is the hydroxyl radical, and OH^- is the hydroxide ion. Mitochondria utilize the energy derived from the reduction of O_2 to drive the pumping of protons out of the inner mitochondrial volume into the space between the inner and outer mitochondrial membranes. The phosphorylation of adenosine diphosphate (ADP) to generate ATP is then driven from the energy stored in the hydrogen ion gradient across the inner mitochondrial membrane. Two aspects of this system that are very important to understanding the role of mitochondria in postischemic reperfusion injury are the single-electron reduction of O_2 and the hydrogen ion gradient across the inner mitochondrial membrane.

Depletion of ATP occurs during ischemia; this develops most rapidly in the brain, where the concentration of ATP is reduced to near zero within approximately 5 minutes of complete ischemia (reviewed by O'Neil et al., 1996). This ATP depletion degrades the energy-dependent maintenance of ionic gradients across the plasmalemma, and sodium ions (Na^+) and calcium ions (Ca^{2+}) enter the cell, and potassium ions (K^+) exits the cell down their respective concentration gradients. The Ca^{2+} concentration in the extracellular fluid and within the endoplasmic reticulum (ER) is about 10^4 greater than the cytosolic Ca^{2+} concentration, and early massive overload of the cytosol with Ca^{2+} is a major consequence of tissue ischemia.

Unlike Na^+ and K^+, Ca^{2+} is both a signaling molecule (Clapham, 1995) and a cofactor for a number of important enzymes. Important enzymatic consequences of ischemia-induced cytosolic Ca^{2+} overload and ER Ca^{2+} depletion are activation of (1) phospholipases, (2) the proteolytic enzyme μ-calpain, (3) the

phosphatase calcineurin, and (4) a kinase responsible for inhibition of the mechanism by which the first amino acid is introduced during initiation of protein synthesis. The consequences of all of these events will be addressed below. Another important consequence of cytosolic Ca^{2+} overloading during early re-perfusion is mitochondrial diversion of the energy derived from O_2 reduction to the sequestration of Ca^{2+} instead of ATP production. In the situation of high cytosolic Ca^{2+} concentrations, mitochondria utilize the proton gradient across the inner mitochondrial membrane to drive the influx of Ca^{2+} rather than to drive the production of ATP (Carafoli and Crompton, 1978).

Free fatty acids, and in particular arachidonate, are released from membrane lipids during brain ischemia as a consequence of the activity of both phospholipase C (Abe et al., 1987) and phospholipase A_2 (Drenth et al., 1976; Moskowitz et al., 1984). The concentration of free arachidonate can reach 180 μM during ischemia and remains elevated during early reperfusion (Bazan, 1970; Katsuki and Okuda, 1995; Rehncrona et al., 1982; Umemura, 1990; Yasuda et al., 1985; Yoshida et al., 1980). Oxidative metabolism of arachidonate occurs during re-perfusion and is an important source of superoxide (Bakhle, 1983), which is also generated by reperfused mitochondria. Superoxide is not a potent oxidizer, but it does readily reduce insoluble iron ion (Fe^{3+}) in storage proteins to soluble ferrous iron (Thomas et al., 1985), which catalyzes the formation of strong oxidants that initiate the lipid peroxidation chain reactions (Aust and White, 1986). During brain reperfusion both the release of iron from high-molecular-weight stores (Krause et al., 1987) and lipid peroxidation in selectively vulnerable neurons (White et al., 1993) occur, and similar evidence exists for peroxidative damage to other vital organs. This lipid peroxidation can generate substantial ultrastructural damage to the plasmalemma such that it is no longer able to partition ions (Kumar et al., 1987).

Although the role of ionic iron in catalyzing radical-mediated damage to tissue macromolecules is well established, the identity of the oxidizing chemical species that initiates the injury has been elusive. Recent observations suggest that during early reperfusion there is a burst of cellular nitric oxide (NO) synthesis and that NO can react directly with superoxide to generate the potent oxidizer peroxynitrite. In addition to initiating radical damage to tissue macromolecules, peroxynitrite can nitrosylate amino acid side chains (Alvarez et al., 1999) and modify the structure and activity of enzymes. Phosphorylation of NO synthase inhibits the activity of the enzyme, which is activated by dephosphorylation by calcineurin, and agents that inhibit calcineurin-mediated activation of NO synthase have neuroprotective effects (Dawson et al., 1993)

Radical damage is normally opposed by superoxide dismutase and catalase; transcription for both of the enzymes is regulated by the SP1 housekeeping promoter sequence. The transcription of another set of important antioxidant enzymes comprising the antielectrophile response is regulated by the AP1 promoter (reviewed by O'Neil et al., 1996). Activator Protein 1 (AP1) is a heterodimer comprising c-*Fos* and c-*Jun*; messenger RNAs (mRNAs) for both these transcription factors are generated in response to brain ischemia and reperfusion

(reviewed by O'Neil et al., 1996) but are not efficiently translated because of inhibited protein synthesis, which is discussed below.

Alterations in Macrophage Function

In the 1880s Ilya Metchnikoff demonstrated in the larva of marine starfish that puncture injury induced a massive response by macrophages, the only type of immunologic cell in this simple creature (Meltzer and Nacy, 1987). Macro-phage ligand receptors for cytokines, their secretion of interleukin-1 (IL-1) and tumor necrosis factor α (TNF-α) in response to cell injury and antigen-antibody complexes, and their phagocytic activity indicate the central roles that they play in inflammation. Macrophages present antigen to T lymphocytes, and this together with IL-1 causes T lymphocytes to produce IL-2, an obligate growth factor for T-cell proliferation. IL-1 also acts as a required signaling factor for B-lymphocyte maturation and antibody production (Meltzer and Nacy, 1987).

Circulating IL-1α and IL-1β are not detectable before or after hemorrhagic shock, but elevated levels of IL-1α occur in tissue in response to hemorrhagic shock and are not reduced by resuscitation (Molina et al., 1997). This evidence implicates activation of tissue macrophages. IL-1 signal transduction involves binding to plasmalemmal receptors followed by intracellular phosphorylation cascades that lead to activation of nuclear factor κB (NFκB) (O'Neill, 1995), which is essential for inducible TNF-α transcription (Im et al., 1997). Hemorrhagic shock induces increased levels of macrophage mRNA for TNF-α and IL-6 and increased levels of mesenteric TNF-α and IL-6, and these effects are exacerbated by fluid resuscitation (Tamion et al., 1997). The levels of TNF-α in other tissues are also elevated by hemorrhagic shock and are not reduced in tissue by resuscitation or during the early postresuscitation period (Molina et al., 1997). Circulating TNF-α is normally undetectable but its levels are markedly elevated in hemorrhagic shock (Molina et al., 1997). Endotoxin shock induces similar alterations in the cytokines, but in this case volume resuscitation does not enhance the effect (Tamion et al, 1997). Kupffer cells also release IL-6 in response to trauma-induced hemorrhage (Wichmann et al., 1997). The initial macrophage-mediated cytokine response is followed by reduced immunocompetence of the macrophages, and it has been shown that the shift to cytokine release by macrophages following hemorrhagic shock is associated with a reduced capacity for antigen presentation by macrophages (Ayala et al, 1996).

Pulmonary infection following hemorrhagic shock can greatly augment leukocyte sequestration in the lung. This effect is associated with alveolar macrophage expression of cytokine-induced chemoattractant (CINC) mRNA and protein but not with expression of macrophage-inflammatory protein 2, and it is inhibited by treatment with an antioxidant or antibody against CINC (Fan et al., 1998). Leukocyte adhesion and penetration into the endothelium also depend on expression of the β-integrins CD11b/CD18 (Sun et al., 1996), which in patients are induced by hemorrhagic shock associated with lactic acidosis

(Botha et al., 1997). Finally, focal injury to some organs can induce a generalized response throughout the body. Brain compression as a primary insult upregulates mRNA expression of lymphocyte and macrophage products in a wide variety of peripheral tissues, and this is a major concern in the use of organs from brain-dead donors (Takada et al., 1998).

A much more detailed knowledge of the signal transduction and of the transcriptional and translational regulation of these phenomena of the inflammatory response is highly desirable. For example, ligand activation of the TNF-α receptor has both pro- and antiapoptotic consequences, and thus, macrophage activation may have a significant impact on the survival of cells in many organs. The development of such detailed knowledge of the regulation of fundamental cellular processes by cytokines is very likely to provide specific molecular approaches to patient management.

Surgical Bleeding Disorders

Hemostasis is the physiologic cessation of bleeding. The ability to achieve adequate hemostasis is critical to the success of surgical operations and recovery from injury. Under normal circumstances, blood maintains its fluidity because of the balance of various procoagulant and anticoagulant influences, including interactions at the blood-endothelium interface and a variety of circulating factors (Pearson, 1994). Hemostatic and coagulation mechanisms allow the prompt repair of a local injury in the microcirculation without progression to a systemic reaction. Injured blood vessels can therefore be repaired and hemorrhage can be controlled locally while blood continues to flow normally in other uninjured areas. Once the injury to the blood vessel has been repaired, the lysis of clots that had formed in the area begins and the vessel may ultimately regain patency. Localized injury to the vascular system thus activates hemostatic mechanisms at the injury site and circulating coagulation mechanisms. Once healing has occurred, a system of fibrinolysis reestablishes patency of the blood vessel and degrades the products of coagulation.

Fundamental Alterations in Transcription and Translation: Apoptosis

During the public presentations made to the committee as background for the preparation of this report, apoptosis was repeatedly implicated in the progression of tissue injury associated with hemorrhagic shock and volume resuscitation. Apoptosis is a complex mechanism of cell suicide and is triggered by intracellular molecular signals that may originate from a variety of subcellular organelles including the plasmalemma, mitochondria, endoplasmic reticulum, and nucleus (Bredesen, 1996; Hale et al., 1995). Apoptosis in the context of tissue ischemia and reperfusion must be viewed in a way that sorts the molecu

lar mechanisms into those involved in (1) actual execution of cell death and (2) signaling that activates the execution program.

In early work, the generation of DNA fragments with lengths that were multiples of the interhistone distance (approximately 180 nucleotides)—reflecting double-strand breaks generated by dioxyribonuclease (DNase) action in internucleosomal regions—was thought to be the hallmark of apoptosis (Arends et al., 1990; Hale et al., 1995). Increased intracellular Ca^{2+} levels were found to be involved in stimulation of the DNase(s) activity (Arends et al., 1990), which is inhibited by zinc. Subsequently, it was recognized that additional morphologic alterations are characteristic of apoptosis; these include chromatin condensation, extensive alterations in the microtubular array, fragmentation of the cell into "apoptotic bodies," and detachment of the cell from its neighbors or the culture substrate (Hale et al., 1995). The alterations in a specific instance of apoptosis are somewhat variable, and it has now become clear that cytoplasmic apoptosis can occur without DNA fragmentation (Jacobson et al., 1994).

During development, the human fetus generates many more cells than are finally needed (Hale et al., 1995), and developmental apoptosis has been extensively studied in *Caenorhabditis elegans*, which generates exactly 1,090 cells, of which 131 undergo apoptosis (Ellis et al., 1991). Particularly important genes of the apoptosis system in this organism include *ced-3* (*C. elegans* death gene), *ced-4*, and *ced-9* (Hale et al., 1995). The peptide products of these genes are homologous to mammalian proteins: CED-3 is homologous to cysteine-dependent aspartate proteases (caspases) (Hale et al., 1995), CED-4 is homologous to apoptosis-activating factor 1 (APAF-1) (Zou et al., 1997), and CED-9 is homologous to the antiapoptotic protein Bcl-2 (Hale et al., 1995).

Mammalian plasma membrane receptors for the activation of caspases include FAS and the tumor necrosis factor receptor (TNFR) (Nagata and Golstein, 1995). Both FAS and TNFR include a cytoplasmic domain of approximately 70 amino acids called the "death domain" (Hale et al., 1995), which is a protein-protein interaction zone also present in the molecules FADD (MORT1) and CRADD (Hale et al., 1995). Ligand binding to FAS or TNFR leads to recruitment of FADD or CRADD by association of death domains (Ahmad et al., 1997). FADD and CRADD are adapter linkers (Ahmad et al., 1997) and recruit Machl (FLICE, caspase 8), which then activates other caspases by cleaving zymogen procaspases into the active proteases (Ahmad et al., 1997). Several viruses encode death domain-containing proteins that inhibit receptor-induced apoptosis (Bertin et al., 1997; Muzio et al., 1997; Zhou et al., 1997), indicating that a key function of the receptor-mediated caspase activation system is to provide a viral defense in which infected cells are induced to die.

The level of the FAS protein is increased approximately 130-fold in post-ischemic myocytes at 2 hours reperfusion following myocardial ischemia (Kajstura et al., 1996), and it is clear that apoptosis is involved in the post-ischemic death of cardiac myocytes (Brömme and Holtz, 1996). The situation with regard to plasmalemma receptor-induced apoptosis during brain reperfusion is different. There is little *FAS* mRNA in normal brains, and even after 30

minutes of ischemia and 6 hours of reperfusion *FAS* mRNA is not found in vulnerable hippocampal or cortical neurons (Matsuyama et al., 1994). Similarly, Chopp's group (Zhang et al., 1997) showed appearance of TNFR as quickly as 1 hour after middle cerebral artery occlusion in mice, but the protein was localized to microvessels. Furthermore, TNFR-knockout mice showed exacerbated brain damage in response to focal ischemia (Brace et al., 1996), possibly because TNFR also leads to activation of the transcription factor NFκB (Der et al., 1997), which counterregulates apoptotic mechanisms.

Although a plasmalemma receptor-activated caspase response is not a primary death mechanism in the vulnerable neurons, the apoptotic mechanisms are more complex than the FAS, FADD/CRADD, and Machl system. For example, although the traditional view has been that caspase expression is constitutive (Muzio et al., 1997), ischemia and reperfusion induce in vulnerable neurons the transcription of CPP32 (Gillardon et al., 1997) (caspase 3, apopain, Yama), which is required for normal fetal brain development (Kuida et al., 1996) but which is essentially absent from the adult neurons (Ni et al., 1997). The relatively specific CPP32 inhibitor *N*-benzyloxycarbonyl-Asp-Glu-Val-Asp-flouromethylketone (where Asp is aspartic acid; Glu is glutamic acid; and Val is valine) decreased the amounts of caspase cleavage products, reduced the level of tissue damage, and improved functional outcome in a stroke model in rats and mice (Hara et al., 1997).

In various model systems CPP32 is activated before several other caspases (Martins et al., 1997) and is the predominant enzyme involved in the very rapid cleavage of the DNA repair enzymes poly(ADP ribose) polymerase (PARP) (Margolin et al., 1997; Van de Craen et al., 1997) and DNA-dependent protein kinase (Le Romancer et al., 1996). PARP is substantially conserved from *Drosophila* to humans (Uchida et al., 1993), recognizes strand nicks in DNA, and marks damaged DNA for repair by generating poly(ADP ribose) chains on adjacent nuclear proteins (Rosenthal et al., 1997). PARP is required for cellular recovery after radical-mediated DNA damage (Shah et al., 1996), upregulates the tumor suppressor p53 (Whitacre et al., 1995), and is required for insertion of retrovirus-derived DNA into the genome (Gaken et al., 1996). CPP32 also cleaves actin to produce a characteristic 15-kilodalton (kDa) peptide recognized by a specific antibody (Mashima et al., 1997), which represents a potentially important assay for apoptosis.

The details of non-receptor-mediated caspase mechanisms involved in an apoptosis occurrence can reflect the initiating event. Nedd2 is another caspase that, like CPP32, is expressed in neural precursor cells, is developmentally downregulated (Allet et al., 1996), and efficiently cleaves PARP (Gu et al., 1995). The processing of Nedd2 from the proenzyme to the active form is inhibited in a neural cell line by Bcl-2 (Srinivasan et al., 1996). Reduction of Nedd2 synthesis by antisense mRNA rescues both sympathetic neurons and PC12 cells from apoptosis caused by growth factor withdrawal (Troy et al., 1997). However, in these same cells apoptosis induced by superoxide dismutase

deficiency is inhibited by fluoromethylketone caspase inhibitors but not by the reduction of Nedd2 (Troy et al., 1997).

The prototypic caspase CED-3 can be activated by direct association with CED-4 (Irmler et al., 1997). Transgenic expression of CED-4 in apoptosis-naive *Schizosaccharomyces pombe* induces chromatin condensation and death associated with immunolocalization of CED-4 to the nucleus (James et al., 1997). In this model system mutation of the nucleotide-binding motif of CED-4 eliminates the lethal effects, and coexpression of CED-4 and CED-9 blocks the nuclear localization of CED-4 and its lethal effects (James et al., 1997) through a direct molecular interaction (Spector et al., 1997). The analogous mammalian system appears to involve activation of CPP-32 by a complex of APAF-1 and cytochrome *c* (Zou et al., 1997) released from mitochondria after the formation of a nonspecific large pore (approximately 2 nm) in the mitochondrial membrane under the simultaneous influence of matrix Ca^{2+} overload and radical stress (Andreeva et al., 1995). It is interesting that the iron chelator deferoxamine blocks radical-associated mitochondrial permeability transition and cell death (Nieminen et al., 1997). The human genes encoding CED-9-related proteins include *Bcl-2, Bax, Bad, Bcl-x, mcl-2, Bak*, and *Bak-2* (Hale et al., 1995). Bcl-2 inhibits the processing of caspase proenzymes to active proteases (Srinivasan et al., 1996) and the release of Ca^{2+} from ER (Lam et al., 1994), an event that, as shown below, is crucial to the activation of intracellular apoptosis mechanisms. Several mammalian viruses carry genes encoding Bcl-2 homologues that inhibit apoptosis (Henderson et al., 1993).

Bax forms ion channels in mitochondrial and ER membranes and promotes apoptosis; Bcl-2 binds to Bax and blocks these channels (Antonsson et al., 1997). The tumor suppressor p53 both upregulates Bax and downregulates Bcl-2 transcription (Hale et al., 1995). These observations suggest that the ratio of Bcl-2 to Bax is important in the regulation of apoptosis.

It is becoming clear that major changes in the translation initiation system for protein synthesis are crucial in apoptosis. During translation initiation, mRNA selection and delivery of the first amino acid (methionine) for the new peptide are two critical steps that are affected by the mechanisms of apoptosis. mRNA is delivered to the translation initiation complex by eukaryotic initiation factor-4 (eIF4). Most mammalian mRNAs include an m^7GTP (where GTP is guanosine triphosphate) cap that is bound by the subunit eIF4E. The eIF4 complex also includes eIF4A and eIF4B, which facilitate unwinding of secondary structure in the 5' leader of the mRNA, and eIF4G, which provides docking sites for the other eIF4 subunits and for attachment via eIF3 to the small ribosomal subunit. Methionine is delivered to the small ribosomal subunit by a ternary complex that comprises eukaryotic initiation factor-2 (eIF2), GTP, and methionine-transfer RNA (met-tRNA) (Merrick, 1992). Translation initiation leads to hydrolysis of the eIF2-associated GTP and generation of an eIF2-GDP (where GDP is guanosine diphosphate) complex. The enzyme eIF2B must then exchange that GDP for another GTP on eIF2 before the next round of translation initiation. This GTP exchange reaction is competitively inhibited by eIF2 con

taining a serine at position 51 (ser-51)-phosphorylated α-subunit [eIF2α(P)] (Rowlands et al., 1988). Because the molar ratio of eIF2 to eIF2B is about 5 to 1, when approximately 20 percent of eIF2 is in the eIF2α(P) form, new protein synthesis is substantially inhibited.

Apoptosis is associated with caspase-mediated degradation of eIF4G (Clemens et al., 1998), which is also degraded during brain ischemia by the calcium-activated protease μ-calpain (Neumar, 1998) and by an unknown protease during early brain reperfusion (DeGracia et al., 1996). Several studies have directly implicated activation of calpain in apoptosis (Estaquier et al., 1996; Jordan et al., 1997; Squier and Cohen, 1997). Furthermore, there is a more than 20-fold increase in the brain eIF2α (P) level so that this isoform represents over 23 percent of total eIF2 within the first 10 minutes of post ischemic reperfusion, and eIF2α(P) maps to selectively vulnerable neurons that display chromatin condensation consistent with early apoptosis (DeGracia et al., 1996, 1997). Kaufman's group has shown that expression of a mutant that substitutes alanine (which cannot be phosphorylated) for the Ser-51 phosphorylation site in eIF2α blocked TNF-α-induced apoptosis (Kaufman and Srivastava, 1996) and induced downregulation of Bax expression (Kaufman et al., 1998). Furthermore, expression of a conditional eIF2α mutant imitating the negative charge at Ser-51-P (by substituting aspartate for Set-51) induced immediate apoptosis (Kaufman and Srivastava, 1996) and activation of CPP32 (Kaufman et al., 1998).

The proteolytic degradation of eIF4G and phosphorylation of eIF2α not only will result in general depression of overall protein synthesis, but will also exert a major effect on message selection for residual translation. Although m^7GTP cap-dependent translation cannot occur without a fully competent eIF4 complex, eukaryotic mRNAs containing internal ribosome entry site sequences can circumvent this limitation. Furthermore, long 5' mRNA leaders before the AUG start codon are known to favor translation in the presence of elevated eIF2α(P) levels because the long leader prolongs ribosomal scanning time and thus the opportunity to find a good ternary complex. Although little is known about the characteristics of proapoptotic mRNAs, these alterations in the translation initiation system could favor them. Indeed, mRNA leaders with these characteristics are present on the message for APAF-1 (Doldwell et al., 1998) and several stress-response proteins including platelet-derived growth factor-2 (Sella et al., 1998), vascular endothelial growth factor (Stein et al., 1998), fibroblast growth factor-2 (Vagner et al., 1996), and the X-linked inhibitor of apoptosis (Holcik et al., 1998).

The mechanism for phosphorylation of eIF2α in response to ischemia and reperfusion is not understood as well as the degradation of eIF4G by proteases. Phosphoprotein phosphatase 1 is responsible for the dephosphorylation of eIF2α(P) (DeGracia et al., 1996), and ischemia and reperfusion do not appear to cause a loss of this phosphatase activity (DeGracia et al., 1998). Three mammalian eIF2α kinases are known to exist (Samuel, 1993; Sood et al., 1998; Wek, 1994): (1) the hemin-regulated inhibitor (HRI kinase), which is also activated by

transition metals and lipid hydroperoxides, (2) the "double-stranded viral RNA-activated" protein kinase (PKR), and (3) GCN2.

A major function of PKR is to block virus-directed protein synthesis (Proud, 1995). In this regard, a number of viruses, including human immunodeficiency virus (HIV) and members of the smallpox virus family, encode proteins that are PKR inhibitors (Jagus and Gray, 1994). Recent observations have revealed a much broader cellular role for PKR (Petryshyn et al., 1994; Proud, 1995) and have identified a number of endogenous PKR inhibitors and activators (Jagus and Gray, 1994). Transcription of the *PKR* gene is induced by interferon, and PKR activity is antagonized by intracellular signaling activated by insulin-family growth factor receptors (Bandyopadhyhay and Sen, 1992). It is particularly interesting that activated PKR appears to be required for induced transcription of the gene for the FAS receptor (Der et al., 1997; Takizawa et al., 1995). Cells from PKR-knockout mice are resistant to apoptosis induced by TNF-α, lipopolysachrides, or viral RNA (Der et al., 1997; Kaufman and Srivastava, 1996). However, phosphorylation of eIF2α during early brain reperfusion is not reduced in PKR knockout mice.

Depletion of Ca^{2+} from the ER leads to a rapid suppression of protein synthesis (Brostrom et al., 1983, 1985, 1989; Chin et al., 1987; Kumar et al., 1989) by phosphorylation of eIF2α (Kimball and Jefferson, 1992; Prostko et al., 1992, 1993); the detailed mechanism of this is unknown. However, ER Ca^{2+} depletion, eIF2α phosphorylation, and suppression of protein synthesis are induced by free arachidonate concentrations above 10 micromolar (μM) in cell culture models (Fleming and Mellow, 1995; Rotman et al., 1992). On the ER, two Ca^{2+} channel-regulating receptors are known: the ryanodine and the inositol triphosphate (IP-3) receptors (RyR and IP-3R). Both receptors are present in vulnerable CA1 neurons, with IP-3R being more prominent in these cells and RyR being more prominent in the vulnerable hilar neurons (Sharp et al., 1993). Arachidonate appears to compete at the ryanodine receptor (Uehara et al., 1996) and has not been studied at the IP-3 receptor. However, it has been shown that IP-3R-deficient T cells are resistant to apoptosis induced by ionizing radiation, FAS ligand, and glucocorticoids (Marks, 1997).

Both RyR and IP-3R are formed by a tetramer of their respective primary proteins, and one 12-kDa peptide that binds to the transplant immunosuppressant tacrolimus (or FK506, thus, the FK-binding protein [FKBP12]) is associated with each monomer of both receptors (Cameron et al., 1995; Marks, 1996). Expression of cloned RyR in otherwise RyR- and FKBP12-naive *Spodoptera frugiperda* generated tetrameric RyR receptors that exhibited only Ca^{2+} subconductance at four distinct levels, but coexpression of FKBP 12 generated channels that exhibited full ligand-activated conductance (Marks, 1996). FK506 dissociates FKBP12 from both receptors (Cameron et al., 1995; Timermann et al., 1995), resulting in only Ca^{2+} subconductance (Marks, 1996). Thus, the overall effect of FK506 is to reduce ER Ca^{2+} conductance in response to these ligands.

FK506 has been shown to salvage vulnerable neurons after brain ischemia (Sharkey and Butcher, 1994) and to allow them to synthesize stress-response

proteins in response to copious transcript formation, which FK506 did not affect during reperfusion (Kamlya et al., 1997; Katayama et al., 1997). FK506 is a macrolide antifungal immunosuppressant (Thomson et al., 1995) structurally related to rapamycin but not to cyclosporin A (Bierer et al., 1991), neither of which are as neuroprotective during reperfusion (Sharkey and Butcher, 1994). Cyclosporin A and FK506 bind to cellular proteins called immunophilins (Thomson et al., 1995), many of which catalyze the *cis-trans* isomerization of peptidyl-prolyl bonds (by means of peptidyl-prolyl isomerase) (Bierer et al., 1991) and in conjunction with heat shock proteins help peptides fold correctly (Fischer et al., 1993; Galat, 1993). Immunophilins are classified as cyclophilins and FK-binding proteins. Cyclophilins A, B, and C are DNases (Montague et al., 1997). The DNase activities of cyclophilins A and B are stimulated by increased Ca^{2+} levels and are inhibited by K^{+} (50 percent inhibitory concentration = 83 millimolar [mM] concentration) (Montague et al., 1997), and NUC 18, thought to be responsible for apoptotic internucleosomal DNA fragmentation (Montague et al., 1994), appears to be identical to cyclophilin A (Wine et al., 1997). Recent evidence suggests that NUC 18 is activated by proteolysis of an approximately 60-kDa precursor (Fraser et al., 1996). An approximately 21-kDa cyclophilin (Tanveer et al., 1996) is an integral part of the pore formed in the mitochondrial membrane under the simultaneous influence of matrix Ca^{2+} overload and radical stress (Andreeva et al., 1995), and both cyclosporin A and FK506 inhibit Ca^{2+} transit of this pore (Schweizer et al., 1993). Complexes of immunophilins and cyclosporin A or FK506 also inhibit calcineurin (Liu et al., 1992), which dephosphorylates and activates NO synthetase (Dawson et al., 1993).

In summary, apoptotic signals originate from cellular organelles. At the plasmalemma this starts with FAS or TNF-α binding to receptors; in the case of mitochondrial damage it involves the release of cytochrome *c* which interacts with APAF-1; at the ER activation of IP-3R or RyR by ligands that may emerge from membrane damage leads to ER Ca^{2+} depletion and eIF2α phosphorylation; and in the nucleus sufficient DNA damage and poly(ADP) ribosylation activate p53, which leads to enhanced *Bax* transcription and repressed *Bcl-2* transcription, with the consequence of pore formation in the ER and mitochondria by Bax. All multicellular eukaryotic organisms appear to have apoptotic systems, but single-celled eukaryotes (yeast) are naive. Finally, viruses have developed a variety of mechanisms that inhibit eukaryotic apoptosis. These observations indicate that both apoptosis and viral resistance to apoptotic mechanisms emerged after the development of multicelled eukaryotes and suggest that execution mechanisms of inhibition of protein synthesis, proteolytic degradation of a DNA recombination mechanism (PARP), and degradation of polynucleotides are all primarily antiviral strategies. This theoretical approach has the advantage of not requiring more or less simultaneous evolution of apoptosis execution and regulation strategies for a primary role in embryogenesis and suggests that the growth factor signal transduction mechanisms that govern developmental apoptosis were developed later in evolution.

From this basic perspective, the complexity of the system arises from the multiple input pathways and the interconnection of the execution mechanisms. For example, Ca^{2+} release from the ER can be induced by activation of IP-3R or RyR or by an excess of Bax over *Bcl-2*. This can lead to PKR activation, the consequent inhibition of protein synthesis, and the stimulus of transcription for FAS, which invites activation of the caspase system followed by degradation of PARP and induction of DNase activity. Similarly, primary DNA damage would induce PARP activity followed by p53 activation, Bax expression, ER Ca^{2+} depletion, formation of pathologic mitochondrial pores, and so on. Membrane receptor-induced caspase activity results in degradation of eIF4G as well as degradation of PARP and cytoskeletal components.

This suggests that apoptosis induced by acute disease processes involves inappropriate activation of an ancient viral defense system that leads to the death of cells essential to survival of the organism. Therapeutic approaches to this system that are beginning to emerge include the use of inhibitors of calpain and caspases and agents that appear to have diverse antiapoptotic activity such as cyclosporin, FK506, and peptide growth factors.

Alterations in Secretion of and Cellular Responsiveness to Growth Factors

Insulin and other growth factors such as nerve growth factor, insulin-like growth factor-1 (IGF-1) (Zhu and Auer, 1994), and fibroblast growth factor have established neuron-sparing effects in the setting of ischemia and reperfusion (reviewed by O'Neil et al., 1996). In the case of insulin, this effect does not involve reduction of blood sugar (Voll and Auer, 1991a), and on the basis of doses thought to be pharmacologically equivalent, insulin is more effective than IGF-1 (Zhu and Auer, 1994). Other studies have also shown that insulin improves neurologic deficit scores and cognitive function and prevents necrotizing brain damage after transient forebrain ischemia (Fukuoka and Yeh, 1989; Strong et al., 1990; Voll and Auer, 1991b).

The evidence presented above suggests that insulin has a neuron-sparing effect that is mediated by direct interaction with receptors in the central nervous system (Zhu and Auer, 1994), even though insulin does not regulate glucose handling in the brain. Insulin is known to bind to only two receptors: the insulin receptor and the IGF-1 receptor. It does not bind to the IGF-2 receptor (Le Roith et al., 1993) or the insulin receptor-related receptor (Jui et al., 1994; Zhang and Roth, 1992). Insulin binds in the dentate and CA1 regions of the hippocampus (Hill et al., 1986; Kar et al., 1993; Werther et al., 1987), and its receptor is found most prominently on the perikarya and processes of the CA1 and CA2 pyramidal cells and the neurons of the dentate gyms; immunohistochemistry does not identify the insulin receptor on glia (Unger et al., 1989). The insulin receptor comprises two covalently linked homodimers formed by its α and β subunits. Ligand binding activates auto-tyrosine phosphorylation of the β subunit fol

lowed by tyrosine phosphorylation of other peptide substrates, including insulin-receptor substrate-1 (IRS-1). Srchomology-2 domains on phosphorylated IRS-1 activate intracellular signaling cascades. Both pancreatic insulin secretion and the kinase activity of the insulin receptor are markedly downregulated by adrenergic signaling mediated through cyclic adenosine monophosphate and protein kinase A (PKA), and hypovolemia is clinically well known to be associated with insulin resistance. Thus, insulin-mediated growth factor signaling is inhibited by ischemia and reperfusion.

Sullivan and colleagues (1998) have recently used simultaneous autoradiography of pulse-labeled protein synthesis and immunohistochemical mapping of eIF2α(P) to confirm the colocalization of inhibited protein synthesis and eIF2α(P) during brain reperfusion. That same study found that 20 units of insulin per kilogram of body weight administered intravenously at reperfusion caused restoration of normal protein synthesis in and elimination of eIF2α(P) from vulnerable hippocampal neurons by 90 minutes of reperfusion after a 10-minute cardiac arrest.

There is precedent for insulin-mediated downregulation of eIF2α kinases; activation of Ras, an intermediate in insulin signaling, leads to activation of a 97-kDa inhibitor of PKR (Bandyopadhyay and Sen, 1992), although as already indicated, this enzyme is not required for phosphorylation of eIF2α during brain reperfusion. The reversal of eIF2α phosphorylation in vulnerable neurons by insulin during early reperfusion also might be due to the activation of an eIF2α(P) phosphatase. PP1, which is activated in response to insulin signaling (Begum, 1995; Srinivasan and Begum, 1994), is the enzyme responsible for the dephosphorylation of eIF2α(P) in vivo (Ernst et al., 1982; Foulkes et al., 1983; Ingebritsen and Cohen, 1983; Redpath and Proud, 1990), and isoforms PP1α and PP1γ are present in the brain and are concentrated in the neocortex and hippocampus (Hubbard and Cohen, 1993; Ouimet et al, 1995; Takizawa et al., 1994).

There are several possible explanations for the high dose of insulin required for neuron sparing during postischemic reperfusion. Adrenergic downregulation of insulin secretion and of the insulin receptor itself has already been mentioned. Glucocorticoids also inhibit insulin transport into the central nervous system (Baura et al., 1996). Alternatively, other signaling mechanisms may also decrease the responsiveness of the insulin receptor. TNF-α levels are elevated in the reperfused brain (Lavine et al., 1998), and TNF-α induces insulin resistance by increasing serine and threonine phosphorylation of the insulin receptor and of the major insulin receptor substrates IRS-1 and IRS-2 (Paz et al., 1997). It is also possible that the neuroprotective effects of insulin occur by activation of the IGF-1 receptor (Gluckman et al., 1993). Although insulin and IGF-1 have significant sequence homology, the affinity of insulin for the IGF-1 receptor is about 100-fold lower than that of IGF-1 (Le Roith et al., 1993). Autoradiographic studies have shown large quantities of [^{125}I]IGF-1 receptors in hippocampal neurons (Bohannon et al., 1988; Kar et al., 1993; Lesniak et al., 1988).

IGF-1 has also been shown to have a neuroprotective effect in transient forebrain ischemia.

It is noted that insulin resistance and hyperglycemia in patients in shock is a well known phenomenon. The above adrenergic and TNF-α signaling mechanisms that down-regulate insulin secretion, the tyrosine kinase activity of the insulin receptor, and the major intracellular substrates of the insulin receptor are in play in shock and are likely involved in the associated insulin resistance and hyperglycemia.

HEMATOLOGIC ABNORMALITIES ASSOCIATED WITH SHOCK AND RESUSCITATION

The administration of blood products and the management of bleeding disorders are important therapeutic modalities used by surgeons caring for patients with a wide variety of acute and chronic problems. When used with a thorough understanding of appropriate indications, risks, and benefits, blood transfusion is safe and effective. Because blood transfusion is lifesaving for many patients, a knowledge of the appropriate indications, potential risks, and available alternatives should allow clinicians to exercise judgment in using this important resource.

Although it is now routine, the ability to successfully transfuse blood is relatively recent. Accounts of bloodletting and phlebotomy, but not blood transfusion, appear in many early historical references and were recommended for many ailments, including insanity. The routine, safe administration of blood products required several important scientific advances. The discovery of the A, B, and O blood types by Landsteiner in 1900 and the AB blood type by Von Decastello and Sturli in 1902 began the era of modem blood transfusion. By the 1940s, techniques of cross-matching, anticoagulation, and storage of blood and the establishment of blood banks made routine blood transfusion possible. The ability to replace blood lost intraoperatively is an important prerequisite in modem surgical practice.

Modem blood banking is based on the concept that a donated unit of blood can be divided into its components and the different components can be applied to the specific needs of a patient, such as a heightened need for clotting factors. Specifically, after donation, platelets whose shelf life is much shorter than red blood cells, are removed, making them available for platelet transfusion. Clotting factors are also removed, and the residual fluid contains mostly red blood cells suspended in plasma. In the military, citrate-preserved blood (i.e., without platelets) has been the standard transfusion fluid for over 50 years. On a practical basis, any stored blood should be assumed to be deficient in both clotting factors and platelets. From time to time, in acute situations, donors provide whole blood for very specific but quite unusual situations. In some past military experiences, however, inaccuracy in identification of the blood type made the possibility of a severe or fatal reaction occurring from mismatched blood.

Transfusion of the Patient in Shock

During World War I, it was believed that vascular collapse in injured patients was caused by toxins (MacLean, 1985). Experiments in the 1930s showed that fluid was lost from the circulation into damaged tissues. In World War II, plasma became the resuscitation fluid of choice. Subsequent experimental work indicated that extracellular fluids shifted into the intracellular space after significant hemorrhage with shock (Canizaro, 1973). The provision of resuscitation fluid in a volume in excess of the volume of blood that had been shed then became an acceptable practice for the maintenance of adequate circulation.

During World War II, acute tubular necrosis was a common consequence of hypovolemic shock. Because fluid resuscitation became more prevalent during the Korean and Vietnam conflicts, the incidence of acute tubular necrosis dramatically decreased. Although acute tubular necrosis after hypovolemic shock became less of a problem with better fluid resuscitation, the *shock lung* syndrome (i.e., adult respiratory distress syndrome) became increasingly common. The lung injury in adult respiratory distress syndrome is a function of the shock state rather than the resuscitation solution used.

The goal of resuscitation from shock is prompt restoration of adequate perfusion and transport of oxygen. Restoration of circulation allows the cell to clear the products of anaerobic metabolism and restore aerobic metabolism. The American College of Surgeons Committee on Trauma developed a classification of shock that permits useful guidelines for resuscitation (1997). Crystalloid is infused at a 3:1 ratio for every unit of red blood cells administered, and therapy is monitored by hemodynamic response. Because crystalloid solutions are universally available and some delay is required for the preparation of blood products, crystalloid is the proper initial resuscitation fluid. Resuscitation then proceeds with the use of blood products, depending on the patient's response. The choice of a colloid solution (e.g., albumin or plasma) or a crystalloid solution (e.g., lactated Ringer's solution) has been controversial. Both can expand the extracellular space and provide effective resuscitation. However, crystalloid solutions are favored because they are less expensive, need not be cross-matched with the patient, do not transmit disease, and probably create less fluid accumulation in the lungs. No experimental data indicate that colloid solutions are less apt to prevent pulmonary edema than are crystalloid solutions. Some work (Holcroft and Trunkey, 1975; Lewis et al., 1979), based on measurements of pulmonary extravascular water volume or lung water measurements, indicates that albumin, if used as a resuscitation fluid, moves across the cell membrane and draws in extracellular fluid by osmosis, thereby exacerbating the pulmonary edema.

Several crystalloid solutions are available for resuscitation, but isotonic solutions should be used to avoid overload of free water. Lactated Ringer's solution has been recommended as initial therapy. Metabolic alkalosis is common after successful resuscitation with lactated Ringer's solution and blood products, because the lactate in Ringer's solution and the citrate in banked blood are both

converted to bicarbonate if the patient has a functioning, perfused liver. Lactated Ringer's solution contains calcium, and if it is mixed with a unit of blood product, the blood may clot in the bag. Normal saline solution is an acceptable alternative to lactated Ringer's solution, but large volumes can produce a hyperchloremic metabolic acidosis, which may complicate the care of the patient in shock.

Massive Transfusion

Massive transfusion has been defined as replacement of the patient's blood volume with stored red blood cells in 24 hours or as transfusion of more than 10 units of blood over a few hours. Massive transfusion can create significant changes in the patient's metabolic status because of the infusion of large volumes of cold citrate-containing blood that has undergone changes during storage (Canizaro, 1973). If a large volume of stored blood is infused rapidly, significant effects may be seen in the recipient, depending on the recipient's metabolic state, including (1) significant degrees of hypothermia, exacerbated in patients who have an open thoracic or abdominal cavity, which accelerates heat loss, increases the affinity of hemoglobin for oxygen, impairs the function of platelets, and increases the potential for hypocalcemia; (2) production of alkalosis with subsequent undesirable effects on myocardial contractility; and (3) changes due to citrate, such as hypotension, narrowed pulse pressure, and elevated left ventricular, end-diastolic, pulmonary artery, and central venous pressures. Other effects include dilutional thrombocytopenia due to the fact that the number of platelets is almost nil in blood stored for 24 hours.

In summary, although a massively resuscitated and transfused patient may develop bleeding disorders because of shock and resuscitation, the major changes in the massively transfused patient are opposite what one might expect on the basis of the changes that occur in blood during storage. The administration of liquid-preserved blood and crystalloid, the usual routine for the resuscitation of hypovolemic patients, results in profound alterations in the amounts of clotting factors, for example. Additionally, platelet counts fall from the normal value of 250,000 to 300,000 µ1 to approximately 50,000 µ1. This phenomenon, called dilutional thrombocytopenia, occurs in the context of lowering primarily clotting Factors V and VIII and prolongs the clotting time. The clotting time, however, in successful resuscitation will usually not be associated with an excess tendency for bleeding. In other words, although laboratory demonstration of the prolongation of clotting time presents a potential problem, in reality, excess bleeding in groups of patients studied in this situation rarely occurs. Similarly, the use of citrate-preserved blood results in a net effect of alkalosis after one pass through the liver following resuscitation. Transient lowering of the ionized calcium is a theoretical problem, but the resuscitated patient will mobilize enough ionized calcium to balance any transient lowering of ionized calcium. Whole blood usually increases levels of potassium because of the progressive death of red blood cells in liquid preservation. This theoretically could

produce a problem in the face of lowered calcium and potassium load. The realities of this are, however, that the metabolic environment of the resuscitation situation is such where sodium is retained and potassium wasted. The net effect of massive transfusion, then, is a relatively normal level of calcium and a somewhat lowered level of sodium because of the lack of isotonicity of the common resuscitation solution.

Risks of Blood Transfusion

The transfusion of blood products can cause numerous serious complications, even death (Linden et al., 1992). A transfusion of incompatible red blood cells is potentially fatal, but other significant concerns exist when a patient receives blood products, including the transfusion of infectious pathogens and immunologic effects. Because a transfusion exposes the recipient to a complex mixture of donor cells and proteins, it is in many ways a transplant. Blood components contain viable lymphocytes that can provoke a graft-versus-host response in severely immunocompromised recipients. Transfusion can modify the recipient's immune response, as has been demonstrated in patients undergoing renal transplantation.

The most severe acute transfusion reactions involve complement-mediated red blood cell destruction. Because the red blood cells are rapidly destroyed intravascularly, peptides derived from complement are released and produce hypotension, compromise renal blood flow, activate the clotting cascade, and lead to disseminated intravascular coagulation (DIC) (see below). Most reported acute fatalities from transfusion reactions result from ABO-incompatible transfusions (Linden et al., 1992; Sazama, 1990). A hemolytic reaction can occur hours to days after transfusion although delayed hemolysis is rarely serious.

Vital and bacterial diseases may be transmitted by blood transfusion. Viruses include HIV, hepatitis viruses, cytomegalovirus, human T-cell leukemia virus types I and II, and Epstein-Barr virus. Bacterial diseases include infection with *Pseudomonas fluorescens, Yersinia enterocolitica, staphylococcus epidermis, staphylococcus aureus*, or *salmonella choleraesuis*. The rate of contamination of units (but not necessarily transmission) is: red cells 0-2 percent and platelets 0-10 percent (Wagner et al., 1994). Advances in the ability to detect the hepatitis C virus and more efficient screening of blood products have made the currently available U.S. supply of blood from volunteer donors extremely safe, and the risk of posttransfusion infection is significantly reduced (Fakhry and Sheldon, 1994).

Blood Substitutes and Alternatives to Transfusion

Red blood cell transfusion is the only acceptable clinical method for acutely increasing the oxygen-carrying capacity. Development of red blood cell substitutes would eliminate the risk of transfer of infectious agents through blood

transfusion and provide a ready source of universally compatible product. Several substances have been considered as red blood cell substitutes and can be divided into two general groups: (1) synthetic molecules, such as the porphyrins and the perfluorocarbon compounds, and (2) molecules that incorporate hemoglobin in their structures, such as the cross-linked and polymerized hemoglobin solutions. Acceptable red blood cell substitutes must be able to carry at least as much oxygen as hemoglobin normally carries (1.34 milliliters of oxygen per gram of hemoglobin). In addition, these molecules should be stable and should have an acceptable half-life. The red blood cell substitute should have properties that allow it to become completely saturated with oxygen at the standard fraction of inspired oxygen, but that allow it to unload substantial portions of its transported oxygen at the partial pressure of oxygen levels found in tissue. In addition, the solutions must be highly purified and free of contaminants and endotoxins (Fakhry and Sheldon, 1994).

Perfluorocarbons efficiently transport significant quantities of oxygen and carbon dioxide and thus have the potential to be an effective red blood cell substitute. Several perfluorocarbon molecules have been tested with humans, with limited success (Seaghell et al., 1990). At present they have no clinical application as red blood cell substitutes.

Early attempts to prepare hemoglobin solutions consisted of pooling outdated blood, breaking the red blood cells open, and extracting the hemoglobin molecules. Because the antigenic properties of red blood cells are associated with the membrane, hemoglobin solutions prepared in this way can be infused into patients with all blood types. This solution is termed *stroma-free hemoglobin*. Limitations to the use of stroma-free hemoglobin include its very short half-life in the circulation, its relatively low oxygen-carrying capacity, and its clearance through the kidneys, which causes significant side effects. Clinical trials are under way to determine the efficacy of the solution for acute blood loss and perioperative applications.

The salvage of intraoperative blood loss effectively minimizes the need for blood transfusion. This technique has had successful applications in various operative procedures, including cardiac surgery, spine surgery, liver transplantation, trauma procedures, and vascular surgery. Reports of intraoperative cell salvage in trauma patients with enteric contamination have demonstrated that the procedure can be used safely in such situations, provided that the cells are washed before reinfusion (Boudreaux et al., 1983). The use of devices for the collection of blood lost from the thoracic cavity through a chest tube can also decrease the amount needed for transfusion.

Disseminated Intravascular Coagulation

DIC is a syndrome rather than a specific disease. Although DIC is generally considered a hemorrhagic disorder because of the obvious bleeding problems that are encountered, it is important to recognize the very serious sequelae re

sulting from microvascular (and sometimes large-vessel) thrombosis that always accompanies true DIC and that leads to end-organ failure and death (Bick, 1994). The disorder may have a spectrum of presentations, from low-grade DIC, with minimal symptoms and minor laboratory abnormalities, to fulminant DIC, with life-threatening bleeding and protein coagulation abnormalities producing end-organ dysfunction and death. Various disorders ranging from sepsis to malignancy have been described in association with DIC. Although the diagnosis of DIC is made in patients receiving massive transfusions, the diagnosis of platelet dysfunction due to hypothermia or a specific factor deficiency should be excluded before making a diagnosis of DIC.

Laboratory abnormalities in DIC are variable and are related to the many diseases that are associated with this condition. Common abnormalities include abnormal prothrombin and activated partial thromboplastin times with depressed fibrinogen levels and abnormal platelet counts. Levels of fibrin-degradation products and D-dimer are commonly elevated. Because of the continued activation of coagulation, thrombin-antithrombin complexes will be formed and their levels can be measured. Levels of thrombin-antithrombin and antithrombin III are depressed. In addition, the levels of various fragments from coagulation factor degradation are elevated, including those of F1.2 and FpA.

Low-grade DIC generally responds to management of the underlying disorder, with some patients requiring heparin therapy. The appropriate therapy for fulminant DIC remains controversial, and this is compounded by the lack of objective studies and the many underlying causes. Despite improved diagnostic and therapeutic modalities, the rate of mortality from DIC remains high and is closely related to the underlying disorder.

CONCLUSIONS AND RECOMMENDATIONS

Traditionally, clinicians have evaluated patients for the presence and progression of cellular injury or death by assay of cellular proteins in serum that are not specific to injury processes. The new understanding of lethal cellular processes suggests that serum should be examined for the products of these processes. Indeed, immunochemical assays now exist for:

- conjugates of lipid peroxidation products and proteins,
- calpain-specific degradation fragments of spectrin,
- caspase-specific degradation products from actin, and
- phosphorylated eIF2α.

These species are all specifically pathologic and, in fact, reveal the presence of pathochemical processes for which emerging therapeutic approaches exist.

Furthermore, the processes reflected by these products (destruction of membranes by lipid peroxidation, degration of the cytoskeleton by calpain, caspase-mediated proteolysis, and apoptosis-associated blockade of normal

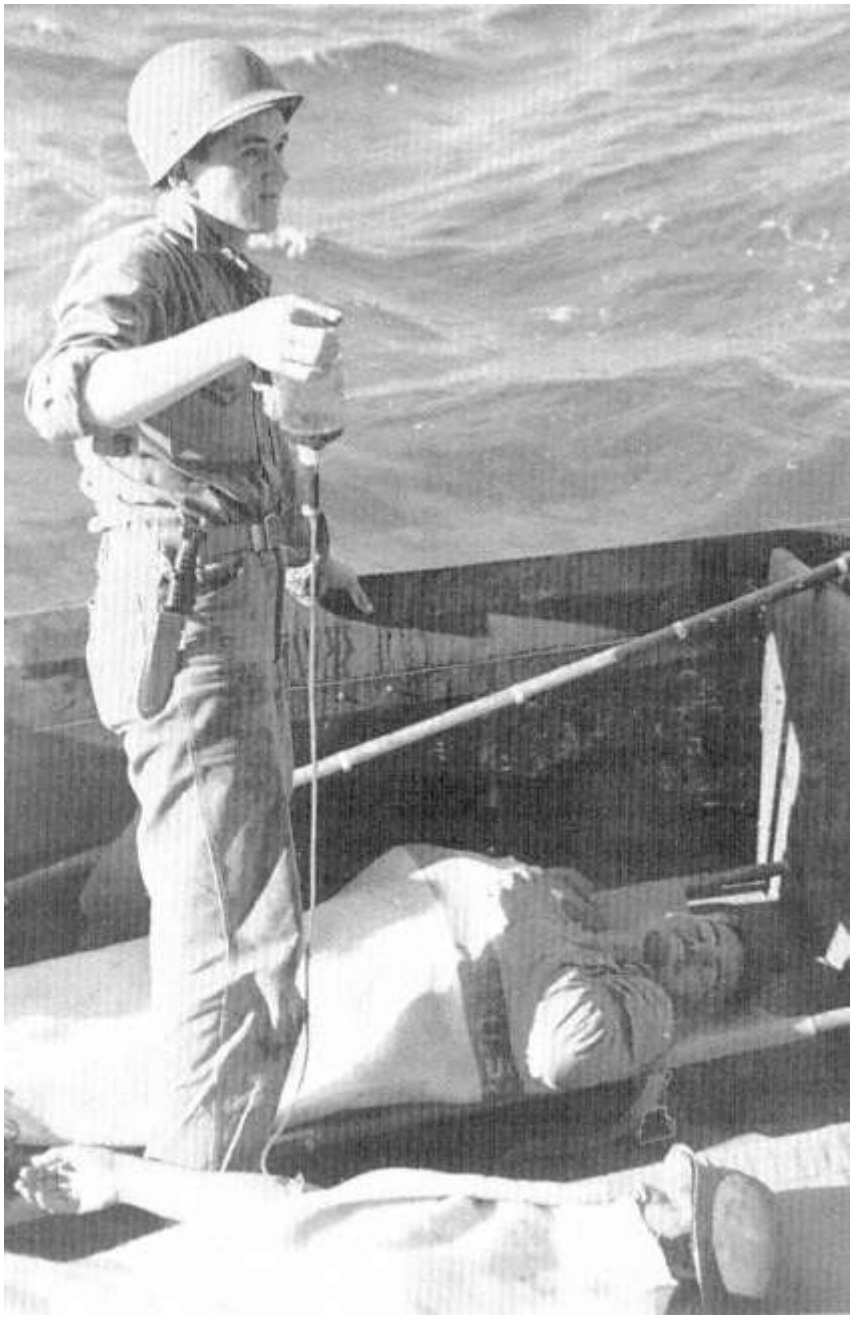
protein synthesis by phosphorylated eIF2α) are probably all independently lethal. In this situation, pharmacologic interference with only a single injury mechanism is unlikely to achieve any dramatic or even clinically noticeable improvement in outcome. The development of polypharmaceutical treatment approaches is a difficult problem, but current cancer chemotherapy shows that this approach can be fruitful. Morever, the availability of assays for individual injury mechanisms indicates that rigorous evaluation of this probably essential therapeutic approach is now possible.

The committee found that traumatic shock is a complex metabolic and cellular process and not just a hemodynamic event. Significant advances in the treatment of traumatic shock are unlikely to result from any simple management protocol alterations directed only at hemodynamic abnormalities. Rather, the major therapeutic advances will result from approaches that address the metabolic and cellular consequences of shock. Therefore, the committee recommends the following:

Recommendation 2.1 **Develop and validate diagnostic assays for substances in serum that indicate the specific mechanisms involved in the molecular processes of cellular injury and cell death induced by shock and resuscitation**.

Recommendation 2.2 **Expand the use of transgenic experimental animals to further evaluate the role of specific proteins and enzymes in cellular injury and death induced by shock and resuscitation**.

Recommendation 2.3 **Study and rigorously evaluate polypharmaceutical approaches directed against the multiple and independent mechanisms of cellular injury and death induced by shock and resuscitation**.



3

Experience With and Complications of Fluid Resuscitation

Controversy regarding the use of salt-containing solutions in surgery and trauma has continued for most of the 20th century. In 1911, Evans wrote:

> The therapeutic value of a physiologic saline solution administered in large amounts either intravenously, hypodermically, or by the intestinal tract in certain pathologic conditions characterized by changes, quantitative or qualitative, in the blood plasma, has been so abundantly demonstrated by clinical experience that it requires no emphasis here. That under certain circumstances saline solutions are productive of great harm to the tissues of the body, and are even capable of producing death, is as true as it is of many other valuable therapeutic procedures. (Evans, 1911)

The use of large-volume isotonic salt solutions has become routine both in postsurgical settings and in the immediate postinjury phase; the use of fluid and electrolyte therapy has been lifesaving. (The electrolyte characteristics of selected resuscitation fluids are listed in Table 3-1.) Despite the impact of salt solutions on survival in shock, numerous questions and concerns persist regarding the composition, rate, and quantity of fluid resuscitation. This chapter gives an overview of colloid and crystalloid resuscitation, examines the complications of fluid resuscitation in general, and then describes the complications of crystalloid and colloid resuscitation specifically.

OVERVIEW OF COLLOID AND CRYSTALLOID RESUSCITATION

Fogelman and Wilson (1960) attributed hypovolemia after trauma to a reduction in the extracellular volume. In their studies, the mortality rate in dogs subjected to 2 hours of hemorrhagic hypotension was 80 percent if the animals were resuscitated with reinfusion of shed blood; however, addition of lactated Ringer's

solution to the return of autologous blood decreased the mortality rate to 40 percent. Shires and colleagues (1960b) extended this work, measuring intraoperative losses in the effective extracellular fluid volume, plasma volume, and red blood cell mass by the use of radioisotopes. Those studies described a 28 percent decrease in extracellular fluid volume that correlated directly with the degree of operative trauma. Shires and colleagues (1961) subsequently extended this work to trauma patients and described similar shock-mediated losses in extracellular fluid volume. Furthermore, resuscitation of hemorrhagic shock with blood or fresh frozen plasma plus blood failed to correct shock-related reductions in extracellular fluid volume, whereas the addition of lactated Ringer's solution to the return of shed blood ablated extracellular fluid volume deficits and improved the survival rate (Middleton et al., 1969; Shires, 1966; Shires et al., 1964).

The phenomenon of fluid redistribution after major trauma involving blood loss was termed "third spacing" and described the translocation of intravascular volume into the peritoneum, bowel, burned tissue, or crush injury sites. Subsequent studies showed that hemorrhagic shock promoted a significant loss of fluid from the extracellular space into the cell, exacerbating third-space losses (Middleton et al., 1969). Fluid was then directed to replace lost intravascular as well as extravascular fluid. Considerable controversy arose, however, regarding the formula or the clinical criteria used to determine the adequacy of fluid resuscitation; in addition, questions arose regarding the type of fluid that was most appropriate for volume replacement. Several studies suggested that normal saline and lactated Ringer's solution were equally effective in maintaining intravascular volume after hemorrhage (Cervera and Moss, 1975; Siegel et al., 1973; Wright, 1974), but complications such as hyponatremia or hypernatremia were reported with the use of 5 percent sodium chloride or molar sodium lactate solutions, respectively. Dillon and colleagues (1966) showed that lactated Ringer's solution (given in a volume that was two to three times the shed blood volume) was as efficacious as 6 percent dextran in saline (given in a volume equal to the shed blood volume) and confirmed that a sodium-containing, colloid-lacking solution could be used effectively to treat blood loss.

Questions arose regarding the effects of large-volume expansion on sodium distribution after hemorrhagic shock as well as the need to correct potassium deficits; although hemorrhage was shown to produce a functional sodium deficit, neither the clinical significance nor the magnitudes of the deficit were determined (Dillon et al., 1966). Large-volume resuscitation with salt-containing solutions gained in popularity because this regimen was consistently associated with improved survival in both clinical and experimental studies of hemorrhagic shock, and few side effects of lactated Ringer's solution were demonstrated. Thereafter research compared the hemodynamic responses to resuscitation with whole blood, plasma expanders, fresh frozen plasma, and saline versus lactated Ringer's solution. In this search for an ideal fluid for adequate restoration of intravascular and extravascular volumes, most studies found no differences in mortality rate or pulmonary function if volume expansion was adequate (Lucas et al., 1986, 1978; Moss et al., 1981).

TABLE 3-1 Electrolyte Characteristics of Selected Resuscitation Fluids and Research Formulations

Fluid	Fluid Compartment	Osmolarity (mOsM/l)	pH	Na^+ (meq/l)	Cl^- (meq/l)	K^+ (meq/l)	Mg^{2+} (meq/l)	Ca^{2+} (meq/l)	Dextrose (g/l)	Buffer
Blood[a]										
Normal serum	Intravascular	308	7.4	140	100	4	0	0	0–4	Protein, bicarbonate
Crystalloid										
0.9% Saline	Extracellular	308	5.0	154	154	0	0	0	0	None
Lactated Ringer's solution	Extracellular	275	6.5	130	109	4	0	3	0	Lactate
Plasmalyte-A, pH 7.4	Extracellular	294	7.4	140	98	5	3	0	0	Acetate, gluconate
Normosol-R	Extracellular	295	5.5–7.0	140	98	5	3	0	0	Acetate, gluconate
7.0% Saline	Extracellular	2,396		1,197	1,197	0	0	0	0	None
5% Dextrose in water	Extracellular	252	4.0	0	0	0	0	0	50	None
Colloid										
Natural										
5% Albumin	Intravascular	309	6.4–7.4	130–160	130–160	≤1	0	0		Sodium bicarbonate, sodium hydroxide, or acetic acid
25% Albumin	Intravascular	312	6.4–7.4	130–160	130–160	≤1	0	0		Sodium bicarbonate, sodium hydroxide, or acetic acid
Frozen plasma	Intravascular	300	Variable	140	110	4	0	0	0–4	Protein, bicarbonate
Synthetic										
6% Hetastarch	Intravascular	310	5.5	154	154	0	0	0	0	None
10% Pentastarch	Intravascular	326	5.0	154	154	0	0	0	0	None
Dextran 40	Intravascular	311	3.5–7.0	154	154	0	0	0	0	None
Dextran 70	Intravascular	310	3.0–7.0	154	154	0	0	0	0	None
Oxypolygelatin	Intravascular	200	7.4	155	100	0	0	1	0	None
Research Formulations										
Carolina Rinse[b]		290–305	6.5	115	122	6	1.2	1.3	1.8	MOPS
Wisconsin Solution[c]	Intravascular	320	7.4	25	0	125	5	0	0	Potassium phosphate
Veech's Fluid		294.3	7	136	106	4	1	2	5	Bicarbonate

[a] Included as a point of comparison.
[b] Contains hydroxyethyl starch (50 grams/liter), allopurinol (1 millimolar [mM]), desferrioxamine (1 mM), glutathione (3 mM), fructose (10 mM), glucose (10 mM), adenosine (200 micromolar [μM]), nicardipine (2 μM), insulin (100 U/liter), 3-[*N*]-morpholinopropanesulfonic acid (20 mM), and glycine (5 mM).
[c] Contains hydroxyethyl starch (50 grams/liter), benisine (5 mM), glutathione (3 mM), allopurinol (1 mM), insulin, penicillin, and dexamethesone.

SOURCE: Adapted from Lemasters et al. (1995), Physicians' Desk Reference (1999), Rudloff and Kirby (1998), and Veech (1986).

Beecher (1955), in the *Surgeon General of the Army's Surgery in War Worm II*, warned that crystalloids should be used primarily to replace body fluid lost through dehydration and stated that "As blood substitutes, these solutions were not effective, and they could be dangerous" (p. 32). Despite this concern, massive transfusion with crystalloid was routine during the Vietnam conflict. Additionally, studies undertaken during and immediately following the Vietnam conflict raised the concern that a large volume of salt-containing solution increased the incidence of acute respiratory distress syndrome (ARDS) and promoted multiple-organ dysfunction syndrome (MODS; see Figure 3-1). A loss of endothelial integrity and capillary leak coupled with the infusion of protein-free fluid, which in turn diluted plasma proteins, could contribute to pulmonary edema. A significant emphasis was subsequently placed on acute respiratory failure as well as ventilatory management in the shock patient with massive blood losses. Despite the lessons learned in Vietnam (increased incidence of pulmonary failure and ARDS with aggressive fluid resuscitation from shock), crystalloid solutions gained increasing acceptance in both clinical and military areas for fluid resuscitation from trauma. Studies with baboons and sheep confirmed that the hypoproteinemia that occurs after resuscitation with salt-containing solutions did not promote water movement into the lung interstitium (Moss et al., 1981). These studies contributed to increased acceptance of crystalloid volume replacement.

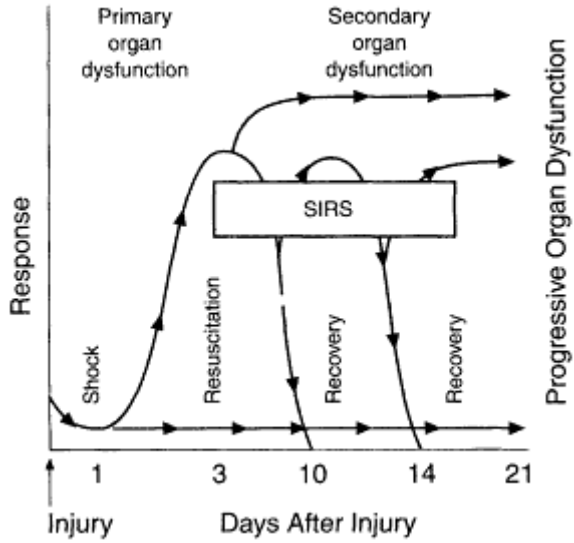


Figure 3-1
Inflammatory and organ dysfunction responses to injury. Normal response to an injury or insult may decrease after 3 to 5 days or be reactivated by a complication. A continuous inflammatory response is seen with systemic inflammatory response syndrome (SIRS) and can eventually progress to organ dysfunction. SOURCE: Reprinted, with permission, from Beal and Cerra (1994). Copyright 1994 by the American Medical Association.

Shoemaker and colleagues (1973, 1976, 1981) examined the effects of fluid resuscitation on tissue oxygenation and suggested that albumin improved hemodynamic and oxygen transport variables more than lactated Ringer's solution, attributing these results to the greater increases in plasma volume expansion and cardiac output with albumin. Similarly, Hauser and colleagues (1980) showed that infusion of 1 liter of lactated Ringer's solution in critically ill surgical patients with shock expanded the plasma volume 194 ± 18 milliliters (ml) whereas 25 grams of 25 percent albumin (100 ml) increased the plasma volume 465 ± 47 ml. Although hemodilution-related falls in the intravascular protein concentration have continued to raise concerns about crystalloid infusion in shock, the adverse effects of albumin administration on extravascular flux of protein and cardiopulmonary function contributed to the continued use of aggressive crystalloid resuscitation (Carey, 1971; Carey et al., 1970; Cloutier et al., 1969; Cochrane Injuries Group Albumin Reviewers, 1998; Lowe et al., 1979; Lowery et al., 1971; Lucas et al., 1980; Metildi et al., 1984; Moss et al., 1969; Poole et al., 1982; Virgilio et al., 1979).

Although the controversy regarding crystalloid versus colloid resuscitation of the shock patient with hemorrhage continues, most investigators agree that acute hemorrhage-induced changes in plasma volume require replacement with crystalloid solutions at volumes at least three times the volume of the shed blood. A major concern with regard to resuscitation of hemorrhage in a military setting is the considerable weight and volume of crystalloid solutions that must be transported in the field. The large bulk of the lactated Ringer's solution that must be transported compromises the resuscitation phase in forward areas of deployment. In addition, patients with hemorrhagic shock in a combat area are frequently dehydrated, presenting an additional problem for successful resuscitation.

COMPLICATIONS OF RESUSCITATION IN GENERAL

Although fluid resuscitation is necessary to assist a patient to recover from a loss of blood, there are complications that occur in the administration of the fluid. Fluid resuscitation can have an adverse effect on coagulation, and cause oxygen toxicity or reperfusion-mediated injury. Additionally, there are further complications associated with late resuscitation.

Effects of Fluid Resuscitation on Coagulation

Prolonged bleeding time has been described in patients with severe anemia (Hellem et al., 1961). A decrease in hematocrit as a consequence of large-volume crystalloid resuscitation produces anemia, thrombocytopenia, reduced plasma, and oncotic, clotting, and opsonic proteins. In addition to altered oxygen and CO_2 transport, hemodilution and a fall in the circulating red blood cell volume reportedly alter several aspects of coagulation, including bleeding time and platelet adhesiveness and have detrimental effects related to excess, unscav

enged nitric oxide production. Previous studies (Valeri et al., 1998) have suggested that the nitric oxide-mediated platelet dysfunction occurs as a result of hemodilution and the lack of availability of adequate red blood cells to scavenge nitric oxide by oxidation or by binding of nitric oxide to the hemoglobin molecule. In addition, red blood cell scavenging of nitric oxide activates platelets to produce thromboxane A_2 and serotonin and stimulates endothelium-derived endothelin production in an effort to restore and maintain microcirculatory hemostasis (C. R. Valeri, personal communication). Anemia-related platelet dysfunction has been described by several laboratories (Blajchman et al., 1994; Duke, 1910; Hellem et al., 1961; Marcus, 1990), and altered bleeding times have been correlated with peripheral hemoglobin and hematocrit contents. The altered bleeding associated with a reduced circulating red blood cell content has been attributed, in part, to the decrease in blood viscosity and increased sheer stress at the levels of the endothelium.

More recently, studies from the Naval Blood Research Laboratory have shown that the hematocrit level and the oxygen state of red blood cells have a greater effect on bleeding time than does the concentration of either platelets or clotting proteins. In correcting the effects of hemodilution on bleeding time, the transfusion of platelets produces only a transient rise in total platelet count related, in part, to the short half-life of this cell type. Transfusion and an increase in the level of circulating red blood cells after aggressive fluid resuscitation from hemorrhagic shock have been shown to have a beneficial effect on platelet function. Red blood cells disperse platelets from the center of the blood vessel, concentrating this cell population near endothelial cells of the vessel walls (Turitto and Weiss, 1980). The ability of red blood cells to stimulate platelet synthesis of thromboxane A_2 has important effects on vasoconstriction and platelet aggregation in the presence of continuing blood loss. Anemia and subsequent platelet dysfunction diminish the level of production of thromboxane A_2, contributing to continued blood loss. These data raise additional concerns regarding large-volume lactated Ringer's solution resuscitation in a subject with continuing blood loss. Dilution of clotting proteins and increased bleeding time would exacerbate hemorrhage-related blood loss as well as microcapillary oozing. Aggressive fluid resuscitation from hemorrhage and the resulting anemia increase the blood flow but also increase the shear stress on vascular endothelial cells, promoting the release of endothelium-derived nitric oxide (Duke and Abelmann, 1969; Griffith, 1987; Ignarro, 1987; Loscalzo, 1995; Palmer et al., 1987). Shear stress on the endothelium is related to both shear rate and whole-blood viscosity. With hemodilution, blood viscosity falls, but the shear rate increases and the net result is increased shear stress. Finally, hemodilution-related increases in shear stress can promote the release of adenosine diphosphate (ADP) from red blood cells, potentiating shear-related platelet aggregation (Alkhamis, 1988; Alkhamis et al., 1990; Bell et al., 1990; Luthje, 1989; Saniabadi et al., 1987; Santos et al., 1991; Valles et al., 1991). Although increased shear stress on endothelial cells enhances nitric oxide production, the subsequent rise in platelet cyclic guanosine monophosphate (cGMP) levels further inhibits platelet function (Azuma et

al., 1986; Cooke et al., 1990; Mendelsohn et al., 1990; Michelson et al., 1996; Radomski et al., 1987).

Since it was recognized that human immunodeficiency virus (HIV) could be transmitted through blood transfusion, blood products have been used conservatively in patients with hemorrhagic shock, and a measure of hemodilution after aggressive crystalloid resuscitation has been accepted in clinical practice. However, the death rate as a result of HIV-related transfusion is extraordinarily low (Valeri et al., 1998). It is clear that the hemodilutional effects of aggressive crystalloid resuscitation extend far beyond the reduced oxygen-transport capacity and include altered endothelial function. These resuscitation-related changes in endothelial cell function may, in turn, exacerbate the capillary leak syndrome recognized to occur with hemorrhagic shock.

Oxygen Toxicity Associated with Resuscitation

Oxygen therapy is frequently used to treat critically ill patients who have pulmonary insufficiency; such patient populations frequently receive supraphysiologic concentrations of O_2 (that is, more than 21 percent O_2). The adverse effects of supranormal oxygen concentrations have been well described, and the lungs of patients who receive oxygen therapy are frequently damaged due to the high level of O_2 exposure (Kazzaz et al., 1996; Morris, 1994; Stogner and Payne, 1992). Similarly, exposure of cells in culture to hyperoxia produces chromosomal breakage, oxidative damage, and cell cycle arrest at the G_2 state (Clement et al., 1992). Hyperoxia-related cell injury has been attributed to the accumulation of oxygen-derived free radicals, which overwhelm cellular antioxidant mechanisms. A number of free radical scavengers including 21 aminosteroids have been shown to provide protection against free-radical-mediated injury and have been confirmed to have cytoprotective effects in hyperoxic insults (Frank and McLaughlin, 1993; Richards et al., 1993). Hyperoxia-mediated lung injury in humans, similar to that seen in ARDS, has been associated with activation of reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and increased levels of hydrogen peroxide release by alveolar macrophages (Kinnula et al., 1992; Vacchiano et al., 1998). These studies suggest that prolonged exposure to a high partial pressure of oxygen produces direct pulmonary injury and exacerbates preexisting lung dysfunction.

Although trauma patients with hemorrhagic shock may receive oxygen therapy during transport and in the emergency department, these patients seldom require supraphysiologic concentrations of oxygen. A primary concern in the treatment of the trauma patient with hemorrhagic shock is aggressive fluid resuscitation and the potential for reperfusion injury related to (1) the return of molecular oxygen to previous ischemic tissues and (2) the toxic cellular effects of oxygen-derived free radicals. An increase in blood oxygen tension after resuscitation regimens that increase the oxygen-transport capabilities of the blood

may promote the increased intracellular production of superoxide, subjecting the endothelial cell to a barrage of deleterious oxygen-derived free radicals. Resuscitation with whole blood produces a burst of superoxide radical release, which may be exacerbated by platelet adherence to the surface of the endothelium and subsequently may promote adherence and activation of leukocytes. This activated cell population, in turn, produces additional toxic O_2 radicals, contributing to endothelial damage. The lung is particularly susceptible to free-radical-mediated injury; in the scenario of endothelial damage from adherent and activated leukocytes, free radicals may migrate to the alveolar space, contributing to the progression of pulmonary oxygen toxicity (Sacks et al., 1978; Shinomiya et al., 1998; Steinberg et al., 1979). The toxic effects of oxygen in animals and humans has been well recognized, but oxygen toxicity has been best characterized with regard to reperfusion injury, in which the return of molecular oxygen to previously ischemic tissues contributes to oxygen free-radical generation via xanthine oxidase activation.

Reperfusion-Mediated Injury

A major concern with regard to fluid resuscitation, that is, the reintroduction of molecular oxygen into previously ischemic tissues, the production of excess oxygen-derived free radicals, and subsequent tissue damage, has been investigated extensively. Most molecular species have pairs of electrons within their outer orbitals; each molecule is stabilized by the opposite spin of each of these electrons. A free radical is a molecule with an unpaired electron which is highly reactive and tends to react with other molecules in an effort to pair its lone electron with another electron. This interaction renders the molecule reactive and unstable, contributing to the extremely short half-lives and difficulty in quantitating the radicals in a biological setting. The recent development of electron paramagnetic resonance (EPR) spectroscopy, alone or in combination with spin trapping, has allowed these radicals to be observed. EPR directly measures the amount of energy absorbed by an unpaired electron in a magnetic field at the ultralow temperatures; free radicals are far too unstable to measure at room temperature since the half-life is approximately 6 to 10 seconds (Fantone and Ward, 1982; Grisham and McCord, 1986; Reilly et al., 1991).

Free radicals are the normal by-products of cellular metabolism, and the most common radicals include superoxide, hydrogen peroxide, the hydroxyl radical, and nitric oxide. During oxidative phosphorylation, molecular oxygen is reduced to water within the mitochondria. However, as the conversion of oxygen to water occurs, between 1 and 5 percent of this oxygen escapes the pathway, producing several toxic intermediates. Other endogenous means or sources of free-radical production include the oxidation of purines including hypoxanthine oxidation through xanthine to urate, the metabolism of arachidonic acid to produce prostaglandins and leukotrienes, and the NADPH-dependent oxidase system on neutrophil membrane surfaces. Endogenous antioxidant systems

serve to scavenge or neutralize free radicals, maintaining effective balance between free-radical production and removal (Babbs, 1988).

Oxygen-Derived Free Radicals

The role of free radicals in hemorrhage and shock arises from the fact that volume replacement or reperfusion of previously ischemic tissues has been recognized to produce significant tissue injury and dysfunction. This phenomenon has been described as the "oxygen paradox" and describes the fact that although the restoration of oxygen delivery to ischemic tissue is essential to the maintenance of function and survival, this oxygen may initiate a cascade of deleterious events, producing tissue injury. The free radicals produced during reperfusion or fluid resuscitation from hemorrhagic shock attack multiple components of the cell, including lipids, nucleic acids, and proteins. Therefore, although hypoperfusion itself will produce cellular death with time, the very act of correcting the perfusion deficits introduces significant and greater injury. McCord and Fridovich (1968) proposed that the major source of free radicals during reperfusion was the enzyme xanthine oxidase, an enzyme that is present in the liver and gut.

A decrease in blood flow limits the available oxygen required for adenosine triphosphate (ATP) production. ATP depletion produces a subsequent rise in the level of adenosine monophosphate (AMP), which, in turn, is catabolized to hypoxanthine. With fluid resuscitation and the return of molecular oxygen to previously hypoperfused tissues, hypoxanthine serves as a substrate for xanthine oxidase. A complicated series of reactions converts hypoxanthine to xanthine and finally to uric acid and in the process generates hydrogen peroxide and superoxide, both of which are powerful oxidizing agents. The resulting increased production of superoxide and hydrogen peroxide overwhelms the capacity of endogenous scavengers. Hemorrhagic shock produces "whole-body" ischemia with inadequate perfusion of most tissues. As ATP levels fall dramatically in several tissues, the levels of hypoxanthine in plasma rise. The role of hypoxanthine in hemorrhagic shock was first suggested by Crowell and associates (1969) and was confirmed by others; those studies found that allopurinol provides significant benefits if it is given during blood loss (Bulkley, 1983; Hess et al., 1982; Parks, 1982; Powell and Tortolani, 1992; Rao et al., 1983). Numerous subsequent studies have confirmed the hemodynamic and cardioprotective effects of free-radical scavengers given during either ischemia or shock with hemorrhage (Bernier et al., 1986; Crowell et al., 1969; Cunningham and Keaveny, 1978; Granger et al., 1986; Lee et al., 1987).

Although a major source of oxygen-derived free radicals in hemorrhagic shock is xanthine oxidase, others have shown that adherent and activated neutrophils produce free radicals. Although this serves an important and necessary role in the scavenging of invading bacteria, a burst of neutrophil-produced free radicals may exacerbate the xanthine oxidase activity, producing significant

tissue damage. In addition to free-radical production, hemorrhagic shock clearly impairs endogenous antioxidant defense mechanisms, rendering the subject more susceptible to the damage caused by toxic oxygen metabolites. Hemorrhagic shock alters nonenzymatic defense mechanisms, decreasing α-tocopherol, β-carotene, ascorbic acid, and vitamin E reserves. These normal antioxidants protect membranes against lipid peroxidation, dissipating free-radical energy and scavenging toxic radicals. In addition, hemorrhage impairs endogenous enzymatic defense mechanisms including those involving superoxide dismutase, catalase, and reduced glutathione, which catalyze the breakdown of superoxide free radicals and hydrogen peroxide, respectively. Shock-mediated downregulation of these defense mechanisms predisposes the cell to injury from an unopposed storm of toxic radicals.

Evidence for oxygen-derived free-radical-mediated injury in hemorrhagic shock has been provided by a host of studies. Hemorrhagic shock reduces the activities of copper, zinc-superoxide dismutase, and glutathione peroxidase in several organs, whereas the activity of glutathione reductase remains unchanged (Makarewicz-Plonska et al., 1998). Other studies have described an oxidative-antioxidative imbalance in experimental hemorrhagic shock as reflected by morphologic changes in peripheral organs accompanied by increased malondialdehyde levels, indicating oxidative tissue injury. A fall in the antioxidative potential has been confirmed by altered sulfhydryl compounds and reduced superoxide dismutase activity (Debek et al., 1998). Kapoor and colleagues (1997) provided evidence that oxygen radicals contribute to the deterioration of cardiovascular function and cellular injury during hemorrhagic shock and resuscitation. Those investigators described the increased oxygen radical-producing activity of polymorphonuclear leukocytes, decreased antioxidant enzyme activities (superoxide dismutase, catalase, and glutathione peroxidase), and a rise in cardiac malondialdehyde concentrations.

In addition, a fall in glutathione, α-tocopherol, and plasma glutathione peroxidase levels paralleled by a rise in the levels of lipid peroxides (expressed as thiobarbituric acid substances) have been described in patients resuscitated from trauma or shock (Kretzschmar, 1998). A progressive loss of plasma sulfhydryl groups and α-tocopherol and a significant increase in the plasma-reduced glutathione level paralleled the development of a multiple-organ failure in this patient population. These data suggest that traumatic injury with hemorrhage produces significant oxidative stress paralleled by a loss of endogenous antioxidants. These factors are major contributing factors to the development of multiple-organ failure after traumatic injury with shock, despite aggressive fluid resuscitation. Other studies have described no significant changes in either superoxide dismutase or catalase levels during hemorrhage alone (Uzuner et al., 1995); however, in those studies, a reduced catalase level after resuscitation suggested reperfusion-mediated injury via the generation of free radicals as well as a fall in antioxidant capacity.

Nitric Oxide

In addition to oxygen-derived free radicals, nitric oxide generated via the nitric oxide synthases contributes to membrane injury and cellular dysfunction during hemorrhagic shock and fluid resuscitation. Numerous studies have shown that hemorrhagic shock upregulates the inflammatory or inducible nitric oxide synthase (NOS2 or iNOS) (Kelly et al., 1997; Thiemermann et al., 1993), suggesting that increased iNOS expression in shock contributes to the proinflammatory response. Nitric oxide mediates cell injury via either direct effects on several intracellular processes or the indirect effects by interaction with superoxide (Szabó, 1996). Attempts to define the contribution of iNOS and nitric oxide to postshock-mediated cell injury have used two approaches: (1) pharmacologic probes (selective iNOS inhibitors as well as inhibitors that block both iNOS and endothelial nitric oxide synthase [eNOS]) and (2) transgenic animals, specifically, animals deficient in iNOS. These studies have suggested that nitric oxide is "a final common mediator" in hemorrhagic shock and a primary mediator of the inflammatory response shown to occur after resuscitation from hemorrhagic shock. However, the role of nitric oxide in shock has remained controversial. The finding that arginine administration provided organ protection in resuscitated hemorrhagic shock suggested that low levels of nitric oxide (likely occurring as a result of eNOS) exert protective effects on organs; decreased arginine availability after resuscitation from hemorrhagic shock could impair basal levels of nitric oxide. Arginine added to fluid used for resuscitation may restore these basal levels of nitric oxide, contributing to the observed organ protection (Chaudry et al., in press).

In contrast, enhanced nitric oxide production during shock via increased iNOS expression produces significant cellular injury including vascular decompensation and nitric oxide-mediated activation of the transcription factor nuclear factor κB (NFκB), providing one mechanism by which nitric oxide modulates oxidant signaling in hemorrhagic shock (see Figure 3-2). Despite this paradigm proposed by Szabó and Billiar (in press), there is abundant evidence that nitric oxide interaction with superoxide radical yields peroxynitrite, exacerbating the injury produced by either nitric oxide or superoxide alone. Peroxynitrite generation occurs particularly during fluid resuscitation from hemorrhagic shock in which the level of production of the superoxide radical exceeds the scavenging capacity of endogenous enzymes. Peroxynitrite contributes to cell injury and cellular death by producing DNA single strand breakage (Szabó and Hoshima, 1997) and activation of the nuclear enzyme poly(ADP ribose) synthetase (PARS), which, in turn, contributes to energy depletion and cellular necrosis. The detrimental effects of peroxynitrite in hemorrhage and resuscitation have been attributed to oxidation of sulfhydryl groups, and nitration of hydroxylation of tyrosine, tryptophan, and guanine, and inhibition of several enzyme systems including membrane sodium potassium adenosine triphosphatase. PARS activation depletes nicotinamide adenine dinucleotide (NAD), altering glycolysis, electron transport, and ATP synthesis; in addition, PARS activates the caspase

cascade, which triggers DNA fragmentation and cellular apoptosis (Endres, 1998; Szabó, 1998; Virág et al., 1998; Yaoita et al., 1998).

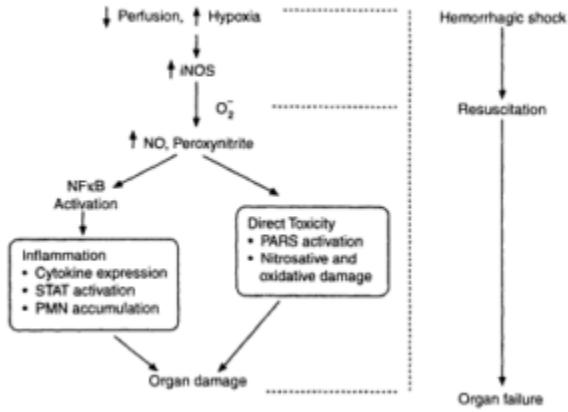


Figure 3-2
Roles of induced NO in hemorrhagic shock. The figure depicts the dual roles of inducible NO synthase-generated NO in hemorrhagic shock. The NO generated during resuscitation combines with the superoxide, forming peroxynitrite. Peroxynitrite generated during resuscitation can exert direct cellular toxicity through oxidative and nitrosative damage and PARS activation. NO and perhaps peroxynitrite can activate inflammatory cascades through the upregulation of NFκB. This results in inflammation manifested by cytokine expression, STAT3 activation, and neutrophil accumulation. Combined, these actions result in organ damage and organ failure. Source: Reprinted, with permission, from Szabó and Billiar (in press). Copyright 1999 by BioMedical Press.

Clearly, hemorrhagic shock produces local and whole-body ischemia; and fluid resuscitation, regardless of the type of fluid administered, increases perfusion of previously ischemic or hypoperfused tissues, triggering the production of numerous free radicals and likely contributing to cellular injury.

Activated Neutrophils

In addition to the generation of free radicals by reperfusion of hemorrhage-induced ischemia, recent attention has focused on the role of activated neutrophils in resuscitation-mediated cellular injury. Intracellular adhesion molecules 1 and 2 (ICAM-1 and ICAM-2, respectively) have been shown to be upregulated by lactated Ringer's fluid resuscitation from hemorrhagic shock (Rhee, 1998). These adhesion molecules are instrumental in binding leukocytes to the

vascular endothelium, and neutrophils are the predominant population of cells that adhere to the vascular endothelium and subsequently migrate into the tissues. Neutrophil-derived free radicals have been shown to play a significant role in tissue injury after resuscitation from hemorrhagic shock, as indicated by organ protection and increased survival in animals treated with monoclonal antibodies specifically directed against ICAM-1 (Mileski et al., 1990, 1991). Similarly, Flynn and colleagues (1996) demonstrated that sialyl Lewis oligosaccharides that block the low affinity selectin ligands (including P, L, and E) were effective in models of trauma and hemorrhagic shock. Pharmacologic strategies designed to inhibit specific adhesion molecules recognized to play a role in the tethering, adherence, and activation of leukocytes provide significant organ protection after hemorrhagic shock. The use of ICAM-1- and P-selectin-knockout mice have provided alternative strategies to confirming the roles of these adhesion molecules and neutrophils in shock-mediated injury. Such transgenic and pharmacologic approaches have been shown to decrease free radical-mediated injury, as indicated by decreased lipid peroxides and malondialdehyde levels and have confirmed that the activated neutrophil is one source of free radicals in resuscitated hemorrhagic shock.

Complications of Late Resuscitation of Shock

In the late phase of shock resuscitation, emphasis is placed on continued volume replacement as well as nutritional support delivered via the enteral or the parenteral route. Metabolic derangements after shock include altered energy production and utilization and altered carbohydrate, lipid, and protein metabolism; in addition, acute-phase protein synthesis, impaired wound healing, impaired glucose oxidation, and accelerated protein catabolism are hallmarks of stress-related injury. Finally, shock alters macrophage and lymphocyte glutamine requirements after burn shock, and several therapies have been directed toward the restoration of shock-mediated changes in tissue glutamine levels. Late treatment of the trauma patient with hemorrhagic shock has been directed toward determination of the patient's metabolic needs. Dietary manipulation after shock has been shown to increase survival in rodent stress models and to improve nitrogen balance and protein synthesis (Manson et al., 1988; Wolfe et al., 1989), to improve cell-mediated immunity in burned guinea pigs (Alexander et al., 1986), and to improve hemodynamic and metabolic function and outcome (Muakkassa, 1991). Nutritional support, however, is considered during the late phase of shock therapy and is not to be confused with front-line therapy in disaster or combat situations, in which the primary concern is restoration of the circulating volume and hematocrit.

COMPLICATIONS OF COLLOID RESUSCITATION

Numerous difficulties with current regimens of colloid fluid replacement in patients with shock have been described. The lack of availability of whole blood in the field, the requirements for typing and cross matching, the danger of transmitting numerous infectious agents (including hepatitis B and hepatitis C viruses, and HIV), reduced concentrations of coagulation factors, and the absence of functioning platelets all complicate the use of whole blood for adequate resuscitation of hemorrhage. The limitations of liquid plasma for resuscitation from shock in the field include the relatively short shelf life, cost (currently $126/200 milliliters of plasma), and the risk of transmitting infectious agents. Although detergent treatment of plasma has been shown to reduce the risk of transmission of lipid-encapsulated viruses such as HIV, the persistent problem of possible contamination of large plasma pools with other infectious agents, for example, nonenveloped viruses, and requirements for storage in a frozen state have dampened enthusiasm for this approach to field resuscitation from hemorrhage (Klein et al., 1998). Finally, numerous studies have confirmed shock-mediated alterations in endothelial cell barrier integrity (Maier and Bolger, 1996).

Administration of albumin-containing solutions in the presence of a persistent capillary leak has been proposed to increase interstitial protein concentrations, promoting the movement of fluid from the intravascular compartment to the interstitium and aggravating hemorrhage-induced hypotension. These factors have resulted in an increased interest in synthetic colloids, agents that can be successfully retained in the intravascular compartment to increase circulating plasma volume. With these synthetic agents (the dextrans, hydroxyethyl starch [HES], and several gelatin preparations), the rare occurrence of anaphylactic reactions, mild inhibition of normal hemostasis, and the long-term retention of these agents within the body have tempered enthusiasm for their clinical use (Adelson et al., 1955; Bergqvist, 1982; Cronberg et al., 1966; Dahn et al., 1979; Leibold et al., 1983; Lucas et al., 1980; Macintyre et al., 1985; Metildi et al., 1984; Mishler, 1982; Stump et al., 1983). However, these agents are cheap to manufacture, are stable with storage at room temperature, and eliminate the infectious risks associated with blood products. Recent studies have shown that artificial colloids increased the levels of expression of adhesion molecules, increased the levels of synthesis of several proinflammatory cytokines, and promoted cellular apoptosis (Coimbra et al., 1996; Junger et al., 1997a,b, Rhee, 1998). Newer colloids that carry oxygen and that can be formulated for a variety of oncotic activities have considerable promise, but considerable research is required before their clinical application as resuscitation fluids.

Glucose-containing solutions have been avoided for resuscitation from hemorrhagic shock in patients with head trauma. The stress-related rise in circulating epinephrine levels decreases the level of insulin released by the pancreas, complicating metabolism of the glucose load. Furthermore, increased glucose levels have been shown to alter potassium-adenosine triphosphate (K-ATP) channels, thus excerbating shock-related ion redistribution across mem

branes. Patients with injury have adrenergic-system-mediated insulin resistance so that the glucose space is expanded. The implications are that with increased gluconeogenesis, glucose-containing fluids are unnecessary and probably harmful.

The debate between colloid and crystalloid resuscitation has recently taken on some new features that merit consideration. A systematic review of 30 randomized trials found no evidence that albumin administration reduced the rate of mortality among critically ill patients with hypovolemia, bums, or hypoalbuminemia and suggested that albumin may in fact increase the rate of mortality (Cochrane Injuries Group Albumin Reviewers, 1998). That analysis has been criticized for its reliance on studies with small numbers of patients and with patients with different diseases and for the use of death as its single, if objective, endpoint. There is also the inherent problem of publication bias in analyses of this kind, and that review should not end the debate. Therapeutic policies determined by such consensus-building mechanisms as the Delphi method, a systematic literature-based consensus process for making complex clinical practice guidelines (Vermeulen et al., 1995), have a place in medical decision making, but they are not a substitute for high-quality clinical trials with large numbers of subjects.

The suggestion that one colloid solution, albumin, has the potential to cause harm further underlines the need for adequate controlled trials. There are several reasons why the use of albumin might become a moot point, including its limited availability and the reluctance of clinicians to use a blood fractionation product. The same may be said for freeze-dried plasma, which cannot yet be treated to eliminate the risk of viral transmission and would require reconstitution under battlefield conditions. Albumin synthesis by recombinant technology is being pursued, but it is not yet an alternative and will likely be expensive. Cardiac decompensation may occur after the rapid infusion of large volumes of a highly concentrated (25 percent) albumin solution or some other colloid, since this leads to a substantial increase in volume retention. Increased filtration of fluids together with proteins into the interstitial space may exceed the ability of the lymphatic system to return extravascular fluids to the circulation (Roberts and Bratton, 1998). However, arguments against the use of colloids rely heavily on older studies with non-human primates that show, for example, that interstitial pulmonary edema develops after albumin administration in hemorrhagic shock (Moss et al., 1979). These studies use conventional colloid formulations. Similarly, studies suggest that conventional albumin resuscitation may impair sodium and water excretion and worsen renal failure (Moon et al., 1989). Furthermore, the timing of resuscitation, in terms of the progression of the shock syndrome, may be as important as the nature and volume of the resuscitation fluid.

Resuscitation with a minimal volume of hypertonic saline has been discussed, but it is important to note that hard evidence of the effectiveness of this strategy exists primarily from the early resuscitation of patients with ruptured aortic abdominal aneurysms and penetrating truncal trauma. Alternative synthetic colloids like hydroxyethyl starch (and other high-molecular-weight starches), long used in Europe, may not leak as readily into the extravascular

space and appear to improve hemodynamics and reduce pulmonary edema compared with saline solutions infused into patients with capillary leak syndrome (Boldt et al., 1998). The mild anticoagulant properties of polydisperse colloids such as starches and dextrans, in part as a result of interference with platelet function, have not been associated with significant bleeding problems. This defect might even prove to be advantageous by reducing the activation and adhesion of platelets and granulocytes to one another and to the vascular endothelium. Similar effects have been reported with the lower-molecular-weight plasma expanders, such as modified gelatin-based colloids, which have also long been used in Europe as resuscitation solutions (Evans et al., 1998). Combinations of colloids, electrolytes, and an energy source in a hypertonic solution merit further clinical study.

COMPLICATIONS OF CRYSTALLOID RESUSCITATION

Effects of Crystalloid Resuscitation on Immune Function

It is well recognized that trauma with hemorrhagic shock produces significant stimulation of the immune system and that neutrophil activation, adherence, and emigration into tissues contribute to the systemic inflammatory response, which frequently culminates in ARDS and MODS. Although the use of crystalloids and artificial colloids has been routine for the resuscitation of patients with acute blood loss, recent studies have raised questions regarding the effects of resuscitation regimens on several aspects of the immune response to hemorrhagic shock. Rhee and colleagues (1998) examined the effects of lactated Ringer's solution on neutrophil function. Neutrophil activation and adherence have been described in trauma patients (Tanaka et al., 1991), and excess superoxide production by activated neutrophils has been implicated in trauma-related capillary endothelial injury and dysfunction (Chen and Christou, 1996; Maier and Bolger, 1996). Rhee and colleagues (1998) used a model with awake swine given a 40 percent volume hemorrhage over 1 hour, followed by resuscitation with either lactated Ringer's solution, shed blood, or 7.5 percent hypertonic saline. A control group included swine given a sham hemorrhage and a lactated Ringer's solution infusion. Flow cytometry was used to detect superoxide burst activity as a measure of neutrophil activation. Hemorrhage-mediated neutrophil activation was exacerbated by lactated Ringer's solution resuscitation, whereas shed blood or hypertonic saline infusion returned neutrophil activity to baseline values. It was of interest that lactated Ringer's solution administration to sham-hemorrhaged swine increased neutrophil activation similar to that seen in the resuscitated shock subjects (see Figure 3-3), emphasizing the need for further research in this totally undefined area.

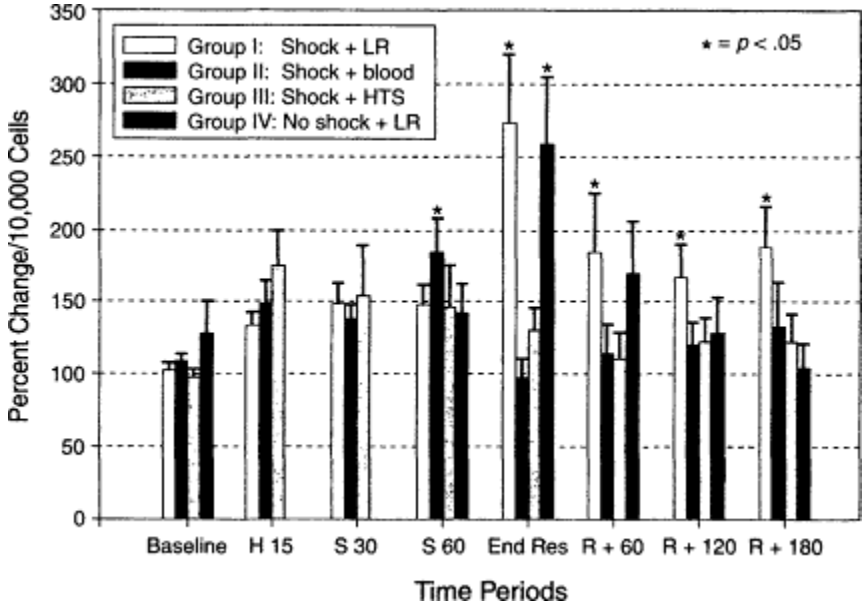


Figure 3-3
Neutrophil fluorescence, percent change from venous baseline values per 10,000 cells ± SEM, (*) = p < 0.05, compared with baseline values, ANOVA with Tu-key's b multiple comparison test. Baseline, before hemorrhage; H 15, end of 15-minute hemorrhage; S 30, 30 minutes into shock period; S 60, 60 minutes into shock period; End Res, end of 60-minute resuscitation period; R + 120, 120 minutes after end of resuscitation; R + 180, 180 minutes after end of resuscitation. Source: Reprinted, with permission, from Rhee et al. (1998). Copyright 1998 by Lippincott Williams & Wilkins.

In a subsequent study, Rhee and colleagues (in press) diluted whole blood from healthy volunteers with various resuscitation fluids to examine the hypothesis that neutrophil adherence and activation correlates with the type of fluid resuscitation used as well as with the degree of hemodilution. Oxidative burst activity was examined with dichlorofluorescein diacetate staining as well as neutrophil adhesion (cell surface receptor expression of CD 18 cells) measured by flow cytometry (flourescence-activated cell sorter analysis). Blood was diluted with several fluids to achieve 10, 25, 50, or 75 percent dilution (see Figures 3-4 and 3-5). The fluids examined included buffered saline, normal saline, lactated Ringer's solution, dextran 40, hespan (6 percent), albumin (25 percent), albumin (5 percent), hypertonic saline (3.5 and 7.5 percent). Blood samples diluted with hypertonic saline were controlled for sodium content so that it was equal to that measured in normal blood samples. These studies showed that crystalloid solutions produced neutrophil adherence, activation, and adhesion in a dose-dependent fashion. Artificial colloid solutions produced neutrophil activation and neutrophil adherence that were similiar to those with crystalloid solutions, whereas 5 percent albumin produced modest activation and 25 percent albumin produced none. It was of interest that

hypertonic saline caused neither neutrophil activation nor neutrophil adherence. The investigators indicated that this cellular activation and adherence was not associated with changes in pH, electrolyte content, hemoglobin, hemodilution, or osmolality. Although many trauma patients with hemorrhagic shock have received larger volumes than necessary for adequate resuscitation with no evidence of inflammatory activation, the data suggest that fluid resuscitation from hemorrhagic shock is not innocuous and that the type of resuscitation fluid may contribute to the inflammatory syndrome.

Subsequent studies (Sun et al., in press) showed that lactated Ringer's solution resuscitation from hemorrhagic shock increased the levels of E-selectin and intracellular adhesion molecule 1 (ICAM-1) expression on isolated neutrophils, raising further serious concerns about the immunologic consequences of crystalloid resuscitation. However, many trauma patients with hemorrhagic shock receive large volumes of resuscitation fluids without evidence of an inflammatory cascade, suggesting that fluids alone are not sufficient to trigger serious immunologic consequences. However, patients who develop systemic inflammatory responses are most frequently those who receive the large volumes of resuscitation fluids, suggesting that the cautious use of fluids should be considered.

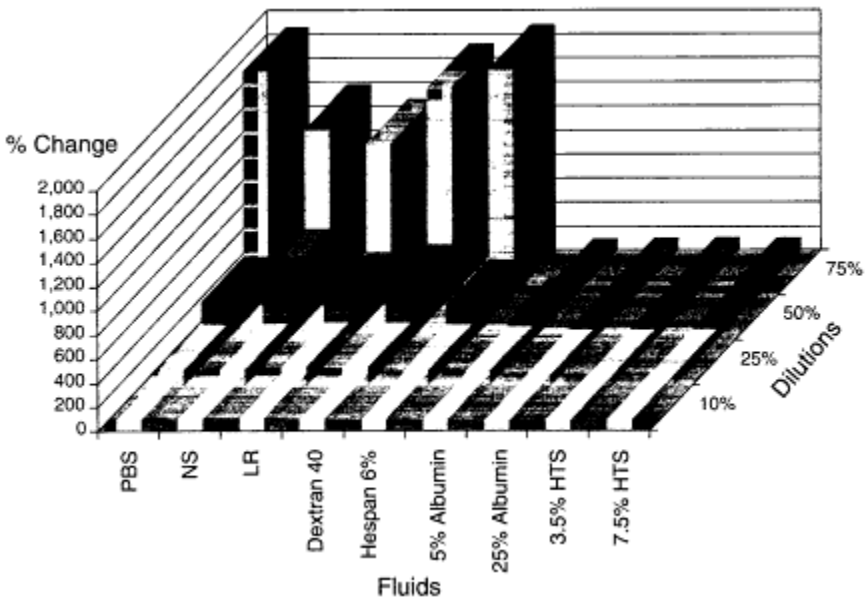


Figure 3-4
Neutrophil fluorescence (activation). Source: Reprinted, with permission, from Rhee et al. (in press). Copyright 1999 by the Society of Critical Care Medicine.

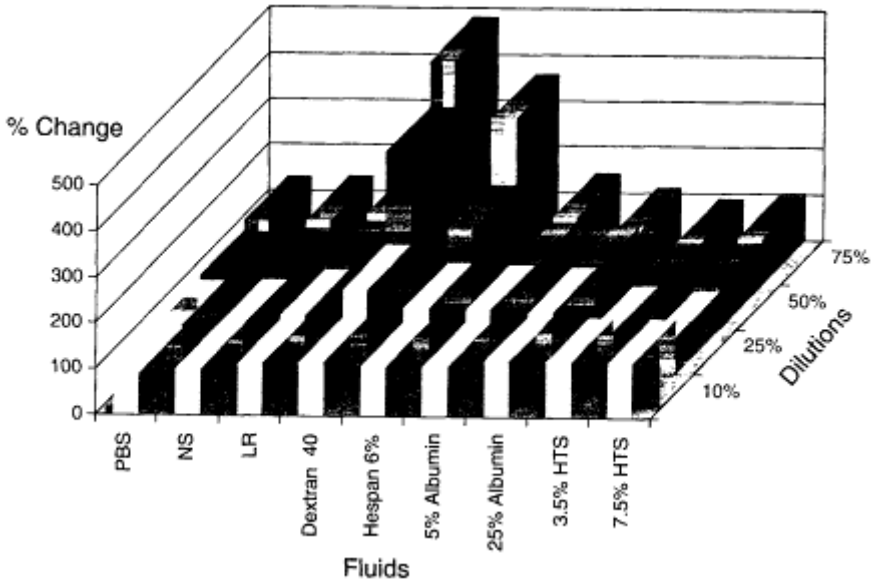


Figure 3-5
Neutrophil CD18 expression (adhesion). Source: Reprinted, with permission, from Rhee et al. (in press). Copyright 1999 by the Society of Critical Care Medicine.

Hemorrhage-induced apoptosis is well recognized (see Chapter 2), and the mechanism of intestinal mucosal immune dysfunction after trauma with hemorrhage has been attributed to apoptosis in Peyer's patches and impaired intestinal mucosal immunity (Xu et al., 1997). Similarly, trauma with hemorrhage has been shown to increase thymic apoptosis, whereas it decreases the level of interleukin-3 (IL-3) release (Xu et al., 1997). Further support for the deleterious effects of lactated Ringer's solution resuscitation from hemorrhage was provided by studies that examined the effects of resuscitation on cellular apoptosis (Deb et al., 1999b). Deb and colleagues examined lactated Ringer's solution resuscitation from hemorrhage in Sprague-Dawley rats (which received a volume of lactated Ringer's solution equivalent to three times the volume of blood that was lost); sham-hemorrhaged rats were given an equal volume of lactated Ringer's solution, and an additional group included hemorrhage rats resuscitated with 7.5 percent hypertonic saline. Lactated Ringer's solution increased the number of apoptotic cells in the mucosa and smooth muscle of the bowel wall and increased the rate of apoptosis in the liver compared to the values for sham-hemorrhaged animals. In contrast, resuscitation with neither shed blood nor hypertonic saline produced significant changes in apoptotic staining in either the mucosa, the bowel wall, or the liver (see Figure 3-6). Furthermore, the mucosa walls and bowel walls of sham-hemorrhaged rats given lactated Ringer's solution produced high levels of apoptotic staining, confirming that large-volume lactated Ringer's solution infusion, even in the absence of hemorrhage, pro

motes apoptosis in highly vulnerable tissues. The lower rate of programmed cell death in rats given whole blood resuscitation may be related to the presence of free-radical scavengers (red blood cells); however, the mechanisms by which hypertonic saline provided cellular protection remain unclear.

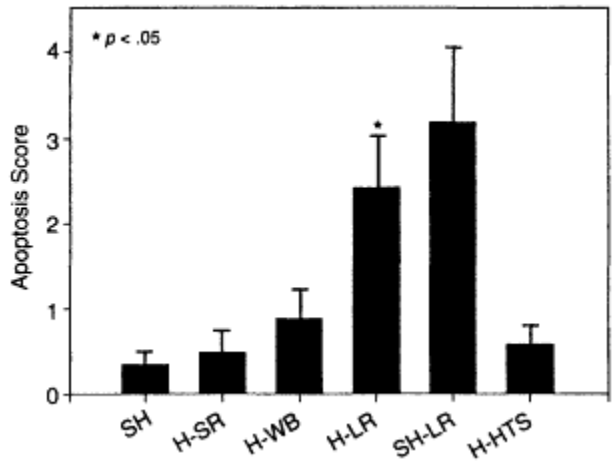


Figure 3-6
Resuscitation with lactated Ringer's solution increased the number of apoptotic cells in the mucosa and smooth muscle of the bowel wall and increased the rate of apoptosis in the liver compared to the values for sham-hemorrhaged rats. Source: reprinted, with permission, from Deb et al. (1999b). Copyright 1999 by the Society of Critical Care Medicine.

In subsequent studies, Deb and colleagues (1999a) examined the effects of various fluid resuscitation regimens on the upregulation of the Bax protein, a member of the *Bcl-2* protein family and a potent inducer of apoptosis (see Chapter 2). In their study, Sprague-Dawley rats were hemorrhaged and maintained through a 75-minute hemorrhagic shock phase; fluid resuscitation consisted of either 5 percent albumin, whole blood, 6 percent hetastarch, dextran 40, or lactated Ringer's solution. The resuscitation volume was equivalent to the hemorrhage volume except in the groups given lactated Ringer's solution, which received lactated Ringer's solution at three times the shed blood volume. Lactated Ringer's solution and hetastarch administration to hemorrhaged rats significantly increased the levels of expression of Bax in the lung compared with those for the sham-hemorrhaged group; in contrast, there was no statistically significant increase in the level of Bax protein expression in the sham-hemorrhaged or albumin-, blood-, or dextran-treated groups (see Figure 3-7). These studies confirm that increased programmed cell death and apoptosis are modulated by the type of fluid resuscitation from shock.

In summary, several studies have provided definitive evidence that distinct immunologic complications including cellular apoptosis are related to the type of fluid resuscitation. Although those studies confirm the deleterious effects of

infusions of large volumes of lactated Ringer's solution, even in the absence of hemorrhage and hypotension, the consequences of initiating fluid resuscitation with a small bolus of hypertonic saline followed by lactated Ringer's solution to maintain mean arterial pressure and cardiac output have not been addressed. Similarly, no studies, to date, have examined the immune consequences of resuscitation with a reduced volume of lactated Ringer's solution rather than the large and aggressive volumes that have become routine for the treatment of the trauma patients with hemorrhagic shock. The absence of evidence-based research in this regard suggests that additional studies are needed to examine both the immune consequence of small-volume lactated Ringer's resuscitation and the immune consequences of small-volume hypertonic saline resuscitation followed by lactated Ringer's resuscitation.

Effects of Crystalloid Resuscitation on Cytokine Response

Recently Hierholzer and colleagues (1998) showed a marked increase in the levels of IL-6 gene expression and peptide secretion after hemorrhage but only in animals that had received aggressive crystalloid resuscitation. Several studies have shown that hemorrhagic shock increases the levels of IL-1, IL-6, and tumor necrosis factor (TNF) production by Kupffer cells (Ayala et al., 1992; Yamashita, 1998; Zhu et al., 1995). Molina and colleagues (1997), using a con

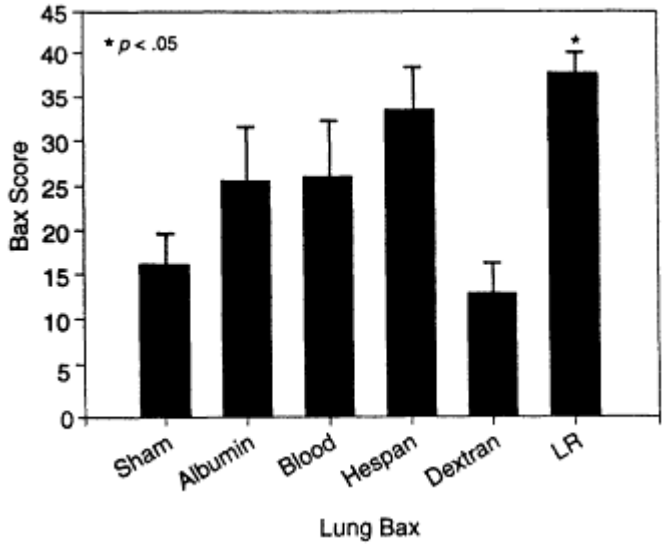


Figure 3-7
Lactated Ringer's solution administered to hemorrhaged rats significantly increased the levels of expression of Bax in the lung compared with those for the sham-hemorrhaged group. Source: Reprinted, with permission, from Rhee (1998).

scious rat model in which mean arterial blood pressure was reduced to 40 mm Hg over 25 minutes, showed significant increases in circulating TNF levels; circulating cytokine levels remained elevated after resuscitation with lactated Ringer's solution (with four times the shed blood volume), despite significant hemodilution. Unresuscitated hemorrhage produced a detectable rise in tissue TNF content (spleen and lung), and cytokine production was exacerbated by crystalloid resuscitation. The cytokine response to hemorrhage resuscitation correlated with sympathetic upregulation. Abraham (1998) confirmed the sympathetic modulation of tissue cytokine response to hemorrhage and fluid resuscitation; the marked elevation in lung IL-1 and TNF messenger RNA (mRNA) levels in pulmonary polymorphonuclear neutrophils persisted after fluid resuscitation from hemorrhage shock but was attenuated by phentolamine and adrenergic blockade. However, studies examining the effects of colloid resuscitation, reduced-volume lactated Ringer's solution resuscitation, or hypertonic saline resuscitation from hemorrhagic shock on cytokine response are lacking.

Adverse Effects of Large-Volume Crystalloid Resuscitation

Although Shires' work proposed that volume resuscitation after hemorrhagic shock should consider extracellular fluid deficits (Shires et al., 1960a, 1961, 1964), others proposed that altered extracellular fluid volume was an inadequate basis for salt and water infusion in critically ill individuals (Bishop et al., 1991; Gattinoni et al., 1986; Lowell, 1990; Ray, 1974; Roth et al., 1969; Simmons et al., 1987). More recently, Lyons (1996) described a significant increase in surgical mortality that was attributed to crystalloid overresuscitation in instances in which resuscitation began during surgery and continued uninterrupted throughout the postoperative period. A review of 37 deaths on the surgical service between January and June 1990 showed that over half of the deaths were related to respiratory failure; a significant portion of these deaths were related to fluid overload on the basis of criteria of continuous intravenous infusions, significant acute weight gain, clinical evidence of abnormal extracellular fluid volume, and "cumulative crystalloid balance far in excess of any physiologic need" (Lyons, 1996, p. 42). Although this was a small clinical study, excess crystalloid administration after hemorrhage or during the perioperative period posed a significant clinical risk of ARDS.

To further address the question of optimal volume of crystalloid for the restoration of intravascular deficits after hemorrhage, Lilly and colleagues (1992) used a large-animal model to confirm that the infusion of normal saline in a volume that was 1.8 times the hemorrhage volume provided significant benefit, even though the mean arterial pressure was unchanged from the levels during the hemorrhage. These investigators proposed that early small-volume resuscitation could provide significant benefit, particularly in patients with uncontrolled hemorrhage, in whom arterial pressure and thus bleeding would be unaffected by this type of resuscitation. The consistent finding of an acute

weight gain of 10 to 20 percent in patients after trauma or surgery suggests a significant risk for pulmonary edema (Lowell, 1990). As described by Lyons (1997), a 10 to 20 percent weight gain in a 70-kilogram (kg) man represents "a 2-3 gallon increase in the intracellular and extracellular fluid compartments." These studies collectively suggest that reassessment of both the composition and the rates of fluid resuscitation in the patient with hemorrhagic shock is warranted.

Adverse Effects of Lactated Ringer's Solution

Although Ringer's solution (Ringer, 1883) has been widely used for the treatment of hemorrhagic shock, bums, and sepsis, the negative effects of Ringer's solution were recognized as early as 1901, when Cushing described "the poisonous effects of both Ringer's and normal saline on nerve muscle preps" (Cushing, 1901). Subsequently, lactated Ringer's solution was described as superior to normal saline for treatment of infant diarrhea since the lactate could be metabolized to carbon dioxide (CO_2) and water (H_2O), providing a safe and acceptable substitute for bicarbonate (Hartmann, 1934). The deleterious effects of normal saline were also recognized in that the kidneys are unable to excrete the excess chloride resulting from the transfusion of large volumes of normal saline, producing hyperchloremic acidosis.

Current research has focused on the electrolyte compositions of fluids and whether current regimens of volume replacement aggravate shock-mediated plasma, extracellular electrolytes, and intracellular electrolyte imbalances. The well-characterized loss of intracellular potassium ion (K^+) concentrations and increases in intracellular sodium ion (Na^+) and calcium ion (Ca^{2+}) concentrations in shock can be exacerbated by sodium chloride (isotonic or hypertonic), producing hyperchloremic acidosis; additionally, Ringer's acetate solution can promote selective sequestration of calcium in the mitochondria, depleting the calcium that is essential for cellular function.

Although lactated Ringer's solution has been used to treat several types of trauma and hemorrhage, concerns regarding the side effects of lactated Ringer's solution in the injured subject with blood loss have been raised (Dronen et al., 1992). Lactated Ringer's solution is a racemic mixture containing two stereo-isomers of lactate: D(-)-lactate and L(+)-lactate. L-lactate is a product of glycolysis, and concentrations in serum (0.5 to 0.6 millimolar) directly correlate with food intake and physical activity levels; D-lactate is produced either from ketone bodies or by microorganisms (Anderson et al., 1997). Metabolism of D-and L-lactate occurs via different pathways and produces distinct metabolic consequences. A rise in serum D-lactate levels alters neurologic function, producing encephalopathy (Thurn et al., 1985). Although the toxicity of D-lactate is well recognized, several limitations of L-lactate alone in solution have been proposed. The L-lactate-pyruvate balance is tightly controlled in normovolemia; however, resuscitation with lactated Ringer's solution after traumatic injury alters the lactate-pyruvate balance, reducing the ratio of the concentration of oxy

dized nicotinamide adenine dinucleotide (NAD^+) to the concentration of reduced nicotinamide adenine dinucleotide (NADH), reducing the cytosolic phosphorylation potential, and depleting cellular energy stores. An altered cellular redox state may alter hormonal function and diminish cardiac filling. In addition, resuscitation with existing regimens of lactated Ringer's solution and normal saline has been shown to lower the cellular phosphorylation potential (Veech et al., 1986) and exacerbate shock-induced intra- and extracellular calcium shifts, worsening shock-mediated cell injury and dysfunction.

The significant advantage of the currently available lactated Ringer's solution is that it provides a source of bicarbonate as a result of the metabolism of lactate to CO_2 and H_2O; and unlike bicarbonate, lactated Ringer's solution does not precipitate calcium when it is added to intravenous fluids. D-Lactate is oxidized to pyruvate via an enzyme that has not been identified in human tissues. Previous studies have shown that D-lactate is metabolized at a slower rate than L-lactate and that D-lactate is excreted in urine (Cori and Cori, 1929). The toxicity of D-lactate has been described in patients undergoing peritoneal dialysis with racemic lactate mixtures (Chan et al., 1994), indicating the limitations and dangers of this solution for select patient populations. However, no incidences of either neurologic impairment or encephalopathy have been attributed directly to lactated Ringer's solution resuscitation from hemorrhagic shock.

However, the cardiotoxicity of lactated Ringer's solution resuscitation from hemorrhagic shock has been examined in adult Sprague-Dawley rats. Ringer's solutions containing L-lactate, D-lactate, or the racemate were compared in conscious unrestrained rats after hemorrhage to a mean arterial blood pressure of 40 millimeters of mercury (mm Hg) over a 10-minute period. The volume of lactated Ringer's solution equaled four times the maximal bleed-out volume. Electrocardiograms were monitored for changes in rhythm, ectopy, ventricular tachycardia, sinus bradycardia, heart block, and asystole. Rats resuscitated with either the racemate- or L-lactated Ringer's solution had no changes in cardiac function, whereas D-lactated Ringer's solution produced various degrees of cardiac arrhythmogenicity, premature ventricular contractions, ventricular tachycardia and bradycardia as well as ventricular fibrillation, third-degree heart block, and asystole (Delman et al., 1996). The absence of cardiotoxicity with the use of either racemate- or L-lactated Ringer's solution is consistent with clinical reports of successful resuscitation of trauma patients with hemorrhage and an absence of serious side effects. For example, the clinical use of lactated Ringer's solution (particularly in burn patient populations who may require 15 to 20 liters of lactated Ringer's solutions during the first 24 hours postburn) has not been associated with notable cardiac or neurologic deficits. These data emphasize the safety of current regimens of lactated Ringer's solution and suggest that the form of lactate in lactated Ringer's solution is not important clinically as long as there is a racemic mixture.

Safety and Efficacy of Hypertonic Saline Solutions

The use of hypertonic saline for resuscitation from hemorrhage was first described in 1980, when Velasco and colleagues and DeFelippe and colleagues reported in separate studies that hyperosmotic sodium chloride rapidly expands plasma volume after major blood loss (DeFelippe et al. 1980; Velasco et al., 1980). Subsequent clinical studies examined the role of concentrated salt solutions in the resuscitation of patients with severe burn injuries (Caldwell and Bowser, 1979; Monafo et al., 1984). Current interest in hypertonic saline solutions for clinical application arose from studies by Kramer and colleagues (1986), and Holcroft and colleagues (1987), who described the use of a small bolus of hypertonic saline-dextran (HSD; 4 ml/kg) to initiate fluid resuscitation from hemorrhagic shock in a sheep model. In their study, the infusion of HSD was followed by the infusion of lactated Ringer's solution to maintain hemodynamic function and urine output at prehemorrhage levels. This small-volume resuscitation was shown to restore blood pressure and cardiac output within 2 minutes of infusion, to maintain both oxygen consumption and urine output above baseline levels during a 30-minute simulated transport period, and to decrease the total volume of lactated Ringer's solution used for resuscitation.

These studies in the early 1980s produced a storm of experimental and clinical studies examining the use of HSD as a pharmacologic intervention for early restoration of blood pressure and cardiac output in the field. Over 300 papers have appeared in the last 10 years, and these studies from several independent laboratories with several animal models of hemorrhagic shock have confirmed the consistent physiologic benefits of small-volume hypertonic saline resuscitation (Coimbra et al., 1997; Dubick and Wade, 1994; Greene et al., 1998; Horton et al., 1995; Kramer et al., 1986; Lilly et al., 1992; Maningas et al., 1986a, b; Nakayama et al., 1985; Pascual et al., 1992; Velasco et al., 1980; Velasco et al., 1989; Wade et al., 1990). These studies have confirmed the safety of HSD, the early volume-sparing benefits, as well as the significant improvements in hemodynamic function. Differences in the composition of hypertonic solutions were studied by a group at the University of California at Davis (Smith et al., 1985); the benefits of hypertonic sodium acetate, sodium chloridemannitol and sodium chloride-6 percent dextran 70, and glucose were compared by using an ovine shock model. These solutions were used as a bolus of 4 ml/kg, and although all solutions were initially successful, the beneficial effects were transient with all solutions except the 7.5 percent saline-dextran 70. This optimal hypertonic solution provided posttrauma cardioprotection, reduced the oxygen debt, and improved the microcirculation.

Since 1986, several clinical trials of HSD for initial resuscitation from hemorrhagic shock have been performed (Holcroft et al., 1987). The initial trial included injured adults who required helicopter transport and who were randomized to receive either HSD or normal saline during transport; HSD significantly improved the overall survival rate, but most importantly, this initial study confirmed that rapid HSD infusion has no adverse effects in the hypovolemic pa

tient. Subsequent prospective studies compared HSD with either hypertonic saline alone or lactated Ringer's solution. Despite the improved survival rate when HSD was used in the field, the application of this resuscitation regimen in the emergency department failed to affect the survival rate (Vassar et al., 1990). Other clinical trials included a multicenter trial at Ben Taub General Hospital, the Denver General Hospital, and the Milwaukee County Medical Complex (Mattox et al., 1991), and included a total of 422 patients enrolled over a 13-month period. Patients were randomized in a blinded fashion to receive an initial bolus of HSD or crystalloid administered during transport by ambulance. Blood pressure was significantly improved in the HSD group upon arrival at the emergency department, and HSD significantly affected the survival rate in that sub-population of patients requiring emergency surgery. This prospective clinical study confirmed the safety of small-volume HSD administration in injured patients, and the major benefit of resuscitation with HSD appeared in the population of patients with craniocerebral trauma, in whom resuscitation with large-volume crystalloid tended to increase intracranial pressure.

To date, eight double-blind randomized trials have evaluated the use of small-bolus HSD for prehospital or emergency department treatment of patients with traumatic hypotension (Holcroft et al., 1987; Shackford et al., 1992; Vassar et al., 1990, 1993a,b; Wade et al., 1997a). Improved rates of survival after discharge were reported with HSD in seven of eight trials, although statistically significant improvement in overall survival was seen in only one trial. Metaanalysis for the evaluation of HSD as the initial treatment for hypovolemic shock included the original records from six trials and included 604 subjects. Overall discharge survival rates were improved with HSD resuscitation, and HSD resuscitation was particularly effective for a subpopulation of subjects with head injury with a discharge survival rate of 38 percent compared with a rate of 27 percent for the control group receiving saline (Wade et al., 1997b). The striking benefits of the HSD solutions have been described recently. Hypertonic saline solutions were shown to provide immunologic protection, whereas hypo-osmolar lactated Ringer's solution as well as artificial colloids upregulated adhesion molecule expression, increased proinflammatory cytokine synthesis, and promoted cellular apoptosis (Coimbra et al., 1996; Junger et al., 1997a,b; Rhee, 1998).

The limits of hypertonic saline resuscitation include the fact that its efficacy is lessened in patients who receive an initial bolus of isotonic lactated Ringer's solution. In addition, rapid administration of HSD may produce profound vasodilation and hypotension. Hypertonic saline-dextran for resuscitating hemorrhagic shock in the presence of head injury has received considerable attention. Despite significant concerns regarding the use of concentrated salt solutions for resuscitation from hemorrhagic shock, current regimens of hypertonic resuscitation produce a transient rise in serum osmolality that remains less than 350 milliosmolar (mosM)/liter. The serum sodium level has been shown to remain less than 160 milliequivalents (meq); the 15-gram dextran load in HSD resuscitation has been associated with no morbidity.

However, Gross and colleagues (1988) raised the question as to whether early resuscitation of the injury patient with HSD increases blood loss in the presence of uncontrolled hemorrhage. With several rodent models, including rats with transected tails or ileocolic artery injury, deleterious effects of HSD in the presence of continuing blood loss were described (Gross et al., 1989). Despite these data, there has been a remarkable absence of deleterious effects with HSD administration in more than 1,000 trauma and surgical patients who have been treated with HSD (Kramer et al., 1995). No incidence of hypernatremic seizures, increased bleeding or blood needs, coagulopathies, renal failure, cardiac arrhythmias, or central pontine myelinolysis have been described in trauma patients. However, HSD administration in patients with significant cardiac dysfunction has been shown to produce hypertension and signs of acute fluid overload. In patients with underlying cardiac dysfunction, titration with smaller volumes such as 1 mg/kg have been recommended (Kien et al., 1997; Welte et al., 1997, 1995).

Although results vary with individual studies, evidence from human trials suggests that both the safety and efficacy of hypertonic (7.5%) saline alone are similar to those for the hypertonic saline-6% Dextran 70 solution. Clinical trials in civilian trauma patients (Vassar et al., 1990, 1993a,b) demonstrated no adverse clinical effects of infusing either solution, and the addition of the colloid did not appear to offer any additional benefit.

Given the information presented above, the safety and efficacy of small-volume hypertonic resuscitation has been confirmed. In light of the continuing concerns of transportation of large crystalloid volumes required for resuscitation, the ability to recover mean arterial pressure and cardiac output in the field with small-volume HSD resuscitation confirms the value of this regimen as the initial treatment for hemorrhagic shock in the field. The added benefits of HSD-related reductions in the total volume of fluid used for resuscitation may contribute to a decrease in the incidence of ARDS, MODS, and systemic inflammatory response syndrome currently associated with crystalloid resuscitation. Finally, the finding that HSD resuscitation did not enhance the cytokine response or alter any aspect of the immune response to hemorrhage are added benefits.

ALTERNATIVE RESUSCITATION APPROACHES

Carolina Rinse Solution, Initially Applied As A Rinse And Storage Solution For Transplanted Organs, Significantly Increased Organ Survival Above That Described With Lactated Ringer's Solution. The Solution Contains Antioxidants, Allopurinol, Desferrioxamine, Nicardipine, Glutathione, Insulin, Low-Concentration Adenosine To Inhibit Kupffer-Cell Activation, And The Cytoprotective Amino Acid Glycine (Lemasters Et Al., 1995). Carolina Rinse Solution Has Been Shown To Provide Considerable Protection Of The Small Bowel And Liver Before Transplantation In Both Humans And Animals (Abdennebi Et Al., 1998; Massberg Et Al., 1998; Gao Et Al., 1991). Compared To Lactated Ringer's Solution, The Carolina Rinse Solution

improved the excretion of indocyanine green which is a known marker of parenchymal and nonparenchymal cell integrity, from bile, improved nutritive perfusion after small-bowel transplantation, attenuated the leukocyte-endothelial cell interaction, and almost completely prevented mucosal ischemia reperfusion injury. Additional studies showed that Ringer's solution containing adenosine (0.1 mM) increased the survival time when it was used to rinse cold-stored liver grafts. Despite the improved survival following liver transplantation, however, Ringer's solution with adenosine did not prevent parenchymal cell injury, suggesting that the adenosine in the Carolina Rinse solution may work via non-hepatic mechanisms (Gao et al., 1991).

In a human clinical trial, the use of the Carolina Rinse solution improved the transaminase release by transplanted human livers. Recently, a modified Carolina Rinse solution has been examined in a cardiac preparation; coronary artery occlusion in open-chest pigs produced myocardial infarction, as expected. Modified Carolina Rinse solution plus cyclosporine, used as the initial solution for reperfusion of the cardiac tissue, decreased creatine kinase release, confirming the significant rescue of ischemic and hypoxic tissue (J. Lemasters, 1998, School of Medicine, University of North Carolina at Chapel Hill, personal communication). The inclusion of an immunosuppressant such as cyclosporine likely modulated the reperfusion injury via several mechanisms, including blockade of the mitochondrial transition pore and altered translation of protein synthesis. Despite the advantages of the Carolina Rinse solution in attenuating postischemic microvascular injury after small-bowel or liver transplantation, the value of this solution for volume replacement after trauma or hemorrhage has not been evaluated. However, the value of adding antioxidants, cytoprotective amino acids, or adenosine to resuscitation fluids warrants further study.

SUMMARY

Numerous recent studies have raised questions about the type, volume, and rate of fluid resuscitation from shock. The incidence of ARDS and MODS in patients who have received large-volume crystalloid resuscitation is a significant concern. The finding that lactated Ringer's solution and artificial colloids increase the levels of expression of adhesion molecules, promote the release of proinflammatory cytokines, and promote cellular apoptosis suggests that fluid resuscitation has significant immunologic consequences. It was of interest that hypertonic saline solutions, shown to stabilize arterial blood pressure and cardiac output with small-volume infusion, have been shown to have no deleterious effects on immune function or to promote programmed cell death. Valid concerns have been raised regarding the composition of lactated Ringer's solution, and the omission of D-lactate with a concomitant reduction in the total L-lactate load appears feasible. The increasing availability of solutions with improved oxygen transport capabilities coupled with therapeutic strategies that limit reperfusion injury (antioxidants, iNOS inhibitors) have great potential. However,

current research and clinical experience caution against the indiscriminate use of crystalloids for resuscitation. As Evans cautioned in 1911, "under certain circumstances saline solutions are productive of great harm to the tissues of the body" (Evans, 1911).

CONCLUSIONS AND RECOMMENDATIONS

The committee found that although the existing resuscitation fluids are rarely questioned in clinical practice, there are at least theoretical disadvantages to these fluids, and many have not been modified for several decades. In contrast, there have been significant advances in organ preservation resulting from new fluid formulations. New resuscitation fluids should be developed and tested as described in the committee's recommendations below. Such fluids should address the metabolic and cellular consequences of traumatic shock and the potential disadvantages of existing fluid formulations.

An ideal fluid for intravenous use should provide adequate control of pH, partial CO_2 pressure/bicarbonate ratio, the phosphorylate potential, the redox state, and osmotic pressure; adequate control of sodium chloride, calcium, and potassium levels; and adequate control of the lactate and pyruvate ratio. Veech and colleagues (1986) proposed modification of existing regimens of fluid resuscitation to more nearly mimic the composition of intravascular fluid. For example, the use of parenteral normochloremic carbonate (HCO_3^+)/CO_2 saline would have a more physiologic ratio of sodium chloride, thus avoiding the risk of hyperchloremia. The stability of such electrolyte solutions could contribute to a long shelf life in both combat situations and civil disasters. Concerns regarding the toxicity of lactated Ringer's solution (which contains 14 mM D-lactate) (Chan et al., 1994) have prompted the recommendation that D-lactate be excluded from current preparations of lactated Ringer's solution and that more physiologic levels of L-lactate be used. Veech (1986) further proposed replacing D- and L-lactate in lactated Ringer's solution with "physiological ratios of L-lactate-pyruvate, HCO_3^-:CO_2, and d-β-hydroxybutyrate:aceloacetate" (p. 547). Inclusion of such compounds would allow tighter control of the phosphorylation state as well as better control of the redistribution of water and electrolytes in shock. The rapid reversal of such shock-mediated shifts in sodium, potassium, and chloride levels should reduce the level of tissue injury. However, evidence-based research demonstrating (1) the ability of such solutions to effectively restore blood volume, (2) the hemodynamic, hematologic, cellular, and immunologic advantages, and (3) an absence of side effects, is lacking. Other concerns, which exist with proposed modifications of existing regimens with saline, include the concern that the unphysiologic concentrations of bicarbonate may produce alkalosis (Veech, 1986, 1991). To guide the development of an ideal fluid, the committee makes the following recommendations.

Recommendation 3.1 **Research involving modifications of existing lactated Ringer's solutions could include:**

1. **elimination of** D-lactate,
2. **reduction of total** L-lactate load,
3. **addition of ketones as an energy source, and**
4. **addition of free-radical scavengers and antioxidants (vitamins E and C, glutathione, or iron chelators).**

Recommendation 3.2 **Studies examining modifications of the existing lactated Ringer's solution formula must include examining the effects of the modified solution on:**

1. **immunologic-related function,**
2. **cellular apoptosis,**
3. **intravascular retention, and**
4. **specific end-organ function such as pulmonary, renal, and cardiac function (i.e., the presence or absence of arrhythmias).**

Recommendation 3.3 **Previous concerns regarding the detrimental effects of aggressive fluid resuscitation with large volumes of crystalloids suggested the need to examine both the immunologic as well as hemodynamic consequences of small-volume lactated Ringer's resuscitation. Studies examining reduced volume of lactated Ringer's solution should examine the effects of this volume on:**

1. **hemodynamic function,**
2. **immunologic-related function,**
3. **cellular apoptosis, and**
4. **specific end-organ function, such as pulmonary, renal, and cardiac function.**

4

Novel Approaches to Treatment of Shock

To stop his wounds, lest he do bleed to death
— Shakespeare, The Merchant of Venice,

Act IV, Scene I

Approximately 80 percent of battlefield casualties experience substantial blood loss, and hypovolemic shock is a major contributor to early mortality from trauma, reportedly the leading cause of death in Americans under the age of 45 years. Improved resuscitation fluids and fluid protocols might reduce the rates of morbidity and mortality both in the far-front battlefield "stay and play" setting and in the civilian setting, where the "scoop and run" strategy might be logistically easier to institute. The different requirements for differing scenarios of trauma seem to argue for a formulary of boutique fluids, perhaps broadly divided into supportive fluids and therapeutic fluids designed to take into account the pathophysiology of the particular injury. The committee recognizes, however, that military staff training and logistical considerations favor simplicity and a single resuscitation fluid with a single resuscitation strategy wherever possible.

The ideal resuscitation fluid would have to have the properties of an elixir of life: a small-volume cocktail that among its virtues improves perfusion, enhances oxygen (O_2) delivery and diffusion, provides adequate metabolic substrates, neutralizes toxic molecules released as a result of tissue injury, provides antimicrobial activity, renders the recipient globally less vulnerable to the effects of hemorrhagic shock, and has prolonged beneficial effects. The solution should further be stable for lengthy periods at a variety of temperatures, be easy to prepare and administer, and, if not inexpensive, be at least affordable. Such a solution is not on the near horizon. However, a variety of research observations and potential novel therapeutics hold promise as additives to some basic resuscitation solutions. Those novel therapeutics that emerge from preclinical testing might be tested as additives to the basic resuscitation fluid in a stepwise, controlled fashion in appropriate animal models and subsequent human studies. Strategic planning for the trauma arena should include a translational research program with goals of rational fluid design and improvement.

Hypovolemic shock results from an acute disturbance in the circulation that leads to an imbalance between O_2 supply and demand in the tissues. The shock syndrome is a final common pathway through which a variety of pathologic processes lead to cellular ischemia and ultimately cell death. Shock syndrome may predispose the individual to other major comorbidities such as acute respiratory distress syndrome, sepsis, systemic inflammatory response syndrome, and multiple-organ dysfunction syndrome (MODS). The complex events that result from the initial insult, tissue injury, and resuscitative attempts provide numerous potential therapeutic targets for novel interventions. The novel therapeutics and research strategies might be conveniently, if somewhat artificially, categorized as those that prevent the early complications of the shock syndrome (prevention), those that treat the complications of shock syndrome and reperfusion injury (intervention), and those that render the subject less vulnerable to hypoxia and its consequences (tolerance). The military should maintain a research interest, if not research support, in each of these areas, recognizing that some are approaching sufficient maturity for clinical trials, whereas others are still at a basic science stage.

PREVENTION

Optimal resuscitation requires a strategy that prevents or limits the course of the complex sequence of molecular events that contribute to the shock syndrome. In theory, such a strategy probably involves use of a solution that (1) optimizes O_2 delivery, (2) supports basic cell function, (3) provides a useful energy source, and (4) prevents oxidative reperfusion injury. The development and testing of components and combinations of such a solution are achievable research goals.

Oxygen Therapeutics

Although oxygen delivery is a critical factor in the pathophysiology of hemorrhagic shock, the role of the oxygen-carrying potential of blood and the point at which transfusion becomes essential is less well documented. The hemoglobin concentration at which a healthy resting human develops tissue hypoxia as a result of inadequate critical oxygen delivery is not known. It likely differs for a young, well-conditioned soldier and an elderly civilian with cardiovascular or pulmonary disease, and certainly differs by organ and by the nature of the acute traumatic injury. Acute isovolemic reduction of the blood hemoglobin concentration to 50 grams per liter (g/liter) in conscious, resting humans does not produce evidence of inadequate systemic critical O_2 delivery, as assessed by O_2 consumption, plasma lactate, and ST changes on an electrocardiogram (Weiskopf et al., 1998). Compensatory mechanisms included a 58 percent increase in systemic vascular resistance and expected increases in heart rate, stroke volume index, and cardiac index. This is consistent with observations

with conscious nonhuman primates (baboons) (Levene et al., 1990) and from clinical experience with Jehovah's Witnesses (Viele and Weiskopf, 1994). Healthy, resting patients tolerate anemia (<10 milliliters [ml] of O_2/kilogram [kg] of body weight/minute), a level reached in healthy humans at a hemoglobin concentration of less than 50 g/liter without systemic acidosis or other apparent adverse metabolic sequelae. Although such studies may underestimate the O_2 requirements of severely wounded soldiers (Cerra, 1987), they do suggest that resuscitation solutions with small enhancements in O_2-carrying capacity might reduce rates of morbidity and mortality.

Several different oxygen therapeutics (formerly referred to as blood substitutes or red blood cell substitutes) are in various stages of preclinical and clinical trials (Gould et al., 1998; Hughes et al., 1996; Keipert, 1998). It is important to consider each of these formulations as different drugs or biologics with different physical characteristics, biologic activities, and adverse reaction profiles. Among the hemoglobin-based molecules, some are derived from human blood, some are derived from bovine red blood cells, and one is from a recombinant *Escherichia coli* source. Tetramers have been chemically modified, polymerized, oligomerized, conjugated, and encapsulated in liposomes. Some formulations are pyridoxylated, glutaraldehyde cross-linked, diaspirin cross-linked, raffinose cross-linked, and polyethylene glycol modified. Sizes range from tetramers to distributions of relatively large polymers. Furthermore, it is possible to customize cell-free hemoglobin with a specified O_2 affinity, molecular size, heme stability, viscosity, and intravascular retention time depending upon the proposed use and the postulated requirements, if they are understood. The optimal physiologic characteristics of the molecule and of its carrying solution are not agreed upon. It is more difficult still to design appropriate clinical trials to test the specific application and define both efficacy and toxicity for hemorrhagic shock. As important as O_2 consumption is to cellular well-being, it is unlikely that these additives will yield Lazarus solutions that raise the dead; rather, they might provide incremental improvements in morbidity and mortality rates among gravely injured subjects.

For military use as a component of a resuscitation fluid, desirable characteristics of the oxygen therapeutics include the ability to deliver O_2 effectively, a lack of toxicity, freedom from infectious disease transmission, stability, low immunogenicity, ease of storage and use, and universal compatibility. Enthusiasm for these drugs as components of a resuscitation fluid has oscillated dramatically in the last year alone. This was due in part to contradictory results in clinical trials and in part to an incomplete understanding about how these drugs deliver O_2, the mechanisms of their side effects, and the fundamental mechanisms in shock, hemorrhage, and the control of blood flow. Currently the only formulation licensed as a red blood cell replacement is the veterinary product Oxyglobin (Biopure Corp., Cambridge, Mass.), which was licensed in January 1998 for the treatment of acute anemia in dogs. A similar bovine hemoglobin-derived oxygen therapeutic for human use is currently in phase III clinical trials.

Small molecules dissolved in plasma likely deliver O_2 in a fundamentally different fashion than red blood cells (erythrocytes) do. Erythrocytes are 7 to 8 micrometers (μm) in diameter, traverse the microcirculation differently than plasma, and release O_2 across a protein membrane (Homer et al., 1981). Erythrocyte O_2 transport is directly related to hemoglobin concentration. A great deal less is known about how different hemoglobin-based O_2 carriers deliver O_2 and about how they are distributed, metabolized, and excreted. The mechanisms of O_2 delivery and the ideal viscosity and concentration in a resuscitation fluid are fertile areas for research.

First-, Second-, and Third-Generation Therapeutics

Recently, resuscitation trials with one first-generation molecule, diaspirin-cross-linked hemoglobin (HemAssist; Baxter International), were halted in the United States and Europe. A full report of these trials has not yet been published. Clinical studies with a first-generation recombinant hemoglobin (Optro; Somatogen, Inc.) were suspended as well. The side effects of these preparations have been attributed by some to the inherent vasoactive properties of these molecules. Although the mechanism of this effect is unclear, it may relate to nitric oxide binding (Abassi et al., 1997), which is well recognized in cell-free hemoglobin solutions. It has not been proved that this property was responsible for excess morbidity or mortality in the clinical studies. In fact, a polyoxyethlene hemoglobin (Apex Bioscience) that acts as a nitric oxide scavenger rather than as an oxygen therapeutic has been proposed as a drug to effect vasoconstriction in shock. A second theory attributes the vasoreactivity to some auto-regulatory reflex induced by increased O_2 available from cell-free hemoglobin (Vandegriff and Winslow, 1995). Whatever the mechanism, larger, polymerized molecules seem to have minimal vasoactivity, and at least one such compound is being studied in a resuscitation trial (Gould et al., 1998). This observation underscores the importance both of considering each of these drugs individually and of continuing studies on later-generation formulations.

Several second- and third-generation oxygen therapeutics are in preclinical studies. Some novel concepts that are being entertained include inclusion of antioxidants such as superoxide dismutase (SOD) and catalase to reduce the effects of ischemia-reperfusion injury resulting from superoxide formation and oxygen radical liberation after reoxygenation. If cell-free heme iron and the lack in these solutions of the antioxidant activity that is found in intact erythrocytes does in fact exacerbate pro-oxidant activity in vivo, it might be advantageous to combine these products with molecules that chelate iron or block the oxidant-mediated injury cascade. Surprisingly few experimental data concerning iron chelation during resuscitation are published, considering that recognition of a connection between iron, free radicals, and tissue damage in hemorrhagic shock goes back almost 30 years (Hedlund and Hallaway, 1991). Polynitroxylation of biomacromolecules (albumin, starch, or hemoglobin) is one example of an approach to scavenging of reactive species to block the oxidant-mediated injury

cascade (Zhang et al., 1998). There are certainly numerous other biochemical possibilities if this concept of prevention proves clinically important.

Perfluorochemicals

Different formulations of perfluorochemicals have been used to dissolve O_2 since Clarke's original report in 1966. Perfluorocarbons are chemically synthetic molecules consisting primarily of carbon and fluorine atoms. Perfluorocarbons are formulated to dissolve large quantities of gases, including oxygen, carbon dioxide, and nitrogen, and they do so in a linear fashion. Because they are hydrophobic, they must be emulsified with a surfactant such as phospholipid before they are suitable for intravenous use. The ability of perfluorocarbons to replace red blood cells as temporary O_2 carriers was demonstrated more than 30 years ago (Geyer et al., 1968). Perfluorocarbons exchange O_2 by simple diffusion; O_2-carrying capacity is directly proportional to the partial pressure of O_2 in arterial blood. There has been limited preclinical experience with the use of these substances used in hemorrhagic shock (Goodin et al., 1994; Stem et al., 1995). Using tissue oxygenation as an endpoint and 100 percent inspired O_2, these studies demonstrate improved survival in dogs compared with that for controls given lactated Ringer's solution. Although these compounds meet many of the requirements of a candidate oxygen carrier, their requirement for supplemental (100 percent) O_2 to work optimally and issues with transport and reconstitution, even with the newer emulsions (e.g., perfluorodichlorooctane and perfluorooctyl bromide emulsions), may make them more suitable candidates for such applications as interoperative hemodilution than as additives to early resuscitation fluids to be used in the field. These emulsions may prove to be more useful in ameliorating cerebral, myocardial, and acute limb ischemia when O_2 is available after transport to a hospital setting.

Liposomes

Liposomes are self-assembling amphiphilic lipids and can be fabricated with a wide variety of compositions appropriate to the planned application. The composition determines the biological response to the infused component. Liposome formulations with extended intravascular persistence have been formulated for the delivery of pharmaceuticals (Parr et al., 1993). Similar success has eluded formulations of liposome-encapsulated hemoglobin. Hemoglobin encapsulation in lipid vesicles (liposomes) has been tested with primates; such a formulation should attenuate vasoconstriction and prolong the intravascular circulation of an O_2 carrier. Given the particle size, recent investigation has concentrated on the interaction of liposomes with the reticuloendothelial system and on the inflammatory cytokine response to infusion. Studies of hemorrhagic shock have been carried out with a rat model. A related approach used encap

sulation of hemoglobin, together with a variety of red blood cell enzymes including SOD, into biodegradable polymer nanocapsules. The physiology and toxicity profiles, including reports of thrombocytopenia, leukocytosis, and complement activation, of such formulations are now being studied. Although these approaches are promising and an encapsulated hemoglobin molecule comes closest to representing a universal red blood cell, the obstacles to clinical use and practical production are formidable.

Other Novel Approaches

Other novel approaches to the delivery of O_2 in the earliest phase of hemorrhagic shock might also qualify as prevention. For example, crocetin, a carotenoid compound derived from the fruit of *Gardenia jaminoides Ellis*, a Chinese medicinal herb, has been investigated for its protective action in quenching free radicals. However, the compound has also been demonstrated to increase O_2 diffusivity, and it has been observed to increase the level of O_2 consumption and the rate of survival in a rat hemorrhagic model (Gainer et al., 1993). A similar molecule, *trans*-sodium crocetinate, persists longer in the circulation (half-life = 25 minutes), improves diffusivity even after it is cleared, and improves the rate of survival of rats in a hemorrhagic model. These data are as yet preliminary and unpublished.

INTERVENTION

During the past decade progressive understanding of the complex pathophysiology of shock and of reperfusion injury after reoxygenation has provided numerous candidate molecular and biochemical targets that might interrupt cellular injury, stabilize the patient's clinical status, and reduce long-term morbidity and mortality. However, recognition of numerous candidate targets has often become an invitation to investigate blindly any and all potential inhibitors of substances whose levels are elevated and phenomena that are observed in the presence of shock. This approach is akin to reversing a waterfall by targeting individual droplets. Worse yet, such targeting may prove harmful because components of these complex responses have undoubtedly been selected through the process of evolution because they have a protective effect. A systematic approach to studying the processes in hopes of reversing or ameliorating those processes that result in deteriorating cellular function should prove more profitable.

Targets for Intervention

It is not possible to describe all of the potential targets for intervention; these are limited only by the imagination. Several examples may be instructive. For instance, investigations might address issues such as mitochondrial function,

which is critical to both energy homeostasis and the regulation of apoptotic cell death. One approach to reducing hypoxic brain injury might concentrate on the elevated calcium and nitric oxide levels that can damage intracellular organelles by reducing excessive synaptic glutamate levels or blocking neuronal glutamate receptors. Adenosine and γ-aminobutyric acid are two such candidate agents. Approaches to blocking nitric-oxide synthase bicalcium, which may protect the central nervous system (CNS) from lipid peroxidation and protein oxidation, might focus on the inhibition of poly-adenosine diphosphate (ADP) ribo-polymerase, overactivation of which during cell injury depletes the cell's normal nicotinamide adenine dinucleotide (NAD) supply. Agents like cyclosporin and FK506 inhibit opening of a high-conductance pore in the mitochondrial inner membrane, preventing necrotic cell death after restoration of the pH following reperfusion, an injury that is not mediated through oxygen free radicals. Similar agents that do not have immunosuppressive effects may likewise inhibit the mitochondrial permeability transition and prevent apoptotic cell death (Lemasters, 1998).

Although the early events in hemorrhagic shock include an increase in circulating as well as tissue concentrations of proinflammatory cytokines (tumor necrosis factor [TNF, interleukin-1 [IL-1], IL-6), the precise mechanism of the cytokine cascade remains elusive. If the cytokine cascade is, as has been hypothesized, a major factor in the development of MODS, which occurs in up to 15 percent of patients who survive the first 48 hours following trauma, a better understanding of the cytokine events and associated neurohormonal alterations should provide novel therapeutic strategies (Molina et al., 1997). Inhibition of individual inflammatory molecules has proved to be less promising in acute inflammation than in such chronic inflammatory conditions as rheumatoid arthritis. Inhibition of certain of the leukotrienes and thromboxanes that are associated with the aggregation of leukocytes and platelets and lipids that contain large amounts of omega-3 polyunsaturated fatty acids may reduce the production of eicosanoids as well as alter IL-1 and TNF release. However, despite promising preclinical data, clinical trials of antagonists of IL-1 (Dinarello, 1997) and TNF-α (Abraham, 1998) with critically ill patients have been disappointing. The multiple and often conflicting signaling pathways of these and other cytokines suggest a number of novel approaches (Ksontini et al., 1998), but better understanding of the process will likely be necessary before rational clinical trials can be contemplated for trauma.

A great deal of attention has been given to preventing the generation of free radicals and the activation of inflammatory cascades at the time of resuscitation. Treatment or reversal of reperfusion injury is a related priority. One mechanistic approach is to investigate control of the inducible inflammatory nitric oxide synthase (iNOS) which is upregulated in human trauma and which is involved in the activation of a variety of stress transcriptional factors. Upregulation appears to be associated with cytokine production and neutrophil accumulation in the lung and liver, at least in some animal models (Hierholzer et al., 1998). Agents such as mercaptoethylguanidine (MEG) that inhibit iNOS and that have a scavenging effect on peroxynitrite, a toxic oxidant formed from nitric oxide

and superoxide, are reported to have beneficial effects in rat and porcine hemorrhage models (Zingarelli et al., 1997). Such agents have broad inhibitory actions and will likely have some unexpected and undesirable consequences. However, the availability of scavengers of excess nitric oxide or inhibitors of iNOS provides opportunities for experimental approaches that may modulate the inflammatory response and ameliorate end-organ damage in hemorrhagic shock.

Therapies for Reperfusion-Mediated Free-Radical Damage

Although free radicals remain difficult to measure in living tissue because of their short half-lives, multiple strategies to the scavenging or neutralization of radicals have provided indirect evidence of increased free-radical production after resuscitation from shock.

Antioxidant or Scavenging Strategies

A plethora of studies have indicated an imbalance in oxidant-antioxidant mechanisms in trauma with hemorrhagic shock. Early studies examined the protective effects of SOD or catalase alone or in combination on both survival and organ injury in the shock state. These scavenging therapies provided modest protection, likely due to the short half-lives of the scavengers as well as their inability to access the intracellular space, the major site of free-radical production. Subsequent studies examined conjugation of either SOD or catalase with polyethylene glycol, a measure that increased the scavenger half-life, significantly improved the survival rate, and reduced the level of tissue injury (Tan et al., 1993; Tominaga et al., 1995).

More recent strategies have focused on the use of SOD and catalase with polyhemoglobin as a new blood substitute for the treatment of hemorrhagic shock. This combined strategy resulted in only a minimal increase in oxygen-radical generation, as measured by the hydroxylation of 4-hydroxybenzoate to 3,4-dihydroxybenzoate (Chang, 1998). Additional modifications to this hemoglobin- and scavenger-containing blood substitute have included the encapsulation of hemoglobin, SOD, and catalase in liposomes or biodegradable nanocapsules. This combined strategy would increase the oxygen carrying capacity of the blood as well as provide significant scavenger activity (see Figure 4-1). Although various degrees of organ protection have been achieved with SOD and catalase, the most promising strategy would appear to be the incorporation of these compounds in a polyhemoglobin compound that would also serve to increase the oxygen-carrying capacity of the blood while scavenging the deleterious by-products of reperfusion.

Another pharmacologic approach to the scavenging of free radicals has been the administration of *N*-acetylcysteine (40 milligrams [mg]/kg/day). Suter and colleagues (1994) described the ability of this scavenger to enhance recov

ery from acute lung injury in a university hospital intensive care unit. *N*-Acetylcysteine improved the survival rate, reduced the time of ventilatory support, and improved the oxygenation index (partial arterial pressure O_2/fraction of inspired O_2); in addition, no adverse effects were observed during *N*-acetylcysteine treatment. *N*-Acetylcysteine has also been shown to provide significant protection in a number of models, including ischemia reperfusion and hemorrhagic shock (Demir and Inal-Erden, 1998; Fan et al., 1998).

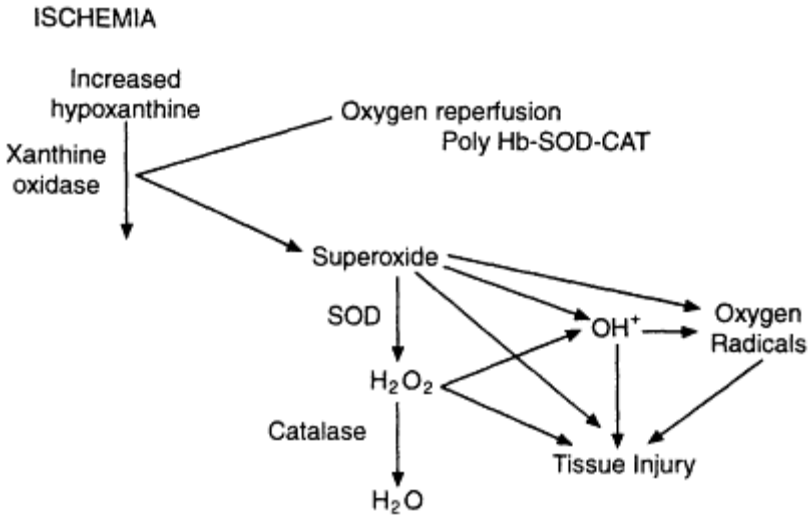


Figure 4-1
Schematic diagram showing the relationship between ischemia and oxygen reperfusion. Source: Reprinted, with permission, from Chang (1998).

In addition to neutralizing or scavenging the free radicals produced during resuscitation from shock, antioxidant therapy has been examined in several experimental models. Trolox, a water-soluble vitamin E antilog, and ascorbic acid (vitamin C) have been studied in hemorrhagic shock in rats (Daughters et al., 1996). The addition of vitamin E but not the addition of vitamin C to fluid used for resuscitation from shock significantly improved the 72-hour survival rate (75 percent compared to 40 percent for vitamin E-treated and vehicle-treated animals). The improved survival rate was not related to differences in either blood pressure or neutrophil adhesion and activation. Zollei and colleagues (1989) showed that the synthetic antioxidant of the dihydroquinoline type provided significant protection against hemorrhagic shock-induced gastric lesions, whereas Ekman and colleagues (1994) showed that low-dose ascorbate protected the gastric mucosa if it was given 15 minutes prior to hemorrhage. Anti-oxidant strategies have also been shown to reduce total fluid requirement in trauma, to improve hemodynamic variables, and to provide significant cardioprotection (Horton et al., 1996; Matsuda et al., 1991, 1995).

Iron Chelation

One biochemical consequence of ischemia followed by reperfusion is the release of iron from intracellular storage sites; although the quantities of iron released during the ischemic process are minute, reintroduction of molecular oxygen with fluid resuscitation promotes iron-oxygen interaction, setting the stage for free-radical formation. Earlier experimental studies of ischemia and reoxygenation have shown that deferoxamine (DFO) conjugates reduced micro-vascular injury after reperfusion, suggesting that chelation of iron during volume expansion provides significant vascular protection. Increased levels of iron in plasma after global ischemia were described as early as the 1950s (Mazur et al., 1955, 1958). Those investigators showed that a rise in plasma iron levels was capable of saturating the available transferrin-binding capacity. However, the contribution of ferrous iron and its role as a catalyst in the decomposition hydrogen peroxide to produce the highly reactive hydroxyl radical were not clearly recognized until 1985 (Thomas et al., 1985).

The role of free catalytically active iron in shock resuscitation-mediated tissue injury has been best defined by the use of iron chelators, which have been shown to reduce injury and provide significant long-term protection. The protective effects of DFO have been attributed to inhibition of iron-dependent free-radical reactions (Halliwell, 1989). Although the number of publications implicating iron chelation in shock resuscitation-related injury is extensive, the concept of adding an iron chelator at the time of fluid resuscitation to reduce injury and to attenuate numerous aspects of the postshock inflammatory response has proven to be exceptionally attractive. DFO administered with fluid for resuscitation from hemorrhagic shock significantly improved cellular function (Sanan et al., 1989) whereas the addition of DFO to cardioplegic solutions provided significant cardioprotective effects in models of ischemia (Illes et al., 1989; Menasché et al., 1988). Yet, the short half-life and the hypotensive effects of intravenously administered DFO have limited the clinical application of this compound. Similarly, the normally occurring iron-binding proteins (transferrin or ferroxidase) provide significant antioxidant effects but have not been studied in models of hemorrhagic shock. Instead, the development of a synthetic transferrin (produced by covalent binding of DFO to colloids) reduced the systemic toxicity of DFO (Hallaway et al., 1989), reduced micro-circulatory disturbances and improved organ function in models of hemorrhage, shock and smoke inhalation (Bauer et al., 1995; Demling et al., 1996)

Restriction of the chelator to the intravascular compartment has not been shown to reduce the efficacy of a colloid-deferoxamine conjugate (Hedlund and Hallaway, 1991). Starch-chelator conjugates developed by Biomedical Frontiers (Hedlund, 1998) have been used in two clinical trials and their safety, suitability for freeze-drying, and suitability for incorporation into resuscitation fluids currently in use in civilian and military settings have been confirmed. Those studies have further shown that this resuscitation fluid is well tolerated, even in patients with iron overload. The suitability of this compound for field use is related to

reconstitute it under field conditions. Currently, preclinical trials are needed to determine the optimal volume expansion, rate of excretion, and optimal chelator concentration for continued use of these compounds.

Other compounds described to inhibit iron chelation include tirilazad mesylate, a 21-aminosteroid. Infusion of these aminosteroids prior to fluid resuscitation from hemorrhagic shock have been shown to preserve endothelial structural integrity and to improve outcome, despite no change in neutrophil influx into tissue (Eversole, 1993; Fleckenstein et al., 1991).

Inhibition of Polymorphonuclear Neutrophil Adherence and Activation

Indirect evidence for the role of neutrophils as a source of free radicals and deleterious mediators in trauma with hemorrhagic shock has been provided by studies that use monoclonal antibodies to inhibit specific adhesion molecules involved in the tethering, adherence, and activation of this cell population. Several studies have shown improved hemodynamic performance, reduced cellular injury, a downregulation of the overall inflammatory response, and improved survival in animals resuscitated from hemorrhagic shock with lactated Ringer's solution plus the R6.5 antibody, a specific inhibitor of intracellular adhesion molecule 1 (Mileski et al., 1990, 1991). This antibody strategy reduced the level of adherence of polymorphonuclear neutrophils in the microcirculation of several organs and prevented the transmigration of activated leukocytes into peripheral tissues (Mileski et al., 1990). The application of these monoclonal antibodies with fluid resuscitation in several other types of trauma confirmed the hemodynamic and cardioprotective effects (Horton et al., 1996).

Nitric Oxide Inhibition

Hemorrhagic shock and resuscitation activate the inducible isoform of nitric oxide synthase (iNOS), and selective inhibition of iNOS provides beneficial effects in several types of circulatory shock. Of particular concern is generation of the highly toxic compound peroxynitrite, which is formed by the interaction of nitric oxide and the superoxide radical. Both pharmacologic approaches and iNOS-knockout animals have confirmed that nitric oxide and peroxynitrite play significant roles in cellular injury and organ dysfunction after fluid resuscitation from hemorrhagic shock. Recent strategies have been directed toward specific scavenging of peroxynitrite in an effort to delay vascular decompensation and to reduce cellular energetic failure in severe hemorrhagic shock. In this regard, MEG is a potent inhibitor of iNOS and effectively scavenges peroxynitrite. MEG added to fluid used for resuscitation from shock diminished the shock-related increase in plasma nitrite/nitrate and 6-keto-prostaglandin F 1-α levels, improved arterial blood pressure, and ablated the vascular hyporeactivity associated with crystalloid resuscitation from shock. Lactated Ringer's solution re

suscitation from shock has been associated with a significant rise in the levels of nitrotyrosine, an indicator of peroxynitrite formation; nitrotyrosine formation was ablated by MEG treatment of shock (Salzman, 1998). The use of MEG in porcine hemorrhagic models provided similar hemodynamic improvement, reduced lipid peroxidation, improved the mean arterial blood pressure and cardiac index, and significantly improved survival.

A host of other studies that have used a number of iNOS inhibitors have been described. Billiar (1998) has described the use of both the iNOS inhibitor N^6-(iminoethyl)-L-lysine in rats and iNOS-knockout mice for studies of hemorrhagic shock. An iNOS deficiency was associated with decreased nuclear factor κB (NFκB) activation and decreased signal transducer and activator of transcription (STAT)-3 activation, reduced IL-6 and granulocyte colony-stimulating factor (GCSF) messenger RNA levels, a reduced level of neutrophil accumulation, and a significantly decreased level of lung and liver cell injury. Similarly, a compound described as NOX (Medidox, San Diego, Calif.) has been shown to efficiently scavenge nitric oxide, reduce the level of lung injury, and decrease the level of neutrophil influx into the lungs with minimal effects on endothelial nitric oxide synthase (eNOS) (Harbrecht, 1998). Although compelling evidence implicates iNOS in the proinflammatory response initiated by fluid resuscitation from hemorrhagic shock, a primary concern regarding nitric oxide blockade is secondary alterations in vascular compensatory mechanisms. In addition, constitutive eNOS is essential to limiting neutrophil adhesion and accumulation in tissue, and preservation of this isoform is essential.

Novel Strategies for Scavenging Free Radicals

Current techniques for confirming increased free-oxygen-radical production in circulatory shock have relied on strategies that inhibited free-radical formation, scavenged excess free radicals, or used transgenic models that were deficient in some component of the oxygen-radical-cellular injury cascade. Recently, spin-trapping nitrones have been used to further define the role of free radicals in experimental shock. This group of compounds inactivates free radicals by forming stable adducts and includes *N-tert*-phenylbutyl-nitrone, α-4-pyridyl—oxide-*N-tert*-butyl-nitrone, and 5,5-dimethyl-l-pyrroline-*N*-oxide. These nitrones have been administered intraperitoneally to adult rats subjected to several types of traumatic or septic shock (Novelli, 1992), and spin probes and electron spin resonance were applied to measure cell membrane stiffness, a well-accepted indicator of peroxidative damage. Membranes obtained from animals subjected to trauma or shock had increased cell membrane rigidity compared to membranes from control animals. However, the administration of the nitrones reduced cell membrane stiffness, producing mitochondrial and microsomal membranes that were typical of those observed in control animals (Novelli, 1992). These studies have confirmed the role of toxic oxygen radicals in shock and

have provided evidence that spin-trapping nitrones may be applicable as a therapeutic tool in models of shock.

Another novel strategy designed to reduce oxygen-radical injury in shock models is melatonin therapy (Shelby, 1998). Melatonin is a neurohormone that plays an important role in several physiologic systems, including regulation of circadian rhythms; however, recent work suggested that melatonin has potent antioxidant activity. The effectiveness of melatonin-mediated detoxification of hydroxyl radicals and reduced lipid peroxidation after shock has been confirmed (Bubenik et al., 1998). Other studies have described that intragastric melatonin administration in shock reduces the number of gastric lesions, attenuates the fall in gastric blood flow, and upregulates other antioxidant systems in the body. Most recently, studies have shown that melatonin prevents activation of the transcription factor NFκB, a factor well recognized to play a pivotal role in the transcription of numerous inflammatory cytokines (Chaudry et al., in press). The administration of melatonin with fluid resuscitation from hemorrhagic shock reduced the total volume of crystalloid required to maintain a stabilized blood pressure, suggesting that this neurohormone may have considerable cytoprotective and antioxidant properties (Shelby, 1998).

Other novel technologies for limiting free-radical-mediated injury after fluid resuscitation from shock have included the development of nitroxides, a group of small, synthetic, metal-free molecules that have been shown to have significant antioxidant and radical-scavenging activity (Krishna and Samuni, 1994; Zhang et al., 1998). Nitroxides act to scavenge the superoxide radical, limiting superoxide-mediated injury to cells and inhibiting the interaction of superoxide with nitric oxide. In addition, nitroxides prevent generation of the hydroxyl radical by their interaction with transition-metal ions (Mohsen et al., 1995), provide significant protection against gut mucosal injury induced by oxidants (Karmeli et al., 1995), and inhibit apoptosis (Slater et al., 1995). The nitroxides provide vascular, interstitial, and intercellular protection, preserve the integrity of the vascular endothelium, inhibit leukocyte-endothelial cell adhesion and leukocyte emigration through the epithelium, and improve survival. Enhanced therapeutic activity of the nitroxides has been accomplished by linking these molecules to a biomacromolecule, providing stable antioxidant compounds. Despite the potential advantage of these antioxidant molecules, the type of macromolecules (for example, albumin, hemoglobin, or starch) has not been resolved; in addition, questions regarding oxygen delivery by the resuscitation regimen and dose-response relationships have not been examined. The polynitroxyl human serum albumin produced by covalently labeling human serum albumin with 40 molar equivalents of the nitroxide 4-acetamido-2,2,6,6-tetramethylpiperidine-1-oxyl has been studied extensively with animal models of ischemic reperfusion, stroke, and hemorrhagic shock, and clinical trials are scheduled for 2000 (C.J. Hsia, Synzyme, personal communication, September 13, 1999).

Hormonal Influences

Reports of the apparent preponderance of morbidity and mortality for males compared to those for females for a variety of diseases including sepsis pique one's interest in hormonal influences during hemorrhagic shock. There is a burgeoning literature, primarily from studies with rodents, concerning the importance of the hypothalamic, pituitary, and adrenal axis, especially regarding the immune response to sepsis, and those studies indicate a survival advantage for female animals. There are reports of adverse effects of testosterone and the potential therapeutic effects of compounds with estrogenic effects (in males), such as estradiol, on the heart and immune system after trauma-hemorrhage and resuscitation. Those studies have illuminated the understanding of some of the pathways that influence hormonal changes following trauma, such as prolactin release. These alterations seem to have important effects in stressed animals and possibly in clinical hemorrhagic shock. Less convincing is the suggestion that compounds such as metatropamide or fluoramide might prove helpful in combating the immunosuppression of shock.

Diagnostic Instrumentation

Because 59 million episodes of injuries were reported in the United States in 1995 and unintentional injury and violence account for 30 percent of all lost years of productive life before age 65 (Institute of Medicine, 1999), research into effective immediate treatment is warranted. Although diagnosis historically precedes treatment, research into portable, front-line diagnostic instrumentation capable of being operated by the far-forward line soldier who will then determine therapy is probably unrealistic. Such technology is far more applicable to the early-care facility. Research into protective equipment would be far more applicable to the far-forward situation. On the other hand, research into the mechanisms of neuropeptide, endogenous opioid, and endocrine activation and into the concept of "internal armor" is more appropriate. Such interventions as cocktails for free-radical scavenging might be given orally immediately prior to battle (i.e., as a prophylaxis) or as part of a resuscitation fluid (i.e., as a therapeutic) to be infused after injury has occurred. Perhaps antioxidants or nitric oxide scavengers that are given prophylactically or as a component of a first resuscitation fluid might lead to an oral medication or fluid additive to ameliorate neural cell injury after trauma.

TOLERANCE

Perhaps the most interesting, possibly the most promising, and certainly the most challenging approach might be to render the soldier globally less vulnerable to the effects of hemorrhagic shock, either prophylactically or at some point

after an injury has occurred. The classic examples might involve reducing metabolic demand through induced hypothermia or induction of a hibernation state. Although study of the former might provide insights that lead to therapeutic strategies, it is unlikely that induction of hypothermia per se will be practical in any battlefield setting. The possibility of a pharmacologically induced state of "hibernation" in which the organism might be less susceptible to the effects of hypoxia and cell injury is an appealing concept. It is unclear whether naturally occurring animal models of hibernation do afford this protection and, if so, whether it is induced by some circulating factor or by a complex of events.

RECOMMENDATIONS

The committee found that much of the research on hemorrhagic shock has remained focused on hemodynamics or has been directed toward correcting a single biochemical abnormality that accompanies hemorrhage. Such strategies are unlikely to be successful. Rather, novel therapies should be aimed at the metabolic and cellular derangements that accompany traumatic shock. These approaches should take advantage of advances in other related fields (such as ischemia-reperfusion research in specific organs) and should be approached in a systematic manner that involves prophylaxis, immediate intervention, or the development of tolerance to global ischemia. Combinations of novel therapies should be explored, because multiple pathways lead to cell death. Prevention of any component of the shock syndrome's cascading pathologic processes is preferable to treating or attempting to reverse the effects of the syndrome. Correcting the imbalance between O_2 supply and tissue demand is highly desirable. Providing a resuscitation fluid with increased O_2-carrying capacity represents a potential target to achieve this goal. Numerous oxygen therapeutic agents have been developed, and several are in different stages of clinical trials. Whether a resuscitation fluid that enhances O_2-carrying capacity or facilitates O_2 delivery would reduce the rate of morbidity or mortality from hemorrhagic shock remains to be demonstrated. Because organ toxicity following hemorrhagic shock results from a complex of interrelated mechanisms that lead to death, it is unlikely that a single drug, vitamin, electrolyte, or other agent would be able to alter organ toxicity significantly. Some markedly altered physiologic states offer protection to cells and organs. Strategies that induce tolerance to hypoxia might improve the survival of patients with shock syndrome.

Recommendation 4.1 **Evaluate the applicability of small-volume, stable oxygen (O_2)-carrying and O_2-facilitating agents that improve and sustain O_2 delivery in the wounded subject for 24 to 48 hours.**

Recommendation 4.2 **Therapeutic agents that target the toxic effects of hypoxic injury (e.g., antioxidants, chelating agents, hormones, and nitric oxide inhibitors) should be studied with animal**

models and subsequently in clinical trials. Combinations of several therapeutic agents should also be investigated.

Recommendation 4.3 **Mechanisms that may induce tolerance to ischemia and cellular hypoxia (e.g., hibernation, ischemic preconditioning, and hypothermia) should be explored with appropriate preclinical models**.

5

Protocols of Care at the Site of Injury

Hemorrhage is the major cause of death in combat casualties.

— Ronald Bellamy, M.D., 1998

A valid approach to fluid resuscitation in the wounded combatant should be based on both military considerations and scientific evidence. In Chapter 1, we reviewed the nature of combat injuries and the importance of treating severe blood loss on the battlefield. Chapter 2 outlined the major pathophysiologic events in hemorrhagic shock. Chapter 3 summarized the laboratory evidence and clinical experiences with various resuscitation fluids. Next, it is necessary to consider the specific needs of the injured combatant in a far-forward area, so that treatment protocols can be developed to address those needs. This chapter provides an overview of the combat environment, describes the resuscitation needs of the injured combatant, and makes recommendations for immediate treatment on the battlefield.

THE COMBAT ENVIRONMENT

As mentioned in Chapter 1, the battlefield of the 21st century will likely differ from that of the past in scale and pace. The U.S. military expects that future battles will be fought by smaller groups of combatants, over shorter time intervals, and with fewer casualties at any point in time (Secretary of Defense, 1999). Future battles, however, are expected to be fought in challenging settings, such as cities and towns or remote areas where air and ground transport may be difficult (Bruttig, 1998). These new battlefield environments have implications for the physical condition of the combatant, and for the type of care that should be provided in the field.

Expected Condition of Combatant on the Battlefield

The U.S. military expects that military operations in the near future will more frequently resemble activities of the Special Operations forces (Secretary of Defense, 1999). That is, conflicts will develop quickly and troops will be delivered to the battlefield on short notice, as compared with prior wars that involved large numbers of ground troops engaged in prolonged conflicts over large areas. Because of the rapid deployment, it is unlikely that combatants will be chronically dehydrated or malnourished. However, engagements may take place in desert or arctic environments as well as in jungles or mountainous regions. Precipitation, humidity, altitude, and extremes of hot and cold can all affect the condition of the combat casualty.

Limits of Battlefield Care for the Injured Combatant

Although many of the approaches to trauma care evolved from clinical research in the civilian sector, there are substantial differences between the injured combatant and the injured civilian. Civilian trauma patients encompass a broad spectrum of age, weight, coexisting disease, and cardiovascular conditioning. In addition, ethanol or illicit drug intoxication is a frequent component of civilian trauma. In contrast, military combatants are uniformly young, healthy, and physically fit, and ethanol or drug intoxication is a rare occurrence on the battlefield. As described in Chapter 1, battlefield injuries differ from those in the civilian sector in that civilian injuries often involve blunt trauma, whereas penetrating wounds are more common on the battlefield. Penetrating injuries in civilians are the result of bullets or knives, whereas penetrating battlefield wounds are usually due to shrapnel. Finally, civilian trauma is commonly associated with very rapid transport to an advanced medical facility (emergency department or trauma center), whereas military medical transport is often delayed for the wounded combatant.

Fluid therapy and other first-line treatments also differ between military and civilian environments. Battlefield care is characterized by limited resources, limited expertise, and delayed transport to medical facilities. Initial combat care is often provided by a medic or other combatant who carries limited medical supplies in addition to weaponry. Approaches to battlefield care and fluid resuscitation must be designed for the remarkably challenging conditions that face the first responders who care for casualties in far-forward deployment areas.

Because of the likely locations of future conflicts, immediate evacuation by air may be difficult or impossible. As a result, initial and even ongoing treatment of casualties may be significantly extended. As mentioned above, lifesaving medical treatment may well come from a fellow combatant or a medic—both of whom are included in the term "first responder." First responders are likely to be young, inexperienced in combat, and without medical training or experience.

Often, they will need to initiate immediate care while under fire, returning fire, or otherwise consumed by the chaos and confusion of battle.

Appropriate fluids and fluid protocols will play an integral part in the preservation of life until the wounded can be evacuated to a site where advanced medical expertise is available. Because the military first responder is severely limited in what he or she can carry into the field, approaches to resuscitation as well as other therapies must be streamlined and efficient. Supplies and equipment must be of low weight and small volume. Initial treatment protocols must consider both the needs of the average wounded combatant and the limitations of the battlefield environment.

Resuscitation Needs of the Injured Combatant on the Battlefield

For the reasons stated above, initial treatment protocols must be simple and should focus on the most critical needs of the typical combat casualty. Those needs are (1) establishing or ensuring an adequate airway and breathing, (2) controlling external hemorrhage, and (3) fluid resuscitation of hypovolemia and shock. In addition, the first responder must initiate and facilitate rapid evacuation to deliver live casualties to organized medical care behind the lines of battle. Critical tasks for resuscitative care include:

- establishment of an adequate airway,
- control of massive hemorrhage,
- circulatory support by intravascular fluid replacement,
- detection and treatment of hemo- or pneumothorax (including tension pneumothorax), and
- immobilization of fractures.

Immediate Versus Delayed Fluid Resuscitation

Regarding the unresolved issue of immediate versus delayed fluid resuscitation, Bickell and colleagues (1994) reported interesting findings in an inner city served by an efficient paramedic system with short times of transport to definitive care. These investigators concluded that the group of patients in whom fluid resuscitation was delayed had a lower mortality rate. Because the transport times were far shorter than they would be in the battlefield, it is not clear that their findings would apply to the military setting. In addition, the methodology of that work has been criticized on the basis of the lack of comparability of the two treatment groups, bias in patient selection, the small difference in the actual amounts of fluids that the two groups received in the prehospital setting, and the differences in the times to operative intervention. In view of the above and the likely delays to definitive therapy in the combat environment, immediate fluid resuscitation seems more appropriate for wounded combatants.

Current Protocols

Certain small subsets of military combatants (notably Special Operations forces corpsmen and medics, referred to as "trained responders" below) receive training based on the Advanced Trauma Life Support® (ATLS®) course (American College of Surgeons, 1997). Essential elements of this approach include control of hemorrhage, airway and breathing support, and intravenous fluid resuscitation.

Because exsanguination is the single major cause of death in potentially salvageable battlefield casualties (see Chapter 1), control of external hemorrhage, preferably by pressure but even by tourniquet if necessary, is an essential and immediate priority. The effectiveness of subsequent fluid resuscitation will depend greatly on the extent to which bleeding is controlled.

According to ATLS® protocol, the trained responder should ensure that the airway is clear. If the wounded combatant is conscious and breathing, then airway intervention is not necessary. If the wounded combatant is unconscious and respiration is labored, efforts should be made to clear the airway. The first approaches involve the chin-lift or jaw-thrust maneuvers. When clearing the airway of the unconscious victim on the battlefield, the first responder should not worry about cervical spine immobilization, because cervical spine injury is uncommon in combatants (Butler et al., 1996). Attention to cervical spine immobilization is secondary to evacuation from the front line.

Tension pneumothorax may also cause inadequate breathing. As noted in Chapter 1, 10 percent of the deaths in the battlefield during the Vietnam conflict were due to tension pneumothorax. Successful treatment of battlefield injuries requires that this condition be addressed by the trained responder (Coats et al., 1995; Deakin et al., 1995; Glinz, 1986; Krome, 1983). As outlined by Butler and colleagues (1996), respiratory distress occurring in a combat casualty with a penetrating chest wound should be assumed to represent tension pneumothorax. The diagnosis of a tension pneumothorax can be strengthened by visually identifying other signs, but this is not essential and may not be possible at night or while under fire. Although the trained responder will likely be inexperienced with needle thoracostomy, any additional trauma caused by this intervention is not expected to worsen the combat casualty significantly, whether or not a tension pneumothorax is present (Cameron et al., 1993). Current protocols used by Special Operations forces medics for treatment of injury on the battlefield are summarized in Box 5-1.

Items 1 through 5 of the protocol in Box 5-1 relate to the special focus of this committee. The committee finds at least two limitations to these approaches: (1) the use of large-volume (1,000 ml) solutions limits their availability on the battlefield, and (2) fluid resuscitation protocols based on intravenous access limit the number of personnel who could provide care on the battlefield. Together, these limitations inevitably restrict fluid resuscitation to only a few of the many who might benefit from such treatment. Both limitations are addressed as part of the committee's conclusions and recommendations.

BOX 5-1 BASIC TACTICAL CASUALTY MANAGEMENT PLAN FOR U.S. SPECIAL FORCES MEDICS

1. Airway management

Chin lift or jaw thrust

Unconscious casualty without airway obstruction: nasopharyngeal airway

Unconscious casualty with airway obstruction; cricothyroidotomy

Cervical spine immobilization is not necessary for casualties with penetrating head or neck trauma

2. Breathing

Consider tension pneumothorax and decompress with needle thoracostomy if a casualty has unilateral penetrating chest trauma and progressive respiratory distress

3. Bleeding

Control any remaining bleeding with a tourniquet or direct pressure

4. IV

Start on 18-gauge IV (heparin or saline lock)

5. Fluid resuscitation

Controlled hemorrhage without shock: no fluids necessary

Controlled hemorrhage with shock: Hespan 1,000 cc

Uncontrolled (intra-abdominal or thoracic) hemorrhage: no IV fluid resuscitation

6. Inspect and dress wound

7. Check for additional wounds

8. Analgesia as necessary

Morphine: 5 mg IV, wait 10 minutes; repeat as necessary

9. Splint fractures and recheck pulse

10. Antibiotics

Cefoxitin: 2 g slow-IV push (over 3-5 minutes) for penetrating abdominal trauma, massive soft-tissue damage, open fractures grossly contaminated wounds, or long delays before casualty evacuation

11. Cardiopulminary resuscitation

Resuscitation on the battlefield for victims of blast or penetrating trauma who have no pulse, no respirations, and no other signs of life will not be successful and should not be attempted

SOURCE: Butler et al., 1196, p. 11.

CONCLUSIONS AND RECOMMENDATIONS

The greatest opportunity for reducing the rate of mortality among combat casualties occurs on the battlefield. Unfortunately, the battlefields of the future may be less accessible than those of the past. Because conflicts in high-density urban or remote locations may lead to delays in evacuation by air, the committee concluded that immediate and subsequent ongoing treatment of casualties in far-forward areas should be improved. The committee's recommendations address approaches to the targets of injury, protocols for treatment in the field, and methods of evaluating and improving these protocols in the future.

Training First Responders

Initial care can be improved substantially by training more combatants to administer lifesaving measures to their fellow soldiers. The Israeli army trains half of its combatants to be medics. Although this may be unrealistic for the U.S. military, small mobile combat units should include a significant number of individuals with emergency medical training to provide the care that is outlined below. Larger units would require a smaller percentage of combatants so trained. Today there are excellent courses—such as the Prehospital Trauma Life Support (PHTLS) advanced trauma life-support course for prehospital providers developed by the American College of Surgeons (McSwain et al., 1994), the Basic Trauma Life Support (BTLS) advanced trauma life support course for prehospital providers developed by the American College of Emergency Physicians (ACEP) (Campbell, 1996), and the British Battlefield Advanced Trauma Life Support (BATLS) course—which could be used as a structure on which to base such training. The signs and symptoms of shock or tension pneumothorax, and all of the medical interventions recommended below, are taught in these courses. In view of the marked differences between the battlefield conditions and the civilian environment, the committee believes that it would be valuable to design a modified ATLS® course (Military Trauma Life Support) to train significant numbers of military first responders who could supplement the efforts of corpsmen and medics.

Recommendation 5.1 **The number of trained first responders in the combat environment should be increased through development of a Military Trauma Life Support course**.

Available Approaches for Treatment of Injury

The injuries that are responsible for the majority of fatalities and that are potentially treatable are exsanguinating hemorrhage, airway compromise, and tension pneumothorax (Bellamy, 1984, 1987b). The resuscitation and maintenance of life in a combat casualty (or civilian trauma victim) requires multiple

skills that could be learned by first responders as described in Recommendation 5.1 and summarized in Box 5-2.

The protocol for the treatment of battlefield casualties by a single first responder is presented in the airway-breathing-circulation format. Most of these recommendations represent a departure from traditional therapies administered in the battlefield. There is ample evidence (from experience in civilian emergency medical systems) that nonphysician first responders can be trained to administer aggressive lifesaving therapies in the field. However, these skills must be taught in carefully constructed training courses and administered by simple and clear protocols. Use of these protocols will represent a new era in battlefield therapy.

Hemorrhage

The proposed protocols for the fluid resuscitation of battlefield casualties are based on a modification of those presented by the medical command of the U.S. Navy Seals (Butler et al., 1996). The goal of therapy will be to stop hemorrhage, expand volume rapidly, increase cardiac output, and sustain effective perfusion.

Based on evidence about available resuscitation fluids that is discussed in Chapter 3, taking into consideration the large number of studies that have demonstrated both safety and efficacy, the need for simplicity, the limited volume that can be carried in the field, and relative cost, the committee concluded that 7.5 percent saline should be used for immediate fluid resuscitation on the battlefield.

Recommendation 5.2 **The initial fluid resuscitation of the hemorrhaging battlefield casualty should be a 250 ml bolus of 7.5 percent saline delivered by a rapid-infusion system**.

First responders in the field should be equipped with a rapid systemic infusion system consisting of a small plastic bag containing a 250 ml bolus of hypertonic (7.5 percent) saline. Both the composition and amount of the initial bolus are based on clinical trials outlined in Chapter 3.

The bag containing hypertonic saline would be placed under low pressure or accompanied by a simple, sturdy pumping device that could be mechanical or electric. Systemic accesses would be achieved via an intraosseous needle (Dubick and Kramer, 1997; Guy et al., 1993), placed into the anterior tibia. In extreme conditions where time and the condition of the wounded combatant dictate, the intraosseous needle with trocar in place could be placed directly through clothing. The possibility of infection is recognized, but this could be treated at a later point. If time and the condition of the wounded combatant allow, clothing could be cut away and a sterile field obtained. Intravenous access

could be provided also, but the committee concluded that the intraosseous route would be easier to teach to nonmedical combatants.

The use of hypertonic saline is recommended only as the initial intervention until definitive fluid resuscitation can be provided by more skilled medical personnel (again, consistent with the approaches used in clinical trials). If future research justifies it, additional compounds, such as those that carry oxygen or other novel therapies (see Chapter 4), could be added to the hypertonic saline solution.

Repeat Administration of Hypertonic Saline

Repeat administration of hyper-tonic saline would depend on the environment and the wounded combatant's physical condition. Regardless, an established limit as to the total amount of hypertonic saline that is infused would be necessary to avoid the complications that could occur with large infusions (Dubick and Wade, 1994; Lyons, 1996; Vassar et al., 1990). It is recommended that a total limit of 500 ml be set, and that the second bolus be given only if there is extended time to evacuation. Since the typical combatant will be a young healthy male, the likelihood is reduced that he will be highly sensitive to perturbations that might occur from a second bolus of hypertonic saline.

Airway and Breathing

The success of any fluid resuscitation depends on continued breathing and oxygenation. Although airway maintenance was not part of the charge to this committee, it was still felt that recommendations to ensure the integrity of the airway and breathing should be made. Without such efforts, any fluid resuscitation protocol would be futile. To this end, first responders should be instructed in securing the airway by administering chin lift, jaw thrust, and the use of adjuncts such as the intubating laryngeal mask airway (LMA). The LMA has been used in Europe for a number of years (Benumof, 1992; Davies et al., 1990; Martin et al., 1993; Pennant and Walker, 1992; Pennant and White, 1993; Reinhart and Simmons, 1994; Smith and Joshi, 1993; Somerson and Sicilia, 1993; Stone et al., 1994; Tolley et al., 1992; Walker et al., 1993). Its use can rapidly be taught to lay personnel, and the success rate maintained at a high level (Davies et al., 1990; Tolley et al., 1992; Walker et al., 1993). Recent work has indicated that after 5 minutes of training, lay personnel trained to use the intubating LMA on mannequins had a 90 percent success rate, a rate maintained over a period of at least 1 week (Richard Levitan, personal communication). Proper placement should be confirmed with an inexpensive disposable end tidal carbon dioxide (CO_2) device (Goldfarb and Cohen, 1990; Vukmir et al., 1991).

Tension pneumothorax is a relatively common problem in combat casualties, accounting for 10 percent of the deaths in the battlefield during the Vietnam conflict (Figure 1-2). A substantial number of civilian paramedic systems in the United States have trained their medics to recognize the major signs of a tension

pneumothorax (i.e., massive jugular venous distention, shifted trachea, and decreased breath sounds) and to treat it successfully with the relatively simple intervention of needle thoracostomy (Cameron et al., 1993; Lavery et al., 1992). In addition, segments of the military already train their medics to treat casualties with needle thoracostomies (Butler et al., 1996). It therefore seems appropriate to train military first responders similarly as a means of reducing mortality from tension pneumothorax. The committee concurs with Butler and colleagues (1996), who recommended that any combatant with severe progressive respiratory distress resulting from a unilateral penetrating chest wound should be considered to have a tension pneumothorax. When under fire, presumed tension pneumothorax should be treated by immediate needle (14 gauge) thoracostomy. When not under fire, the signs of a tension pneumothorax could be evaluated before therapy.

Recommendation 5.3 **First responders should ensure that the airway of a battlefield casualty is patent and that breathing is adequate.**

BOX 5-2 SUMMARY OF TREATMENT PROTOCOL FOR FIRST RESPONDERS IN THE COMBAT ENVIRONMENT

1. Airway. In the semiconscious or unconscious wounded combatant, the airway should be secured by chin lift, jaw thrust, or the use of the LMA.
2. Breathing.

a. Apnea. If the wounded combatant is apneic, the LMA should be inserted and breathing controlled by use of a small, portable self-inflating bag.

b. Tension pneumothorax. Tension pneumothorax should be treated by immediate needle (14 gauge) thoracostomy.

3. Hemorrhage. The first line of therapy should be to employ every effort to control and stop hemorrhage through the use of direct pressure.

a. In controlled hemorrhage without shock, withhold all fluid therapy and support the wounded combatant. If the setting allows, an intravenous catheter (heparin lock) should be placed.

b. In controlled hemorrhage with shock. Treat the wounded combatant with 250 ml of hypertonic (7.5 percent) saline via the intraosseous route at the anterior tibia. If evacuation to definitive care will be delayed, a second bolus (250 ml) of hypertonic saline may be administered, but the total should not exceed 500 ml.

c. Uncontrolled hemorrhage with shock. If resuscitation fluids are available and evacuation to definitive care will occur promptly, these wounded combatants should be treated as described for controlled hemorrhage with shock.

Additional Considerations

In addition to the treatment protocols and training recommendations made above, the committee identified some additional considerations. These include the need for prompt evacuation and the development and use of miniaturized physiological monitoring equipment.

Prompt Aeromedical Evacuation

The United States and many parts of Europe have seen the development of extensive sophisticated civilian aeromedical emergency care and helicopter evacuation systems (Mabry et al., 1993). In some settings, trauma patients are no more than 5 minutes from the closest aeromedical units. These systems have treated and evacuated thousands of trauma victims and have saved countless lives (DeLorenzo, 1997; Gearhart et al., 1997; Hotvedt et al., 1996; Young et al., 1998; Zalstein and Cameron, 1997). Unless conditions are totally prohibitive, the committee encourages the military to emulate the civilian sector in this regard. The long evacuation times from the battlefield that occurred as recently as Operation Desert Storm, deserve substantial attention and remediation. Resuscitation of casualties in the field will never replace definitive surgical intervention, and if mortality is to be reduced in the future, aggressive field resuscitation must be followed immediately by aggressive aeromedical evacuation.

Recommendation 5.4 **If accessible, all severely injured battlefield casualties should be evacuated to a front-line high-echelon care site in less than 1 hour**.

Monitoring

The committee endorses the use of miniaturized physiological monitoring equipment as well as the continued research into its further development (Gopinath et al., 1995; Robertson et al., 1995, 1997). These devices could provide passive monitoring of the most critical vital signs of all combatants. Specific modalities that should be monitored are:

a. systolic blood pressure,
b. pulse,
c. oxygen saturation,
d. electrocardiogram, and
e. end tidal CO_2.

Other potentially useful monitoring systems are under development. These include cardiac output monitors and the self-contained monitoring medical litter.

These seem more suited for secondary evacuation and transport. At this time, it is unclear whether having these litters on the battlefield would be realistic. In addition, systems are needed to monitor real-time physiological data at remote sites and, in turn, to direct medical care on the battlefield based on these data.

6

Future Directions

ANIMAL MODELS

The majority of what is known about the physiology of hemorrhagic shock has been learned from studies with animal models—primarily dogs and rats. Determination of the degree to which experimental results from studies with animal models can be extrapolated to the human shock condition must consider several factors, including (1) the species, (2) the initial experimental condition of the animal, (3) the shock model (i.e., how do you go about inducing the blood loss and what do you do after the initial blood loss is induced?), and (4) the dynamic character of the shock process itself. For example, anesthetics have been shown to play a vital role in affecting the way that an animal responds to the shock process; that is, the type of anesthesia influences central nervous system responses as well as peripheral vasomotor responses to blood loss.

Choice of species can have a major impact on outcomes. For example, greater blood loss has been exhibited in studies of isobaric models of hemorrhagic shock in species with a large splenic red blood cell reserve, such as the dog. This variable can also affect the severity of the shock when the blood loss is based on percent body weight in fixed-volume models of hemorrhagic shock. The pig, although noted for the similarity of its cardiovascular system responsiveness to that of the human cardiovascular system, is quite dissimilar with respect to the hemoglobin P_{50} (the partial pressure of oxygen where hemoglobin is 50 percent saturated with oxygen) which is substantially higher than that for the human hemoglobin (e.g., 38 versus 28-30 millimeters of mercury [mm Hg]). Rats demonstrate a very small oxygen extraction reserve in skeletal muscle beds compared with that for dogs, which have an extraordinarily large extraction reserve. The magnitude of this reserve affects the degree of tolerable flow decrement during hypotension, which in turn affects the magnitude of the systemic vascular resistance response observed during hemorrhage.

Canine models typically develop portal hypertension during shock and bloody diarrhea as a postresuscitation complication that other species do not exhibit. Baboons appear to exhibit a more robust compensatory response to hemorrhagic shock compared with the responses of humans and most other species, and are able to tolerate a mean arterial blood pressure of 40 mm Hg for over 8 hours before they exhibit signs of decompensation.

Models of hemorrhagic shock are of two basic types: controlled and uncontrolled with respect to the manner in which ongoing blood loss is allowed to proceed. Controlled models are either of the fixed-volume or constant-pressure varieties and are generally more reproducible than uncontrolled bleeding models, which usually involve laceration, puncture, or transection. When an experimental hypothesis includes the treatment effect on homeostasis, uncontrolled bleeding models are relevant. Since shock is a dynamic process involving dramatic changes in cardiovascular and metabolic states that vary with time, species, laboratory, and even investigator, it is important to evaluate the effectiveness of various resuscitative regimens not simply on the basis of time but also on the basis of a more complete definition of the stage of physiologic compensation at the time that treatment is initiated. It has been shown that the type of anesthesia, state of hydration, nutrition status, core temperature, and use of heparin all affect the time course and degree of compensatory capacity of the animal in response to blood loss.

Effects of Extent of Hypotension and Rates of Hemorrhage on Immune Function in Mouse Models

Available information indicates that even transient hypotension in the absence of significant tissue trauma is sufficient to produce marked suppression of both specific and nonspecific immune responses. This appears to be the case irrespective of whether the model of hemorrhage used is one of a fixed-pressure versus a fixed-volume bleed, a bleedout over a relatively brief period (less than 5 minutes of cardiac stick/fixed-volume model), an intermediate period (approximately 5 to 15 minutes of hemorrhage to fixed pressure), or a protracted period (tail vein laceration or uncontrolled hemorrhage models), as well as whether the animal is anesthetized or unanesthetized. Also, although many of these models are typically nonlethal, they render the animal highly susceptible to subsequent lethal septic challenge. This appears to be the case irrespective of the administration of standard fluid resuscitation (with or without blood), although it can be modified by the rate of fluid administration and by the nature of the resuscitation fluid (e.g., lactated Ringer's solution versus hypertonic saline).

Nonetheless, trauma appears to provide a modulatory effect in the sense that it can be additive or prolongs the duration of immune aberrations encountered in these models. Another significant modifier frequently present in many models is an anticoagulant, such as heparin. Studies indicate that this agent can reduce the severity of insult produced in these models. Interestingly, microbial

translocation and the release of endotoxin do not appear to be significant mediators of these immune suppressive effects in short-term (up to 1.5-hour) models but may be a factor in longer-term (more severe) models.

Mechanistically, the process by which cell, immune, or organ function is altered appears to be biphasic. The initial early phase is characterized by acute cellular changes (metabolic dyshomeostatis, which consists of a decrease in adenosine triphosphate [ATP] levels and pH, increases in calcium ion [CA^{2+}]I, etc.) associated with systemic proinflammation (an increase in tumor necrosis factor [TNF], interleukin-1 [IL-1], and IL-6 levels, etc.), which accounts for the early depression in the cellular response. This transitions over time to a chronic phase of mediator-induced (an increase in anti-inflammatory cytokine, nitric oxide [NO], and oxygen [O_2] levels, etc.) or endocrine system-induced (prolactin, androgens versus estrogens, etc.) sustained immune system or organ dysfunction.

The advantage of mouse models in studying both the pathologic changes that occur during shock and the physiologic responses to acute hemorrhage is that inbred strains of mice have greater uniformity at the species level. Also, transgenic or gene-knockout strains provide animal models that are deficient in various mediators and that therefore add important information that often is not available by traditional pharmacologic approaches.

Swine Models of Combined Hemorrhage and Injury

Research conducted by Proctor (1998) relies on models in which tissue injury is superimposed on hemorrhagic shock, followed by administration of the same type of fluid resuscitation that would be available in the combat or civilian setting. The strength of the model is that injury is always associated with hemorrhage. The addition of tissue injury activates the inflammatory process (neurohumoral factors), which alters the response to hemorrhage.

Trauma and shock produce whole-body ischemia, and resuscitation produces a reperfusion injury. During the traumatic insult, there is decreased blood flow and oxygen delivery and breakdown of ATP to adenosine. Proctor (1998) is investigating in a swine model the roles of increased adenosine levels and altered neutophil activation. The data collected in that research, however, are relevant only to those who survive to the point of first aid. Nonetheless, Proctor has found that there is, in fact, secondary injury caused by the activated white blood cells, which can be affected by altering CD 18 cells or granulocyte colony-stimulating factor (GCSF). The only way that these changes can occur, however, is by administering a secondary insult. These studies have also shown that liquid ventilation is able to produce the same protective effects as positive end expiratory pressure without the negative hemodynamic actions.

Value of Animal Models

A great deal of literature exists with regard to canine, swine, and rodent models, but somewhat less information regarding simian or monkey (baboon) models is available. There are some significant interspecies variations in terms of biochemical responses, focus on organ injury, immune response, and neural-element (sympathetic nervous system) involvement. In the canine model there appears to be a significant bowel and splanchnic organ focus of injury, with a combination of endotoxin-like and toxic peptide autodigestive components contributing to the irreversible nature of shock. There is also an early and prominent sympathetic nervous system and adrenal cortical and medullary response. In the canine model splanchnic response to sympathetic nervous system activation and the increase in plasma catecholamines levels is augmentation of the circulating blood volume by autotransfusion from a large splenic reservoir, which does not exist to the same extent in other species.

The appearance of pulmonary injury occurs in a more delayed fashion. Some investigators have preferred the swine model and believe that the hemodynamic responses and cardiovascular reserve capacity more closely relate to the human pathophysiology of hemorrhagic shock. The rodent models provide easier access to large amounts of data, although the technical difficulties of complete cardiovascular monitoring are greater because of the size of the animals and limits to the technology for obtaining certain measurements in small animals. One advantage of the rodent model is the availability of a large number of genetic variants and gene-knockout models, making possible assessment of various tissue mediators, hormones, and neural components of the shock response. The use of simian models has been based on the fact that the animals' anatomy and physiology more closely approximate those of humans and their pathophysiologic responses to hemorrhagic hypotension or shock more closely resemble the human response. The simian models exhibit a more robust response, and the animals usually sustain a more prolonged survival and better outcome with exposure to the same level of hemorrhagic stress to which humans are exposed.

Technical Models

Two principal models of hemorrhagic shock exist: the controlled hemorrhage model and the uncontrolled hemorrhage model. The controlled hemorrhage model uses either bleeding to a predetermined pressure or a predetermined volume as a percentage of blood volume and body weight. In the Wiggers model (Wiggers, 1950), hemorrhage to a mean arterial blood pressure of 40 mm Hg is maintained for a predetermined period of time with measurement of the shed blood volume, various hemodynamic attributes, regional blood flow, biochemical markers of organ function, sympathetic nervous system activity, and the circulating concentrations in plasma of catecholamine and various hormonal indicators of stress such as cortisol. After the predetermined period the animals

are reinfused with the shed blood or various resuscitation fluids, and survival as well as physiologic measurements can be monitored at intervals to determine responses and rates of survival versus death. A number of criticisms have indicated the shortcomings of this model. These shortcomings stem from such things as pretreatment with agents that interfere with the sympathetic nervous system (e.g., ganglionic blockers or adrenergic antagonists) or the use of potent general anesthetics that decrease the level of the shed blood volume at which the desired mean arterial pressure is reached and that lower the catecholamine and hormonal responses, indicating a lower level of hemorrhagic stress. In other words, the mechanics of the experimental design may influence the endpoints of research that uses this model.

When a controlled hemorrhage by percentage of blood volume or body weight is used, the indicators of the response rather than the shed blood volume are the blood pressure and other hemodynamic measurements as well as other measurements of stress, as indicated above. The animals are held for a period of time and are then reinfused with shed blood or the fluid resuscitation being tested, or a combination of both. This technique also permits measurements of various response parameters and can be used to obtain survival data. The common criticism of both of these controlled hemorrhage models is that they do not mimic actual shock conditions in humans.

The uncontrolled hemorrhage model can be totally uncontrolled hemorrhage from either a catheter or a rent in the aorta or major vessel. In this model, controlled rates of bleeding are sometimes used or controlled rates of bleeding are combined with a partially controlled bleeding model with further hemorrhage being uncontrolled. Various hemodynamic measurements as well as other organ function measurements can be made as described above for the controlled hemorrhage model. It has been stated that the model that most closely approximates battlefield casualty conditions is one that uses the uncontrolled hemorrhage in the absence of anesthesia in subjects who were previously dehydrated and exposed to various stresses. Although this might be a closer approximation to battlefield conditions, the ability to measure responses and outcomes would appear to be better with controlled hemorrhage models, simply because additional experimental variability is introduced by uncontrolled hemorrhage.

The combat injury is usually a combination of hemorrhage and soft-tissue injury. The soft-tissue injury component is most often a penetrating injury that produces a number of subsequent reactions that can influence the outcome in different ways. The release of cytokines and other substances from injured tissue contributes significantly to the organ function disorders associated with shock. The animal model that most closely approximates the battlefield injury should include not only an acute hemorrhage but also some aspects of tissue injury such as a penetrating or crush injury to an extremity. Penetrating injury of visceral organs may introduce other complicating factors such as septic shock introduced by penetrating injuries of the gut and other abdominal visceral organs. Penetrating wounds of the chest may add additional injuries to the heart or lungs,

resulting in earlier cardiovascular collapse because of cardiac injury or early respiratory decompensation because of lung injury.

Role of Anesthesia

All of the currently used inhalational anesthetics including halothane, enflurane, isoflurane, desflurane, and sevoflurane may exhibit depressant effects on the cardiovascular system when they are administered at doses of between 1.0 and 2.0 the minimum alveolar concentrations. The cardiac depressant effects of halothane, enflurane, isoflurane, desflurane, and sevoflurane have been demonstrated in isolated heart preparations and in the unanesthetized chronically instrumented dog (Pagel et al., 1991a,b). These studies have indicated that these anesthetics have negative inotropic effects and impair ventricular diastolic function (Pagel et al., 1991c). Among the intravenous anesthetics, barbiturates, the benzodiazepines, etomidate, and propofol, all produce dose-dependent cardiovascular effects (Merin, 1996). Greater controversy exists with regard to ketamine, which may produce transient stimulating effects on the heart and peripheral circulation, but these effects are eliminated in animal models when the sympathetic nervous system is blocked or inhibited (Pagel et al., 1992). The minimal depressant effects of newer narcotics such as fentanyl and its derivatives have been confirmed by studies with animals and humans, but such efforts are found only at higher narcotic levels if ventilation is maintained since respiratory depression is a common finding at higher doses (Merin, 1996).

Among the physiologic responses to acute hemorrhage that occur while receiving potent inhalational anesthetics and many intravenous anesthetics are depression of (1) both high-pressure and cardiac low-pressure reflexes, (2) the chemoreceptor reflex, and (3) sympathetic nervous system responses. Studies with human volunteers have demonstrated inhibition of carotid sinus and aortic sinus reflexes by halothane (Duke et al., 1977), enflurane (Morton et al., 1980), and isoflurane (Kotrly et al., 1984). Other studies with humans have demonstrated that halothane has an inhibitory effect on cardiopulmonary reflex regulation of limb and vascular resistance (Kotrly et al., 1985). The potent anesthetics halothane and isoflurane have been shown (Seagard et al., 1983, 1985) to inhibit the carotid sinus reflex at multiple sites including the central nervous system, sympathetic pre- and postganglionic sites, and neuroeffector junctions in the heart and in the arteries and venules. Direct inhibitory effects of halothane on sympathetic ganglia have also been demonstrated (Bosnjak et al., 1988).

Inhibition of chemoreflex regulation of the cardiovascular system by potent volatile anesthetics has been demonstrated in animal models during halothane (Stekiel et al., 1992) and isoflurane (Stekiel et al., 1995) anesthesia. These effects were demonstrated for arterial resistance vessels as well as venous capacitance vessels.

In summary, direct depressant effects of potent volatile anesthetic agents and many intravenous anesthetics on the heart and peripheral blood vessels as a

baseline effect have been demonstrated. Of equal or greater concern are the depressant effects of most general anesthetics on the major physiologic regulatory systems that are activated in response to acute hemorrhage. The depressant effects have been described for high-pressure baroreflexes, cardiopulmonary baroreflexes, chemoreflexes, and the sympathetic nervous system. These anesthetic-induced alterations in basic physiologic control mechanisms result in subsequent alterations in blood flow, oxygen delivery, tissue oxygenation, and even survival in hemorrhaged animals (Longnecker and Sturgill, 1976; Longnecker et al., 1982; Seyde and Longnecker, 1984; Seyde et al., 1985; Weiskopf et al., 1981). Furthermore, these responses vary by organ and by species (Longnecker and Seyde, 1986), and thus, no single anesthetic regimen can be recommended for all studies involving traumatic shock. Rather, the choice of anesthesia will depend on the experimental design, the animal species, and the measured outcome variables. However, the ubiquitous nature of these effects must be taken into account both in the design of studies and in the analysis of the data. Animal protocols that do not require anesthesia are preferred, when feasible, for they most closely mimic the clinical scenario of battlefield trauma. When anesthesia is required, agents and techniques that are reproducible and that minimize the effects on cardiopulmonary control systems that are activated by acute hemorrhage should be selected.

Animal models should be selected for specific reasons on the basis of the research questions being asked. If survival is an outcome measurement, survival for 5 to 7 days,, not just 12 to 24 hours, should be the endpoint. Experimental designs should look realistically at establishing a repeatable 50 percent lethal dose for the control group. If animal models are chosen to develop a consensus for application of novel treatment regimens and agents to potential human trials, then these interventions should be applied in an identical animal model with identical experimental protocols with defined endpoints or outcomes.

CLINICAL TRIALS

Role of Clinical Trials in Development of Therapies

At the outset of this discussion, the committee differentiates between clinical research and clinical trials. *Clinical research* has recently been considered to be a term applicable to three major areas of biomedical investigation (National Institutes of Health Director's Panel on Clinical Research, 1997):

1. Patient-oriented research, that is, research conducted with human subjects (or with material of human origin, such as tissues, specimens, and cognitive phenomena) and in which an investigator directly interacts with human subjects. This area of research includes (a) mechanisms of human disease, (b) therapeutic interventions, (c) development of new technologies, and (d) clinical trials.

2. Epidemiological and behavioral studies.
3. Outcomes research and health services research.

Clinical trials, which are listed as a subset of clinical research, have been defined as prospective studies comparing the effect and value of an intervention(s) in human subjects against those in a control (Friedman et al., 1985, p. 2). This definition implies that the intervention may be prophylactic, therapeutic, or diagnostic, but it is a necessary component. Without active intervention (e.g., follow-up of subjects over a period of time), the study is merely observational—no experiment is performed and the study would not properly be termed a clinical trial.

Clinical trials often provide the most definitive proof of the safety and efficacy of diagnostic and therapeutic interventions in humans. Although studies with animals and uncontrolled clinical observations contribute to the understanding of a clinical entity, such studies usually cannot definitively demonstrate whether a new treatment has made a difference in clinical outcome. The well-designed clinical trial, in which the treatment group is comparable in every way to the control group except for the intervention studied, provides the means for such definitive demonstration.

Endpoints and Indications

Because of the power of controlled clinical trials, they are an integral part of the regulatory approval system for drugs, biologics, and medical devices. The pertinent federal regulations require that the safety and efficacy of new agents be demonstrated by controlled clinical trials. Regarding efficacy, the regulations (21 CFR 601.25(d)(2)) state:

> Effectiveness means a reasonable expectation that, in a significant proportion of the target population, the pharmacological or other effect of the product ... will serve a clinically significant function in the diagnosis, cure, mitigation, treatment or prevention of disease.

Although this regulation pertains to approvals by the U.S. Food and Drug Administration (FDA), the underlying concept is generally accepted. *Clinically significant function* means that the intervention under evaluation must be shown to benefit clinically the patient population under study. Put another way, the primary endpoint of the pivotal clinical trial that is intended to support approval must be a direct measurement of the clinical benefit of the intervention. A limited number of types of endpoints satisfy the definition given above:

- increased survival of the study population,
- measurable symptomatic relief to the study population, or
- prevention or slowing of the progression of a disease process.

In many cases, it is not possible to measure such direct endpoints, and investigators seek to substitute a more readily measured entity as a surrogate for a clinical endpoint. Such surrogate endpoints in clinical trials are usually laboratory measurements or physical signs that are substitutes for and that are expected to correlate with a clinically meaningful endpoint that directly measures how a patient feels, functions, or survives. There are two principal risks involved with use of surrogate endpoints:

- The clinically meaningful endpoint may not actually correlate with the proposed surrogate.
- The surrogate may correspond to a real benefit, but the intervention may have serious undesirable consequences, complicating evaluation of the true risk-benefit ratio.

In general, surrogate endpoints can be considered when there is sufficient knowledge of the disease entity and of the intervention under investigation, when the feasibility of performing meaningful clinical trials is poor, and when the overall risk-benefit situation justifies the use of such endpoints. Hypertension is a surrogate marker for hypertensive cardiovascular disease, on the basis of extensive research on and experience with these entities. A decrease in the blood pressure of hypertensive patients has been used as a surrogate endpoint in the clinical trial of antihypertensive agents, and this is an example of a validated surrogate endpoint.

The endpoints chosen for a clinical trial are a critical element of the protocol, for they will determine the duration, complexity, and perhaps the success or failure of a trial. Selection of endpoints for trauma trials is especially difficult, and when a clinical endpoint is desired, mortality rate is often chosen. At a recent conference on blood substitutes and oxygen therapeutics (BCI Conference on Blood Substitutes and Oxygen Therapeutics, November 19 and 20, 1998, Bethesda, Md.), a speaker from FDA stated that mortality will be the endpoint of choice for clinical trials on hemorrhagic shock or exsanguinating hemorrhage. If a resuscitation solution studied in a clinical trial on trauma is not anticipated to improve the rate of mortality associated with trauma, then the ability of such a product to improve a major cause of morbidity can be used to demonstrate the efficacy of the product, whereas a solution that does not worsen the mortality rate but that results in major irreversible morbidity in the survivors would not be judged to be effective. Again, the validation of these endpoints will provide a challenge to investigators.

The use of surrogate endpoints in clinical trials on trauma is not as acceptable as is the case with the example of hypertension given above. The clinical conditions associated with trauma and the interventions of interest are more complex and heterogeneous and the relationship between them is less well understood. The use of surrogate endpoints (such as blood pressure, lactate levels, base deficit, or organ functional assessments, among others) must be validated as correlating with survival in hemorrhagic shock or exsanguinating hemorrhage

before use in lieu of a mortality endpoint. Scoring systems developed to evaluate the severity of an injury, such as the APACHE score, have been proposed as the basis for endpoints in trials on trauma. The applicability of these scores as surrogate endpoints has been the subject of considerable debate.

Evaluation of Resuscitation Protocols

The existing trauma indexing systems have contributed a great deal to the triage of trauma patients and to the development of systems for assessment of quality of care (Champion et al., 1996, 1990, 1989, 1983, 1981; Copes et al., 1990, 1988; Gennarelli et al., 1994, 1989; Sacco et al., 1988, 1984); however, the current trauma indexing systems are inadequate for use in the evaluation of future research (Brenneman et al., 1998; Demetriades et al., 1998; Hoyt, 1998; Roorda et al., 1996; Rutledge and Osler, 1998; Rutledge et al., 1998). The injury severity score (ISS) does not accurately stratify patients according to injury because it was not designed to evaluate penetrating injury and is inaccurate in its ability to categorize head injury. The ISS was developed to categorize blunt injuries sustained in motor vehicle accidents (American Association for Automotive Medicine, 1985). In addition, there may be reason to believe that injuries as defined by ISS do not correlate with the actual demand for resources. There are also problems with the ability of physiologic indexing systems, such as the Revised Trauma Score (RTS), to predict resource need. Casualties with scores that imply a minor injury may have penetrating abdominal injuries that will nonetheless require surgery.

There are also problems with the trauma and injury severity score (TRISS) assessment methodology, which has become the benchmark for the evaluation of trauma care. The TRISS model functions as follows:

The probability of survival P_s is computed by the following equation:

$$P_s = 1/1 + e - b ,$$

where

$$b = b_0 + b_1(\mathrm{RTS}) + b_2(\mathrm{ISS}) + b_3(\mathrm{AGE}) ,$$

where b_n are regression coefficients obtained from the large multithousand patient database that the American College of Surgeons (Baker et al., 1974; Champion et al., 1980a,b, 1981; Flora, 1978; Walker and Duncan, 1967) has collected for over 15 years and analyzed by regression analysis.

Comparison of the predicted outcome with the realized outcome (Z) is calculated by:

$$Z = D_i - \sum Q_i / \sqrt{\sum P_i Q_i} ,$$

where P_t is the probability of survival for each patient *i*. As calculated above, Q_t, equals 1 - P_t (the probability of death of patient *i*), and D_t equals the actual number of patients who died. The methodology essentially compares the number of patients who the regression process would predict would die on the basis of national experience of mortality from those injury severities versus the number who actually died.

There are two problems with this methodology. First, there is no matching of injury. Although one can run a Mantel-Haenszel statistic to demonstrate that the distribution of the P_s between the national patient database and a comparative group is statistically the same, there is no way actually to match the types of injuries themselves. For example, if one is testing a new fluid resuscitation protocol with a group of patients using the TRISS methodology to measure a difference in effect, the following could take place: 10 percent of the national group might have had closed head injuries with a Glasgow Coma Score of less than 8 along with other minor injuries, whereas 40 percent of the experimental group might have closed head injuries with a Glasgow Coma Score of less than 8. Since it is known that patients with closed head injuries and Glasgow Coma Scores of less than 8 have a significantly higher mortality rate than patients with minor head injuries and the same ISS, there will be a major bias against the experimental group by TRISS. The TRISS method achieves standardization of injury by use of the RTS and ISS. Although the Glasgow Coma Score affects RTS and the score has a significant influence on the probability of survival, it may still not be sufficient to control for this confounding. In addition, the American College of Surgeons Penetrating Injury database comprises data for civilian penetrating trauma victims who have mainly sustained knife or gunshot wounds. As noted in Chapter 1, the majority of penetrating injuries on the battlefield are due to shrapnel from explosive munitions. This may make the regression coefficients obtained from data for civilians invalid for the latter group.

The second problem is that as many as 90 percent of all patients who are initially triaged as major trauma victims, at least in the civilian sector, do not, as it turns out, have major life-threatening injuries (Bellamy, 1995). The analysis of performance gained by use of the TRISS methodology is statistically based on the outcomes for the remaining 10 percent of patients. The TRISS methodology is troubled because of the sigmoid nature of the distribution of the P_s of trauma patients. The mathematics of the method are such that it is overly affected by either the death or the survival of a very few of these severely injured patients. The use of this type of analysis to evaluate the effectiveness of a therapy is fraught with difficulty because one or two patients can drastically change the results.

Evaluation of the effectiveness of these measures requires the development of a new model to assess injury severity. One approach may be to use some of the newer statistical paradigms that are based on parallel distributed processing that use nonlinear statistics and that appear to be able to take into account multiple confounding effects (Armoni, 1998; Dombi et al., 1995; Forsstrom and Dalton, 1995; Obana and Fukui, 1996; Rutledge et al., 1998). Finally, trauma

outcome research should strive to compare like patients with similar injuries. A standardized data collection system should also be developed for all human studies that encompassed standardized definitions and standardized datum points. The Utstein system (American Heart Association, 1998), designed by investigators in cardiopulmonary resuscitation research, could be used as a template for this development.

Unique Problems of Clinical Trials of Trauma

Heterogeneity of Subject Population

The heterogeneity of the subject population has been alluded to before in this report, but it is a central issue and warrants attention. The patients who would be studied in a clinical trial designed to test an intervention for hemorrhagic shock might have suffered blunt or penetrating trauma or a combination of these; they may have a discrete injury to a single organ or multiorgan lesions; the trauma might be a small lesion in a major vessel or a large lesion affecting a diffuse vascular bed. Comparison of such diversity will demand an understanding or at least a recognition of some unifying principles within the study groups.

Informed Consent

In a clinical trial the subjects or their authorized representatives must be clearly and completely informed of the risks and benefits associated with their participation in the trial. Because trauma victims may not be capable of giving meaningful consent, investigators faced a special problem with clinical trials with this population. To correct this situation, the federal regulation pertaining to the protection of human subjects (21 CFR, Part 50) has recently been amended to provide a narrow exception to the requirement for obtaining and documenting informed consent from each human subject or his or her authorized representative in certain situations. The exception would apply to a limited class of research activities involving human subjects who are in need of emergency medical intervention but who cannot give informed consent because of their life-threatening medical condition and who do not have a legally authorized person to represent them (*Federal Register*, 1996). It should be noted that concern over this change in regulations has been expressed (Moreno et al., 1998). The issues that cause concern include the concept and implementation of community consent, the ability of the system to exert oversight, and the appropriate use of consent from authorized representatives.

The regulations require the study sponsor to provide information about the trial to the community before and after the study is performed. These regulatory changes apply to the civilian population, and investigators using military populations for trauma studies must obtain permission directly from the patient or from an authorized representative.

Applicability of Civilian Clinical Data to Military Needs

Examples supporting or refuting the applicability of civilian clinical data relevant to fluid resuscitation of trauma victims to military needs could be given. The primary difference between the battlefield and civilian settings is that the vast majority of combat injuries are penetrating, whereas those encountered in civilian practice are blunt. Furthermore, not only are the majority of lethal injuries in the battlefield penetrating, but many are caused not by bullets but by shrapnel from explosive munitions.

Transportation and logistics may also differ, in that evacuation of a casualty in a combat setting as opposed to that in the civilian environment is frequently not rapid and the casualty is not routinely brought to a setting where definitive care can be administered. It is therefore not clear whether the results of studies performed under relatively controlled conditions (i.e., in a modem civilian trauma service) could be extrapolated to military field conditions. On the other hand, there are questions for which data are simply lacking, irrespective of the circumstances. Even though fundamental differences exist between combat and civilian settings, it is noteworthy that trauma is the leading cause of morbidity and mortality among teenagers and young adults in Central Europe, and in the United States data from U.S. trauma centers indicate that for approximately 40 percent of fatal trauma cases, death is due to exsanguination or its sequelae. For such clinical situations, data collected in civilian trauma services would be an advance over the current data availability situation, and such data could later be complemented by experience gained in military situations. In this regard, it is noteworthy that civilian trauma facilities are now used for the training of military medical personnel.

Clinical Research and Clinical Trials in Trauma Centers

The United States has regional trauma centers that are mostly self-designated and has accredited and verified trauma centers that are very organized. Research is done at such centers, and multi-institute trials are of interest to several professional organizations (including the American Association for Surgery in Trauma, Multi-Institute Trial Committee; the Eastern Association for Surgery in Trauma; and the Western Trauma Society). These groups have formulated trials for voluntary participation. The multicenter study on blunt aortic trauma recently reported by Fabian and colleagues (1997) is an example of such a study.

No ongoing, formal, funded groups for clinical studies on trauma analogous to those on, for example, oncology, currently exist. For example, at present, three major cooperative groups perform multi-institute trials on various diagnostic and therapeutic approaches to cancer. These are funded by the National Cancer Institute and by the pharmaceutical industry, foundations, and donations from individuals. The groups are the Cancer and Leukemia Group B, the Eastern Cooperative Oncology Group, and the Southwest Oncology Group. Studies

are performed at medical centers and clinics throughout the country, and the results of those studies are constantly reported in the open literature. A group or groups that would organize trauma studies suitable for multicenter implementation at existing trauma centers have the potential to address important questions and generate valuable data. A funding mechanism would be needed to cover the costs of the research that would be above and beyond the costs of care already expended for these patients.

CONCLUSIONS AND RECOMMENDATIONS

Military-civilian research and education opportunities should be expanded and facilitated. The Civilian Level I and Level II Trauma Centers have the potential to evaluate outcomes and costs, to transport trauma patients, and to score the severity of injuries. Although severe limitations on the comparability of the civilian and military situations exist, the best available models are the civilian trauma centers. Progress in the civilian sector, for example, has had military applications in the following areas:

- preservation of blood,
- magnitude and type of fluid therapy,
- helicopter transportation, and
- treatment of burn injuries.

Civilian trauma centers should be used as an educational resource for military residency programs as well as for continuing medical education (CME) for career officers.

The committee found that much of the earlier work in the field of traumatic shock has been tainted by the failure to recognize the differences between pure hemorrhagic shock and traumatic shock associated with tissue injury, the failure to standardize animal models with regard to anesthesia, and the failure to observe subjects for longer-term survival. Clinical research has been hampered by the lack of an organized national approach to trauma research that takes advantage of the considerable clinical material and research expertise among the regional trauma centers. Advances in the treatment of traumatic shock will be enhanced significantly by improved approaches to research performed in studies with both animals and humans.

More specifically, the committee found that animal models in shock research have been broadly selected for convenience or availability rather than specific species-related reasons. It also found that the period of observation has been too short to justify the drawing of any conclusions about survival or mortality. Furthermore, although the use of anesthesia is appropriate for invasive protocols with animals, there is strong evidence that inhalational and intravenous anesthetics as well as many related drugs produce alterations in baseline cardiovascular functions. In addition, they inhibit the physiologic responses to hemorrhage in a significant way.

Many different technical models for the evaluation of hypovolemic shock are available. However, the two major technical models, the controlled and the uncontrolled hemorrhage models, differ significantly in their reproducibilities. Furthermore, many shock protocols are not able to define the beneficial effects of therapies since they have not established clear-cut and reproducible mortality rates. The majority of experimental hypovolemic shock studies deal solely with blood loss and do not address the problem of coincidental tissue injury, which is more typical of the injury sustained on the battlefield. Clinical trials often provide the most definitive proof of the safety and efficacy of diagnostic and therapeutic interventions for humans and are an integral part of the regulatory approval system for new agents. There is a large volume of civilian trauma in the United States, and many of the trauma patients are treated in well-organized trauma centers. Although there are identifiable differences between civilian and military trauma, there are also basic questions that could be approached in the civilian setting to obtain data useful to the military.

Finally, approaches to both current treatment and future research are hampered by inadequate methods for classification of the severity of trauma; such classifications are essential to evaluations of the efficacies of new treatment protocols that involve modifications in fluid formulations or novel therapies. Current trauma indexing systems are inadequate for use in future trauma research. Therefore, the committee makes the following recommendations.

Recommendation 6.1 **Laboratory research should be reproducible and relevant to the clinical scenario. For fluid resuscitation research, the experimental design of animal research should be guided by the following principles**:

- **when feasible, the experimental model should include soft-tissue injury in addition to hemorrhage**;
- **controlled hemorrhage protocols are preferred to uncontrolled hemorrhage models**;
- **when feasible, protocols that do not require anesthesia are preferred. If anesthesia is required, the depth of anesthesia should be reproducible, and the anesthetic agent should be selected to minimize alterations in the physiologic responses to hemorrhage**;
- **experimental animal species should be selected on the basis of clinical relevance, and will vary depending on the research question;**
- **if survival is an endpoint, mortality should be measured for at least 5 days; and**
- **the experimental design should establish a reliable 50 percent lethal dose (LD_{50})** for the control group.

Recommendation 6.2 **A national study group should be convened to develop and implement clinical research, including multicenter clinical trials on selected topics at existing regional trauma centers**.

Federal agencies, including the U.S. Department of Defense, the U.S. Department of Veterans Affairs, and the National Institutes of Health, and national professional organizations, should collaborate with each other and with the private sector in this activity.

Recommendation 6.3 **A new system for categorizing injury in trauma care should be developed**.

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Appendixes

A

Acknowledgments

The committee thanks all those who took the time to attend and participate in its meetings and to share their views with the committee either verbally or as written comments. Conference speakers and attendees are listed below, followed by a list of those who contributed verbal or written comments to the committee.

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B

Acronyms

ABC—	airway-breathing-circulation
ACEP—	American College of Emergency Physicians
ACTH—	adrenal corticotropin hormone
ADH—	anti-diuretic hormone
ADP—	adenosine diphosphate
AMP—	adenosine monophosphate
APACHE—	acute physiology and chronic health evaluation
APAF- 1—	apoptosis-activating factor 1
AP 1—	activator protein 1
ARDS—	acute respiratory distress syndrome
ATLS®—	Advanced Trauma Life Support®
ATP—	adenosine triphosphate
ATPase—	adenosine triphosphatase
BTLS—	basic trauma life support
c-Fos—	protein
c-Jun—	protein
ced C.elegens—	death gene
Ca^{2+}—	calcium
CD—	family of leukocyte adhesion molecules
cGMP—	cyclic guanosine monophosphate
CINC—	cytokine-induced chemoattractant
CNS—	central nervous system
CO_2—	carbon dioxide
CPP32—	caspase 3
CPR—	cardiopulmonary resuscitation
CRADD—	a novel human apoptotic adaptor molecule for caspase-2

DCLHb—	diaspirin cross-linked hemoglobin
DFO—	desferoxamine
DHEA—	dihydroepiandrosterone
DIC—	disseminated intravascular coagulation
DNA—	deoxyribonucleic acid
DNase—	dioxyribonuclease
ECF—	extracellular fluid
eIF—	eukaryotic initiation factor
EKG—	electrocardiogram
EMS—	emergency medical services
eNOS—	endothelial nitric oxide synthase
EPR—	electron paramagnetic resonance
ER—	endoplasmic reticulum
FACS—	flow cytometry
FADD—	Fas-associated death domain
FDA—	U.S. Food and Drug Administration
Fe^{3+}—	iron
FKBP12—	FK-binding protein
FLICE—	alternate name for caspase-8
GCSF—	granulocyte colony-stimulating factor
GCN2—	an eIF2α kinase
GSSH—	oxidized glutathione
H_2—	hydrogen
H_2O—	water
HES—	hydroxyethyl starch
HIV—	human immunodeficiency virus
HRI—	hemin regulated inhibitor
HSD—	hypertonic saline dextran
HTLV—	human T-cell leukemia virus
ICAM-1—	intracellular adhesion molecule 1
ICAM-2—	intracellular adhesion molecule 2
ICU—	intensive care unit
IGF—	insulin-like growth factor
IL—	interleukin
iNOS—	inducible NO synthase
IOM—	Institute of Medicine
IP-3—	inositol triphosphate
IP-3R—	inositol triphosphate receptors
IRES—	internal ribosome entry site
IRR—	insulin receptor-related receptor

IRS-1—	insulin-receptor substrate-1
ISS—	injury severity score
IV—	intravenous
K^+—	potassium
K ATP	potassium-adenosine triphosphate
kDa—	kilodalton
kg—	kilogram
LD_{50}—	fifty percent lethal dose
LMA—	intubating laryngeal mask airway
M—	molar
MEG—	mercaptoethylguanidine
meq—	milliequivalents
ml—	milliliters
mmHG—	millimeters of mercury
MODS—	multiple-organ dysfunction syndrome
MORT1—	a human protein that binds to the death domain of FAS/APO1 and induces apoptosis.
mosM—	milliosmolar
m^7GTP—	where GTP is guanosine triphosphate
mRNA—	messenger ribonucleic acid
MTLS—	military trauma life support
mV—	millivolts
ρM—	micromolar
Na^+—	sodium
NAD—	nicotinamide adenine dinucleotide
NADPH—	reduced nicotinamide adeninedinucleotide phosphate
NAS—	National Academy of Sciences
NEDD 2—	alternate name for caspase-2
NFκB—	nuclear factor κB
NO —	nitric oxide
NOS2—	inflammatory NO synthase
NRC—	National Research Council
NVC18	
O_2—	oxygen
OH—	hydroxide ion
p53 —	p53 gene
PARP—	poly(ADP ribose)polymerase
PARS—	poly(ADP ribose)synthase

PHTLS—	pre-hospital trauma life support
PKA—	protein kinase A
PKR—	protein kinase
PNA—	polynitroxyl human serum albumin
PP—	phosphatase
RES—	reticuloendothelial system
RTS—	revised trauma score
RyR—	ryanodine receptor
ser-51—	serine at position 51
SH—	src homology
SOD—	superoxide dismutase
STAT-3—	signal transducer and activator of transcription
TNF-α—	tumor necrosis factor alpha
TNFR—	Tumor necrosis factor receptor
tRNA—	transfer ribonucleic acid
TRISS—	trauma injury severity score

C

Conference Agenda

Conference on Resuscitation Fluid Design and Resuscitation Protocols for Combat Casualties

National Academy of Sciences Auditorium
2101 Constitution Avenue, N.W.
Washington, D.C.

Thursday, September 17, 1998

8:30 a.m. **WELCOMING REMARKS AND OVERVIEW OF THE CONFERENCE**
Clyde J. Behney
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Head, Human Systems Department
Office of Naval Research
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9:00	**PANEL 1: OVERVIEW OF FLUID RESUSCITATION**
	(15-minute presentations, each followed by 5 minutes of discussion)
	Ronald F. Bellamy, M.D., Colonel, USA, Retired
	Borden Institute, Walter Reed Army Institute of Research
	Trauma Epidemiology of Combat Casualties
	Howard Champion, M.D.
	University of Maryland, Baltimore
	Classical Shock Research vs. Resuscitation Needs
	Uwe Kreimeier, M.D.
	Department of Anesthesiology, University of Munich
	Resuscitation Research in Europe
10:00	BREAK
10:20	**PANEL 2: TREATMENT OF BATTLEFIELD TRAUMA**
	(15-minute presentations, each followed by 5 minutes of discussion)
	Issues
	Steven P. Bruttig, Ph.D.
	Novel Technologies, Inc.
	Issues in Trauma Treatment on the Battlefield
	Frank Butler, Jr., M.D., Captain, USN
	Naval Special Warfare Command Detachment Pensacola
	Issues of Trauma Treatment for Special Forces
	Geoffrey S. F. Ling, M.D., Ph.D.
	Uniformed Services University of the Health Sciences
	Neurological Aspects of Battlefield Trauma
	Strategies
	Michael M. Krausz, M.D.
	RAMBAM Medical Center, Haifa, Israel
	Resuscitation Strategies in the Israeli Army
	George C. Kramer, Ph.D.
	University of Texas, Galveston
	Hypertonic Saline/Dextran Resuscitation: Novel Methods of Delivering Fluids on the Battlefield

	Monitoring
	Frederick J. Pearce, Ph.D.
	Walter Reed Army Institute of Research
	Casualty Resuscitation and Monitoring Devices Under Development by the U.S. Army
12:20 p.m.	LUNCH
1:20	**AGENDA ACCOMODATION I**
	(15-minute presentations, each followed by 5 minutes of discussion)
	Jane Shelby, Ph.D.
	University of Utah
	Melatonin Therapy in Acute Trauma (from Panel 7)
	Richard McCarron, M.D.
	Naval Medical Research Institute
	Hibernation as a Model for Tolerance to Ischemia (from Panel 8)
2:00	**PANEL 3: ANIMAL MODELS OF HEMORHAGIC SHOCK**
	(15-minute presentations, each followed by 5 minutes of discussion)
	Frederick J. Pearce, Ph.D.
	Walter Reed Army Institute of Research
	Animal Models of Hemorrhagic Shock and Physiological Responses to Hemorrhage
	Kenneth G. Proctor, Ph.D.
	University of Tennessee
	Models of Combined Hemorrhage and Injury
	Alfred Ayala, Ph.D.
	Rhode Island Hospital
	Effects of the Extent of Hypotension and Rates of Hemorrhage and Outcome in Animal Models
3:00	BREAK
3:20	**PANEL 4: PATHOPHYSIOLOGY AND METABOLIC SEQUELAE**
	(15-minute presentations, each followed by 5 minutes of discussion)

	Richard Veech, M.D., D.Phil. National Institute on Alcohol Abuse and Alcoholism National Institutes of Health *Cellular Energetics and Fluid Design Issues* Ajay Verma, M.D., Ph.D., Major, USA Uniformed Services University of the Health Sciences *Strategies for Supporting Energy Demand During Resuscitation* Edwin A. Deitch, M.D. New Jersey Medical School *Sequelae of Hemorrhagic Shock (Intestinal Ischemia, Bacterial Translocation, ARDS)* Irshad H. Chaudry, Ph.D. Rhode Island Hospital *Immune and Endocrine Aspects of Hemorrhage* Patricia E. Molina, M.D., Ph.D. North Shore University Hospital *Cytokines in Hemorrhagic Shock* Timothy R. Billiar, M.D. University of Pittsburgh *Role of Induced Nitric Oxide Synthase in Hemorrhagic Shock*
5:20	**CLOSING REMARKS** David E. Longnecker, M.D. Committee Chair
5:45	**ADJOURN**

National Academy of Sciences Auditorium
2101 Constitution Avenue, N.W.
Washington, D.C.

Friday, September 18, 1998

8:15 a.m.	**OPENING REMARKS**
	David E. Longnecker, M.D.
	Committee Chair
8:25	**PANEL 5: COMPLICATIONS OF RESUSCITATION**
	(15-minute presentations, each followed by 5 minutes of discussion)
	Richard Veech, M.D., D.Phil.
	National Institutes of Health
	(for Kieran Clarke, Ph.D., Oxford University)
	Acute Toxicity of Lactate
	C. Robert Valeri, M.D.
	Boston University
	Red Blood Cells and Platelets in Hemostasis
	Peter Rhee, M.D.
	Uniformed Services University of the Health Sciences
	Stimulation of Inflammation by Standard Resuscitation Fluids
	Y. John Wang, Ph.D.
	Synzyme Tech., Inc., Irvine, Calif.
	Polynitroxyl-Albumin for Treatment of Reperfusion Injury
	Kenneth Mattox, M.D.
	Baylor University
	Complications of Fluid Resuscitation
10:05	BREAK
10:20	**PANEL 6: NOVEL THERAPIES FOR HEMORRHAGE**
	(15-minute presentations, each followed by 5 minutes of discussion)
	Alan S. Rudolph, Ph.D.
	Naval Research Laboratory
	Oxygen-Carrying Resuscitation Fluids

	Carleton J. C. Hsia, Ph.D. Synzyme Tech., Inc., Irvine, Calif. *Reperfusion Injury in Hemorrhage as a Therapeutic Target* Paul Segall, Ph.D. BioTime, Inc., Berkeley, Calif. *Using Hextend to Treat Hemorrhagic Shock* Bo E. Hedlund, Ph.D. Biomedical Frontiers, Inc. *Beyond Volume Expansion: Treatment of Reperfusion Injury by Iron Chelation* John L. Gainer, Ph.D. University of Virginia *Fluid Additive for Promoting Oxygen Consumption* T.M.S. Chang, M.D., Ph.D., M.R.C.P. McGill University, Montreal, Canada *Polyhemoglobin-Superoxide Dismutase-Catalase: A New Blood Substitute*
12:20 p.m.	LUNCH
1:20	**AGENDA ACCOMODATION II** (15-minute presentations, each followed by 5 minutes of discussion) William P. Wiesmann, M.D., Colonel, USA, Retired Biostar *Occult Hemorrhage Detection on the Battlefield by Means of Novel Microwave Sensors/Detectors* (from Panel 2) John J. Lemasters, M.D., Ph.D. University of North Carolina *Cellular Responses to Hypoxia* (from Panel 4)
2:00	**PANEL 7: NOVEL THERAPIES FOR HEMORRHAGE** (15-minute presentations, each followed by 5 minutes of discussion) Andrew Salzman, M.D. Cincinnati Children's Hospital *Treatment of Hemorrhage with MEG*

Alan Kim Johnson, Ph.D.
University of Iowa
Treatment of Hemorrhage with Melanocyte-Stimulating Hormones
William R. Millington, Ph.D.
University of Missouri, Kansas City
Neurobiology of Hemorrhagic Shock
Florence M. Rollwagen, Ph.D.
Uniformed Services University of the Health Sciences
Oral Cytokines for Treatment of Hemorrhagic Shock
Radha K. Maheshwari, Ph.D.
Uniformed Services University of the Health Sciences
Picroliv: Beneficial Effects in Hypoxia/Ischemia
Barbara A. Araneo, Ph.D.
Pharmadigm Biosciences Inc., Salt Lake City, Utah
Intravenous DHEA in the Treatment of Burn Syndrome

4:20 **PANEL 8: HYPOTHERMIA IN TREATMENT OF HEMORRHAGE**
(15-minute presentations, each followed by 5 minutes of discussion)
Samuel Tisherman, M.D.
University of Pittsburgh
Hypotensive Hypothermic Fluid Resuscitation During Uncontrolled Hemorrhagic Shock in Rats and Hypothermic Strategies for Suspended Animation with Delayed Resuscitation in Dogs
Peter Safar, M.D.
University of Pittsburgh
Pharmacologic-Hypothermic Suspended Animation

5:00 **GENERAL DISCUSSION AND CLOSING REMARKS**
David E. Longnecker, M.D.
Committee Chair

5:30 **ADJOURN**

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Committee and Staff Biographies

Committee Biographies

DAVID E. LONGNECKER, M.D., is the Robert Dunning Dripps Professor and Chair of the University of Pennsylvania Department of Anesthesia. Dr. Longnecker received his M.D. degree in 1964 from Indiana University School of Medicine, where he completed residency training in anesthesiology in 1967. Following a National Institutes of Health (NIH) Special Research Fellowship in Physiology, he continued clinical and laboratory research at the NIH Clinical Center, where he served as a Clinical Associate from 1968 to 1970. He has received numerous NIH research grants and a Research Career Development Award for research involving the effects of anesthetics on the microcirculation, oxygen delivery to tissue, oxygen therapeutics, endothelium-dependent circulatory control, and health services research. Dr. Longnecker is the author and coauthor of over 175 scientific abstracts and original scientific articles, 29 chapters, and five textbooks. Dr. Longnecker was the Harold Carron Professor of Anesthesiology at the University of Virginia, where he served from 1974 to 1988, and an Assistant Professor of Anesthesiology and Physiology at the University of Missouri from 1970 to 1973. Dr. Longnecker is a member of the Institute of Medicine.

WILLIAM G. BAXT, M.D., is the Chief of Emergency Medicine Services at the University of Pennsylvania Medical Center. Dr. Baxt received his B.A. at Brown University before later earning his M.D. at Yale University. Dr. Baxt specializes in emergency medicine, prehospital care, and informatics. Specifically, his research involves nonlinear statistics using the artificial neural network as a paradigm for decision analysis and clinical diagnosis. Dr. Baxt is also Professor and Chair of the Department of Emergency Medicine at the University of Pennsylvania Medical Center. Dr. Baxt is a member of the Institute of Medicine.

JOSEPH C. FRATANTONI, M.D., is the Vice President of Biologics at C. L. McIntosh and Associates, Inc., which provides consulting services to companies that manufacture biologic products, including blood products, diagnostics, vaccines, and therapeutics. Dr. Fratantoni earned his M.D. degree from Cornell University Medical College, an M.A. degree in chemistry from Harvard University, and a B.S. degree in chemistry from Fordham College. Dr. Fratantoni's areas of expertise include regulatory, quality, and product development. Dr. Fratantoni was the Director of the Division of Hematology at the U.S. Food and Drug Administration's (FDA's) Office of Blood Research and Review. In addition to his participation in many FDA policy committees, Dr. Fratantoni has served on the Scientific Program Committee of the American Association of Blood Banks, the Biomedical Excellence for Safe Transfusion Working Party of the International Society of Blood Transfusion, the Naval Research Advisory Committee's Special Panel on Blood Substitutes, and the Institute of Medicine Forum on Blood Safety and Availability.

JURETA W. HORTON, Ph.D., is Director of the Surgical CORE Research Facility and a professor in the Department of Surgery at the University of Texas Southwestern Medical Center in Dallas, Texas. She is also a full member of the graduate faculty in Biomedical Engineering and serves as CoDirector of the STARS program for Dallas County Teachers. Dr. Horton received her B.A. from Our Lady of the Lake in San Antonio, Texas, and her Ph.D. from the University of Texas Southwestern Medical Center at Dallas. Dr. Horton is on the Editorial Board of *SHOCK. Molecular, Cellular, and Systemic Pathobiological Aspects and Therapeutic Approaches* as well as on the Editorial Board of *Current Science*. She is also a member of the American Burn Association, the American Heart Association, the American Heart Council on Circulation, the Shock Society, the American Association for the Surgery of Trauma, the American Physiology Society, and the Society of Critical Care. Dr. Horton is a reviewer for the *Journal of Surgical Research*, for the *American Journal of Physiology*, and for *Critical Care Medicine.* Dr. Horton has served as a member of the Special Emphasis National Institutes of Health (NIH) Study Section and as an NIH site visitor to evaluate training and research programs.

JOHN P. KAMPINE, M.D., Ph.D., is Professor and Chair of the Department of Anesthesiology at the Medical College of Wisconsin. He is also a Staff Anesthesiologist at Zablocki Veterans Administration Hospital and Froedtert Memorial Hospital and the Director of Anesthesiology and Operating Room Services at Milwaukee County Medical Complex. Dr. Kampine received his M.D. and Ph.D. from Marquette University with a focus on physiology. He is Past President of the Association of University Anesthesiologists and Past President of the Society of Academic Anesthesia Chairmen. He has been a member of the Surgery, Anesthesia and Trauma study section and ad hoc member of the Experimental Cardiovascular Sciences Section of National Institutes of Health. The author or coauthor of more than 600 original papers and published abstracts, Dr. Kampine's research

interests are in cardiovascular regulation during anesthesia. Dr. Kampine is a member of the Institute of Medicine.

HARVEY G. KLEIN, M.D., is currently the Chief of the Department of Transfusion Medicine at the Warren G. Magnuson Clinical Center, National Institutes of Health. Dr. Klein received his A.B. degree from Harvard College and his Doctor of Medicine from the Johns Hopkins School of Medicine. He is board certified in internal medicine, in hematology, and in blood banking and immunohematology. Dr. Klein has authored or coauthored more than 150 publications pertaining to blood transfusion, including transfusion-transmitted disease, the management of immunosuppressive effects of blood transfusion, and the impact of biotechnology on transfusion medicine. Dr. Klein is Editor-in-Chief emeritus of the *Journal of Clinical Apheresis*, has served on the Editorial Board of *Blood*, and is currently an editor of *Transfusion and Transfusion Medicine Reviews*. Dr. Klein has been a member of the Institute of Medicine Forum on Blood Safety, Chairman of the Standards Committee of the American Association of Blood Banks, Chairman of the Blood and Blood Products Committee of the U.S. Pharmacopoeia, and Chairman of the Transfusion Medicine Subcommittee of the American Society of Hematology. He was formerly Council President of the National Marrow Donor Program.

JOSEPH E. RALL, M.D., is a Senior Scientist at the National Institute of Diabetes and Digestive and Kidney Diseases of the National Institutes of Health. He received his B.A. from North Central College and his M.A. from Northwestern University. Dr. Rall received his M.D. from Northwestern University Medical School and his Ph.D. from the University of Minnesota. His research has involved endocrine research on the thyroid and more recently isolation and cloning of the steroid/thyroid nuclear hormone receptors. Recent publications are in *Development* and the *Proceedings of the National Academy of Sciences*. Dr. Rall is a member of the National Academy of Sciences and has served as a member on a number of committees.

GEORGE F. SHELDON, M.D., is the Zack D. Owens Distinguished Professor of Surgery and chairman of the Department of Surgery at the North Carolina School of Medicine. Dr. Sheldon received his A.B. degree and his M.D. degree at Kansas University in 1957 and 1961, respectively. He was a Fellow in Internal Medicine at the Mayo Clinic prior to doing his surgical residency at the University of California, San Francisco, from 1964 to 1969. He completed 2 years of research fellowship at Harvard Medical School in 1971. Dr. Sheldon has been Chairman of the American Board of Surgery, a Regent of the American College of Surgeons, President of the American Association for the Surgery of Trauma, Secretary and President of the American Surgical Association, and is currently President of the Society of Surgical Chairman and President of the American College of Surgeons. He has been named an Honorary Member of the Royal College of Surgeons of Edinburgh, the Association of Surgeons of Great Britain and Ireland, and the

European Surgical Association and is Chair of the Council of Academic Societies of the Association of American Medical Colleges. In 1993 he received the National Safety Council's Surgeon's Award for Distinguished Service to Safety. His bibliography includes over 200 journal articles, and he is editor or coeditor of eight books. He has been on 14 editorial boards. Dr. Sheldon is a member of the Institute of Medicine.

BLAINE C. WHITE, M.D., is the Director of Basic Science Research and a Professor of Emergency Medicine, Physiology, and Molecular Medicine at Wayne State University (WSU). Dr. White was designated a WSU Distinguished Scholar by the University Board of Governors in 1992, received the first Center for Excellence Grant from the Emergency Medicine Foundation and Genentech in 1993, was recipient of the American College of Emergency Physicians Award for Outstanding Contributions to Research and Education in 1988, and has received awards as an outstanding teacher from the medical students at both WSU and Michigan State University. As a member of the Graduate School faculty, he supervises the doctoral work performed in laboratories and is an investigator on an National Institutes of Health grant.

Iom Project Staff

ANDREW M. POPE, Ph.D., is the Director of the Health Sciences Policy Program at the Institute of Medicine. With expertise in physiology, toxicology, and epidemiology, his primary interests focus on environmental and occupational influences on human health. As a research fellow in the Division of Pharmacology and Toxicology at the U.S. Food and Drug Administration, Dr. Pope's research focused on the biochemical, neuroendocrine, and reproductive effects of various environmental substances on food-producing animals. During his tenure at the National Academy of Sciences and since 1989 at the Institute of Medicine, Dr. Pope has directed and edited numerous reports on environmental and occupational issues; topics include injury control, disability prevention, biologic markers, neurotoxicology, indoor allergens, and the inclusion of environmental and occupational health content in medical and nursing school curricula.

GEOFFREY S. FRENCH is a Project Officer in the Health Sciences Policy Program. He has been with the Institute of Medicine (IOM) for 3 years, having supported the Office of Finance and Administration and the IOM committees that produced the reports *Scientific Opportunities and Public Needs: Improving Priority Setting and Public Input at the National Institutes of Health, Enabling America: Assessing the Role of Rehabilitation Science and Engineering*, and *Halcion: An Independent Assessment of Safety and Efficacy Data*. His undergraduate degree is in history and anthropology, and he completed his master's degree in national security studies at Georgetown University.

CHARLES H. EVANS, JR., M.D., Ph.D., is the Head of the Health Sciences Section in the Institute of Medicine. Dr. Evans joined the staff of the Institute of Medicine in March 1998. As Head of the new Health Sciences Section, Dr. Evans has management responsibility for all scientific, administrative, and financial affairs of the Health Sciences Section, which includes the Health Sciences Policy Program and the Neuroscience and Behavioral Health Program and their respective boards in the Institute of Medicine. Dr. Evans is a pediatrician and immunologist and holds the rank of Captain (retired) in the U.S. Public Health Service with 27 years of service as a medical scientist at the National Institutes of Health. He received his B.S. (biology) degree from Union College in 1962 and M.D. and Ph.D. (microbiology) degrees from the University of Virginia in 1969. He was an intern and resident in pediatrics at the University of Virginia from 1969 to 1971 and from 1971 to 1998 served as a Medical Officer in the U.S. Public Health Service Commissioned Corps and concurrently from 1976 to 1998 was Chief of the Tumor Biology Section at the National Cancer Institute. An expert in carcinogenesis and the normal immune system defenses to the development of cancer, he is the author of more than 250 scientific publications. He and his laboratory colleagues discovered the cytokine leukoregulin in 1983 and were awarded three U.S. patents. Dr. Evans has been active as an adviser to community medicine and higher education through his service on the Board of Trustees of Suburban Hospital Health System (1988 to present) and on the Arts and Sciences Alumni Council at the University of Virginia (1987 to 1997). He is the recipient of numerous scientific awards including the Outstanding Service Medal from the U.S. Public Health Service and the Wellcome Medal and Prize. Dr. Evans has been a member of the editorial boards of several scientific journals, has served on a variety of scientific advisory committees, and is a Fellow of the American Institute of Chemists and a credentialed Fellow in Health Systems Administration of the American Academy of Medical Administrators.

KATHI E. HANNA, Ph.D., is a science and health policy consultant specializing in biomedical research policy, specifically, genetics, cancer, and reproductive technologies. Most recently, Dr. Hanna served as Senior Advisor to the National Bioethics Advisory Commission in its response to the president's request for recommendations regarding human cloning. Prior to that she was Senior Advisor to the President's Advisory Committee on Gulf War Veterans Illnesses, in which she assessed the effects of military service on the reproductive health of veterans. Dr. Hanna was a senior analyst at the congressional Office of Technology Assessment for 7 years and contributed to numerous science policy studies requested by committees of the U.S. Congress on biotechnology, human genetics, women's health, reproductive technologies, and bioethics. In 1989, Dr. Hanna spent a year at the Institute of Medicine where she edited a book about the interface between biomedical research and politics. In the past decade, Dr. Hanna has also served as a consultant to the Howard Hughes Medical Institute, the National Institutes of Health, the Institute of Medicine, the Federation of American Societies of Experimental Biology, and several academic

health centers. Prior to her work in Washington, D.C., Dr. Hanna was the Genetics Coordinator at Children's Memorial Hospital in Chicago. Dr. Hanna received an A.B. in biology from Lafayette College, an M.S. in human genetics from Sarah Lawrence College, and a doctorate from the School of Business and Public Management, George Washington University.

SARAH PITLUCK, M.S. is an Administrative Assistant in the Health Sciences Policy Program of the Institute of Medicine (IOM) and a Research Assistant for the IOM's Roundtable on Environmental Health Sciences, Research, and Medicine. She completed her undergraduate degree in political science at Washington University in St. Louis before completing her Master's degree in Public Policy and Public Administration at the London School of Economics and Political Science. Sarah's Masters thesis addresses the sources of divergent public policies toward prostate cancer screening in the United States and United Kingdom.

GLEN SHAPIRO is a Project Assistant/Research Assistant in the Health Sciences Policy Program. As an undergraduate at Wesleyan University, Middletown, Connecticut, he completed a degree in Russian Language and Literature as well as fulfilled the premed requirements.

MELVIN H. WORTH, JR., M.D., is a Scholar-in-Residence at the Institute of Medicine. Dr. Worth completed his surgery residency at New York University-Bellevue in 1961 and remained on that faculty for 18 years. He founded the Bellevue Trauma Service in 1966 and continued as Director until 1979, when he left to become Director of Surgery at the Staten Island University Hospital. He served for 15 years with the New York State Office of Professional Medical Conduct and 8 years as a member of the New York State Hospital Review and Planning Council (for which he was Chair in 1993). He is a Fellow of the American College of Surgeons, the American College of Gastroenterology, and the International Society for Surgery and holds memberships in the American Association for the Surgery of Trauma, the Society for Critical Care Medicine, the Association for Academic Surgery, New York Surgical Society (for which he was President in 1979), and other academic and professional organizations. Dr. Worth retains his appointment at New York University, and is Clinical Professor of Surgery at the State University of New York Downstate (Brooklyn) and the Uniformed Services University of the Health Sciences.

Index

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