ANNUAL RESEARCH PROGRESS REPORT
FOR FISCAL YEAR 1987
1 OCTOBER 1986 - 30 SEPTEMBER 1987

PREPARED BY: US ARMY INSTITUTE OF SURGICAL RESEARCH
FORT SAM HOUSTON
SAN ANTONIO, TEXAS 78234-5012

PREPARED FOR: US ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
FORT DETRICK
FREDERICK, MARYLAND 21701-5012

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1 October 1987

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Human subjects participated in these studies after giving their free and informed voluntary consent. Investigators adhered to Army Regulation 70-25 and US Army Medical Research and Development Command Regulation 70-25 on the use of volunteers in research.

In conducting the research described in this report, the investigators adhered to the Animal Welfare Act and other Federal statutes and regulations relating to animals and experiments involving animals and with the Guide for the Care and Use of Laboratory Animals, National Institutes of Health Publication 86-23.

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This publication was compiled and edited by Christine C. Davis, Research Protocol Coordinator, US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas 78234-5012.
**Title:** US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Year 1987 (U)

**Personal Author(s):** Pruitt, Basil A. Jr.

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**Date of Report:** 1 October 1987

**Page Count:** 307

**Abstract:**

US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Year 1987 (U)
SGRD-USZ

1 October 1987

MEMORANDUM: SEE DISTRIBUTION

SUBJECT: US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Year 1987


[Signature]

BASIL A. PRUITT, JR., MD, FACS
Colonel, MC
Commander and Director
Much of liability law and economic theory are based on the concept of the prudent man. The assumption that human actions will be governed by rational decisions based on available data is applicable to physician supply and career choice as well and explains, in large part, shortages of medical specialists in the military and the difficulty in recruiting and retaining physician and scientists on the staff of the biomedical research institutes of the uniformed services in general and this Institute in particular (1). A principal negative influence on the prudent surgeon's career decision is the discrepancy in salary vis-a-vis civilian peers. One can predict the decision of a prudent surgeon freshly graduated from a residency program who will receive, as a Captain, total compensation of $53,234 including bonuses, as contrasted to the mean total compensation of $85,700 received by entry level full-time assistant professors of surgery at US medical schools during the 1985-86 academic year and the even greater income of private practice surgeons (2,3). This discrepancy is accentuated by the fringe benefits of journal subscriptions, professional association dues, dependent tuition, and generous travel allowances available to the civilian analogue of a military surgeon.

The prudent surgeon contemplating initiation or continuation of a military career must also, on the basis of his personal interests and preferences, evaluate other aspects of the career of the military physician scientist. The limited duration of assignments will be compared to the relative, if not absolute, permanence of residence of a civilian peer, and the paucity of research opportunities at some of those assignments will be contrasted to continuity of research opportunity in the civilian sphere. The dissociation of scholarly productivity and advancement in the military will also have a chilling effect. A retention decision on the part of the prudent surgeon investigators has been further prejudiced by a unique and unusual preoccupation with body size and physical prowess. No other organization in the world gives any consideration to weight or running speed as employment criteria for physicians or scientists. One ordinarily selects a physician and/or scientist on the basis of intellectual capability and professional skills, not on the basis of physical endurance. It is generally believed that Walter Reed's accomplishments were not the result of outrunning mosquitoes.

Fortunately, the economic and other disincentives have been, to a certain degree, counterbalanced in the decision process of the surgical investigators of this Institute by the availability of research support funds provided by the US Army Medical Research and Development Command. Those funds permit the young investigator to initiate his research career without involvement in the NIH grant application process. In that process, only slightly more than one in three applications are
approved and those, on the average, after three review iterations. The work reported in this volume has resulted from the efforts of these dedicated physicians and scientists for whom this Institute's clinical, teaching, and research opportunities have made possible their professional productivity and counterbalanced the economic and other negative factors noted above. The results of their research have increased our understanding of the multisystem organ response to injury and led to the improvements in clinical management that have reduced the morbidity and increased the survival of seriously injured soldiers.

Unfortunately, retention of such productive investigators and maintenance of research momentum and continuity are ever more tenuous, since the noted disincentives are similar for mid-career surgical scientists with established research track records. A lieutenant colonel must compare his total compensation of $79,217 to the mean compensation of $109,700 for a full-time associate professor of surgery. The commonly observed resignation and migration to medical school faculty positions of such individuals is preordained. The unresponsiveness of retention to cosmetic pay increases in the past has led some to the opinion that increased remuneration doesn't influence retention, but the existing pay discrepancies indicate that salary comparability has never been attempted, let alone evaluated, and that there is no basis for that opinion. Appropriate remuneration and treatment of physicians as physicians give promise of arresting the medical department's brain drain and increasing clinical, educational, and research productivity by enhancing staff continuity (4). Those actions, whose time has come, will maintain the vitality of this Institute.

BASIL A. PRUITT, JR., MD, FACS
Colonel, MC
Commander and Director

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**Title:** Clinical Operation, Center for Treatment of Burned Soldiers

**Subject Areas:**
- Medicine and Medical Research
- Microbiology
- Pharmacology

**Start Date:** 06 07

**Estimated Completion Date:** 99 09

**Fiscal Years:**
- 87: 53.0
- 88: 53.0

**Funds (in thousands):**
- 2,115
- 2,231

**Contract Grant:** DA LR89AP

**Keywords (Provide each with Security Classification Code):**
- Heterograft:
- Resuscitation:
- Homograft:
- Air Evacuation:
- Thermal Injury:
- Topical Therapy:

**Technical Objective:**
24. (Continued) (U) Volunteers; (U) Autograft; (U) RAI

23. (U) The Clinical Division of this Institute is the major treatment center for thermally injured military personnel of all services as well as other eligible beneficiaries. The goals of the Division, in addition to the specialized care of severely injured patients, include the investigation of diagnostic and therapeutic techniques to improve the survival and function of injured patients as well as promulgation of scientific medical information to health professionals. A literature search is conducted for each protocol initiated.

24. (U) Thermally injured patients from the Continental United States and throughout the world are transported to this Institute for intensive, specialized treatment. Carefully controlled evaluation of new treatment techniques is conducted by the professional staff.

25. (U) 8601 - 8612. Two hundred and six seriously burned patients were admitted and treated at this Institute during calendar year 1986. Current clinical research activities include host resistance studies, endocrine changes following injury, development of optimal nutritional support of the burned patient, the use of skin substitutes, and studies in the control of postinjury infection.
ANNUAL RESEARCH PROGRESS REPORT

PROJECT NUMBER: 3S162787A874-00, APPLIED RESEARCH

PROJECT TITLE: CLINICAL OPERATION, CENTER FOR TREATMENT OF BURNED SOLDIERS

US ARMY INSTITUTE OF SURGICAL RESEARCH
FORT SAM HOUSTON
SAN ANTONIO, TEXAS 78234-5012

1 January 1986 - 31 December 1986

INVESTIGATORS

William F. McManus, MD, Colonel, MC
Khan Z. Shirani, MD, Lieutenant Colonel, MC
George M. Vaughan, MD, Lieutenant Colonel, MC
Donna W. Kyzar, RN, Lieutenant Colonel, AN
Thomas B. Dougherty, MD, Major, MC
James C. McKay, MD, Major, MC
Anne E. Missavage, MD, Major, MC
Steven I. Schmidt, MD, Major, MC
Anthony A. Smith, MD, Major, MC
William G. Cioffi, Jr., MD, Captain, MC
Gary R. Culbertson, MD, Captain, MC
Theresa A. Graves, MD, Captain, MC
Mark R. Jackson, MD, Captain, MC
Judson C. Lively, MD, Captain, MC
David W. Mozingo, MD, Captain, MC
L. Willis Roberts, MD, Captain, MC
Frank M. Robertson, MD, Captain, MC
Patricia S. Latona, RN, Major, AN
Rosendo T. Gutierrez, Captain, SP
Stephen H. Luster, Captain, SP
Kathleen F. McCoy, Captain, SP
Susan W. Wilson, Captain, SP
Basil A. Pruitt, Jr., MD, Colonel, MC
ABSTRACT

PROJECT NUMBER: 35162787A874-00, APPLIED RESEARCH

PROJECT TITLE: CLINICAL OPERATION, CENTER FOR TREATMENT OF BURNED SOLDIERS

INSTITUTION: US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas 78234-5012

PERIOD COVERED IN THIS REPORT: 1 Jan 86 through 31 Dec 86

INVESTIGATORS: William F. McManus, MD, Colonel, MC
Khan Z. Shirani, MD, Lieutenant Colonel, MC
George M. Vaughan, MD, Lieutenant Colonel, MC
Donna W. Kyzar, RN, Lieutenant Colonel, AN
Thomas B. Dougherty, MD, Major, MC
James C. McKay, MD, Major, MC
Anne E. Missavage, MD, Major, MC
Steven I. Schmidt, MD, Major, MC
Anthony A. Smith, MD, Major, MC
William G. Cioffi, Jr., MD, Captain, MC
Gary R. Culbertson, MD, Captain, MC
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Kathleen F. McCoy, Captain, SP
Susan W. Wilson, Captain, SP
Basil A. Pruitt, Jr., MD, Colonel, MC

Two hundred and six patients were admitted to this Institute during calendar year 1986. Principal activities included care of severely burned patients, research to improve survival and function of such patients, and education and training of health care professionals and paraprofessionals. The areas of research included a study to evaluate the effectiveness and safety of Artificial Skin in the treatment of third degree flame or scald injuries, an ongoing study of 5% aqueous mafenide acetate soaks for the topical treatment of burn wounds following grafting, studies of neuroendocrine abnormalities in burn injuries, evaluation of imipenem-cilastatin sodium for prophylactic activity against bacterial pneumonias in burned patients with inhalation injury, a clinical study of the safety and efficacy of ceftazidime in the parenteral therapy of infections in hospitalized burn patients, evaluations of immunoglobulin G and thyroxine therapy,
studies of thiamine levels, and antithrombin deficiency in thermally injured patients, and a project to characterize certain biochemical indicators of infection in the thermally injured.
INTRODUCTION

During calendar year 1986, 206 patients were admitted to this Institute and there were 207 patient dispositions during the same period. Statistical data are based on the 207 patient dispositions during calendar year 1986. There were 168 males and 39 females with an average age of 27.8 yr, ranging from 6 wk to 83 yr of age. Fifty-three patients were <15 yr of age (25.6%) and 34 patients were >45 yr of age (16.4%). The average total burn size of the entire population was 27.1% of the total body surface area, with a 12.1% average extent of full-thickness injury. The average hospital stay of all patients, excluding convalescent leave for active duty military patients, was 44.6 days. One hundred and fifty-one patients were admitted within 48 h of injury (73.0%).

During calendar year 1986, 791 operative procedures were performed on 143 patients, an average of 5.53 operative procedures per patient. Four hundred and ten anesthetics were given to 143 patients for an average of 2.9 anesthetics per patient. One hundred and thirty-six patients received a total of 619,025 ml of blood for an average of 4,551.7 ml of blood per patient.

ADMISSIONS DATA

The Clinical Division of this Institute admitted 206 soldiers and other authorized patients with thermal, chemical, or electrical injury during calendar year 1986. Aeromedical teams from the Institute conducted 85 missions within the Continental United States to transfer 97 of the 206 patients (47.1%) admitted. Twenty-two missions were carried out by rotary-wing aircraft (25.9%) and 63 by fixed-wing aircraft (74.1%). One hundred and thirty-eight of the 206 patients (67.0%) were admitted within 24 h of injury and 151 (72.8%) were admitted within 48 h of injury. One hundred and sixty-three patients were male and 43 were female.

DISPOSITIONS DATA

The following statistics are based on 207 patient dispositions during calendar year 1986. The ages of these 207 patients ranged from 6 wk to 83 yr, with an average age of 27.8 yr. Burn sizes averaged 27.1% of the total body surface area, with an average full-thickness component of 12.1%. Fifty-three patients were in the pediatric age group (age 15 and under), with an average age of 5.6 yr and an average burn size of 23.2% of the total body surface area. The average hospital stay of all dispositions was 44.6 days when convalescent leave for active duty military patients was included in the calculation.
and 42.6 days when convalescent leave was excluded. There were 8 patients with high voltage electrical injury and 3 patients with chemical injury. The sources of admission are identified in Table 1 and the causes of burn injury are detailed in Table 2.

Five patients required hemodialysis for acute renal failure. Acute myocardial infarctions were seen in 5 patients and acute pulmonary emboli in 6 patients. Inhalation injury was identified in 60 patients (29.0% based on admissions). Eighty patients (38.8%) had some associated injury (includes 60 patients with inhalation injury) which included fractures or dislocations in 4 patients and lacerations in 9 patients.

Morbidity and Mortality. Twenty-nine of the 207 dispositions (14.1%) died during calendar year 1986. Autopsies were performed in 19 (65.5%) of these hospital deaths. The average burn size of patients who died was 59.8% of the total body surface area and the full-thickness average was 41.4% of the total body surface area. Age ranged from 6 wk to 83 yr. Fourteen of these patients (48.3%) had inhalation injury as a primary or contributing cause of death. Nine patients had burn injuries exceeding 80% of the total body surface area (31.0%). Five patients died with acute myocardial infarctions. Five of the 29 deaths (17.2%) occurred in pediatric patients. These children had an average total body surface area burn of 61.6% and an average full-thickness burn of 49.7%. The average age of children who died was 19 months (6 wk through 4 yr). Two of these children had autopsies.

Infection was once again the most common complication following thermal injury, with bacterial pneumonia occurring in 49 patients. The most common organisms isolated in patients with bacterial pneumonia were Staphylococcus aureus in 19 patients, Pseudomonas aeruginosa in 12 patients, and Klebsiella species in 10 patients. However, only 13 patients demonstrated bacterial septicemia and no patient had bacterial invasion of the burn wound identified during this calendar year.

Table 3 lists the effect of age and extent of injury on survival and Table 4 lists mortality rates associated with increments of 10% of the total body surface area for the years 1979 through 1986. Table 5 summarizes the survival of patients with extensive burns from 1958 through 1986. Table 6 compares mortality before and after the use of topical chemotherapy on the burn wound. Table 7 lists the causes of death for calendar year 1986.

EDUCATIONAL ACTIVITIES

During calendar year 1986, the professional staff of the Clinical Division continued to provide education to professional and paraprofessional groups at the local, national, and international levels. A total of 31 resident
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*Autopsy not performed.
TABLE 7 (Continued)

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*Autopsy not performed.
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*Autopsy not performed.
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<td>93 Total 20</td>
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<td>*93% total body surface area burn and multiple organ failure.</td>
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*Autopsy not performed.
physicians were attached for periods of 1-3 months, including 4 each from Letterman Army Medical Center, Wilford Hall Medical Center, William Beaumont Hospital (Royal Oak, Michigan), and the Naval Aerospace Medical Institute, 3 each from Providence Hospital (Southfield, Michigan) and Fitzsimons Army Medical Center, 2 each from William Beaumont Army Medical Center and Travis Air Force Base Medical Center, and 1 each from the Louisiana State Hospital System, Brooke Army Medical Center, Walter Reed Army Medical Center, Butterworth Hospital (Grand Rapids, Michigan), and UMD - University Hospital (Newark, New Jersey). A total of 17 medical students rotated through the Institute, including 7 health profession scholarship medical students, 3 students from the Indiana University School of Medicine, 2 students from Columbia University, and 1 student each from the University of Iowa, the University of Michigan, East Tennessee State University, Texas Tech University, and Oral Roberts University. A total of 18 physicians visited from foreign countries for periods ranging from 1 day to 1 year, which included 4 from the Dominican Republic, 3 from Pakistan, 2 each from China, Japan, and Great Britain, and 1 each from Israel, Sweden, the Philippines, Norway, and Spain. The Respiratory Therapy Branch had 120 trainees, the Physical Therapy Branch had 26 trainees, and the Occupational Therapy Branch had 32 trainees. Fifteen scientific publications appeared in refereed medical journals and approximately 131 scientific presentations were conducted for military and civilian audiences. Numerous scientific presentations were made at the Academy of Health Sciences and various military installations throughout the continental United States, to include support of the Battlefield Medicine Course for the United States Air Force and the Combat Casualty Care Courses for the United States Army. In addition, weekly professional staff conferences were conducted for and by Institute personnel.

PRESENTATIONS


Pruitt BA Jr: Opportunistic infections in injured patients. Presented as Visiting Professor to the Department of Surgery, Cedars-Sinai Medical Center, Los Angeles, California, 12 March 1986.


Pruitt BA Jr: Fluid resuscitation of the burn patient. Presented as Visiting Professor at the University of Illinois College of Medicine, Champaign, Illinois, 20-21 March 1986.


Jordan BS: Team effort with burn care management. Presented at the INTRACORP Seminar, Dallas, Texas, 1-2 May 1986.


McCoy KF: Team effort with burn care management. Presented at the INTRACORP Seminar, Dallas, Texas, 1-2 May 1986.


Pruitt BA Jr: Local burn wound infection. Presented as Invited Speaker to the Burns Management Course, New Delhi, India, 23 June-4 July 1986.


McCoy KF: Care of the thermally injured patient. Presented to the Physical Therapy Specialist Course (91J),


Pruitt BA Jr: Treatment of thermal injuries. Presented as Visiting Professor to the Department of Surgery, University of South Alabama College of Medicine, Mobile, Alabama, 8 August 1986.


Pruitt BA Jr: The burn patient as a trauma model. Presented as the Curtis Artz Lecturer to the South Carolina Chapter of the American College of Surgeons, Greenville, South Carolina, 12-13 September 1986.


Pruitt BA Jr: Diagnosis and treatment of inhalation injury and other pulmonary complications. Presented at the Buffalo Burn Symposium, Department of Surgery, State University of New York at Buffalo, Buffalo, New York, 10-11 October 1986.


21
McManus WF: Burns. Presented to the Officers' Basic
Academy of Health Sciences, Fort Sam Houston, San
Antonio, Texas, 23 October 1986.

McCoy KF: Care of the thermally injured patient.
Presented to the Physical Therapy Specialist Course (91J),
Academy of Health Sciences, Fort Sam Houston, San Antonio,
Texas, 23 October 1986.

McManus WF: Thermal injury. Presented to Federal Drug
Enforcement Agency pilots, Randolph Air Force Base, San

to the Association of Military Surgeons of the United States,
San Antonio, Texas, 3 November 1986.

McCoy KF: Physical therapy in burn care. Presented to
students from Southwest Texas State University, Fort Sam
Houston, San Antonio, Texas, 3 November 1986.

Cioffi WG Jr: Pulmonary and systemic vascular reactivity
in thermal injury. Presented at the Twentieth Annual Meeting
of the Association for Academic Surgery, Washington, DC, 5
November 1986.

Pruitt BA Jr: Infection and burn problems. Presented to
the Physician's Assistant Course (C4A), Academy of Health
Sciences, Fort Sam Houston, San Antonio, Texas, 6 November
1986.

Summers TM: Introduction to the hospital ministry course.
Presented at Brooke Army Medical Center, Fort Sam Houston, San
Antonio, Texas, 10 November 1986.

McManus WF: Resuscitation of the thermally injured
patient. Presented at the Trauma Conference, William Beaumont
Army Medical Center, El Paso, Texas, 13 November 1986.

Mozingo DW: Chemical burns. Presented at the Trauma
Conference, William Beaumont Army Medical Center, El Paso,
Texas, 13 November 1986.

McManus WF: Massive burn injury. Presented at the
Ninety-Fourth Annual Meeting of the Western Surgical
Association, Dearborn, Michigan, 18 November 1986.

Kyzar DW: Nursing administrator overview of the US Army
Institute of Surgical Research. Presented to the United States
Army Recruiting Service Nursing Educator Tour, Fort Sam

Pruitt BA Jr: Fluid resuscitation and wound care.
Presented to the Department of Surgery, University of Oklahoma
College of Medicine, Oklahoma City, Oklahoma, 11 November 1986.


PUBLICATIONS


MOTION PICTURES

ANNUAL RESEARCH PROGRESS REPORT

PROJECT NUMBER: 3S162787A874-00, APPLIED RESEARCH

PROJECT TITLE: CLINICAL OPERATION, CENTER FOR TREATMENT OF BURNED SOLDIERS: Anesthesiology

US ARMY INSTITUTE OF SURGICAL RESEARCH
FORT SAM HOUSTON
SAN ANTONIO, TEXAS 78234-5012

1 January 1986 - 31 December 1986

INVESTIGATORS

Thomas B. Dougherty, MD, PhD, Major, MC
Steven I. Schmidt, MD, Major, MC
Charles P. Kingsley, MD, Captain, MC
William F. McManus, MD, Colonel, MC
Basil A. Pruitt, Jr., MD, Colonel, MC
ABSTRACT

PROJECT NUMBER: 3516278/A874-00, APPLIED RESEARCH

PROJECT TITLE: CLINICAL OPERATION, CENTER FOR TREATMENT OF BURNED SOLDIERS: Anesthesiology

INSTITUTION: US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas 78234-5012

PERIOD COVERED IN THIS REPORT: 1 Jan 86 through 31 Dec 86

INVESTIGATORS: Thomas B. Dougherty, MD, PhD, Major, MC
Steven I. Schmidt, MD, Major, MC
Charles P. Kingsley, MD, Captain, MC
William F. McManus, MD, Colonel, MC
Basil A. Pruitt, Jr., MD, Colonel, MC

During the period of this report, 410 anesthetics were administered to 143 patients, an average of 2.87 anesthetics per patient. The most commonly used anesthetic agent was enflurane (66.34%), followed by ketamine (15.37%), halothane (8.54%), and isoflurane (5.61%). Due to the nature and combinations of procedures now performed, regional anesthesia is no longer used.
ANESTHESIOLOGY

PREOPERATIVE PROCEDURES

Evaluation. Most burn patients are several days postinjury when first seen by the anesthesiologist. In the immediate postburn period, time is used to gain abundant physiologic data from routine monitoring of various indices, i.e., hematologic (hematocrit, electrolytes, liver and renal function tests), pulmonary (arterial blood gases, respiratory rate, chest roentgenograms), cardiovascular (blood pressure, central venous pressure, cardiac output), and renal (urine output, urine chemistry), in addition to the usual preoperative patient interview and physical examination. All patients, regardless of age, who have electrical injuries are required to have a preoperative electrocardiogram performed to rule out possible myocardial damage.

Preparation. All patients are placed on NPO status after 2400 h the day prior to surgery with the exception of children, who may receive clear liquids up to 5 h prior to surgery. Due to extraordinary fluid requirements in most burn patients, an intravenous infusion, if not already in place, is begun the evening prior to surgery.

Premedication. Glycopyrrolate (Robinul\textsuperscript{R}), from 0.005 mg/kg to a maximum dose of 0.4 mg/kg, is given intramuscularly 30 min prior to anesthesia or intravenously upon entering the operating room. No other premedications are routinely used with the exception of diazepam preceding ketamine anesthetic.

Fluids. All fluids, except hyperalimentation solutions, are changed to 5% glucose in water or Ringer’s lactate on arrival in the operating room. Hyperalimentation solutions are continued throughout operative procedures.

TYPES OF ANESTHESIA

The pattern of anesthetic administration has changed from previous years and involves a greater use of enflurane, having been used in more than 60% of all anesthetic procedures. Ketamine, halothane, and isoflurane are used, but to a much lesser extent (Table 1).

Enflurane (Ethrane\textsuperscript{R}). Enflurane is a halogenated ether which provides rapid induction and good muscle relaxation. Biotransformation amounts to 2 to 2.5% of an inhaled dose, which perhaps accounts for the few clinical toxic effects observed. Plasma fluoride levels in hypermetabolic burn patients during and after enflurane administration have been measured and found not to be in the toxic range. Enflurane is presently the most commonly used anesthetic agent at this Institute.
### TABLE 1. Pattern of Anesthesia Administration (1983-1986)

<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>%</td>
<td>Number</td>
<td>%</td>
</tr>
<tr>
<td>Enflurane</td>
<td>184</td>
<td>63.23</td>
<td>290</td>
<td>62.91</td>
</tr>
<tr>
<td>Ketamine</td>
<td>66</td>
<td>22.68</td>
<td>88</td>
<td>19.09</td>
</tr>
<tr>
<td>Halothane</td>
<td>0</td>
<td>0.00</td>
<td>0</td>
<td>0.00</td>
</tr>
<tr>
<td>Isoflurane</td>
<td>0</td>
<td>0.00</td>
<td>0</td>
<td>0.00</td>
</tr>
<tr>
<td>Local</td>
<td>13</td>
<td>4.47</td>
<td>14</td>
<td>3.04</td>
</tr>
<tr>
<td>Other</td>
<td>6</td>
<td>2.00</td>
<td>42</td>
<td>9.11</td>
</tr>
<tr>
<td>Nitrous oxide</td>
<td>22</td>
<td>7.56</td>
<td>27</td>
<td>5.86</td>
</tr>
<tr>
<td>TOTAL</td>
<td>291</td>
<td>100.00</td>
<td>461</td>
<td>100.00</td>
</tr>
</tbody>
</table>
Halothane (Fluothane\textsuperscript{R}). Halothane is a halogenated alkane that has met with only limited use over the last 4 yr. Biotransformation can account for as much as 25\% of an inhaled dose. Halothane hepatitis, although rare, fortunately has not been reported in burn patients. Since the successful introduction of enflurane, few indications for halothane’s use exist in this patient population that may be predisposed to hepatitis from multiple transfusions with blood products. However, its use is indicated primarily in the burned pediatric patient who requires that his airway be secured by an endotracheal tube. Halothane hepatitis has not been reported to be an issue in the pediatric population. Therefore, its use was increased in the burned pediatric patient population over the past year.

Isoflurane (Forane\textsuperscript{R}). Isoflurane, which is an isomer of enflurane, is the most recent halogenated ether to be introduced. Biotransformation amounts to only 0.25\% of an inhaled dose and no toxic reactions to the metabolic products have been reported to date. It has a rather pungent odor that tends to limit its use for inhalational induction. It is noted for producing minimal myocardial depression and a marked reduction in systemic vascular resistance. At this time, isoflurane has found limited use at this Institute, but as more experience with this agent is gained, its use will probably increase.

Nitrous Oxide. This agent is used in concentrations of 50 to 60\% with oxygen. It is used mainly in conjunction with other analgesic or anesthetic agents.

Ketamine. This agent is used both intramuscularly and intravenously to produce its characteristic dissociative state with preservation of basal functions and laryngeal reflexes plus stimulation of the cardiovascular system. Unfortunately, ketamine shares with its parent compound, phencyclidine, the production of a high incidence of unpleasant hallucinogenic side effects. There seems to have been a "batch" difference in ketamine and that possessed by this Institute in the past has produced an almost 100\% incidence of these side effects. New methods of administering the drug as well as various methods of premedication and patient preparation appear to have reduced the unpleasant emergence reactions to a level where they are of little consideration in the well-selected patient. Laryngospasm, airway obstruction, and regurgitation can occur with ketamine. Pronounced blepharospasm prevents its use in eye cases. All ketamine anesthetics, other than in children, are preceded by intravenous administration of diazepam (0.15 to 0.2 mg/kg).

Sucinylcholine. Succinylcholine has not been used for any purpose at this Institute for more than 10 yr. On the other hand, nondepolarizing muscle relaxants (vecuronium bromide,
pancuronium bromide, and atracurium besylate) have been used in 22% of the operative cases over the past year.

Regional Anesthetics. Regional anesthetics are generally considered one of the safest methods available, but its use in the thermally injured patient is limited for several reasons. Sepsis and infection of the skin over or near the site of injection are contraindications for use and multiple-site operations also limit the practicality of this method.

MONITORING TECHNIQUES

Cardiovascular System. Monitoring includes the precordial and/or esophageal stethoscope, peripheral pulse, blood pressure, central venous pressure, Swan-Ganz catheter, electrocardiogram, and urine output.

The Dinamap blood pressure instrument is routinely used for intraoperative blood pressure monitoring. Since it can be used over dressings and is noninvasive, it is a most practical method of monitoring blood pressure in our patient population. Usually, blood pressure is monitored at two sites. Direct arterial lines are used when necessary.

Respiratory System. Monitoring includes the rate, auscultation, arterial blood gases, pulmonary functions (pre and intraoperative), hemoglobin oxygen saturation, and end tidal carbon dioxide. During the past year, the introduction of new noninvasive monitors has made a significant contribution to the management of the thermally injured patient. The measurement of hemoglobin oxygen saturation by pulse oximetry, end-tidal carbon dioxide, and pulmonary function parameters all represent no risk to the patient, are easily obtainable, and are accurate. These monitors have become standard in our anesthetic care of the burn patient.

Body Temperature. In most cases, a temperature monitor is employed. Because of the greatly increased evaporative losses in burn patients, hypothermia is a serious problem. Several methods are employed to maintain body temperature during anesthesia. Ambient temperatures were maintained between 82 and 87°F in the past; however, patient cooling still occurred. Maintaining the room temperature above 88°F appears to have corrected the problem. Anesthetic gases are heated and humidified. Radiant heat lamps used alone have been found to have little effect in preventing patient cooling. The K-thermia heating blanket is also sometimes used. It is probably used most effectively on children weighing <10 kg and febrile patients.

RESULTS

Complications. There were no intraoperative complications during this reporting period.
Patient Data. Tables 2 and 3 provide overall anesthetic patient data.

Operative Procedures. Table 4 illustrates recent trends in operative procedures.

PRESENTATIONS/PUBLICATIONS

TABLE 2. Frequencies of Use for Selected Intraoperative Monitors/Parameters (1986)

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<tr>
<th>Monitor/Parameter</th>
<th>Number of Intraoperative Uses</th>
<th>Total Anesthetics (%)</th>
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<tbody>
<tr>
<td>Electrocardiogram</td>
<td>410</td>
<td>100.00</td>
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<tr>
<td>Dinamap\textsuperscript{R} (blood pressure)</td>
<td>408</td>
<td>99.51</td>
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<tr>
<td>Temperature</td>
<td>397</td>
<td>96.83</td>
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<tr>
<td>Pulse oximeter (hemoglobin saturation)</td>
<td>370</td>
<td>90.24</td>
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<td>End-tidal carbon dioxide</td>
<td>356</td>
<td>86.83</td>
</tr>
<tr>
<td>Inspired oxygen concentration</td>
<td>356</td>
<td>86.83</td>
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<tr>
<td>Pulmonary function</td>
<td>332</td>
<td>80.98</td>
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<tr>
<td>Arterial line</td>
<td>38</td>
<td>9.27</td>
</tr>
<tr>
<td>Central venous pressure</td>
<td>9</td>
<td>2.20</td>
</tr>
<tr>
<td>Swan-Ganz catheter</td>
<td>9</td>
<td>2.20</td>
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TABLE 3. Overall Anesthetic Patient Data (1971-1986)

<table>
<thead>
<tr>
<th>Year</th>
<th>Number of Patients</th>
<th>Number of Patients Anesthetized</th>
<th>% of All Patients</th>
<th>Total Anesthetics Given</th>
<th>Average Anesthetics Per Patients Anesthetized</th>
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<tbody>
<tr>
<td>1971</td>
<td>301</td>
<td>179</td>
<td>59.47</td>
<td>475</td>
<td>2.65</td>
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<td>1972</td>
<td>301</td>
<td>183</td>
<td>60.80</td>
<td>575</td>
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<tr>
<td>1973</td>
<td>273</td>
<td>141</td>
<td>51.65</td>
<td>377</td>
<td>2.67</td>
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<td>1974</td>
<td>226</td>
<td>123</td>
<td>54.42</td>
<td>380</td>
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<td>1975</td>
<td>254</td>
<td>142</td>
<td>55.91</td>
<td>490</td>
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<td>1976</td>
<td>277</td>
<td>139</td>
<td>50.18</td>
<td>476</td>
<td>3.42</td>
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<tr>
<td>1977</td>
<td>242</td>
<td>129</td>
<td>53.30</td>
<td>344</td>
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<td>1978</td>
<td>268</td>
<td>151</td>
<td>56.34</td>
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<td>179</td>
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<td>54.75</td>
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<td>1984</td>
<td>190</td>
<td>139</td>
<td>73.16</td>
<td>461</td>
<td>3.32</td>
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<tr>
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<td>197</td>
<td>133</td>
<td>67.51</td>
<td>388</td>
<td>2.92</td>
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<tr>
<td>1986</td>
<td>207</td>
<td>143</td>
<td>69.08</td>
<td>410</td>
<td>2.87</td>
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<td>-------------------</td>
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<td>-------</td>
</tr>
<tr>
<td></td>
<td>Number</td>
<td>%</td>
<td>Number</td>
<td>%</td>
<td>Number</td>
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<tr>
<td>Excision</td>
<td>257</td>
<td>33.29</td>
<td>196</td>
<td>42.06</td>
<td>323</td>
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<tr>
<td>Autograft</td>
<td>405</td>
<td>52.46</td>
<td>203</td>
<td>43.56</td>
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<td>Orthopedic</td>
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<td>Chondrectomy</td>
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<td>Eye and lid</td>
<td>14</td>
<td>1.81</td>
<td>8</td>
<td>1.72</td>
<td>18</td>
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<tr>
<td>Intra-abdominal</td>
<td>6</td>
<td>0.78</td>
<td>2</td>
<td>0.43</td>
<td>5</td>
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<tr>
<td>Plastic</td>
<td>15</td>
<td>1.94</td>
<td>2</td>
<td>0.43</td>
<td>5</td>
</tr>
<tr>
<td>Other</td>
<td>44</td>
<td>5.70</td>
<td>31</td>
<td>6.65</td>
<td>31</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td>772</td>
<td>100.00</td>
<td>466</td>
<td>100.00</td>
<td>787</td>
</tr>
</tbody>
</table>

**TABLE 4.** Recent Trends in Operative Procedures (1982-1986)
22. (Continued) (U) Autograft; (U) RAFI

23. (U) The cause of infection in the wounds of burn patients continues to be a major area of study in order to improve the survival of the severely burned soldier. This study is designed to evaluate the efficacy of 5% aqueous mafenide acetate-soaked dressings, employed either for final debridement of burn wounds or following application of meshed cutaneous autograft, to prevent infection and desiccation of the tissue exposed in the interstices of such grafts. A literature search is conducted for each protocol initiated.

24. (U) Patients admitted to this Institute for care following thermal, chemical, or electric injury are treated with 5% aqueous mafenide acetate soaks daily.

25. (U) 8601 - 8612. One hundred and forty-one patients were treated with 5% aqueous mafenide acetate soaks during calendar year 1986. Nineteen of these 141 patients exhibited mild cutaneous atopy. This low incidence of mild side effects of 5% aqueous mafenide acetate and its continued clinical effectiveness speak for the continued use of this valuable therapeutic agent.
ANNUAL RESEARCH PROGRESS REPORT

PROJECT NUMBER: 3S162787A874-00, APPLIED RESEARCH

PROJECT TITLE: EVALUATION OF BURN WOUND CARE IN TROOPS WITH BURN INJURY: E-Z Derm™ and Biobrane®: A Comparative Study

US ARMY INSTITUTE OF SURGICAL RESEARCH
FORT SAM HOUSTON
SAN ANTONIO, TEXAS 78234-5012

1 October 1986 - 30 September 1987

INVESTIGATORS

Stephen M. Pratt, MD
L. Willis Roberts, MD, Captain, MC
William F. McManus, MD, Colonel, MC
Arthur D. Mason, Jr., MD
Basil A. Pruitt, Jr., MD, Colonel, MC
ABSTRACT

PROJECT NUMBER: 3S162787A874-00, APPLIED RESEARCH

PROJECT TITLE: EVALUATION OF BURN WOUND CARE IN TROOPS WITH BURN INJURY: E-Z Derm™ and Biobrane™: A Comparative Study

INSTITUTION: US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas 78234-5012

PERIOD COVERED IN THIS REPORT: 1 Oct 86 through 30 Sep 87

INVESTIGATORS: Stephen M. Pratt, MD
L. Willis Roberts, MD
Bryan S. Jordan, RN, Captain, AN
William F. McManus, MD, Colonel, MC
Arthur D. Mason, Jr., MD
Basil A. Pruitt, Jr., MD, Colonel, MC

Biologic and synthetic dressings offer utility in excised burn wounds when immediate autografting cannot be performed. Traditionally, allograft has been of demonstrated efficacy for this purpose, yet inherent limitations have prompted a search for alternative materials. A controlled prospective trial was conducted comparing a semisynthetic bilaminate (Biobrane™) and an aldehyde-treated xenograft preparation (E-Z Derm™) on paired excised burn wounds prior to autografting. Each material was evaluated using a wound dressing index to numerically rate five factors, i.e., adherence, conformation, pliability, granulation tissue formation, and suppuration. Both materials were highly rated in each category and thus appear to be suitable substitutes for allograft. E-Z Derm™ appeared to promote formation of a better and more graftable wound bed (P=0.001) and Biobrane was judged more pliable (P=0.03). No significant differences were noted in the other three categories or in autograft take following dressing removal. However, considerable variability between sites was noted for both materials.

A consistent and objective means for evaluating wound dressings is presented and used to assess the efficacy of E-Z Derm™ and Biobrane® as temporary covers on excised burn wounds prior to autografting. The wound dressing index presented facilitates comparison of various materials and assists in defining the utility of each dressing in burn care.
INTRODUCTION

Closure of the burn wound is sine qua non to the recovery of the thermally injured patient. Because lack of available donor sites often delays wound closure, alternatives to immediate autografting have been advanced as temporizing measures. Allograft has been widely regarded as the "gold standard" for temporary wound closure since its use in burns was first advanced by Pollack in 1870 (1). Control of fluid, electrolyte, and protein loss from the wound, support of the production of a graftable fibrovascular bed, control of microbial proliferation, and pain control are among the recognized benefits of allograft use (2,3). Additionally, increased survival with early burn wound excision and allograft coverage has been documented in an animal model (4); however, matching allograft availability with need, the diminished effectiveness of frozen and lyophilized preparations, eventual rejection, and storage requirements have imposed limitations on its use. More recently, the potential for disease transmission bodes ill for the widespread medical use of almost any human material. As a result of these limitations, xenograft has been utilized as an alternative to allograft for many years, with porcine skin comprising the bulk of commercially available products today. However, less than optimum durability and wound adherence properties along with an incommensurate antimicrobial effect have been drawbacks of porcine skin graft use. For these reasons, a great deal of interest has been generated in the development of modified biologic dressings and synthetic materials to serve as temporary substitutes for autograft. A collagen-synthetic bilaminate membrane has been documented to be efficacious when compared with porcine xenograft and human allograft. Recently, E-Z Derm\textsuperscript{TM}, an aldehyde-treated porcine dermal preparation, has been introduced and reportedly shares some of the desirable properties of xenograft with the added advantages of room temperature storage, longer wound retention times, and decreased antigenicity (5,6). Both of these materials would therefore appear to be useful alternatives to allograft use in the treatment of partial-thickness wounds and as temporizing measures in excised full-thickness wounds when donor site availability limits definitive autografting.

To evaluate the later, a controlled prospective comparative trial of these two dressings on excised burn wounds prior to autografting was conducted. Utilizing a previously described wound dressing index to quantify differences in each of five categories regarded as essential properties of wound dressings (7), meaningful data was obtained to facilitate efficacy determinations. Use of this system allows for comparison of the various biologic and synthetic dressings which are becoming available for clinical use.
MATERIALS AND METHODS

Sixteen sites were compared in 11 patients with a mean age of 32 yr (range 24-68 yr) and a mean total body surface area (TBSA) burn of 54% (range 42-88%). Sites were comparable with respect to anatomic location, method and depth of excision, and size. In all cases, tangential excision (11 sites) or fascial excision (5 sites) to the level of viable tissue was followed by immediate dressing placement after hemostasis was obtained (Table 1). E-Z Derm® (Genetics Laboratories, St. Paul, MN) was applied in 3-in wide strips, with either side placed against the wound surface. These sites were subsequently wrapped with light gauze dressings for 18 to 24 h to prevent early mechanical disruption during transport and recovery from anesthesia, following which the test sites were left open for observation. Biobrane® (Winthrop Pharmaceuticals, Santa Anna, CA) of appropriate size was expanded until taut and wrinkle-free and stapled into place. All excesses were trimmed as necessary. An occlusive dressing was subsequently applied for 24-36 h to allow for initial adherence of the Biobrane® to the wound.

Evaluation. Conformation of the dressing to the wound surface was assessed by the patient's primary operating surgeon at the initial dressing application and numerically rated as deemed appropriate (Table 1). Dressing pliability was likewise rated from observations made at application and during the course of treatment. Daily observations were recorded by the patient's primary physician for the purpose of assessing adherence of the test dressing and the presence of air pockets, fluid, or frank suppuration beneath the material. Large areas of nonadherence or suppuration beneath the dressing necessitated removal, culture, and treatment of the site with topical agents (mafenide acetate and silver sulfadiazine) applied twice daily. Small areas of fluid accumulation were locally excised and treated topically until reapplication of the dressing was clinically indicated. When adequate donor sites were available and the patient's condition permitted, dressing removal and autografting were performed. At this time, the formation of a graftable bed of granulation tissue at the wound dressing interface was assessed. Each dressing was therefore compared with respect to these five properties essential to the biologic and mechanical function of the material. Additionally, autograft "take" on each test site was recorded.

Statistical Analysis. The Wilcoxon matched-pair signed-rank's test and the Friedman two-way analysis of variance were used to test against the null hypothesis that no difference exists between the two test materials in each category assessed.
### TABLE 1. Definition of Grading Scales

#### Formation of Granulation Tissue

1 = None  
2 = Scanty with irregular distribution and wound debris  
3 = Irregular surface with wound debris or superficial exudate  
4 = Clean granulating surface with minimal fibrosis or debris  
5 = Beefy red, uniformly smooth, clean

#### Presence of Suppuration

1 = Frankly purulent collection beneath dressing  
2 = Moderate seropurulent collection beneath dressing  
3 = Slight seropurulent collection beneath dressing  
4 = Slight serous collection beneath dressing  
5 = None

#### Adherence to Test Dressing

1 = Slides off test site spontaneously  
2 = Easily dislodged  
3 = Easily removed with forceps  
4 = Strips off with resistance  
5 = Strips off with resistance and brisk bleeding

#### Conformation to Wound Surface

1 = Buckled, wrinkled, poor wound contact  
2 = Conforms only to flat wound surface  
3 = Torsion and tension must be applied to gain maximal wound surface contact with minimal wrinkling present  
4 = Torsion and tension must be applied to gain maximal wound surface contact with no wrinkling present  
5 = Mimics skin

#### Pliability of Test Dressing

1 = Stiff prior to wound application  
2 = Loses pliability unrelated to recipient test site condition  
3 = Loses pliability related to absorption of wound exudate  
4 = Maintains most of its pliability despite absorption of wound exudate  
5 = Mimics skin

For each of the five factors listed, a rank of 1 is the least desirable rank and 5 is the most desirable rank. These ranks have been tailored for evaluation of Biobrane® and porcine.

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RESULTS

The mean scores in each of the five categories assessed in the 16 paired sites and the percent graft take in the 10 sites which were grafted are presented in Table 2. Statistically, significant differences were noted in two categories when comparing E-Z Derm™ and Biobrane®.

Granulation tissue formation beneath the dressing at the time of definitive autografting was rated better on the E-Z Derm™ sites (P=0.001). This difference was particularly notable over wound beds excised to fat. Nonhypertrophic, beefy red granulation tissue was felt to represent an optimum site for autografting. Prior experience has demonstrated that removal of adherent dressing on the "best" sites was accompanied by brisk bleeding and some resistance to the removal of the test dressing. Graft "take" on each of these sites which progressed to autografting was evaluated, resulting in 94 and 85% for E-Z Derm™ and Biobrane®, respectively, although this difference failed to reach statistical significance in the 10 sites which were grafted. The control of wound suppuration was essentially equal for both materials, with both materials controlling wound exudate relatively well for the initial 14 days of application. After 14 days postapplication, both materials were noted to have small serous fluid collections to a greater extent, necessitating local debridement. Additionally, frank purulence on three separate Biobrane® study sites was noted on postapplication days 15, 17, and 18, necessitating total Biobrane® removal from two sites and partial removal in the third. All fluid collections were cultured and correlated with the clinical course. Similarly, in two patients with E-Z Derm™ placed on nonexposed surfaces (posterior thighs and legs), nonadherence as a result of shear and fluid at the wound-dressing interface necessitated removal. Biobrane® was rated higher in pliability than E-Z Derm™, conforming to any surface well. This was particularly advantageous over joints where Biobrane® could be applied, permitting continued joint motion with little disruption of the wound bed.

Both materials were highly rated with regard to conformation to the wound surface, receiving equivalent scores. Likewise, adherence was comparable. In these categories, however, considerable site specificity was noted. E-Z Derm™ performed better on flat anterior surfaces while Biobrane® performed better on dependent surfaces, particularly when applied circumferentially.

Dressings were permitted to remain in place until autografting, patient death, or suppuration. E-Z Derm™ remained adherent an average of 18.5 days (range 8-46 days) and Biobrane® 13 days (range 7-21 days). Suppuration beneath the dressing constituted the major reason for removal of dressings prior to autografting.
TABLE 2. *E-Z Derm™* and Biobrane®: Wound Dressing Index Values

<table>
<thead>
<tr>
<th>Factors</th>
<th>Sites</th>
<th>E-Z Derm™</th>
<th>Biobrane®</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adherence</td>
<td>16</td>
<td>3.9</td>
<td>3.6</td>
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<tr>
<td>Conformation</td>
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<td>3.8</td>
<td>4.1</td>
</tr>
<tr>
<td>Granulation tissue</td>
<td>16</td>
<td>4.5*</td>
<td>2.9*</td>
</tr>
<tr>
<td>Pliability</td>
<td>16</td>
<td>3.4**</td>
<td>4.3**</td>
</tr>
<tr>
<td>Suppuration</td>
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<td>4.3</td>
<td>3.4</td>
</tr>
<tr>
<td>Graft take</td>
<td>10</td>
<td>94%</td>
<td>85%</td>
</tr>
</tbody>
</table>

*P=0.005.

**P=0.03.

In one patient with septicemia and pneumonia, *Pseudomonas aeruginosa* was cultured from beneath both dressing sites after removal for progressive suppuration. Positive cultures necessitated wet-to-dry treatment of the wound with mafenide acetate-soaked dressings twice daily until such time as biologic dressing could again be applied.

When primary physician preference was recorded at completion of each study patient, there was no significant difference noted between the dressings (Table 3). No adverse local or systemic reactions were noted during the course of the study.

**DISCUSSION**

In recent years, biomedical technology has afforded the clinician caring for the thermally injured patient with a number of biologic and synthetic dressings for use when wound coverage is problematic (4,8,9). This group includes patients where adequate donor sites or general condition prohibits definitive autografting following excision of nonviable tissue.

The necessary mechanical and physical properties of skin substitutes and biosynthetic materials available for topical wound closure have been outlined. Roberts et al (7) has previously defined characteristics important to temporary burn wound dressings and outlined a wound dressing index for use in comparing various materials. A biologic dressing should
control water evaporation and protein loss from the wound, support production of a graftable fibrovascular tissue bed, facilitate infection control at the wound interface, remain adherent, and conform to the wound surface until definitive wound autografting can be effected. Optimally, these materials should be easily stored, applied, and maintained. Autograft has been traditionally used for this purpose, but availability and cost can greatly limit its utility (10). Xenograft has thus subsequently enjoyed wide use and is generally more available than allograft, although this material, too, is not without certain limitations (11). Storage requirements, the need for frequent replacement, and the less than optimal handling characteristic of lyophilized and frozen preparations have inspired the search for alternative materials. Numerous biologic materials, synthetic polymers, and combinations of these have been marketed with fervor in recent years. A great deal of interest has been generated comparing the properties of the many new dressings now available. The recent advances in the field, however, militate for a standardized set of criteria for use in comparing various biologic dressings and skin substitutes. Only then can consistency be achieved in evaluations within and between burn centers. Data controlled with regard to each dressing can thus be analyzed more effectively to define important properties of each material and optimize their use.

In the current study, both dressings appear to be suitable substitutes for allograft. On certain excised sites, each dressing has been shown to be advantageous as outlined. E-Z Derm™ proved superior to Biobrane® in the promotion of a graftable fibrovascular bed. This was particularly noteworthy on wounds excised to fat. Likewise, patient needs would be better served by the use of Biobrane® on dependent, nonexposed surfaces and over joints.

Using a standardized wound dressing index, current and new biologic and synthetic dressings can be systematically

<table>
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<tr>
<th>PHYSICIAN PREFERENCE FOR FUTURE USE</th>
<th>E-Z Derm™</th>
<th>Biobrane®</th>
</tr>
</thead>
<tbody>
<tr>
<td>Definitively</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>Probably</td>
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<td>3</td>
</tr>
<tr>
<td>Maybe</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>No</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>
evaluated in a prospective fashion with better definition of the advantages and limitations of each. The use of a uniform grading system will allow for a more meaningful analysis of results.

PRESENTATIONS/PUBLICATIONS

None.

REFERENCES


ANNUAL RESEARCH PROGRESS REPORT

PROJECT NUMBER: 3S162787A874-00, APPLIED RESEARCH

PROJECT TITLE: EVALUATION OF BURN WOUND CARE IN TROOPS WITH BURN INJURY: A Study to Evaluate the Effectiveness and Safety of Artificial Skin in the Treatment of Third Degree Flame or Scald Injuries

US ARMY INSTITUTE OF SURGICAL RESEARCH
FORT SAM HOUSTON
SAN ANTONIO, TEXAS 78234-5012

1 October 1986 - 30 September 1987

INVESTIGATORS

Stephen M. Pratt, MD
Bryan S. Jordan, RN, Captain, AN
William F. McManus, MD, Colonel, MC
Arthur D. Mason, Jr., MD
Basil A. Pruitt, Jr., MD, Colonel, MC
ABSTRACT

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INSTITUTION: US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas 78234-5012

PERIOD COVERED IN THIS REPORT: 1 Oct 86 through 30 Sep 87

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The evaluation of a collagen-based synthetic wound dressing in the treatment of full-thickness burn injury was conducted at this Institute as part of a multicenter study of this material. Artificial Skin, a bilaminate membrane which consists of a distinct dermal and epidermal portion, was compared to autograft and allograft after excision of full-thickness burn wounds.

Seven patients were entered into the study since its inception, including 3 patients during the period of this report. The average total body surface area burn was 45% (range 29-70%). Artificial Skin was used on sites averaging 9% of the total body surface area (range 6-13%). Autograft served as the control site in 6 patients and allograft was used as a temporary wound dressing on the control site in 1 patient. Mean healing time from initial excision was 61 days (range 51-81 days) for the Artificial Skin sites after definitive epidermal autografting, 35 days (range 14-63 days) for autografted control sites, and 67 days for the site initially covered with allograft prior to autografting.

Complications with the use of Artificial Skin included total loss of the material secondary to infection (1 patient), premature separation of a portion of the Silastic component (4 patients), shallow wrinkling in the initial postoperative period (1 patient), hematoma beneath the collagen matrix requiring evacuation and replacement of the dressing (1 patient), significant loss of the epidermal grafts (1 patient), and failure of the neodermis to vascularize sufficiently to accept epidermal grafts (1 patient). No deaths or septic complications attributable to the material were observed.
As a dressing on excised burn wounds prior to definitive autografting, Artificial Skin served to protect the wound from infection (6/7 patients) and provide a graftable fibrovascular bed (4/7 patients). Additionally, donor site healing time for the thin "epidermal" grafts was significantly shorter than the standard 0.004-in split-thickness grafts, 9.6 days vs. 14 days, respectively (P<0.001).
A STUDY TO EVALUATE THE EFFECTIVENESS AND SAFETY OF ARTIFICIAL SKIN IN THE TREATMENT OF THIRD DEGREE FLAME OR SCALD INJURIES

INTRODUCTION

Tangential excision of full-thickness burn wounds with immediate autografting are the standard in modern burn care. However, in extensive burn injury, the limitation of donor site availability is often problematic. Additionally, repeated autograft harvesting and the application of widely meshed autograft to achieve coverage provide for less than optimal long-term coverage. The promotion of a "scar epithelium" is the end result, with a tendency for contracture, breakdown, and poor cosmesis. To date, no acceptable permanent replacement for autograft has been developed, although biologic dressings and skin substitutes are now extensively used to meet the immediate needs of wound coverage when autograft is not available (1-7).

Artificial Skin (Integra®, Marion Laboratories, Kansas City, MS) was designed to achieve the advantages of immediate coverage and to provide for a long-term analogue to skin (8). Artificial Skin is a bilaminate material consisting of distinct dermal and epidermal analogues. Composed of a polymer of chondroitin-6-sulfate and collagen, the dermal portion provides a template for the ingrowth of capillaries and fibroblasts with, in theory, the formation of a neodermis onto which definitive "epidermal" grafts can be placed to effect final wound closure (9-11). The outer "silastic" portion, which is ultimately removed prior to epidermal grafting, is designed to meet the immediate short-term needs of a temporary wound cover, i.e., control of water and protein loss, reduction of heat loss, provision of a barrier to bacterial invasion, and pain control. Composed of a 0.1-mm Silastic® sheet, it is designed to be removed after formation of the neodermis and at a time when the patient’s condition permits epidermal autografting. Unlike autografting in the traditional sense, which has a significant dermal element, the recipient neodermis requires only a thin epidermal graft (0.004 in). The end result is coverage with a better dermal base than could be achieved normally when donor sites are limited. The donor site is also reported to heal more rapidly, allowing for more frequent epidermal harvests (9-11).

Artificial Skin was used in this study in a prospective trial on paired full-thickness burn wounds following excision. Objectives assessed included the ability of Artificial Skin to effect immediate wound coverage, the healing time of Artificial Skin sites, the quality of healed sites, adverse reactions, and effects on patient management. Subjective evaluations for each site by each patient and the investigator were also recorded one month and two months following definitive closure.
MATERIALS AND METHODS

Patient Criteria. Patients eligible for entry into this study were those hospitalized with extensive thermal injuries which were life-threatening and/or covered at least 10% of the total body surface area and, in the opinion of the investigator, would not heal within 3 wk and were amenable to excisional therapy. All patients were hospitalized within 24 h of the burn and underwent excisional therapy of their wounds. The wounds utilized for evaluation were similar in size and site. Excision of the wound was initiated within 7 days of injury and was completed within 21 days of injury.

Wound Management. Burn wounds were excised to the level of viable tissue as judged by color, texture, and the presence of punctate bleeding. Both tangential and fascial excision were employed as appropriate. Tourniquets, topical thrombin, and warm laparotomy sponges were used to effect hemostasis as needed.

Using a computer-generated randomization scheme, comparative sites were designated to receive either Artificial Skin or coverage with autograft or allograft. Autograft was meshed either 1.5:1 or 3.0:1 and expanded as appropriate. Prior to coverage, each site was biopsied for microbiologic assessment. Autograft and Artificial Skin are stapled into place using small surgical clips prior to coverage with an occlusive dressing treated with mafenide acetate. Immobilization as appropriate was also employed.

Autografted site dressings were removed on either the third or fourth postoperative day and as necessary thereafter. Artificial Skin dressings were removed on the third postoperative day and subsequently left exposed. Fluid collections beneath the dermal element or the SilasticR were aspirated and cultured. Nonadherent areas were sharply debrided. Control autograft sites were routinely treated. At approximately 14 days after application, epidermal autografting was performed. Under general anesthesia, epidermal grafts were taken with the Padgett dermatome set at 0.004 inch. These were meshed 1.5:1 and applied to the neodermis after SilasticR removal. Epidermal grafts were then dressed with a single layer of fine-mesh gauze followed by an occlusive layer of coarse-mesh gauze treated with mafenide acetate. Because of the extremely friable nature of these thin grafts, dressings were allowed to remain in place until the fifth postoperative day, at which time the course-mesh layer was removed. Clean fine-mesh gauze was permitted to remain in place until the seventh to tenth postoperative day. Occlusive dressings were employed at this stage for approximately two more weeks.

Effectiveness Evaluation. Artificial Skin was compared to conventional covers. The primary end point for evaluating the efficacy of Artificial Skin was the time from placement of the
definitive epidermal autograft to final closure. Additionally, the "take" of Artificial Skin to the wound bed, the maintenance of the Silastic® "epidermis" to the "dermal" element, and the "take" of the final epidermal graft to the neodermis were evaluated for Artificial Skin. The "take" of the other dressing to the wound bed and the "take" of the definitive autograft were evaluated. The method of removal of nonautograft dressing and the difficulties encountered in their removal were recorded. Another parameter assessed was infection at the sites. Following excision the wounds were cultured. Following removal of the Silastic, the dermis was cultured and after removal of the comparative cover at the time of autograft, the wound was also cultured. At any time when clinical evidence of sepsis or infection was apparent, a culture of both the Artificial Skin and the comparative site were obtained from the area that was clinically determined most likely to be infected.

Rates of wound healing were compared between the two materials used. Both wounds were rated after final healing as to percent of autoepidermal or autograft "take" and serviceability of the cover. Comparisons also included the cosmetic appearance of the wound sites. Photographic documentation of all sites was obtained. Subjective evaluations by the participating patient and the investigator were made at one and two-month periods after initial placement of the material.

Safety Evaluation. Patient safety was evaluated by obtaining vital signs at a minimum of every 6 h until the patient was felt to be clinically normal. Intake and output were continuously monitored at a minimum of every 24 h. SMAC profiles, routine hematologies, and urinalysis were obtained just prior to the first application of Artificial Skin, weekly thereafter until all comparative and the noncomparative study sites were grafted and healed, and on the last day of hospitalization. Each patient had blood samples drawn for determination of antibody formation to Artificial Skin, bovine collagen types I, II, and III, and human collagen types I, II, and III. Tissue samples were taken by biopsy from the sites at which Artificial Skin was applied for histologic evaluation of the neodermis at defined intervals during the initial two months after application. Adverse reactions, including both clinical events and laboratory values attributable to the use of Artificial Skin, were noted.

Analysis of Results. The various assessment parameters that were considered in this study included time to final wound cover, percent "take" of autograft, percent "take" of Artificial Skin, level of bacterial invasion at each site, relationship of septicemia to site invasion, incidence of adverse reactions, immunological changes (tissue and/or blood), final cosmetic outcome, and patient's subjective assessment. Application of the Fisher sign test, in most cases, was used to
test against the null hypothesis that no difference exists between Artificial Skin and control materials. The student’s t-test was used where applicable to healing times.

RESULTS

Seven patients (6 male, 1 female) were entered into the study. Mean patient age was 30 yr (range 21-38 yr). Average total body surface area burn size was 47% (range 24-70%) with an average full-thickness burn size of 31% (range 18-45%). Study sites ranged in size from 6 to 13% of the total body surface area (mean 9%). All patients received initial placement of the Artificial Skin and control cover within 6 days postburn. Control sites were covered with 0.015-in thick split-thickness autografts in 6 patients and allograft in 1 patient (see Table 1).

Figure 1 shows the excision to viable tissue, while Figure 2 depicts the postoperative result in one patient who underwent autograft placement to the right chest (control site) and Artificial Skin application to the left chest. Following initial excision and Artificial Skin placement, definitive autografting of the neodermis was conducted as determined by an obligatory 10 to 14-day period for graftable neodermis formation, availability of donor sites, remaining wound status, and overall patient condition. Neodermis following Silastic removal has the appearance of a smooth, uniform fibrovascular bed (Fig 3) to which harvested epidermal autografts (0.004 in thick) were applied (Figs 4 and 5). Although vigilance was necessary so as not to damage these extremely delicate grafts, technique was otherwise similar to traditional autografting.

Table 2 depicts the number of days postburn for excision and application of Artificial Skin and the control cover, the number of days after Artificial Skin application for epidermal grafting, and the number of days postburn and postexcision to achieve a healed wound for each site. The end point of final closure denotes 100% epithelial coverage exclusive of subsequent breakdown secondary to trauma, blistering, etc., which in some cases resulted in prolonged hospitalization or the need for further operative intervention. In all cases, the achievement of a healed wound was effected sooner on the control site as compared to the Artificial Skin site, an observation which is not surprising given the results achieved with the current method of split-thickness skin grafting. The final coverage achieved with epidermal autografting, however, appeared to be much the same as traditional autografting in the early period after wound closure (Fig 6). No differences were noted in wound texture, pliability, tendency for breakdown, edema, or blistering by the investigator or the patient at one and two months following definitive closure. Artificial Skin and autograft "take" to the excised bed, final epidermal graft "take" to the neodermis on the Artificial Skin sites, and
TABLE 1. Extents of Injury and Treatment Sites

<table>
<thead>
<tr>
<th>Patient Number</th>
<th>Total Body Surface Area Burn (%)</th>
<th>Total</th>
<th>Artificial Skin Site</th>
<th>Control Site</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>23.5 7.5</td>
<td>31.0</td>
<td>6.0</td>
<td>6.0</td>
</tr>
<tr>
<td>2</td>
<td>26.0 4.0</td>
<td>30.0</td>
<td>9.5</td>
<td>9.0</td>
</tr>
<tr>
<td>3</td>
<td>55.0 14.5</td>
<td>69.5</td>
<td>10.5</td>
<td>10.5</td>
</tr>
<tr>
<td>4</td>
<td>39.5 22.8</td>
<td>62.3</td>
<td>10.5</td>
<td>10.5</td>
</tr>
<tr>
<td>5</td>
<td>23.0 20.5</td>
<td>43.5</td>
<td>13.0</td>
<td>13.0</td>
</tr>
<tr>
<td>6</td>
<td>7.5 21.5</td>
<td>29.0</td>
<td>6.0</td>
<td>6.0</td>
</tr>
<tr>
<td>7</td>
<td>5.0 48.0</td>
<td>53.0</td>
<td>6.5</td>
<td>6.5*</td>
</tr>
</tbody>
</table>

*Allograft.
Figure 1. Patient after temporal resection to level of viable tissue.
FIGURE 2. In accordance to the randomization scheme, artificial skin has been applied to the left chest and control material (in this case autograft) has been placed on the left chest.
FIGURE 3. Neodermis after Silastic® removal 19 days after Artificial Skin placement. The bed has a characteristic appearance not unlike granulation tissue and appears to be the ready recipient of a graft.
FIGURE 4. Epidermal autograft harvested with the Padgett dermatome set at 0.004 in. The composition of this thin graft is mostly epidermis with very little dermis present.
FIGURE 5. Epidermal autografts applied to the neodermis and stapled into place prior to dressing. Note the control site on the right chest that has already healed.
<table>
<thead>
<tr>
<th>Patient Number</th>
<th>Postburn Day of Excision</th>
<th>Artificial Skin Time from Epidermal Grafting</th>
<th>Healed Wound Time from Burn Wound Excision</th>
<th>Control Site Healed Wound Time From Burn Wound Excision</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5</td>
<td>26</td>
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<td>3</td>
<td>35</td>
<td>64</td>
<td>67</td>
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</table>
FIGURE 6. Control and Artificial Skin sites 3 wk after final epidermal autografting. Differences in color are the result of grafts taken from previously harvested donor sites.
autograft "take" to the control site initially prepared with allograft (patient 7) are noted in Table 3.

Complications. Fluid collection beneath the Silastic® layer and separation of the Silastic® from the dermal analogue® were major complications (Table 3). Near total Silastic® separation before epidermal autografting occurred in patients 1 and 2, necessitating coverage of the neodermis with biofilm dressing and the utilization of 5% mafenide acetate soaks. Silastic® separation in patients 3 and 4 prior to epidermal autografting was, however, only 10% (Fig 1). Small fluid collections have usually been in areas of wrinkling and have been sterile in all cases. In patient 3, who had Artificial Skin placement on postburn day 3, wrinkling was a prominent feature and attributed to a postresuscitation decrease in peripheral edema. "Take" of Artificial Skin to the excised bed was unaffected in this patient, yet wrinkling of the Silastic® required frequent evacuation of fluid and sharp debridement of the wrinkled areas.

In patient 1, postoperative bleeding required evacuation of a hematoma from a previously dry graft bid on control and Artificial Skin sites one day postoperatively. Reapplication of autograft and Artificial Skin resulted in 100% "take" on both sites. No septic complications have resulted to date. Complications are summarized in Table 4.

Donor Sites. Donor site healing times were recorded in all cases for the purpose of comparing differences between the traditional thickness autografts (0.015 in) and epidermal autograft (0.004 in) used to graft the neodermis. In most cases, because control sites were autografted at the initial operative procedure placement, most of these donor sites were previously unharvested (8 sites) or had only one prior harvest (2 sites). Epidermal autografts to the neodermis were initial harvest (1 site), second harvests (6 sites), third harvests (5 sites), and four harvests (1 case) of the same site. Healing times for each donor site thickness are depicted in Table 5. Donor site healing was constant regardless of the number of prior harvests. Without regard to prior harvests, thin autograft donor sites healed faster than thick autograft donor sites, 9.6 days and 13.6 days, respectively (P<0.001).

Subjective Evaluation. No significant site preference was noted at the one-month or two-month physician evaluation. Two patients preferred control autograft sites to Artificial Skin sites, 1 patient preferred the Artificial Skin site, and 1 patient expressed no preference.

Immunology and Biopsy Results. No significant host antibody response to human collagen or bovine collagen at 3 and 6 wk postapplication was noted.
<table>
<thead>
<tr>
<th>Patient Number</th>
<th>Artificial Skin &quot;Take&quot;</th>
<th>Control Graft &quot;Take&quot;</th>
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<tbody>
<tr>
<td></td>
<td>Collagen Matrix</td>
<td>Epidermal Grafting</td>
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<tr>
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<tr>
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<td>100</td>
<td>10</td>
</tr>
<tr>
<td>7</td>
<td>0</td>
<td>N/A**</td>
</tr>
</tbody>
</table>

*Initial cover was autograft in 6 of 7 cases. Patient 7 was initially covered with allograft.

**Collagen matrix not sufficiently vascularized and thus no epidermal grafting was performed.
<table>
<thead>
<tr>
<th>Patient Number</th>
<th>Premature Silastic Loss (%)</th>
<th>Fluid Accumulation Beneath Silastic</th>
<th>Wrinkling of Artificial Skin</th>
<th>Hematoma</th>
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<tr>
<td>1</td>
<td>86%</td>
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<tr>
<td>2</td>
<td>90%</td>
<td>2X</td>
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<td>3</td>
<td>10%</td>
<td>6X</td>
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<td>4</td>
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</tr>
<tr>
<td>5</td>
<td>60%</td>
<td>&gt;10X</td>
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<td>No</td>
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<td>7</td>
<td>17%</td>
<td>7X</td>
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TABLE 5. Donor Site Comparisons

<table>
<thead>
<tr>
<th>Prior Harvesting</th>
<th>Epidermal Autograft (0.004 In)</th>
<th>Standard Autograft (0.015 In)</th>
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<tr>
<td>0</td>
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<td>8/13.5</td>
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<td>6/9</td>
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<td>3</td>
<td>1/11</td>
<td>-</td>
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<tr>
<td>TOTAL</td>
<td>13/9.6*</td>
<td>10/13.6*</td>
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</table>

DISCUSSION

The relatively small size of our study population precludes reaching any definitive conclusions regarding the overall safety and efficacy of Artificial Skin. However, when analyzed from the multicenter study population of 150 patients, results should provide for more conclusive observations as to the utility of this wound dressing.

Experience to date does show that this material may be useful in patients with very large burns where excision is desirable, yet donor site availability is limiting with respect to wound closure. In these patients, Artificial Skin that requires a minimum of care until final closure can be effected may provide a means for covering large surface areas of excised wound. Thin "epidermal" grafts appear to provide adequate coverage with the added advantage of more rapid donor site healing.

Subjectively, Artificial Skin appeared to be more successful on large, relatively flat, nondependent, nonmobile surfaces. Excess wrinkling of the collagen mat and silastic layer was observed about joints. Shear of the material was observed on dependent surfaces.

While infection beneath the material was not a major problem at this center and septic complications as a result of Artificial Skin placement were not observed, Artificial Skin was observed to separate rapidly from an infected wound bed (patient 5). In this patient, 40% of the collagen mat required removal from the study site and near total autograft loss on the control site was observed.

More widespread use of this skin substitute will permit definition of the patient population for which Artificial Skin has utility, identification of the optimum manner in which it
is to be used to maximize its unique properties, and
delineation of its overall impact on patient management and
outcome. Only by further prospective analysis can these and
other important questions be answered in a meaningful fashion,
with the inclusion or exclusion of Artificial Skin to the
developing treatment armamenture.

PRESENTATIONS/PUBLICATIONS

None.

REFERENCES


ANNUAL RESEARCH PROGRESS REPORT

PROJECT NUMBER: 3362787A874-00, APPLIED RESEARCH

PROJECT TITLE: EVALUATION OF BURN WOUND CARE IN TROOPS WITH BURN INJURY: 5% Aqueous Sulfamylon® Soaks Used in Topical Treatment of Burned Soldiers

US ARMY INSTITUTE OF SURGICAL RESEARCH
FORT SAM HOUSTON
SAN ANTONIO, TEXAS 78234-5012

1 October 1986 - 30 September 1987

INVESTIGATORS

William F. McManus, MD, Colonel, MC
Basil A. Pruitt, Jr., MD, Colonel, MC
ABSTRACT

PROJECT NUMBER: 3S162787A874-00, APPLIED RESEARCH

PROJECT TITLE: EVALUATION OF BURN WOUND CARE IN TROOPS WITH BURN INJURY: 5% Aqueous Sulfamylon Soaks Used in Topical Treatment of Burned Soldiers

INSTITUTION: US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas 78234-5012

PERIOD COVERED IN THIS REPORT: 1 Oct 86 through 30 Sep 87

INVESTIGATORS: William F. McManus, MD, Colonel, MC
Basil A. Pruitt, Jr., MD, Colonel, MC

During this reporting period, 5% aqueous mafenide acetate dressings have continued to be an efficacious treatment modality in the care of the burn wound. One hundred and seventy-five patients were treated with 5% aqueous mafenide acetate dressings, employed either for final debridement of the wound or following application of meshed cutaneous autograft to prevent desiccation of tissue exposed in the interstices of such grafts. An 11.4% incidence of skin rash (atopy) was noted as the only adverse reaction. The clinical results achieved by the use of 5% aqueous mafenide acetate solution strongly support its continued use.
5% AQUEOUS SULFAMYLYON® SOAKS USED IN TOPICAL TREATMENT OF BURNED SOLDIERS

During this reporting period, the evaluation of 5% aqueous mafenide acetate solution for topical treatment of the burn wound has continued at this Institute where it was used in 175 of 216 patients (81.2%). The 5% aqueous mafenide acetate-soaked dressings are used as wet-to-dry dressings to debride nonviable tissue elements in preparation for split-thickness autograft procedures or as continuous wet dressings to protect freshly excised wounds that are not autografted. In addition, when meshed cutaneous autografts are applied, dressings are soaked with 5% aqueous mafenide acetate solution to decrease the rate of bacterial growth and to prevent desiccation of tissue exposed in the interstices of such grafts.

Twenty patients (11.4%) demonstrated allergic reactions (atopy) with the use of 5% aqueous mafenide acetate solution and these 20 patients demonstrated rapid resolution of the atopic reaction following administration of an antihistamine and/or discontinuation of the 5% aqueous mafenide acetate-soaked dressings. Saline or other aqueous topical antimicrobial agents were substituted when 5% aqueous mafenide acetate-soaked dressings were discontinued and no other adverse reactions were noted in this group of patients.

The use of 5% aqueous mafenide acetate-soaked dressings has continued to be efficacious, both in the preparation of the burn wound for cutaneous autografting and in the prevention of desiccation of ungrafted granulation tissue. In addition, 5% aqueous mafenide acetate solution is most helpful in preventing desiccation or premature bacterial colonization of meshed cutaneous autografts. The dressings over such meshed autografted skin can be left in place for an average of 3 days, allowing development of good adherence of the autografts prior to the first dressing change. The efficacy and the low incidence of adverse side effects speaks for continued use of this solution.

PRESENTATIONS/PUBLICATIONS

None.
Studies of the Neuroendocrine Abnormalities in Burn Injury

I. Overview:

Subject Areas:
- Basic Medical Research
- Pharmacology

II. Start Date:

14 March 1986

III. Estimating Completion Date:

9 September 1986

IV. Funding Organization:

DA

V. Performance Method:

C

VI. Control Grant Number:

CAG 69670

VII. Total Amount Awarded:

$120,000

VIII. Total Amount Used:

$100,000

IX. Name of Responsible Individual:

Lt. Col. L. A. Thompson, USA

X. Name of Principal Investigator:

W. M. Vaughn

XI. Telephone Number:

112-221-5416

XII. General Use - Title:

MILITARY CIVILIAN APPLICATION

XIII. Keywords:

(1) Pineal; (2) Indoles; (3) Catecholamines; (4) Voleantors; (5) Rod Animal; (6) Lab Animal; (7) Rats; (8) Hamsters; (9) Guinea Pigs; (10) Rats; (11) Hams; (12) Guinea Pigs; (13) Rats

XIV. Technical Objective:

To characterize sympathetic control of pineal activity in burned patients in order to improve survival. A literature search was conducted.

XV. Progress:

1. To assess biological rhythms under nontrauma stress.

2. (1) 6719 - 7099. Seven healthy males, 25-31 yrs old, were studied at intervals around the clock in two sessions, one before (S1) and one 6 months after (S2) beginning a surgical residency. Stress was monitored by standard tests of mood and simple reaction time for visual vigilance and auditory vigilance. The end of nocturnal sleep and the rhythms of measured sleepiness, overall mood score, oral temperature, and urinary Na+K+ ratio were all significantly phase advanced in S2 vs. S1. Scores for mood and vigilance (24-h pooled estimates) were both worse in S2 vs. S1. Though urinary cortisol excretion exhibited a raised curve average in S2 vs. S1, there was no difference in the amplitude of timing of the rhythm. The 24-h rhythm of urinary melatonin sulfate (reflecting pineal melatonin secretion) was unaffected in S2 vs. S1. Control of the timing for rhythms of melatonin and cortisol can be exerted independently from the rhythms of temperature, sleep, and mood. Nontrauma stress can result in desynchronization of biorhythms, raising the question of the role of possible synchonization in severe traumatic stress.
ANNUAL RESEARCH PROGRESS REPORT

PROJECT NUMBER: 3S162787A874-00, APPLIED RESEARCH

PROJECT TITLE: STUDIES OF THE NEUROENDOCRINE ABNORMALITIES IN BURN INJURY: Alterations of Temperature, Sleepiness, Mood, and Performance in A Nontrauma, Nonillness Stress Model Are Not Associated With Changes in Sulfatoxymelatonin Excretion

US ARMY INSTITUTE OF SURGICAL RESEARCH
FORT SAM HOUSTON
SAN ANTONIO, TEXAS 78234-5012

1 October 1986 - 30 September 1987

INVESTIGATORS

George M. Vaughan, MD, Lieutenant Colonel, MC
Katherine A. Sharp, PhD*
David J. Kennaway, PhD**
Basil A. Pruitt, Jr., MD, Colonel, MC

*Medical Resource Group
Blue Bell, Pennsylvania

**University of Adelaide Medical School
Adelaide, South Australia
ABSTRACT

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Residency training may disrupt normal sleep/wake cycles, resulting in mood and performance deficits and alterations in biological rhythms. To characterize such disturbances and determine whether they are associated with an alteration in the day/night pattern of melatonin excretion, measurements were obtained around-the-clock in 7 male subjects, each studied in two 48-h sessions. Session 1 was conducted during the week before beginning a residency, and Session 2 at 6 months into a first-year surgical residency. The mean time of the end of nocturnal sleep and the timing of the temperature rhythm were both approximately 2.3 h earlier in Session 2 (P<0.01 vs. Session 1). The sleepiness rhythm and the overall mood score rhythm were also phase-advanced in Session 2 (P<0.05). The mean value of mood around-the-clock was significantly worse due to increased anger, tension, confusion, depression, and fatigue in Session 2. Vigilance, tested by simple reaction time, did not exhibit a 24-h rhythm and was worse in Session 2 with an increase in lapse time (microsleep, >1 sec latency, P<0.01). The urinary cortisol rhythm exhibited a raised curve average value (mesor) in Session 2 (P<0.05 vs. Session 1), but no difference was revealed in amplitude or acrophase. Urinary excretion of Na+, K+, and Cl− did not differ between sessions, though the Na+/K+ ratio peaked earlier in Session 2 (P<0.05).

The urinary 6-sulfatoxymelatonin rhythm did not differ in timing, amplitude, or mesor between sessions. A residency training schedule can be associated with altered timing in rhythms of sleep, sleepiness, temperature, and mood, and deterioration of mood and performance without detectable alteration of the endogenous melatonin pattern as exhibited by the excretion rate of the principal urinary metabolite.
Nontrauma stress can result in desynchronizing of biorhythms, raising the possibility of dyssynchrony in severe traumatic stress as well.
ALTERATIONS OF TEMPERATURE, SLEEPINESS, MOOD, AND PERFORMANCE IN A NONTRAUMA, NONILLNESS STRESS MODEL ARE NOT ASSOCIATED WITH CHANGES IN SULFATOXYMELATONIN EXCRETION

INTRODUCTION

The pineal gland has a profound influence on thyroid and reproductive function in experimental animals (1), though pineal control of these systems in humans has not yet been documented. However, burn injury causes marked suppression of the thyroid and reproductive axes in humans (2), as well as suppression of the nocturnal surge of melatonin (3), so far the only major pineal hormone recognized. Whether these phenomena are connected in a mechanistic way has not yet been determined.

First, it was necessary to address the question of whether the alteration of the melatonin rhythm in burn injury is related to injury/illness or is a nonspecific concomitant of stress in general. In this report, we have utilized a model (residency training) that contains many elements of stress not connected with trauma or illness per se in order to assess potential changes in the excretion of the principle melatonin metabolite, which has been shown to be an excellent marker for pineal function.

Serum concentrations and urinary excretion of the pineal hormone, melatonin (N-acetyl-5-methoxytryptamine (aMT)) and its major metabolite, 6-sulfatoxymelatonin (aMT.6s), are highest at night (4). Although administration of aMT reportedly induces sleepiness, apparently normal sleep during the day or night can occur without an acute surge of aMT, and sleep deprivation does not acutely disturb the nocturnal aMT surge (5,6). However, the rhythm shifts over 1-2 wk to accommodate a change in the timing of sleep and darkness (7,8). On the basis of (a) deteriorations of mood observed after acute (9) or chronic (10) administration of large doses of aMT, (b) the ability of exogenous aMT to induce sleepiness (11-13), (c) the blunted endogenous nocturnal surge of aMT seen in depressed patients (14-17), (d) the occurrence of jet lag symptoms early in the course of phase-shifting the aMT rhythm (18), and (e) the covariation of urinary aMT, fatigue, and depressed performance within a 24-h time frame (19), the question arises as to whether temperature, sleepiness, mood, or performance might be controlled by the rhythm or endogenous levels of aMT. The literature on sleep deprivation in residents is scarce and to our knowledge no study has been conducted using circadian principles. It is well known, however, that residency training requires a diurnal working schedule with additional night work.

Negative mood changes and impaired performance have been reported in this population as a result of rigorous training schedules (20-28). We have therefore tested the hypothesis that changes in sleepiness, mood, and performance in residency...
training might be associated with a detectable alteration of the melatonin metabolite excretion rhythm. Because the urinary aMT.6s rhythm is a good reflection of the serum aMT rhythm and it was not possible to obtain blood samples in this study, we have used urinary aMT.6s to assess the melatonin rhythm. We also assessed the characteristics of temperature, cortisol, and electrolyte fluctuation under the same conditions.

MATERIALS AND METHODS

This investigation was conducted in two 48-h sessions for each subject, i.e., within the week prior to a first-year surgical residency (Session 1) and approximately 6 months into residency (Session 2). The protocol was approved by the Institutional Review Board of the Youngstown Hospital Association, Youngstown, Ohio, where 5 subjects were studied. Two subjects were studied at the Harrisburg Polyclinic, Harrisburg, Pennsylvania, with an identical protocol. All subjects provided informed consent.

Subjects. Seven males entering a first-year surgical residency volunteered for this study. The residency program involved at least one on-call night every 4 days, including approximately 2 weekends per month, without a day off duty after an on-call night, resulting in 85 to 130 h of service per week. Subjects were considered healthy by means of clinical and laboratory examinations and ranged in age from 25-34 yr, in weight from 59-108 kg, and height from 165-189 cm. Prior to residency, they did not have a history of trouble sleeping. No subjects reported any mental illness during their lives or in their families. No subjects had crossed time zones within 2 wk prior to Session 1.

Sleep/Wake Log. Each subject kept a sleep/wake log in which he recorded his sleep/wake schedule for 2 to 3 days prior to Session 1 and for 1-2 wk prior to Session 2. Assessments of sleep related to a session were based on mean or pooled sleep data for a given subject over this period. During each session, light exposure (100-1000 lux in the hospital) was uncontrolled with the exception of subject testing rooms (<100 lux). Sleep (in the dark) was interrupted for nocturnal testing unless the subjects were already awake in relation to work.

Stanford Sleepiness Scale (SSS). This scale was designed to quantify stages of the alertness-sleepiness continuum (29). At 3-h intervals concurrent with temperature recording and urine voiding, subjects circled 1 item in a scale with values from 1 (feeling active and vital, wide awake) to 7 (almost in reverie, sleep onset soon, lost struggle to remain awake), with actual sleep time being 8.
Temperature (Temp). Oral temp was recorded at 3-h intervals throughout each 48-h session. The subject was instructed not to exercise, smoke, or drink within 0.5 h before temp measurement.

Urinary Collection. Under close supervision, each subject was provided with sterile plastic containers without added chemicals and was instructed to collect all urine for each separate 3-h interval with a voiding at the end of each interval. After storage at 4°C for up to 3 h, volumes were recorded and specimens were aliquoted into polypropylene tubes and stored at -60°C until analysis.

The Profile of Mood States (POMS). A standardized, self-rated 5-point scale (ranging from 0 = "not at all" to 4 = "extremely") for 65 mood adjectives, this test (30) was administered every 6 h, taking approximately 2 min. It yields 6 component variables, i.e., tension-anxiety, depression-dejection, anger-hostility, vigor-activity, fatigue-inertia, confusion-bewilderment, and an integrated overall score representing a global index of mood and constituting the 7th POMS variable.

The Serial Search Test (SST). The SST (31) was given at 6-h intervals and required approximately 1 min. The subject was presented with a line of 30 random upper case letters on the video screen and searched for the occurrence of the letter "E". The time (to the nearest 10 msec) required to signal its presence or absence was recorded for 32 trials per test to calculate a mean latency. The very few errors were not excluded from the analyzed data.

The Verbal Reasoning Task (VRT). The VRT (31) included a randomly shuffled set of 32 statements describing a letter pair, e.g., "M does not precede C. CM."). Approximately 2 min were required to obtain a set of responses ("true" or "false") for which a mean latency (not excluding the very few errors) was calculated at 6-h intervals.

The Wilkinson's Unprepared Simple Reaction Time Test (USRT). The USRT is a 15-min, 150-trial response latency test of vigilance with the advantage of being relatively free of any practice effects (32). At random intertrial intervals ranging from 1-10 sec, a randomly chosen 3-digit number on a video screen began rapidly incrementing by 1, providing a stimulus for the subject to press a button as quickly as possible. The resulting response times (to the nearest 10 msec) were electronically stored for computation of mean latency. Errors of commission (button pressed prior to the stimulus onset, false hits), and errors of omission (lapse times or "microsleeps", latencies >1 sec) were excluded from the results of mean latency per test. This test was given at 6-h intervals.
Protocol. During each of the two 48-h sessions, the procedures occurring at 3-h intervals (SSS, urine voiding, and Temp measurement) together required about 3-5 min and were scheduled for 0800, 1100, 1400, 1700, 2000, 2300, 0200, and 0500 h; those at 6-h intervals (POMS, SST, VRT, USRT) required about 20 min and were performed at 0930, 1530, 2130, and 0330 h. These latter tests were administered on Apple Macintosh computers. The subjects responded by moving the videoscreen cursor with a positioning device ("mouse") and pressing the mouse button. For the USRT, subjects simply pressed the mouse button. At no time were subjects required to use the computer keyboard. Videoscreen brightness was controlled to maintain approximately 100 lux in front of the screen. To reduce practice effects, the computer operation and the tests were thoroughly explained and the subjects practiced the battery of tests until they were familiar with the computer and the tests. During Session 2, each subject was on-call at least one of the two nights, but this did not interfere with sampling or testing.

Assays. Separate aliquots of each 3-h urine sample were used for determination of Na+ and K+ by ion selective electrode and Cl- by thiocyanate colorimetry; cortisol (33) by methylene chloride extraction, HPLC separation, UV detection, and recovery correction for each sample with 6-OH-progesterone as an internal standard (performed at the Nichols Institute (San Juan Capistrano, California); and αMT.6s by a sensitive and specific RIA (34) on samples diluted 1:50. The αMT.6s antibody was generously supplied by Dr. J. Arendt, and the standard was prepared by the method of Fellenberg et al (35).

Analysis. Measurements made during 48-h around-the-clock were subjected to 24-h periodic regression (36) which yields a mesor (average predicted ordinate value of the best-fit curve over time as the abscissa), amplitude (best-fit curve peak minus mesor), and acrophase (time of the best-fit curve peak). Urinary data were assigned the times of the midpoints of the urine collections. The cosinor system software (generously provided by Mark Vokac (37) of Oslo, Norway for the Apple II) or the BMDP software for the VAX 11/780 (38) were used for the analyses. The significance of the rhythm was assessed by a standard regessional analysis of variance determining likelihood of a zero amplitude, the acrophase provides an index of the timing of the rhythm, and the mesor reflects the central value for the entire sampling period. Each of these parameters could be obtained for each subject and separately for the pooled data among subjects for a given session. If a rhythm was not detected in the pooled data, overall between-subject (mesor) variation was removed to test for significance of the group rhythm. If the group rhythm of a variable was significant in both sessions, a paired t-test was used to evaluate individual subjects’ amplitudes and acrophases for a difference between sessions for that variable. Individual
mesors were used to assess the difference (paired t-test) in overall magnitude between sessions.

RESULTS

Prior to residency, all subjects reported regular nocturnal sleeping habits of 6-8 h per 24-h period. The group mean sleep obtained from sleep/wake records before Session 1 was 7.35 h per 24-h period (Fig 1). The mean sleep after 6 months of residency, still almost entirely at night, was 4.57 h per 24 h period in the 1-2 wk prior to Session 2 (including on-call and off-call times), indicating a significant reduction of 2.48 h per 24-h period (P=0.014, paired t-test). The mean sleep episode duration (Fig 1) was also reduced prior to Session 2, and the number of sleep episodes per 24-h period (not shown) did not differ significantly between sessions. The end of the last episode of nocturnal sleep was earlier for Session 2 than for Session 1 (Figs 2 and 3), though there was no difference in the beginning time of nocturnal sleep.

Cosi or results are indicated in Table 1. Most variables were rhythmic in both sessions, with the exception of SST, VRT, USRT, and some POMS components. There were no differences in amplitude for any variable between sessions. Cortisol excretion and POMS Overall, Tension, and Anger exhibited significantly higher mesors in Session 2. (For the Overall POMS and each POMS component except Vigor, a higher score indicates worse mood.) USRT (excluding latencies >1 sec) exhibited a higher latency mesor in Session 2. Among the variables with significant group rhythms in both sessions, sleepiness (SSS), temp, and the POMS Overall score were significantly phase-advanced in Session 2 (vs. Session 1).
Figure 2. Mean + SE for temperature and urinary variables and best-fit pooled 24-h periodic regression curves. Session 1 = closed circles, unbroken lines; Session 2 = open circles, broken lines. Arrows (Session 1 = unbroken; Session 2 = broken) above the abscissa in 2nd and 4th panels (applicable to each panel) indicate the mean time between sleep onset and the end of the last nocturnal sleep episode.

Figure 2 shows the group patterns for temp and urinary hormones and ratio of Na+/K+ concentration and indicates the highly rhythmic nature of these variables (each P<0.001 in both sessions). However, between-subject variation in rhythm timing (acrophase) does not allow this display adequately to represent between-session changes in timing. Figure 3, based on the between-session acrophase mean for individuals, shows that whereas temp and Na+/K+ ratio became phase-advanced in Session 2, there was no such change in the urinary cortisol or aAMT.6s rhythms.

Figure 4 highlights the between-session differences in the POMS variables. A mean value for each subject over the entire testing time was used to calculate a group mean for each session. Paired t-tests indicate that each component except Vigor was significantly worse in Session 2. Since there were only four measurements per 24-h period for these variables and the actual times of the tests occasionally varied from an exact
<table>
<thead>
<tr>
<th>Variable</th>
<th>Mesor Session 1</th>
<th>Mesor Session 2</th>
<th>Amplitude Session 1</th>
<th>Amplitude Session 2</th>
<th>Acrophase (h) Session 1</th>
<th>Acrophase (h) Session 2</th>
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<tr>
<td>Temp (°F)</td>
<td>97.5</td>
<td>97.2</td>
<td>0.72*</td>
<td>0.55*</td>
<td>1752</td>
<td>1531**</td>
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<tr>
<td>SSS</td>
<td>3.30</td>
<td>3.30</td>
<td>2.34*</td>
<td>1.93*</td>
<td>0320</td>
<td>0154***</td>
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<td>Urinary Na+ (meq/L)</td>
<td>8.24</td>
<td>9.61</td>
<td>1.29***</td>
<td>2.43**</td>
<td>1858</td>
<td>1723</td>
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<td>Urinary K+ (meq/L)</td>
<td>2.61</td>
<td>2.87</td>
<td>1.19*</td>
<td>1.38*</td>
<td>1437</td>
<td>1250</td>
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<tr>
<td>Urinary Cl- (meq/L)</td>
<td>7.73</td>
<td>8.23</td>
<td>1.40*</td>
<td>2.13**</td>
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<td>1421</td>
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<tr>
<td>Urinary Cortisol (g/h)</td>
<td>1.10</td>
<td>1.40***</td>
<td>0.95*</td>
<td>1.00*</td>
<td>0919</td>
<td>0829</td>
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<td>Urinary aMT6s (g/h)</td>
<td>1.17</td>
<td>1.21</td>
<td>1.24*</td>
<td>1.30*</td>
<td>0426</td>
<td>0320</td>
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<td>POMS Overall (%)</td>
<td>16.4</td>
<td>20.9***</td>
<td>5.9*</td>
<td>4.7***</td>
<td>0323</td>
<td>0102***</td>
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<td>POMS Tension (%)</td>
<td>10.8</td>
<td>18.1***</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>POMS Anger (%)</td>
<td>3.54</td>
<td>8.13</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>POMS Fatigue (%)</td>
<td>27.5</td>
<td>35.4</td>
<td>17.2*</td>
<td>-</td>
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<td>-</td>
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<td>POMS Vigor (%)</td>
<td>46.6</td>
<td>43.1</td>
<td>17.5*</td>
<td>17.7*</td>
<td>1501</td>
<td>1316</td>
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<tr>
<td>POMS Confusion (%)</td>
<td>21.3</td>
<td>24.2</td>
<td>11.1**</td>
<td>7.2*</td>
<td>0312</td>
<td>0215</td>
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<td>POMS Depression (%)</td>
<td>4.0</td>
<td>6.0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>SST (msec)</td>
<td>3396</td>
<td>3350</td>
<td>-</td>
<td>291***</td>
<td>-</td>
<td>0617</td>
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<tr>
<td>VRT (msec)</td>
<td>4057</td>
<td>4158</td>
<td>-</td>
<td>394***</td>
<td>-</td>
<td>0509</td>
</tr>
<tr>
<td>USRT (msec)</td>
<td>334</td>
<td>365***</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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</table>

*P<0.001, **P<0.01, ***P<0.05 (for mesor and acrophase, comparison of Session 2 vs. Session 1; for amplitude, within-session significance of the group rhythm). No amplitudes were significantly different between sessions. Acrophase is given as clock hour (h and min), mesor and amplitude as units associated with the variable name. Differences between sessions were tested by use of parameters from regressions for individuals in each session. For parameters significantly different between sessions, the mean difference is given in Table 2.
<table>
<thead>
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<tr>
<td>01 Oct 87</td>
<td>US ARMY INSTITUTE OF SURGICAL RESEARCH ANNUAL RESEARCH PROGRESS REPORT FOR FISCAL YEAR 1987(U) ARMY INST OF SURGICAL RESEARCH FORT SAM HOUSTON TX B A PRUITT</td>
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FIGURE 3. The end of the last nocturnal sleep episode and the acrophases (time of best-fit curve maxima) for oral temperature (temp) and urinary Na+/K+ ratio, cortisol, and aMT.6s, expressed for the group as mean time ± SE (abscissa) before or after the within-subject midpoint (mean) between each's respective two values (one for each session). *P<0.05, **P<0.01 for phase-advancement in Session 2 (paired t-test on the actual clock values). The abscissal time zero (mean within subject mid-value between sessions) is indicated above each panel.

6-h interval, the mesors (Table 1) and overall means vary slightly in their representation of the central tendency in the data. Analyses of the mesors indicate worsening of Tension and Overall POMS; whereas, analyses of the overall means indicate worsening of Anger, Fatigue, Confusion, and Depression as well.

Figure 5 shows that the overall SST and VRT scores were not different between sessions, but in Session 2, there developed a significant rhythm in speed of response not detected in Session 1 (Table 1). USRT performance, excluding latencies >1 sec, was significantly worse in Session 2 (Figure 5) and did not exhibit a 24-h rhythm (Table 1). When the latency difference for each subject between sessions was calculated (not shown), this difference exhibited a 12-h rhythm (P<0.02). Lapse time (microsleep, latency >1 sec) was significantly greater in Session 2 (Fig 5).

DISCUSSION

The times used in these observations were read directly from the clock that was reset off daylight savings time about two months prior to the measurements in Session 2. This resetting ("fall back") automatically phase-delays the clock and all activities synchronized to it (arising, going to work, going to bed, etc.) by 1 h, and this is normally reflected in a similar delay in the aMT/6.s and cortisol excretion rhythm (34). In the present observations, such a phenomenon is recorded as a phase change of zero and probably explains no observed between-session phase change in aMT.6s and cortisol,
Profile of Mood States

Overall

Tension

Anger

Fatigue

Vigor

Confusion

Depression

FIGURE 4. Mean ± SE for POMS components by hour of testing and 24-h means. Ordinate units are expressed as percent of the minimum/maximum range for each variable. *P<0.05, **P<0.01, ***P<0.001 (Session 2 vs. 1).
FIGURE 5. Mean ± SE for SST, VRT, and USRT response latencies and USRT mean of total lapse time (latency >1 sec) per test (msec). *P<0.05, **P<0.01 (Session 2 vs. 1).
both likely phase-delayed along with the autumn clock resetting and the timing of activity and onset of sleep and dark exposure, well before the measurements in Session 2. Our use of the actual clock times allows the between-session comparison to include the same influence that the timing of the clock had for both sessions.

It is not possible to completely separate influences of the light/dark and sleep/wake cycles in sighted humans, because subjects typically sleep in the dark with eyes closed. In these studies, we focused on the sleep/wake cycle because it was not possible to control or monitor 24-h light exposure in the days prior to the physiological and psychological measurements taken. We assessed sleep prior to the measurements because circadian rhythms are entrained to the sleep/wake and/or dark/light cycle as it is in the days leading up to the measurements (6,7,18).

The reduction in amount of sleep for Session 2 can be viewed as chronic sleep loss. Thus, the increased USRT latencies are consistent with decreased alertness measured by self-ratings in shift workers with a sustained 2-3 h/day sleep loss (39,40). Our results suggest that a performance deficit in residents more reflects vigilance during a routine and reflexive task rather than activity requiring a level of conscious thinking effort, as in the SST and VRT. Disrupted sleep and chronic sleep deprivation enhance the secondary arousal, though that normally occurs in the afternoon (41). In our subjects, this likely was reflected in greater USRT latency at 0330 and at 1530 h in Session 2 (vs. Session 1) and a bimodal (12-h) rhythm in the latency difference between sessions. Sleep deprivation has previously been associated with an increased frequency of brief lapses of consciousness or "microsleeps" (42). Thus, in addition to a general deterioration in response latency, more lapses of >1 sec were apparent in Session 2. It is likely that sleep deprivation during residency was a major contributor to decrements in performance and mood and to elevated cortisol excretion as a reflection of stress.

During residency training (Session 2), sleep tended to end earlier in the morning. Since the SSS included actual sleep as the most intense indicator of sleepiness, the measurement of sleepiness tended to confirm a phase advancement of sleep rhythm in Session 2. The earlier end of nocturnal sleep suggests advancement of the activity rhythm. Along with this advancement in the sleep/activity rhythm, the rhythms of temp, Overall mood, and the urinary Na+/K+ ratio were similarly advanced in Session 2, indicating phase advancement of a substantial element of the circadian system in residency training.

Cortisol levels have been shown to be unchanged or decreased in sleep deprivation (43,44), possibly as a result of
boredom in laboratory-controlled sleep deprivation. In contrast, residents are required to maintain a high level of psychological activation and alertness, which may provide stress in attempting to override fatigue and thus may have contributed to higher cortisol mesors. It is possible that the altered timing of the Na+/K+ ratio in Session 2 partly reflected aldosterone secretion in that this hormone tends to restrict Na+ excretion and promote K+ excretion. The assessment of excretion rhythms of electrolytes is difficult in the present study because dietary intake was not measured or kept constant.

The magnitudes of the residency-induced alterations in timing that were observed in those rhythms with significant altered timing were small (Table 2). Lack of detectable changes in melatonin and cortisol rhythm timing does not exclude the possibility that greater phase alteration of temperature, sleepiness, and mood produced by some other technique would then change melatonin and cortisol phasing. More frequent sampling of circulating melatonin or cortisol might have more sensitively revealed residency-induced small changes in rhythm timing. However, since sulfatoxymelatonin and cortisol were sampled at least as frequently as for those variables showing a changed rhythm phasing, sampling frequency is not likely a major factor in the apparently different responsiveness of rhythm timing to residency training among measured variables. Further, in that the changes observed in the residents involved more than altered timing of several rhythms and included sleep deprivation, an earlier final awakening time, overall worsening of mood and vigilance performance, and raised cortisol levels, it is important to note that the overall level and pattern of melatonin excretion was not significantly changed. These results do not support the notion that rhythm changes in or deteriorations of sleep, mood, and vigilance seen in residency training provoke or depend upon a major alteration of the melatonin rhythm. Changes in melatonin that occur in illness or injury may thus result from factors other than those associated with the general aspects of stress. However, nontrauma stress can result in desynchronizing of biorhythms, raising the possibility of dyssynchrony in traumatic stress as well.
TABLE 2. Mean Within-Subject, Between Session Difference ±SE in the 7 Subjects for Cosinor Parameters Significantly Different Between Sessions (from Table 1)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Parameter</th>
<th>Difference (Session 2 Minus Session 1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temp</td>
<td>Acrophase*</td>
<td>-2.26 ± 0.48++</td>
</tr>
<tr>
<td>SSS</td>
<td>Acrophase**</td>
<td>-1.55 ± 0.62+</td>
</tr>
<tr>
<td>POMS Overall</td>
<td>Acrophase***</td>
<td>-3.10 ± 1.18+</td>
</tr>
<tr>
<td>Urinary Cortisol</td>
<td>Mesor</td>
<td>0.304 ± 0.12+</td>
</tr>
<tr>
<td>POMS Overall</td>
<td>Mesor</td>
<td>5.53 ± 1.71+</td>
</tr>
<tr>
<td>POMS Tension</td>
<td>Mesor</td>
<td>7.69 ± 2.99+</td>
</tr>
<tr>
<td>USRT</td>
<td>Mesor</td>
<td>30.7 ± 8.5+</td>
</tr>
</tbody>
</table>

*Rhythmicity regressional variation \( r^2 \) as % of total variation in individual subjects (mean ± SE): Session 1, 62±5; Session 2, 50±9 (all P<0.05 for rhythmicity in both sessions except for 1 subject in Session 2). **r^2**: Session 1, 74±5; Session 2, 56±2 (all P<0.05 in both sessions). ***r^2**: Session 1, 47±8 (P<0.05 in 1 of 7 subjects); Session 2, 47±8 (P<0.05 in 4 of 7 subjects). Acrophase computations for all subjects were included in the between-session tests, because in both sessions, the group rhythms were significant (Table 1). +P<0.05, ++P<0.01 (Session 2 vs. Session 1, paired t-test). Acrophase difference is in decimal h, negative numbers meaning earlier timing (phase advance) in Session 2. Mesor units (as in Table 1) are those for the measurements of the respective variables.

PRESENTATIONS/PUBLICATIONS


REFERENCES


87


It was thought that high frequency ventilation in the treatment of respiratory failure in patients with inhalation injury with associated pneumonia, time of initial onset, and duration of ventilation, total duration of ventilatory support, and the age and burn size of the patient. A literature search was conducted.

12. Initial high frequency ventilation support will be established by the medical team in the treatment of respiratory failure in patients with inhalation injury with associated pneumonia. Time of initial onset and duration of ventilation, total duration of ventilatory support, and the age and burn size will be evaluated in the study group.

13. In 1983-1984, this project was approved by the Subcommittees on Research Review Board on 26 February 1987. One patient has been entered into the study. After the entry of 30 patients, the effect of high frequency ventilation on the outcome after inhalation injury will be compared to several well-defined factors.
The objectives of this study are to design and construct a dynamic splint device to monitor selected characteristics of the hand during therapy and to quantify initial stiffness, laxity of joints, and changes in joint characteristics as a response to changes in treatment regimens and to develop guidelines for the appropriate use of dynamic splints. Data from this study will enhance the knowledge and therefore the quality of hand rehabilitation of injured soldiers as well as others. A literature search provided only anecdotal information regarding the forces applied to joints during splinting procedures.

A dynamic splint force-angle monitoring device will be designed and developed. Patients will be entered into the study after meeting the patient entry criteria and giving their signed consent. A classification of the burned hand will be made by the attending physician and an occupational therapist based upon the depth and location of the burn. Baseline data will be collected for each patient, to include burn location, joint range-of-motion at the target joints, digit circumference measurements, two-point discrimination sensation at the distal tips, electromyographic activity of the extensor digitorum communis muscle group, and the patient's perception of pain using a visual analog scale. The patient will then be placed in the splint device. Within each group, subjects will be randomly assigned to treatment groups in which force being
exerted by the splint is 5, 25, or 50 g greater than the measured stiffness of the metacarpophalangeal joint. The time required to cause observed changes, electromyograph readings associated with the change, and force history leading to the changes will be measured. A 4 (qualitative hand classification) X 3 (levels of force) analysis of variance will be used in analyzing the effects of the splint treatments. A stepwise multiple regression analysis will be performed on the data.

25. (U) 8610 - 8709. This project was approved as a minimal risk protocol by the US Army Institute of Surgical Research Human Use Committee on 11 September 1987.
### RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

<table>
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### 11. TITLE
Studies of Infection and Microbiologic Surveillance of Troops with Thermal Injury

### 12. SUBJECT AREAS
- 06 03 Medicine and Medical Research
- 06 13 Microbiology
- 06 15 Pharmacology

### 13. START DATE
1976 10

### 14. ESTIMATED COMPLETION DATE
1980 09

### 15. FUNDING ORGANIZATION
DA

### 16. PERFORMANCE METHOD
C

### 17. CONTRACT GRANT
X

### 18. RELEVANCY CERTIFIED
Y

### 19. RESPONSIBLE DOD ORGANIZATION
US Army Institute of Surgical Research

### 20. PERFORMING ORGANIZATION
US Army Institute of Surgical Research

### 21. GENERAL USE

#### MILITARY CIVILIAN APPLICATION
M

### 22. KEYWORDS
- (U) Pseudomonas
- (U) Klebsiella
- (U) Staphylococcus
- (U) Wound Infection
- (U) Antibiotic Resistance

### 23. TECHNICAL OBJECTIVE
24. APPROACH
25. PROGRESS

#### 23. (Continued)

(24) Burns constitute a large component of military injuries sustained in combat. Military relevance of this research lies in the fact that infection and ensuing sepsis are major problems among burned soldiers. Control of surface infection is a major objective and species of organisms causing sepsis, epidemiology, response of significant species to topical chemotherapy modalities, and relationship of antibiotics to sepsis control are major study areas. A literature search is conducted for each protocol initiated.

#### 24. (U) Cultures of human wounds, tissues, and body fluids are carried out with precise strain speciation and differentiation being employed. Virulence is assessed in burn wound models which are also used to assess effectiveness of experimental drugs, both topical and systemic.

#### 25. (U) Microbiologic surveillance was carried out on 200 of the 206 admitted and discharged burn patients. More than 8,307 isolates were recovered. As has been the experience in recent years, relative isolation of Gram-negative organisms continued to be low. The most common Gram-negative isolate was Pseudomonas aeruginosa, but was recovered in <3%
of the patients and 8% of the total flora. Positive blood cultures were recovered from 20 patients, with Staphylococcus aureus being the most common offender.
ANNUAL RESEARCH PROGRESS REPORT

PROJECT NUMBER: 3M161102BS14-00, BASIC RESEARCH

PROJECT TITLE: STUDIES OF INFECTION AND MICROBIOLOGIC SURVEILLANCE OF TROOPS WITH THERMAL INJURY

US ARMY INSTITUTE OF SURGICAL RESEARCH
FORT SAM HOUSTON
SAN ANTONIO, TEXAS 78234-5012

1 January 1986 - 31 December 1986

INVESTIGATORS

Albert T. McManus, PhD
Jack R. Henderson, PhD
Timothy E. Lawson, Staff Sergeant
Charles H. Guymon, Sergeant
Aldo H. Reyes, Staff Sergeant
Kenneth Hawthorne, Staff Sergeant
Anne Lasko, Sergeant
Arthur D. Mason, Jr., MD
Basil A. Pruitt, Jr., MD, Colonel, MC
ABSTRACT

PROJECT NUMBER: 3M161102BS14-00, BASIC RESEARCH

PROJECT TITLE: STUDIES OF INFECTION AND MICROBIOLOGIC SURVEILLANCE OF TROOPS WITH THERMAL INJURY

INSTITUTION: US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas 78234-5012

PERIOD COVERED IN THIS REPORT: 1 Jan 86 through 31 Dec 86

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During calendar year 1986, 200 burned patients were cultured and 8,223 isolates were identified. A relatively low colonization frequency (<25%) with Gram-negative organisms has continued for the fifth reporting period. This was also reflected in an increase in Gram-positive organisms in blood cultures. Staphylococcus aureus and Staphylococcus epidermidis represented 39.7% of the bacteremia cases. The computerized microbial culture surveillance system has been extended to include infection control and antibiotic usage data bases. This system is being evaluated for its use in predicting infecting organisms from previous sites of colonization and antibiotic usage.
STUDIES OF INFECTION AND MICROBIOLOGIC SURVEILLANCE
OF TROOPS WITH THERMAL INJURY

INTRODUCTION

This report is produced from microbiology data collected for patients admitted during calendar year 1986. Data were collected from admission through disposition. This is the second report that is based on calendar year rather than fiscal year. This change more nearly aligns culture results with the annual research progress report produced by the Clinical Division for the same patient population.

AUTOMATED MICROBIOLOGY DATA BASE

The microbiology data base now contains complete surveillance data for >1,000 burn patient admissions. Epidemiologic use of these data has resulted in several publications. The microbiology data base has been aligned with antibiotic use and infection control data bases. This has improved the utility of the system for prospective use in identifying outbreaks and aiding empiric therapy by predicting on a statistical basis the probable antibiotic sensitivity patterns of infecting organisms.

ANTIBIOTIC SENSITIVITY DETERMINATION

The 1986 antibiotic testing panels are presented in Table 1. Bacterial organisms were tested by agar overlay disc diffusion. Broth dilution minimal inhibitory concentrations and minimal bactericidal concentrations were available upon specific request. The protocol for selecting organisms for in vitro sensitivities was isolation from blood cultures, predominant organisms in biopsy cultures, predominant organisms in sputum and in urine cultures with >10^5 cfu/ml, Staphyloccoccus aureus isolates, Pseudomonas aeruginosa isolates, and other organisms as requested.

MICROBIAL SURVEILLANCE

The microbial surveillance protocol established in fiscal year 1983 was continued in calendar year 1986 (1). Patients were cultured from wound, sputum, urine, and rectum on admission. Thereafter, sputum and urine were cultured three times per week and stools and wound surfaces twice per week. Patients transferred to the convalescent ward and hospitalized >30 days were cultured once per week. Gentamicin-resistant Gram-negative organisms from sputum or stool specimens were screened by plating on MacConkey agar containing gentamicin sulfate (25 µg/ml).
<table>
<thead>
<tr>
<th><strong>Enteric Organisms</strong></th>
<th><strong>Nonenteric Gram-Negative Organisms</strong></th>
<th><strong>Gram-Positive Organisms</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Amikacin (^{a,b})</td>
<td>1. Amikacin (^{a,b})</td>
<td>1. Amikacin (^{a,b})</td>
</tr>
<tr>
<td>2. Gentamicin (^{a,b})</td>
<td>2. Gentamicin (^{a,b})</td>
<td>2. Gentamicin (^{a,b})</td>
</tr>
<tr>
<td>3. Tobramycin (^{a})</td>
<td>3. Tobramycin (^{a,b})</td>
<td>3. Tobramycin (^{a,b})</td>
</tr>
<tr>
<td>4. Ticarcillin (^{a})</td>
<td>4. Ticarcillin (^{a})</td>
<td>4. Ticarcillin</td>
</tr>
<tr>
<td>5. Mezlocillin (^{a,b})</td>
<td>5. Mezlocillin (^{a,b})</td>
<td>5. Mezlocillin (^{a,b})</td>
</tr>
<tr>
<td>6. Piperacillin (^{a,b})</td>
<td>6. Piperacillin (^{a,b})</td>
<td>6. Piperacillin (^{a,b})</td>
</tr>
<tr>
<td>7. Moxalactam (^{a,b})</td>
<td>7. Moxalactam (^{a,b})</td>
<td>7. Moxalactam</td>
</tr>
<tr>
<td>8. Cefotaxime (^{a})</td>
<td>8. Cefotaxime (^{a})</td>
<td>8. Cefotaxime</td>
</tr>
<tr>
<td>10. Sulfadiazine</td>
<td>10. Cefsulodin (^{a})</td>
<td>10. Cefsulodin</td>
</tr>
<tr>
<td>12. Kanamycin</td>
<td>12. Sulfadizine (^{a})</td>
<td>12. Oxacillin (^{a})</td>
</tr>
<tr>
<td>15. Cefoxitin (^{a})</td>
<td>15. Chloramphenicol</td>
<td>15. Kanamycin</td>
</tr>
<tr>
<td>16. Cefamandole (^{a})</td>
<td>16. Tetracycline</td>
<td>16. Chloramphenicol (^{a})</td>
</tr>
<tr>
<td>17. Ampicillin (^{a})</td>
<td>17. Imipenem-Cilastatin (^{b})</td>
<td>17. Tetracycline</td>
</tr>
<tr>
<td>18. Trimethoprim</td>
<td>18. Azlocillin (^{a})</td>
<td>18. Ampicillin</td>
</tr>
<tr>
<td>22. Streptomycin</td>
<td>22. Erythromycin (^{a})</td>
<td>22. Streptomycin</td>
</tr>
<tr>
<td>24. Norfloxacin</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^{a}\)Reported daily on daily clinical microbiology report (hard copy).
\(^{b}\)Reported on computer screen from patient data base.
A total of 200 patients admitted in 1986 were cultured. Species isolated and number of patients yielding each species are presented in Table 2. Because of the decreased host resistance of the patient population, no organism is considered "normal" flora and all isolated organisms are reported to the physician. A summary of the 10 most common isolates is presented in Table 3. The table contains 81% of the species identified. The relative frequencies of sites of isolation are presented in Figure 1. The relative frequencies of sites of isolation of Gram-negative organisms, Gram-positive organisms, and yeast are shown in Figure 2.

FLORA RECOVERED FROM RESPIRATORY SYSTEM SPECIMENS

A total of 6,211 organisms were recovered from respiratory system specimens. The majority of these were sputum cultures collected in the surveillance program. The 10 most frequent species are presented in Table 4, which represents 81.61% of the respiratory isolates. Of particular note is the continued decline of Gram-negative isolates. Pseudomonas aeruginosa was not in the top 10 organisms, with only 28 of the 184 patients colonized. This frequency was not significantly different from calendar years 1984 and 1985.

FLORA RECOVERED FROM WOUND SURFACE SPECIMENS

A total of 1,138 contact plate surface cultures were taken and 452 isolates were made. Relative frequencies of isolated species are presented in Figure 3. Subsurface flora, as measured by biopsy specimens, was measured in 162 biopsies taken from 35 patients. Organisms were recovered from 20 of the biopsied patients. The 10 most common organisms are presented in Table 5. Filamentous fungi remained the principal isolate with Aspergillus being the most common fungal genus. Pseudomonas aeruginosa was recovered from 9 biopsies taken from 4 patients. The continued decrease in recovery of wound bacteria is best correlated with the decrease in resistance to topical and parenteral antimicrobial agents. The loss of competitive bacterial flora is a reasonable basis for the increased frequency of fungal isolates.

FLORA RECOVERED FROM URINARY TRACT SPECIMENS

Urine specimens from 191 patients yielded 836 isolates. The 10 most common species are presented in Table 6. The top 10 organisms isolated from urine specimens with >10 xfu/ml are presented in Table 7.

FLORA RECOVERED FROM BLOOD CULTURES

Blood cultures were obtained from 110 patients for a total of 890 cultures. The principal organisms recovered are listed
<table>
<thead>
<tr>
<th>Organism</th>
<th>Number of Isolates</th>
<th>Number of Patients Colonized</th>
<th>Organism</th>
<th>Number of Isolates</th>
<th>Number of Patients Colonized</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acinetobacter anitratus</td>
<td>137</td>
<td>14</td>
<td>Pseudomonas aeruginosa</td>
<td>567</td>
<td>64</td>
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<td>Acinetobacter lwoffi</td>
<td>7</td>
<td>7</td>
<td>Pseudomonas cepacia</td>
<td>8</td>
<td>5</td>
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<tr>
<td>Aspergillus flavus</td>
<td>9</td>
<td>4</td>
<td>Pseudomonas maltophilia</td>
<td>2</td>
<td>2</td>
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<tr>
<td>Aeromonas hydrophila</td>
<td>4</td>
<td>1</td>
<td>Pseudomonas putida</td>
<td>3</td>
<td>2</td>
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<tr>
<td>Bacillus</td>
<td>13</td>
<td>17</td>
<td>Serratia marcescens</td>
<td>37</td>
<td>13</td>
</tr>
<tr>
<td>Branhamella catarrhalis</td>
<td>30</td>
<td>10</td>
<td>Staphylococcus aureus</td>
<td>1,455</td>
<td>146</td>
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<tr>
<td>Candida albicans</td>
<td>236</td>
<td>60</td>
<td>Staphylococcus epidermidis</td>
<td>270</td>
<td>105</td>
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<td>Candida parapsilosis</td>
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<td>3</td>
<td>Staphylococcus saprophyticus</td>
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<td>Candida rugosa</td>
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<td>13</td>
<td>Alpha Streptococcus</td>
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<td>13</td>
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<td>Candida tropicalis</td>
<td>31</td>
<td>13</td>
<td>Beta Streptococcus, Not</td>
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<tr>
<td>Citrobacter freundii</td>
<td>17</td>
<td>9</td>
<td>Group A, B, or D</td>
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<td>Citrobacter diversus</td>
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<td>14</td>
<td>Group A Nonhemolytic Beta</td>
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<td>1</td>
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<td>91</td>
<td>31</td>
<td>Streptococcus</td>
<td></td>
<td></td>
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<tr>
<td>Enterobacter agglomerans</td>
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<td>14</td>
<td>Group D Streptococcus, Not</td>
<td>82</td>
<td>51</td>
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<td>Enterobacter cloacae</td>
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<td>24</td>
<td>Enterococcus</td>
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<td>Escherichia coli</td>
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<td>Group D Enterococcus</td>
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<td>12</td>
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<td>Haemophilus parainfluenza</td>
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<td>2</td>
<td>Nonhemolytic Streptococcus, Not</td>
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<tr>
<td>Klebsiella oxytoca</td>
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<td>20</td>
<td>Streptococcus pneumonia</td>
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<td>Klebsiella pneumoniae</td>
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<td>Streptococcus viridans</td>
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<td>Morganella morganii</td>
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<td>True Fungi Species (Other)</td>
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<td>Neisseria mucosa</td>
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<td>Yeast Species (Other)</td>
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<td>1</td>
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<td>Proteus mirabilis</td>
<td>296</td>
<td>53</td>
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</table>

Total Number of Isolates = 8,223  
Total Number of Patients = 200
<table>
<thead>
<tr>
<th>Organism</th>
<th>Number of Patients Colonized</th>
<th>% Patients</th>
<th>Number of Isolates</th>
<th>% Total Isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonhemolytic Streptococcus, Not Group D</td>
<td>200</td>
<td>94.7</td>
<td>1,198</td>
<td>14.6</td>
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<tr>
<td>Streptococcus viridans</td>
<td>194</td>
<td>92.0</td>
<td>1,832</td>
<td>22.2</td>
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<tr>
<td>Staphylococcus aureus</td>
<td>146</td>
<td>69.0</td>
<td>1,455</td>
<td>17.7</td>
</tr>
<tr>
<td>Staphylococcus epidermidis</td>
<td>105</td>
<td>49.8</td>
<td>270</td>
<td>3.2</td>
</tr>
<tr>
<td>Neisseria mucosa</td>
<td>99</td>
<td>46.9</td>
<td>292</td>
<td>3.6</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>79</td>
<td>37.0</td>
<td>350</td>
<td>4.3</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>79</td>
<td>37.4</td>
<td>330</td>
<td>4.0</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>64</td>
<td>30.3</td>
<td>567</td>
<td>6.7</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>60</td>
<td>28.4</td>
<td>236</td>
<td>2.9</td>
</tr>
<tr>
<td>Beta Streptococcus, Not Group A, B, or D</td>
<td>60</td>
<td>23.4</td>
<td>153</td>
<td>1.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>6,692</td>
<td>81.0</td>
</tr>
</tbody>
</table>

Total Number of Patients Cultured = 211
Total Number of Isolates = 8,223
FIGURE 1. Display of the relative frequency of specimen sources yielding isolates in 1986.
FIGURE 2A. Display of the relative frequency of specimen sources yielding Gram-negative organisms.
FIGURE 2B. Display of the relative frequency of specimen sources yielding Gram-positive organisms.
<table>
<thead>
<tr>
<th>Organism</th>
<th>Number of Patients Colonized</th>
<th>% Patients</th>
<th>Number of Isolates</th>
<th>% Total Isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Streptococcus viridans</td>
<td>190</td>
<td>97.9</td>
<td>1,807</td>
<td>29.1</td>
</tr>
<tr>
<td>Nonhemolytic Streptococcus, Not Group D</td>
<td>182</td>
<td>93.8</td>
<td>1,154</td>
<td>18.6</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>128</td>
<td>65.9</td>
<td>1,102</td>
<td>17.7</td>
</tr>
<tr>
<td>Neisseria mucosa</td>
<td>99</td>
<td>51.0</td>
<td>291</td>
<td>4.7</td>
</tr>
<tr>
<td>Staphylococcus epidermidis</td>
<td>61</td>
<td>31.4</td>
<td>136</td>
<td>2.2</td>
</tr>
<tr>
<td>Beta-Hemolytic Streptococcus, Not Group A, B, or D</td>
<td>58</td>
<td>29.9</td>
<td>151</td>
<td>2.4</td>
</tr>
<tr>
<td>Group D Streptococcus, Not Enterococcus</td>
<td>52</td>
<td>26.8</td>
<td>82</td>
<td>1.3</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>44</td>
<td>22.7</td>
<td>180</td>
<td>2.9</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>43</td>
<td>22.2</td>
<td>104</td>
<td>1.7</td>
</tr>
<tr>
<td>Enterococcus species</td>
<td>31</td>
<td>15.9</td>
<td>63</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Total Number of Patients Cultured = 194
Total Number of Isolates = 6,211
FIGURE 3. Display of the relative frequency of organism types isolated from surface wound cultures.
<table>
<thead>
<tr>
<th>Organism</th>
<th>Number of Patients Colonized</th>
<th>% Patients</th>
<th>Number of Isolates</th>
<th>% Total Isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Filamentous fungi</td>
<td>11</td>
<td>31.4</td>
<td>32</td>
<td>38.5</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>5</td>
<td>14.3</td>
<td>8</td>
<td>9.6</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>4</td>
<td>11.4</td>
<td>6</td>
<td>7.2</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>4</td>
<td>11.4</td>
<td>7</td>
<td>8.4</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>2</td>
<td>6.1</td>
<td>3</td>
<td>3.6</td>
</tr>
<tr>
<td>Candida rugosa</td>
<td>2</td>
<td>5.7</td>
<td>8</td>
<td>9.6</td>
</tr>
<tr>
<td>Group D Enterococcus</td>
<td>2</td>
<td>5.7</td>
<td>4</td>
<td>4.8</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>2</td>
<td>5.7</td>
<td>4</td>
<td>4.9</td>
</tr>
<tr>
<td>Proteus mirabilis</td>
<td>2</td>
<td>5.7</td>
<td>4</td>
<td>4.8</td>
</tr>
<tr>
<td>Aeromonas hydrophila</td>
<td>1</td>
<td>2.8</td>
<td>3</td>
<td>3.6</td>
</tr>
<tr>
<td>Citrobacter freundii</td>
<td>1</td>
<td>2.8</td>
<td>2</td>
<td>2.4</td>
</tr>
</tbody>
</table>

Total Number of Patients Biopsied = 35
Total Number of Isolates = 83
Biopsies Taken = 162
### TABLE 6. Ten Most Frequent Organisms from Urinary Specimens (1986)

<table>
<thead>
<tr>
<th>Organism</th>
<th>Number of Patients Colonized</th>
<th>% Patients</th>
<th>Number of Isolates</th>
<th>% Total Isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Klebsiella pneumoniae</td>
<td>53</td>
<td>27.7</td>
<td>106</td>
<td>12.7</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>47</td>
<td>24.6</td>
<td>110</td>
<td>13.2</td>
</tr>
<tr>
<td>Proteus mirabilis</td>
<td>39</td>
<td>20.4</td>
<td>142</td>
<td>16.9</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>31</td>
<td>16.2</td>
<td>112</td>
<td>13.4</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>24</td>
<td>12.6</td>
<td>95</td>
<td>11.4</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>22</td>
<td>11.5</td>
<td>34</td>
<td>4.1</td>
</tr>
<tr>
<td>Group D Enterococcus</td>
<td>19</td>
<td>9.9</td>
<td>32</td>
<td>3.8</td>
</tr>
<tr>
<td>Nonhemolytic Streptococcus, Not Group D</td>
<td>17</td>
<td>8.9</td>
<td>19</td>
<td>2.3</td>
</tr>
<tr>
<td>Streptococcus epidermidis</td>
<td>14</td>
<td>7.3</td>
<td>18</td>
<td>2.2</td>
</tr>
<tr>
<td>Morganella morganii</td>
<td>11</td>
<td>5.8</td>
<td>18</td>
<td>2.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>686</td>
<td>82.1</td>
</tr>
</tbody>
</table>

Total Number of Patients Cultured = 191  
Total Number of Isolates = 836
<table>
<thead>
<tr>
<th>Organism</th>
<th>Number of Patients Colonized</th>
<th>% Patients</th>
<th>Number of Isolates</th>
<th>% Total Isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Klebsiella pneumoniae</td>
<td>35</td>
<td>36.0</td>
<td>60</td>
<td>13.5</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>32</td>
<td>32.9</td>
<td>63</td>
<td>14.2</td>
</tr>
<tr>
<td>Proteus mirabilis</td>
<td>28</td>
<td>28.9</td>
<td>84</td>
<td>18.9</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>22</td>
<td>22.7</td>
<td>55</td>
<td>12.4</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>16</td>
<td>16.5</td>
<td>44</td>
<td>9.9</td>
</tr>
<tr>
<td>Morganella morganii</td>
<td>9</td>
<td>9.3</td>
<td>13</td>
<td>2.9</td>
</tr>
<tr>
<td>Nonhemolytic Streptococcus, Not Group D</td>
<td>9</td>
<td>9.3</td>
<td>9</td>
<td>2.0</td>
</tr>
<tr>
<td>Acinetobacter anitratus</td>
<td>6</td>
<td>6.2</td>
<td>21</td>
<td>4.7</td>
</tr>
<tr>
<td>Enterobacter aerogenes</td>
<td>6</td>
<td>6.2</td>
<td>13</td>
<td>2.9</td>
</tr>
<tr>
<td>Group D Enterococcus</td>
<td>6</td>
<td>6.2</td>
<td>9</td>
<td>2.0</td>
</tr>
</tbody>
</table>

Total Number of Patients Cultured = 97
Total Number of Isolates = 443
Positive cultures were obtained from 39 patients and 85 isolates were made from 82 positive cultures. Fifty-three cases of bacteremia were noted. A case of bacteremia was defined as isolation of an organism once or more than once with a 30-day period.

Intravenous catheter tips were cultured from 95 patients. Isolations were made from 47 patients and 159 isolates were made. Data are presented in Table 9. These data show an unexpectedly high incidence of contamination.

**SUMMARY OF ANTIBIOTIC TESTING**

A total of 3,003 bacterial isolates were tested for in vitro sensitivity to antibiotics. A comparison of sources of tested strains is presented in Figure 4. The relative frequency of tested organisms is presented in Figure 5.

Gentamicin resistance was again used as a plasmid surveillance marker. Testing was done on 2,997 isolates. Figure 6 displays the relative frequency of tested organisms. Figure 7 displays the frequency of resistant species. Only 100 Gram-negative isolates of 1,548 strains tested were resistant to gentamicin (6.5%). This is the lowest percentage ever reported from the Institute and is a direct marker of the success of infection control isolation techniques to prevent the accumulation of a resistant Gram-negative flora.

**Staphylococcus aureus.** The sources of Staphylococcus aureus strains tested for in vitro activity are presented in Figure 8. The incidence of multiply resistant Staphylococcus aureus was 46% of isolates and these strains were isolated from 83 patients. The resistant strains are multiply resistant with expression of gentamicin, erythromycin, oxacillin, and streptomycin resistance. Multiply resistant Staphylococcus aureus and gentamicin-sensitive strains are displayed separately in Table 10 and histograms are shown in Figure 9.

**Pseudomonas aeruginosa.** The frequency of sources of Pseudomonas aeruginosa strains tested in vitro is presented in Figure 10. The results of testing are presented in Table 11. Sensitivity to aminoglycoside antibiotics has remained high. The relative frequency of gentamicin resistance for recent reporting periods is presented in Figure 11. The relative frequency of sulfonamide resistance for recent reporting periods is presented in Figure 12. Histogram displays of the distributions of zone sizes for selected antibiotics are presented in Figure 13.

Klebsiella pneumoniae. A total of 215 isolates were tested for in vitro sensitivities to antibiotics. The sources of isolation for tested strains are presented in Figure 14. The results of in vitro antibiotic testing are presented in
<table>
<thead>
<tr>
<th>Organism</th>
<th>Number of Patients</th>
<th>% Patients Cultured</th>
<th>Number of Cases</th>
<th>% Cases</th>
<th>Number of Isolates</th>
<th>% Total Isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus aureus</td>
<td>11</td>
<td>10.0</td>
<td>11</td>
<td>20.3</td>
<td>18</td>
<td>21.2</td>
</tr>
<tr>
<td>Staphylococcus epidermidis</td>
<td>10</td>
<td>9.1</td>
<td>10</td>
<td>18.9</td>
<td>16</td>
<td>18.8</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>4</td>
<td>3.6</td>
<td>4</td>
<td>7.5</td>
<td>7</td>
<td>4.7</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>4</td>
<td>3.6</td>
<td>4</td>
<td>7.5</td>
<td>7</td>
<td>4.7</td>
</tr>
<tr>
<td>Candida rugosa</td>
<td>3</td>
<td>2.7</td>
<td>3</td>
<td>5.7</td>
<td>13</td>
<td>15.3</td>
</tr>
<tr>
<td>Proteus mirabilis</td>
<td>3</td>
<td>2.7</td>
<td>3</td>
<td>5.7</td>
<td>5</td>
<td>3.6</td>
</tr>
<tr>
<td>Acinetobacter anitratus</td>
<td>2</td>
<td>1.8</td>
<td>2</td>
<td>3.8</td>
<td>2</td>
<td>2.4</td>
</tr>
<tr>
<td>Micrococcus lutea</td>
<td>2</td>
<td>1.8</td>
<td>2</td>
<td>3.8</td>
<td>2</td>
<td>2.4</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>2</td>
<td>1.8</td>
<td>2</td>
<td>1.9</td>
<td>2</td>
<td>2.4</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>1</td>
<td>0.9</td>
<td>1</td>
<td>1.9</td>
<td>1</td>
<td>1.2</td>
</tr>
<tr>
<td>Serratia marcescens</td>
<td>1</td>
<td>0.9</td>
<td>1</td>
<td>5.7</td>
<td>1</td>
<td>1.2</td>
</tr>
</tbody>
</table>

Total Number of Patients Cultured = 110
Total Number of Isolates = 85
Total Number of Cultures = 890

Total Number of Patient Positives = 41
Total Cases (Patients/Species) = 53
<table>
<thead>
<tr>
<th>Organism</th>
<th>Number of Patients Colonized</th>
<th>% Patients</th>
<th>Number of Isolates</th>
<th>% Total Isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus aureus</td>
<td>24</td>
<td>25.3</td>
<td>40</td>
<td>25.2</td>
</tr>
<tr>
<td>Staphylococcus epidermidis</td>
<td>18</td>
<td>18.9</td>
<td>22</td>
<td>13.8</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>9</td>
<td>9.4</td>
<td>12</td>
<td>7.5</td>
</tr>
<tr>
<td>Group D Enterococcus</td>
<td>7</td>
<td>7.4</td>
<td>8</td>
<td>5.0</td>
</tr>
<tr>
<td>Proteus mirabilis</td>
<td>7</td>
<td>7.4</td>
<td>10</td>
<td>6.3</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>7</td>
<td>7.4</td>
<td>11</td>
<td>6.9</td>
</tr>
<tr>
<td>Candida rugosa</td>
<td>6</td>
<td>6.3</td>
<td>15</td>
<td>9.4</td>
</tr>
<tr>
<td>Acinetobacter anitatus</td>
<td>4</td>
<td>4.2</td>
<td>8</td>
<td>5.0</td>
</tr>
<tr>
<td>Enterobacter aerogenes</td>
<td>4</td>
<td>4.2</td>
<td>6</td>
<td>3.8</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>5</td>
<td>5.2</td>
<td>6</td>
<td>3.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>138</td>
<td>86.8</td>
</tr>
</tbody>
</table>

Total Number of Patients Cultured = 95
Total Number of Isolates = 159
FIGURE 5. Display of the relative frequency of organisms tested for in vitro sensitivity to antibiotics in 1986.
FIGURE 7. Display of the relative frequency of gentamicin-resistant organisms isolated in 1986.
FIGURE 8. Display of the relative frequency of sources yielding *Staphylococcus aureus* tested for *in vitro* sensitivity to antibiotics in 1986.
TABLE 10A. Antibiotic Sensitivity Data for *Staphylococcus aureus* Sensitive to Oxacillin (1986)

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Resistant (%)</th>
<th>Intermediate (%)</th>
<th>Sensitive (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amikacin</td>
<td>2</td>
<td>2</td>
<td>96</td>
</tr>
<tr>
<td>Cephalothin</td>
<td>-</td>
<td>1</td>
<td>99</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>1</td>
<td>-</td>
<td>99</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>7</td>
<td>4</td>
<td>89</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>10</td>
<td>-</td>
<td>90</td>
</tr>
<tr>
<td>Oxacillin</td>
<td>-</td>
<td>-</td>
<td>100</td>
</tr>
<tr>
<td>Penicillin</td>
<td>90</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>9</td>
<td>1</td>
<td>90</td>
</tr>
<tr>
<td>Sulfadiazine</td>
<td>11</td>
<td>14</td>
<td>75</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>7</td>
<td>-</td>
<td>93</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>-</td>
<td>-</td>
<td>100</td>
</tr>
</tbody>
</table>

TABLE 10B. Antibiotic Sensitivity Data for *Staphylococcus aureus* Resistant to Oxacillin (1986)

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Resistant (%)</th>
<th>Intermediate (%)</th>
<th>Sensitive (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amikacin</td>
<td>1</td>
<td>32</td>
<td>67</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>1</td>
<td>1</td>
<td>98</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>4</td>
<td>-</td>
<td>96</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>38</td>
<td>26</td>
<td>37</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>92</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>Oxacillin</td>
<td>83</td>
<td>17</td>
<td>-</td>
</tr>
<tr>
<td>Penicillin</td>
<td>100</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>93</td>
<td>-</td>
<td>7</td>
</tr>
<tr>
<td>Sulfadiazine</td>
<td>42</td>
<td>39</td>
<td>18</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>1</td>
<td>-</td>
<td>99</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>-</td>
<td>-</td>
<td>100</td>
</tr>
</tbody>
</table>
FIGURE 9A. Multiple resistant *Staphylococcus aureus*. 120
FIGURE 9A. Multiple resistant Staphylococcus aureus (continued).
FIGURE 9A. Multiple resistant *Staphylococcus aureus* (continued).
FIGURE 9A. Multiple resistant Staphylococcus aureus (continued).
FIGURE 9B. Histogram display of the distribution of zones of inhibition of growth of Staphylococcus aureus.
FIGURE 9B. Histogram display of the distribution of zones of inhibition of growth of Staphylococcus aureus (continued).
FIGURE 9B. Histogram display of the distribution of zones of inhibition of growth of *Staphylococcus aureus* (continued).
FIGURE 9B. Histogram display of the distribution of zones of inhibition of growth of *Staphylococcus aureus* (continued).

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FIGURE 10. Display of the relative frequency of sources yielding *Pseudomonas aeruginosa* tested for *in vitro* sensitivity to antibiotics in 1986.
<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>RESISTANT</th>
<th>INTERMEDIATE</th>
<th>SENSITIVE</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>Number</td>
<td>%</td>
<td>Number</td>
</tr>
<tr>
<td>Amikacin</td>
<td>1.60</td>
<td>7</td>
<td>9.61</td>
<td>42</td>
</tr>
<tr>
<td>Azlocillin</td>
<td>13.50</td>
<td>71</td>
<td>2.85</td>
<td>15</td>
</tr>
<tr>
<td>Aztreonam</td>
<td>10.08</td>
<td>53</td>
<td>10.84</td>
<td>57</td>
</tr>
<tr>
<td>Cefoperazone</td>
<td>12.59</td>
<td>55</td>
<td>6.86</td>
<td>30</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>21.48</td>
<td>113</td>
<td>10.24</td>
<td>1</td>
</tr>
<tr>
<td>Cefosulodin</td>
<td>12.02</td>
<td>50</td>
<td>22.62</td>
<td>119</td>
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FIGURE 13. Histogram display of the distribution of zones of inhibition of growth of *Pseudomonas aeruginosa.*
FIGURE 13. Histogram display of the distribution of zones of inhibition of growth of *Pseudomonas aeruginosa* (continued).
FIGURE 13. Histogram display of the distribution of zones of inhibition of growth of Pseudomonas aeruginosa (continued).
FIGURE 13. Histogram display of the distribution of zones of inhibition of growth of Pseudomonas aeruginosa (continued).
Table 12. Histogram displays of the distributions of zone sizes for selected antibiotics are presented in Figure 15.

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<td>1</td>
<td>93.95</td>
<td>202</td>
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FIGURE 15. Histogram display of the distribution of zones of inhibition of growth of *Klebsiella pneumoniae*. 

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FIGURE 15. Histogram display of the distribution of zones of inhibition of growth of Klebsiella pneumoniae (continued).
FIGURE 15. Histogram display of the distribution of zones of inhibition of growth of Klebsiella pneumoniae (continued).
PRESENTATIONS/PUBLICATIONS


REFERENCES

ANNUAL RESEARCH PROGRESS REPORT

PROJECT NUMBER: 3M161102BS14-00, BASIC RESEARCH

PROJECT TITLE: STUDIES OF INFECTION AND MICROBIOLOGIC SURVEILLANCE OF TROOPS WITH THERMAL INJURY: A Clinical Study of the Efficacy of Ceftazidime in the Parenteral Therapy of Infections in Hospitalized Burn Patients

US ARMY INSTITUTE OF SURGICAL RESEARCH
FORT SAM HOUSTON
SAN ANTONIO, TEXAS 78234-5012

1 October 1986 - 30 September 1987

INVESTIGATORS

Leslie B. Scorza, MD, Captain, MC
Albert T. McManus, PhD
William F. McManus, MD, Colonel, MC
Basil A. Pruitt, Jr., MD, Colonel, MC
ABSTRACT

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INSTITUTION: US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas 78234-5012

PERIOD COVERED IN THIS REPORT: 1 Oct 86 through 30 Sep 87

INVESTIGATORS: Leslie B. Scorza, MD, Captain, MC
Albert T. McManus, PhD
William F. McManus, MD, Colonel, MC
Basil A. Pruitt, Jr., MD, Colonel, MC

This study is designed to evaluate the efficacy of ceftazidime in comparison to agents currently used in combination in the treatment of acute bacterial infections in burned patients (piperacillin, amikacin, and/or vancomycin). Patients entered into this study are adult patients with burn injuries and clinical or laboratory indications of bacterial infection that require the use of intravenous antibiotics. The organism(s) causing the infection are proven or presumed susceptible to ceftazidime and a combination treatment prior to initiation of therapy. Patients entering this study are randomly assigned to either ceftazidime (n=25) or a drug combination (n=25). Evaluations will be made concerning the relative bacteriologic and clinical effectiveness as well as relative safety and tolerance of the treatments. The clinical and bacteriologic course of each patient is followed and documented. Laboratory and clinical data to assess safety is obtained before, during, and after treatment. Twelve patients were entered into this study during this reporting period.
A CLINICAL STUDY OF THE EFFICACY OF CEFTAZIDIME IN THE PARENTERAL THERAPY OF INFECTIONS IN HOSPITALIZED BURN PATIENTS

INTRODUCTION

Ceftazidime is an extended spectrum cephalosporin antibiotic for parenteral administration. It offers both Gram-positive activity and activity against a wide range of Gram-negative organisms, including bacteria that are resistant to first and second generation cephalosporins and aminoglycosides. Ceftazidime is distinguished by its anti-Pseudomonal activity. Additionally, it demonstrates excellent stability to beta lactamases produced by many clinically important organisms.

Ceftazidime exhibits an excellent pharmacokinetic profile. The antibiotic is rapidly distributed throughout the body following parenteral administration. Ceftazidime is not metabolized; excretion is via glomerular filtration. Protein binding is low. The compound has a half-life of 1.90 h, making it possible to employ the drug on an every 8-12 h schedule.

Ceftazidime has proven to be efficacious in the treatment of nosocomial and community-acquired infections. The compound has been evaluated in worldwide clinical trials in over 5,000 patients with a variety of infection problems including lower respiratory tract, urinary tract, bone and joint, intra-abdominal, gynecologic, skin, and skin structure infections as well as bacterial septicemia and meningitis. Overall, 93% of patients treated in United States trials were clinically cured or improved (cured - 68%, improved - 25%). A bacterial cure (eradication of the initial pathogen) was achieved in 89% of ceftazidime-susceptible organisms isolated in United States clinical studies.

The objective of this study is to evaluate the efficacy of ceftazidime as monotherapy in the treatment of serious infections in burned patients and to compare the efficacy of ceftazidime to antibacterial agents currently used in combination in the treatment of serious infections in burned patients.

MATERIALS AND METHODS

This study will randomize 50 consecutive burned patients requiring the first use of intravenous therapy for bacterial infection. Twenty-five patients will be assigned ceftazidime and 25 patients will be assigned to a combination therapy. Patients randomized to ceftazidime or to a combination that does not include vancomycin that develop a clinical need for the addition of vancomycin will be considered failures of the initial therapy. The assigned treatment will be maintained (plus vancomycin) for 8-14 days. Patients under study who
require surgery will be maintained on the assigned treatment during the perioperative period.

MATERIALS AND METHODS

Selection of Patients. Patients who have clinical and/or laboratory indications of bacterial infection are eligible for entry into the study. Intravenous antibiotic therapy must be indicated. Bacteria isolated from the site of infection must be sensitive or presumed sensitive to ceftazidime and a combination therapy prior to randomization. Patients who have a history of allergy or adverse reaction to penicillins, cephalosporins, aminoglycosides, or vancomycin are excluded. Also, patients who have been treated for bacterial infection with intravenous antibiotics (except penicillin) during the current hospitalization are excluded.

Procedures Prior to Treatment. A chest roentgenogram and a medical history are obtained within 72 hours prior to the initiation of treatment and a pertinent physical examination is performed. A laboratory profile, to include hematology, standard chemistries, and urinalysis, is obtained.

Randomization. Randomization is by a random number chart maintained in the pharmacy.

Dosage and Administration. Ceftazidime is administered in a dose of 250, 500, 750, or 1,000 mg 2-4 times daily, with the usual dose being 1,000 mg every 8-12 h. Standard therapy is administered as is currently practiced. The amikacin dose is based on 1.5 mg/kg/day divided into 2-3 equal doses per day given at equal intervals and adjusted as appropriate for renal function. The total daily dose of amikacin does not exceed 1.5 mg/kg/day. Piperacillin is administered in a dose of 3-4 g every 4-6 h not to exceed 24 g/day. Vancomycin is given as 500 mg every 6 h or 1 g every 12 h.

Procedures During Treatment. Clinical signs and symptoms of infection are assessed daily and are used as a measure of the efficacy of the study treatment. Serial chest roentgenograms and other procedures are performed as appropriate to assess clinical status. All adverse effects are recorded. Cultures are taken from the site of infection on a daily basis, with the exception of blood cultures which are taken for clinical indications.

Procedures After Treatment. The clinical effectiveness of the assigned treatment is documented upon completion or withdrawal of treatment. This record indicates the clinical result of the original treatment and reasons, if any, for withdrawal. Seven to 10 days after completion of the treatment, a laboratory profile, to include hematology, standard chemistries, and urinalysis, and a pertinent physical examination are performed.
RESULTS

Six of the 12 patients entered into the study died. However, all of these patients had completed a course of therapy and had clinical and microbiological improvement of the treated infection. One patient of the remaining 6 patients failed on combination therapy and improved on ceftazidime. All other patients also had improvement. Several patients randomized to ceftazidime therapy required the addition of vancomycin and therefore must be deemed failures of ceftazidime therapy. There have been no obvious side effects noted as yet and there have been no withdrawals from the study.

DISCUSSION

Although it is too early at this point to draw statistically significant conclusions, there have been several patients randomized to the ceftazidime group who required the addition of vancomycin. Vancomycin was added when there was lack of improvement as documented either by microbiological or clinical means. Once vancomycin was added, improvements were noted in each patient.

PRESENTATIONS/PUBLICATIONS

None.
In the field of Metabolism and Nutritional Science, the Research and Technology Work Unit Summary provides a detailed overview of ongoing projects and investigations. The summary outlines the project's objectives, methodology, and expected outcomes, highlighting the importance of understanding the cellular mechanisms of postburn metabolism in order to develop effective nutritional interventions for burn victims.

The research focuses on the cellular mechanisms of postburn hypermetabolism, which is a critical consideration in burn injury recovery. A literature search was conducted to identify relevant studies and methodologies. This project aims to explore the contribution of fatty acid metabolism in patients with postburn hypermetabolism.

Tasks include:
- Conducting experiments on animal models to study postburn hypermetabolism.
- Developing nutritional interventions for burn victims.
- Collaborating with other research teams to enhance understanding of metabolic responses to burn injury.

The project is expected to conclude in the upcoming year with the presentation of findings at a national scientific meeting.

This summary is part of a broader initiative to enhance research capabilities in Metabolism and Nutritional Science, aiming to improve patient outcomes and advance scientific knowledge in this critical area.
RESEARCH COMPLETION REPORT

PROJECT NUMBER: 3M161102BS14-00, BASIC RESEARCH

PROJECT TITLE: THE STUDY OF METABOLISM AND NUTRITIONAL EFFECTS OF BURN INJURY IN SOLDIERS: Thiamine Levels in the Acutely Burned Patient

US ARMY INSTITUTE OF SURGICAL RESEARCH
FORT SAM HOUSTON
SAN ANTONIO, TEXAS 78234-5012

1 October 1986 - 30 June 1987

INVESTIGATORS

Debra Ann Reilly, MD
Ronald L. Shippee, PhD, Captain, MS
Arthur D. Mason, Jr., MD
Basil A. Pruitt, Jr., MD, Colonel, MC
ABSTRACT

PROJECT NUMBER: 3M161102BS14-00, BASIC RESEARCH

PROJECT TITLE: THE STUDY OF METABOLISM AND NUTRITIONAL EFFECTS OF BURN INJURY IN SOLDIERS: Thiamine Levels in the Acutely Burned Patient

INSTITUTION: US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas 78234-5012

PERIOD COVERED IN THIS REPORT: 1 Oct 86 through 30 Jun 87

INVESTIGATORS: Debra Ann Reilly, MD
Ronald L. Shippee, PhD, Captain, MS
Arthur D. Mason, Jr., MD
Basil A. Pruitt, Jr., MD, Colonel, MC

Nineteen adult patients admitted to the Institute between February and June 1987 with >20% total body surface area burns were studied to determine their thiamine levels during the first month of their hospital course. Early analysis of the data for 15 of these patients reveals an initial drop in their blood thiamine content below normal followed by a rebound to supranormal levels by the end of the study period. Based on this early analysis, thiamine supplementation for this patient population is recommended during the immediate postburn week.
THIAMINE LEVELS IN THE ACUTELY BURNED PATIENT

INTRODUCTION

The objective of this study was to determine thiamine levels in patients sustaining severe thermal injury (1-6). Blood samples were taken during the first 4 wk postburn. The medical application was to recommend a supplemental dose of thiamine for this patient population (7).

MATERIALS AND METHODS

Nineteen patients and 10 controls were entered into the study during this reporting period. All controls were studied once to determine a baseline level of thiamine in a healthy patient population based upon our assay techniques.

Prior to beginning this study, about 60 units of packed red blood cells, which were randomly transfused in the operating room, were tested for their thiamine content relative to their hemoglobin content, age, and blood type.

All burn patients with total body surface area burns >20% were eligible for admission into the study. Patients entered into the study had 1.5 cc of heparinized blood drawn on admission and weekly thereafter for the first month postburn or until expiration. In addition, all transfused units of packed red blood cells to be received by the patient were tested for thiamine content. Weekly charts were kept to record each patient's nutritional and supplemental thiamine intake, other medical problems, amount of skin grafting done, and subjective and objective mental status changes (8).

Sample preparations were separated by centrifugation and the plasma was aspirated off. The packed cells were then washed, lysed, and frozen at -70°C until assayed. After thawing, the samples were prepared for ion pairing reverse phase liquid chromatography and the thiamine elution peak recorded (9-12).

RESULTS

The randomly sampled units of packed red blood cells were found to have varying levels of both hemoglobin and thiamine present per unit of blood. Therefore, the amount of thiamine transfused into each of our patients could not be assumed, but was measured for each unit of blood received during the study period.

Control subjects were found to have an average hemoglobin content of 14 (similar to the level at which the study patients were kept) and an average thiamine content of 160.
Fifteen of the 19 patients completed 3-4 wk of the study and were found to have a markedly low thiamine blood content upon admission (>2SD vs. control). By the third week postburn, they then rebounded to above normal levels (>2SD vs. control) before returning to normal. Serum hemoglobin levels did not vary much during the study period.

**DISCUSSION**

Total thiamine content in the blood of acutely burned patients appears to be markedly low during the initial phase of burn resuscitation. As the patient's wounds heal and are grafted and nutritional intake improves, the serum thiamine rebounds to above the normal average. Further analysis of the data needs to be performed to determine if the burn size, age, and number of packed red blood cell transfusions or nutritional status have any influence on these values. In addition, the patient's subjective responses and mental status should be examined. It does, however, appear that early thiamine supplementation should be recommended for this patient population.

**PRESENTATIONS/PUBLICATIONS**

None.

**REFERENCES**


### RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

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#### 5. SUMMARYISTORY
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#### 6. WORK SECURITY
- CX

#### 12. CONTRACT GRANT NUMBER
- DA-RRDAPR-PY88-01

#### 16. RESPONSIBLE DOD ORGANIZATION
- US Army Institute of Surgical Research

#### 19. NAME OF PRINCIPAL INVESTIGATOR
- MC Manus, A.T.

#### 21. GENERAL USE
- FINA

#### 23. CODES
- 10: MILITARY-CIVILIAN
- 12: PRIMARY
- 13: CONTRIBUTING
- 14: CONTRIBUTING

#### 24. APPROACH
- (Continued) Effects; (U) Volunteers; (U) RAI

#### 25. (U) 9601 - 8612. The clinical trial of the parenteral antibiotic ceftazidime as monotherapy in infected burn patients is in progress. Isoelectric-focusing techniques to identify specific and chromosomal beta-lactamase activities of multiple resistant Gram-negative burn patient isolates have been developed. A standard set of known plasmid-mediated beta-lactamase genes has been established. A probe to detect one of the two common plasmid-mediated sulfonamide resistance genes has been developed. The second probe is expected to be finished in the next reporting period.

---

**Description:**

- **Title:** Alteration of Host Resistance in Burned Soldiers
- **Subjects:** Medicine and Medical Research, Pharmacology
- **Contract-Grant Number:** DA-RRDAPR-PY88-01
- **Responsible DOD Organization:** US Army Institute of Surgical Research
- **Principal Investigator:** MC Manus, A.T.

**Main Research Focus:***

- characterize the microbial basis of opportunistic infection in susceptible burned soldiers, identify specific mechanisms of decreased host resistance that are targeted by opportunistic pathogens, and develop and evaluate countermeasures. A literature search was conducted for each protocol initiated.

- the high susceptibility of burned rats to experimental infection with Pseudomonas aeruginosa and Proteus mirabilis will be investigated. The effect of in vitro alterations of specific microbial characteristics on infection will be investigated. Specific antimicrobial and immunostimulatory therapies will be examined.

- The clinical trial of the parenteral antibiotic ceftazidime as monotherapy in infected burn patients is in progress. Isoelectric-focusing techniques to identify specific and chromosomal beta-lactamase activities of multiple resistant Gram-negative burn patient isolates have been developed. A standard set of known plasmid-mediated beta-lactamase genes has been established. A probe to detect one of the two common plasmid-mediated sulfonamide resistance genes has been developed. The second probe is expected to be finished in the next reporting period.
ANNUAL RESEARCH PROGRESS REPORT

PROJECT NUMBER: 3M161102BS14-00, BASIC RESEARCH

PROJECT TITLE: ALTERATION OF HOST RESISTANCE IN BURNED SOLDIERS

US ARMY INSTITUTE OF SURGICAL RESEARCH
FORT SAM HOUSTON
SAN ANTONIO, TEXAS 78234-5012

1 October 1986 - 30 September 1987

INVESTIGATORS

Albert T. McManus, PhD
Virginia C. English, MS
Camille L. Denton, MA
Charles H. Guymon, MS, SGT
Aldo H. Reyes, SSG
Arthur D. Mason, Jr., MD
Basil A. Pruitt, Jr., MD, Colonel, MC
ABSTRACT

PROJECT NUMBER: 3M161102BS14-00, BASIC RESEARCH

PROJECT TITLE: ALTERATION OF HOST RESISTANCE IN BURNED SOLDIERS

INSTITUTION: US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas 78234-5012

PERIOD COVERED IN THIS REPORT: 1 Oct 86 through 30 Sep 87

INVESTIGATORS: Albert T. McManus, PhD
Virginia C. English, MS
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Arthur D. Mason, Jr., MD
Basil A. Pruitt, Jr., MD, Colonel, MC

The clinical trial and in vitro evaluation of the parenteral antibiotic ceftazidime as monotherapy in infected burn patients is in progress. Twelve patients were entered into the study during this reporting period. Sensitivity of Gram-negative organisms to ceftazidime has remained high (>93%) during the trial. A beta-lactamase capable of inactivating ceftazidime and all other tested cephalosporins has been recovered from several treated patients. The characteristics and spread of this enzyme are being investigated. A proposed topical antimicrobial agent, Alcide*, has been evaluated in the standardized Pseudomonas-infected burned rat model. This compound was found to have minimal activity when compared to silver sulfadiazine. Evaluation of agar well diffusion technique with in vitro sensitivity to silver sulfadiazine has shown that the agar well diffusion technique can be easily replaced by standardized and much cheaper sulfonamide disc sensitivity testing.
ALTERATION OF HOST RESISTANCE IN BURNED SOLDIERS

EXPERIMENTAL PARENTERAL AGENTS

Measurement of in vitro activity against *Pseudomonas aeruginosa* of the investigational cephalosporin class antibiotic cefsulodin sodium (Abbott-46811) continued during this reporting period. In 264 tested isolates of *Pseudomonas aeruginosa*, 33 resistant strains were found. A comparison of these results to previous findings is presented in Table 1. This compound, although available in most other areas of the world, remains unlicensed in the United States. The activity of this drug against *Pseudomonas aeruginosa* is similar to newer and licensed cephalosporins (see below) and the very limited spectrum and limited need of cefsulodin sodium for specific antipseudomonal activity makes it of questionable future value. Testing of this compound, unless specific need arises, will end with this report.

The in vitro activities of newly approved antibiotics are presented in Table 2. A clinical trial of ceftazidime as monotherapy for infected burn patients is in progress. Ceftriaxone and aztreonam have not been clinically used to date.

Cross resistance to all tested cephalosporins has been observed. The mechanism of resistance appears to be a newly described beta-lactamase enzyme (1). Examination of resistant strains by isoelectric-focusing techniques has shown them to contain multiple beta-lactamase activities. One activity at an isoelectric point of 6.3 appears to be common among the strains. Investigations into the location (plasmid and/or chromosomal) of the gene responsible for this enzyme are being conducted. The multiply resistant phenotype has been found in 3 enteric Gram-negative species isolated from 7 burn patients.

EXPERIMENTAL TOPICAL AGENTS

Alcide®, a proposed topical antimicrobial agent, has been tested for antipseudomonal activity in the standard infected, burned rat model. The compound was tested at 4 concentrations and treatment was once per day for 10 days. Initial treatment was started 24 h after burning and inoculation with *Pseudomonas aeruginosa* (Strain 59-1244). Silver sulfadiazine, an effective burn topical, was used as a reference for positive therapeutic activity. Burned and infected animals without antimicrobial treatment were used as expected mortality controls. The results of this study are presented in Table 3.

Five-percent mafenide acetate was examined for in vitro activity against *Pseudomonas aeruginosa* isolated from 32 burn patients. Agar dilution minimal inhibitory concentration (MIC) assays were done on 79 strains. The mean MIC was 0.2987 g/100
TABLE 1. Cefsulodin Sodium Activity Against Burn Patient Pseudomonas aeruginosa

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<td>Resistant</td>
<td>143 (17.9)</td>
<td>49 (12.1)</td>
<td>36 (7.8)</td>
<td>59 (10.3)</td>
<td>48 (9.7)</td>
<td>33 (14.3)</td>
</tr>
<tr>
<td>Sensitive</td>
<td>655</td>
<td>355</td>
<td>463</td>
<td>571</td>
<td>444</td>
<td>231</td>
</tr>
</tbody>
</table>

( ) = Percent resistant.
TABLE 2. Activity of Experimental Antibiotics for Fiscal Year 1987

<table>
<thead>
<tr>
<th></th>
<th>Ceftazidime(^a)</th>
<th>Ceftriaxone(^a)</th>
<th>Aztreonam(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resistant</td>
<td>80 (6.7%)</td>
<td>167 (14.4%)</td>
<td>81 (7.1%)</td>
</tr>
<tr>
<td>Sensitive</td>
<td>1,106</td>
<td>1,163</td>
<td>1,051</td>
</tr>
</tbody>
</table>

\(^a\)Against all flora except oxacillin-resistant Staphylococcus aureus.
\(^b\)Against Gram-negative aerobic flora.

( ) = Percent resistant.

ml. The median MIC was 0.319 g/100 ml. Data comparing fiscal years 1986 and 1987 are presented in Table 4.

SEROLOGIC TYPES OF Pseudomonas aeruginosa ISOLATED FROM BURN PATIENTS

Pseudomonas aeruginosa isolates from 26 patients were serotyped using the Difco International Typing Sera™ set and autoclaved bacterial suspensions. Stains were selected on the basis of having a distinct antibiotic sensitivity pattern for each patient. A total of 147 strains were typed. Data are presented as the total number of patients with each serotype and the total number of isolates per serotype in Figure 1. Serotypes 09 and 11 were the predominant types identified.

EFFECT OF SULFONAMIDE ACTIVITY ON AGAR WELL DIFFUSION MEASUREMENTS OF SILVER SULFADIAZINE

The effect of sulfonamide activity on agar well diffusion inhibition zones of silver sulfadiazine was examined by plasmid transfer. Clinical isolates with known transferrable sulfonamide resistance were mated to separate clones of Escherichia coli k12 c600. Matings were done on agar and selection was for transfer of sulfonamide resistance to naladixic acid resistance c600 hosts. Plasmid from 33 strains were successfully transferred. Strains of c600 with a transferred plasmid were then tested against the same parent c600 without the plasmid. Strains were examined for zones of inhibition against 1% silver sulfadizine, the silver salt of the sulfadiazine analog benzenesulfonamidopyrimidine and standard sulfonamide disc (250 mg). The effect of transfer of sulfonamide resistance plasmids on silver sulfadiazine zones is presented in Figure 2. The average inhibition zone without the
**TABLE 3. Examination of Alcide\(^R\) in *Pseudomonas aeruginosa*-Infected Rats**

<table>
<thead>
<tr>
<th></th>
<th>Untreated</th>
<th>Control</th>
<th>C1X</th>
<th>C2X</th>
<th>C3X</th>
<th>C4X</th>
<th>Silver Sulfadiazine</th>
</tr>
</thead>
<tbody>
<tr>
<td>First Study</td>
<td>7/8</td>
<td>6/8</td>
<td>4/7</td>
<td>5/8</td>
<td>2/8</td>
<td>4/8</td>
<td>0/8</td>
</tr>
<tr>
<td>Second Study</td>
<td>8/8</td>
<td>8/8</td>
<td>8/8</td>
<td>8/8</td>
<td>7/8</td>
<td>5/8</td>
<td>2/8</td>
</tr>
<tr>
<td>TOTAL</td>
<td>15/16</td>
<td>14/16</td>
<td>12/15</td>
<td>13/16</td>
<td>9/16</td>
<td>9/16</td>
<td>2/16</td>
</tr>
</tbody>
</table>

Mortality (%)     94% 88% 80% 81% 56%* 56%* 13%

*Less effective than silver sulfadiazine (P<0.023, Fisher exact test).

C = Concentration.
FIGURE 1. Number of patients with each serotype and isolates per serotype.
<table>
<thead>
<tr>
<th>Mafenide Acetate Concentration (g/100 ml)</th>
<th>Number of Strains Fiscal Year 1986</th>
<th>Number of Strains Fiscal Year 1987</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.019</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>0.035</td>
<td>9</td>
<td>5</td>
</tr>
<tr>
<td>0.078</td>
<td>28</td>
<td>12</td>
</tr>
<tr>
<td>0.156</td>
<td>47</td>
<td>21</td>
</tr>
<tr>
<td>0.312</td>
<td>83</td>
<td>21</td>
</tr>
<tr>
<td>0.625</td>
<td>15</td>
<td>19</td>
</tr>
<tr>
<td><strong>TOTAL NUMBER OF STRAINS</strong></td>
<td><strong>189</strong></td>
<td><strong>79</strong></td>
</tr>
</tbody>
</table>

Plasmid was changed from a mean zone of 24.9 mm to a mean zone of 17.3 mm (P<0.001). The activity of the same strains against the activity of silver at silver benzenesulfonamidopyrimidine is presented in Figure 3. There was no significant change between plasmid-containing (15.93 mm) and plasmid-free (15.81 mm) strains of c600. The transfer of sulfonamide activity alone was responsible for the significant change in silver sulfadiazine agar well diffusion. Figure 4 shows the control study in which the sulfonamide-sensitive parent strains were converted to sulfonamide resistance using disc technique.
FIGURE 2. Effect of sulfonamide plasmids on silver sulfadiazine diffusion zones.
FIGURE 3. Effect of sulfonamide plasmids on silver diffusion zones.
FIGURE 4. Effect of sulfonamide plasmids on sulfadiazine diffusion zones.
ANNUAL RESEARCH PROGRESS REPORT

PROJECT NUMBER: 3M161102BS14-00, BASIC RESEARCH

PROJECT TITLE: ALTERATION OF HOST RESISTANCE IN BURNED SOLDIERS: Characterization of Biochemical Indicators of Infection in the Thermally Injured

US ARMY INSTITUTE OF SURGICAL RESEARCH
FORT SAM HOUSTON
SAN ANTONIO, TEXAS 78234-5012

1 October 1986 - 30 September 1987

INVESTIGATORS

David G. Burleson, PhD, Lieutenant Colonel, MS
Avery A. Johnson, BS
Arthur D. Mason, Jr., MD
Basil A. Pruitt, Jr., MD, Colonel, MC
ABSTRACT

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INVESTIGATORS: David G. Burleson, PhD, Lieutenant Colonel, MS Avery A. Johnson, BS Arthur D. Mason, Jr., MD Basil A. Pruitt, Jr., MD, Colonel, MC

We have previously described factors found in perchloric acid (PCA) filtrates of the blood of burned-infected rats. One of these factors has a maximum emission at 420 nm at 355 nm excitation (355ex/420em). This factor was subsequently found in the blood of many burned patients. The factor consists of several fluorescent substances that can be resolved by high-pressure liquid chromatography (HPLC). We have analyzed the level of one of the fluorescent components that has an identical HPLC retention time to neopterin. The factor is chromatographically and spectrally similar to neopterin, yet its identity has not been verified by a separate chemical technique. The concentration of the compound quantitated by HPLC was found to be highly correlated to the level of 355ex/420em fluorescence found in many patient blood samples. This suggests that the HPLC-identified substances contribute significantly to the fluorescence measured in the PCA filtrates and that the conditions that lead to the appearance of these factors in the blood of burned patients lead to the appearance of these HPLC-identified substances as well.
CHARACTERIZATION OF BIOCHEMICAL INDICATORS OF INFECTION IN THE THERMALLY INJURED

INTRODUCTION

Infection continues to be a serious complication in severely burned patients and contributes heavily to mortality. Sepsis can be difficult to diagnose in a timely manner in severe trauma. The increase in white blood cell count and fever induced by burn injury hamper the detection of sepsis and make objective diagnosis more difficult. Abnormal levels of hormones (1,2), acute phase proteins in serum (3,4), and fluorescent substances (5,6) in blood and plasma are associated with the presence of inflammation and/or infection in human burn patients and animal burn models. The presence of these substances in blood and plasma likely reflect the metabolic response that the stress of trauma and infection place on the host.

A specific measure of pathogens is desirable, but the wide range of possible microbes that would have to be detected at the low levels present in blood early after infection limit the applicability of microbe-specific biochemical indicators. A biochemical measurement of specific products of the immune system produced in response to invading organisms would provide a means of differentiating the metabolic response induced by trauma from the response induced by activation of the immune system. If this test could be detected by biochemical means, it could be performed in less than an hour and be timely enough to alert the clinician to sepsis in a more timely and objective manner than currently possible with standard microbiology techniques.

We are attempting to find clinically useful indicators to use as an adjunct to standard microbiological methods of assessing the presence of sepsis (5-7). In this report, we describe further characterization of the nature of the previously reported 355ex/420em fluorescent substances found in the blood of burn victims.

MATERIALS AND METHODS

Measurement of Fluorescent Indicators in Blood. One ml of anticoagulated blood or plasma was mixed with 4 ml of cold (4°C) PCA (0.8 M). After incubating for 10 min, the mixture was centrifuged at 4°C for 10 min at 3000 g. The supernatant was recentrifuged at 20000 g for 30 min. The clear supernatant was transferred to another tube and fluorescence was then measured using an Aminco Bowman spectrofluorometer at (355ex/420em). The fluorometer was standardized by using a calibration standard (fluorescence intensity block).
HPLC Determination of the 355ex/420em Factor in Serum. Serum (100 μl) was deproteinized by incubating at 100°C in an oil bath for 20 min after the addition of 200 μl of 0.2 M potassium phosphate buffer (pH 4.5). The mixture was then centrifuged at 20000 g for 20 min and the supernatant was injected directly on the HPLC. HPLC was performed on an Hewlett-Packard liquid chromatograph (Model 1090) with a Biophase ODS reverse phase, 4.6 X 250 mm column (Bioanalytical Systems). The mobile phase consisted of 0.05 M ammonium acetate at a pH of 7.0. The column temperature was maintained at 45°C and the flow rate was 1.0 ml/min. The HPLC was equipped with a Kratos fluorescence detector (Model 980) with a 25 μl flow cell. The excitation monochromometer was set at 350 nm and the emission cutoff filter was at 389 nm. The retention time for standard pterins (Sigma Chemical) were determined using 10 μl of a standard solution of pterins (10 ng/ml). The amount of each fluorescent substance present was measured on a Hewlett-Packard integrator (Model 3392A).

Mass Spectral Analysis of the 355ex/420em Factor. Mass spectral analysis of neopterin was attempted by derivatization of purified standard material (Sigma Chemical) to make it volatile enough for gas chromatography separation and introduction into the mass spectrometer. The mass spectral analysis was attempted on a Hewlett Packard Model 5985. The gas-liquid chromatography column was either a packed column with particles coated with nonpolar silicone as a liquid phase or a 15 m-fused silica capillary column with a nonpolar bonded phase. The gas phase consisted of helium at a flow rate appropriate for the column being used.

RESULTS

Determination of the 355ex/420em Substance in Patient Samples. The conditions under which the fluorescence of the 355ex/420em factor are measured in the PCA filtrates (0.7M PCA, pH <1) and after separation by HPLC (NH₃ acetate buffer, pH 7.4) are quite different. Since the chemical environment can greatly effect fluorescence intensity of a substance, it was not known whether the fluorescence of the HPLC-purified components of the 355ex/420em factor contributed significantly to the fluorescence measured in the PCA filtrates.

A number of samples were analyzed to determine if the level of the fluorescent material with a neopterin HPLC retention time was correlated with the level of fluorescence in the PCA filtrates. The relative amount of material found by HPLC, using neopterin as a standard, was determined by integration of the HPLC peak in randomly selected patient samples. This result was plotted against the log of the fluorescence, which corresponds to concentration, of the same unpurified filtrates at 355ex/420em. Figure 1 depicts the correlation of the neopterin-like substance to the concentration of 355ex/420em factors in blood and plasma. The correlation coefficient ($r^2$)
FIGURE 1. Correlation of the amount of neopterin-like substance determined by HPLC with the amount 355ex/420em fluorescence in the PCA filtrate. Neopterin-like substance was determined by comparing the integrated peak area of fluorescence for the unknown sample to the peak area for the fluorescence determined for a purified neopterin standard. The concentrations determined for the neopterin-like substance by HPLC were compared to the fluorescence measured in the original PCA filtrates.

was >0.8 for both blood and plasma. This suggests that the pterin-like substances contribute significantly to the fluorescence of the 355ex/420em factor and are induced by the same sequence of events that cause the factor to be elevated.

The level of neopterin (peak area) determined by HPLC in patient samples was compared with fluorescence of the samples at 355ex/420em. The level of neopterin was plotted along with fluorescence as a function of postburn day. The plots obtained for 3 different patients are shown in Figure 2. Perusal of the figures show that, in general, the trend of 355ex/420em fluorescence was similar to the level of neopterin determined by HPLC for the same day. This is further evidence that a portion of the fluorescence at 355ex/420em is due to neopterin.
FIGURE 2. Comparison of the level of neopterin-like substance determined by HPLC to the level of fluorescence in PCA filtrates. Fluorescence in serial patient samples were determined after PCA protein precipitation for 3 patients. A portion of whole blood from the sample was deproteinized and the amount of neopterin-like substance was determined by HPLC. Both determinations were then plotted for that patient vs. postburn day. HPLC peak area (ng/ml), log fluorescence in PCA filtrates (arbitrary units).
Gas Chromatography-Mass Spectrometry (GC-MS) analysis was attempted to measure neopterin by GC-MS and electrospray mass spectrometry had met with limited success. The limited vapor pressure of neopterin made detection by direct insertion probe impractical, so many other attempts were made to synthesize a derivative that would have sufficient volatility for GC-MS analysis. Bis-trimethylsilylacetamide, bis-trimethylsilyl-trifluoroacetamide, tri-methylchlorosilane, N-trimethylsilylimidazole, hexamethyldisilane, and hexamethylchlorosilane were used in many combinations and under multiple reaction conditions to find the conditions that would result in a sufficiently volatile derivative of standard neopterin to allow analysis by GC-MS. No suitable combination was found. Bis-trimethylsilylacetamide and pyridine gave some initial success, but could not be repeated consistently.

With the acquisition of a thermospray HPLC-mass spectrometry instrument, we hope to be able to purify sufficient quantities of the neopterin-like material to verify its chemical composition without the necessity of making volatile derivatives. Direct analysis of the material that elutes from the HPLC column should be possible.

**DISCUSSION**

We have separated four components that are consistently found in extracts of patient serum that have highly fluorescent PCA filtrates. These substances have fluorescent spectral characteristics similar to nucleotide derivatives such as the pterins. Three of the components copurify with and behave chromatographically similar to pterins. We have not yet established chemical identity with any of the commonly found pterin derivatives with which they have been compared.

It is difficult to accurately determine how much of the fluorescence seen in the original PCA supernatant extracts from patient serum can be accounted for by these four chromatographically separated substances. Fluorescence is highly dependent on chemical structure as well as environmental factors such as pH. Since the pH of the detector chamber is approximately 7.4 and the fluorescent factors were originally measured in concentrated PCA solution, it is not possible to quantitatively compare the fluorescence. We cannot assume that all of the fluorescent substances present in the supernatant extracts are detectable under the chromatographic conditions employed.

Several attempts have been made to derivatize the material and obtain ionization fragmentation patterns by GC-MS, which is the most sensitive method of determination of chemical structure available. Suitable derivatives have not yet been attained.
In biochemical characterization may not be possible on the small amount of material of limited purity that we have obtained to date. Future attempts at identification of these fluorescent substances will be attempted after collection and purification of milligram quantities of these substances.

PRESENTATIONS/PUBLICATIONS

None.

REFERENCES


ANNUAL RESEARCH PROGRESS REPORT

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1 October 1986 - 30 September 1987

INVESTIGATORS

Robert G. Eagon, PhD*
Albert T. McManus, PhD
Basil A. Pruitt, Jr., MD, Colonel, MC

*Participant, US Army Summer Faculty Research and Engineering Program
Department of Microbiology
University of Georgia
Athens, Georgia  30602
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Outer membrane proteins approximating the molecular weights of proteins D1 and D2 were observed in wild-type, prototrophic PA irrespective of the growth medium, i.e., whether glucose, succinate, or casamino acids served as the carbon and energy sources. This is in contrast to previous reports that outer membrane protein D1 in PA was glucose-inducible and succinate-repressible. The significance of outer membrane protein D1 is that it is considered to function as a diffusion channel through the outer membrane of PA by the beta-lactam antibiotic, imipenem, which is considered to account for the susceptibility of PA to imipenem.

In an imipenem-resistant clinical isolate of PA, however, outer membrane proteins with molecular weights approximating that of outer membrane proteins D1 and D2 were repressed by growth on casamino acids but not by growth on succinate. Moreover, well-defined mutants of PA blocked in glucose catabolism, glucose transmembrane transport, or glucose periplasmic binding protein showed wild-type susceptibility to imipenem. That is, outer membrane protein D1 synthesis would have been expected to be repressed in these mutants if outer membrane protein D1 were genetically regulated similarly to systems that control glucose utilization. Thus, the data suggested that the acquisition of imipenem resistance in PA involved genetic factors other than those which are involved in glucose transport and glucose catabolism and in the synthesis of outer membrane protein D1.
STUDIES ON THE ROLE THAT OUTER MEMBRANE PROTEINS PLAY IN THE SUSCEPTIBILITY OF Pseudomonas aeruginosa (PA) TO IMIPENEM

INTRODUCTION

Imipenem is a broad spectrum, beta-lactam-type antibiotic that is especially effective against PA. This is surprising since PA is inherently resistant to a large variety of antibiotics, especially most beta-lactam antibiotics. According to current concepts, the outer membrane of PA acts as a diffusion barrier against antibiotics, thus preventing the antibiotics from reaching their physiological targets (1-4).

Hydrophilic solutes in general are considered to diffuse through specific proteins in the Gram-negative bacterial outer membrane which form water-filled diffusion channels. The trivial term, porin, has been coined for such proteins. The outer membrane of Escherichia coli, which has been intensively studied, has two major porins, OmpF and OmpC, with diameters of 1.2 and 1.1 nm, respectively (4). The major porin of PA, protein F, however, has a diameter of 2.0 nm. Thus, it would be expected that the outer membrane of PA would be more impermeable than that of Escherichia coli. Such is not the case. Instead, even though PA has about $3 \times 10^5$ copies of protein F in its outer membrane, <1% of the porins are considered to be in an open, functional state (1-4). This, then, is considered to account for the highly impermeable nature of the outer membrane of PA and, as a consequence, its intrinsic resistance to antimicrobial agents.

Imipenem is effective against PA because it is considered to be able to diffuse through outer membrane protein D1 in addition to outer membrane protein F. This supposition is based on recent observations that imipenem-resistant isolates of PA have reduced amounts of an outer membrane protein whose molecular weight approximates that of outer membrane protein D1 (5,6).

Evidence has been presented that PA outer membrane protein D1 is glucose inducible (7,8) and that it may function as a more or less specific diffusion channel for glucose and related sugars. Thus, outer membrane protein D1 may be physiologically analogous to the lamB gene product in Escherichia coli which functions as a specific diffusion channel for maltose and maltodextrin.

Glucose is catabolized in PA by the Entner-Doudoroff pathway, which is an inducible system. Moreover, glucose transport in PA is a shock-sensitive system, meaning that a periplasmic glucose binding protein is involved in addition to a specific glucose transport protein in the cell membrane. Both of the latter two proteins are also glucose inducible.
Organic acids appear to be the preferred energy source for PA and not carbohydrates. Succinate, for example, acts as a catabolite repressor of the glucose catabolic enzymes and transport proteins in PA (9).

Considerable information is available on the position of the genes and on the genetic regulatory factors for the transport and catabolism of glucose and related compounds in PA (9). Thus, the purpose of the studies described herein was to determine whether the synthesis of outer membrane protein D1 was genetically regulated similarly to that of the carbohydrate catabolic enzymes, transport proteins, and binding proteins in PA and to provide further information on the presumed role of outer membrane protein D1 as a diffusion channel for imipenem.

MATERIALS AND METHODS

Organisms. The strains of PA and their descriptions used in these studies are shown in Table 1.

Media. The organisms were grown in either Mueller-Hinton (MH) medium or in the chemically defined basal salt medium (EPBS) previously described (10) and supplemented, in final concentration, with 20 mM glucose (Glc), 20 mM sodium succinate (Suc), or 0.5% casamino acids (CA). As required, these media were solidified with 1.5% agar-agar (in final concentration).

Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC). MIC determinations were normally carried out by the Kirby-Bauer standard disk diffusion overlay technique. In some instances, however, the tube dilution technique was used to determine MIC and MBC. The chemotherapeutic agents used included amikacin (AM), gentamicin (GM), mezlocillin (MZ), piperacillin (PIP), tobramycin (NN), ticarcillin (TIC), moxalactam (Mox), cefotaxime (CTX), cefoperazone (CEP), cefsulodin (CEF), sulfadiazine (SD), azlocillin (AZ), kanamycin (K), chloramphenicol (C), tetracycline (TE), colistin (CL), netilmicin (NET), imipenem (IMP), norfloxacin (NOR), aztreonam (ATM), ticarcillin/clavulanate (TIM), ceftazidime (CAZ), ceftriaxone (CRO), and carbenicillin (CB).

Preparation of Outer Membranes of PA and Extraction of Outer Membrane Proteins. Prototrophic, wild-type PA (Strain PA01(PVP)) and an imipenem-resistant clinical isolate (Strain 87-05-11-004) were used in these studies. The cells were grown overnight in EPBS-Glc, EPBS-Suc, and EPBS-CA, harvested by centrifuging, washed once in EPBS, and harvested again by centrifuging. The cells were ruptured by sonicating as follows: 1 g wet weight of the cell pellets was suspended in 8.5 ml of EPBS minus the (NH₄)₂SO₄ component. Then, 0.5 ml of a mixture of DNase and RNase (2 mg/ml each) was added. The cell suspension was then placed in an ice bath and sonicated for 6 repetitions of 20 sec followed by 40 sec of cooling.
TABLE 1. Strains of Pseudomonas aeruginosa Used

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype/Phenotype</th>
<th>Derivative/Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>PA01(PVP)</td>
<td>Prototroph</td>
<td>From Phibbs' Collection</td>
</tr>
<tr>
<td>PFB311</td>
<td>glcT-4</td>
<td>PFB57; glc- (Spontaneous Revertant)</td>
</tr>
<tr>
<td>PFB331</td>
<td>glcT-2</td>
<td>PFB311; glc- (EMS Mutagenesis)</td>
</tr>
<tr>
<td>PFB360</td>
<td>glcB1</td>
<td>PFB311; glc- (EMS Mutagenesis)</td>
</tr>
<tr>
<td>PFB362</td>
<td>glcT-3</td>
<td>PFB311; glc- (EMS Mutagenesis)</td>
</tr>
<tr>
<td>PFB363</td>
<td>glcT</td>
<td>PFB311; glc- (EMS Mutagenesis)</td>
</tr>
<tr>
<td>A10</td>
<td></td>
<td>PFB311; glc- (EMS Mutagenesis)</td>
</tr>
<tr>
<td>MB723</td>
<td>trp gdh Tglc</td>
<td>See Reference 12</td>
</tr>
<tr>
<td>PA01(HN)</td>
<td>Prototroph</td>
<td>From Nikaido's Collection</td>
</tr>
<tr>
<td>87-05-15-004</td>
<td>Imipenem</td>
<td>Clinical Isolate (From McManus' Collection)</td>
</tr>
<tr>
<td>87-05-11-004</td>
<td>Imipenem</td>
<td>Clinical Isolate (From McManus' Collection)</td>
</tr>
<tr>
<td>1244 (ATCC 27317)</td>
<td>&quot;Standard Virulent Strain&quot;</td>
<td>Clinical Isolate (From McManus' Collection)</td>
</tr>
<tr>
<td>ATCC 27853</td>
<td>&quot;Standard Strain&quot;</td>
<td>American Type Culture Collection</td>
</tr>
</tbody>
</table>

Strains PA01(PVP), PFB311, PFB331, PFB360, PFB362, PFB363, A10, and MB723 were generously provided by Dr. Paul V. Phibbs, Jr., although Strain MB723 was originally acquired by Dr. Phibbs from another laboratory (12).

PFB311 is a spontaneous revertant of PFB57, i.e., glc- to glc+. For a description of PFB57, see reference 13.

Strains PFB331, PFB360, PFB362, PFB363, and A10 were developed by mutagenizing PFB311 with ethylmethanesulfonate (EMS). The parental strain, PFB311, was glc+ (phosphorylative pathway only), mannotol transport negative, glucose and gluconate dehydrogenase negative, gluconate transport negative, gluconokinase negative, and 2-ketogluconate negative.

PFB362 is suspected of having an inactive glucose periplasmic binding protein, whereas MB723 has been reported to be glucose periplasmic binding protein deficient.

A10 is glucose transport negative but the mutation is leaky.

PFB360 is also a leaky mutant.

MB723 is a tryptophane auxotroph, glucose dehydrogenase negative and glucose transport negative.

Strains 87-05-15-004 and 87-05-11-004 are imipenem-resistant clinical isolates from burn wound tissue.

Strain 1244 (ATCC 27317) was a clinical isolate, also originally from burn wound tissue, which is widely used in a control sense as the "standard virulent strain."

ATCC 27853 is a standard strain of PA from the American Type Culture Collection which is also widely used for control purposes.
Whole cells and cellular debris were removed by centrifuging at 4°C for 20 min at 7000 x g. Cell envelopes were then sedimented from the supernatant by centrifuging at 4°C for 45 min at 100000 x g. The cell envelopes were suspended in 10 ml of water and sedimented again at the same centrifugal force and time. Next, the cell envelopes were suspended in 0.15 ml of water and an equal volume of 1% sodium N-laurylsarcosine was added to selectively solubilize the cytoplasmic membrane (13). The suspensions were incubated at ambient temperature for 20 min. Outer membranes were collected by centrifuging at 4°C for 45 min at 100000 x g, washed once in 2.5 ml of water, and collected again by centrifuging in the same manner. This was essentially the same procedure used by others to prepare outer membranes from Gram-negative bacteria (14).

The outer membranes were then suspended in 0.1 ml of a solubilization reduction mixture of the following composition: 0.5 M tris(hydroxymethyl)aminomethane (Tris)-HCl, pH 6.8, 12.5 ml; 10% sodium dodecyl sulfate (SDS), 20 ml; mercaptoethanol, 5 ml; glycerol, 10 ml; and bromphenol blue to 0.001% (final concentration). The suspension was then placed in a boiling water bath for 3 min.

Electrophoresis. The outer membrane proteins were separated by SDS polycrylamide gel electrophoresis (SDS-PAGE) using 14% acrylamide (final concentration) in the separating gel. Electrophoresis was run for 9 h at 25-30 mA.

Molecular weight standards (Bio-Rad Laboratories, Richmond, CA) were: phosphorylase B, 97,400; bovine serum albumin, 66,200; ovalbumin, 42,699; carbonic anhydrase, 31,000; soybean trypsin inhibitor, 21,500; and lysozyme, 14,400.

RESULTS

Susceptibility of Various PA Strains to Imipenem. Susceptibility of the various strains of PA to imipenem when MH, EPBS-Glc, EPBS-Suc, and EPBS-CA were used both as the growth media and as the susceptibility test media is shown in Table 2. When MH medium, which is the accepted antibiotic susceptibility test medium, was used, only two clinical isolates (Strains 87-05-11-004 and 87-05-15-004) were found to be resistant.

The diameters of the zones of inhibition on the other three media were, with two exceptions, wider than those on MH medium for the various antibiotics. Nevertheless, the diameters of the zones of inhibition by imipenem were most narrow for the imipenem-resistant clinical isolates (Strains 87-05-11-04 and 87-05-15-004). Thus, it was concluded that the imipenem resistance patterns did not vary irrespective of what media were used for the growth and susceptibility test media. However, when zones of inhibition noted for media other than MH medium were compared, the evidence suggested that the clinical
TABLE 2. Susceptibility of the Various Strains of PA by Imipenem on MH, EPBS-Glc, EPBS-Suc, and EPBS-CA Media Determined by the Kirby-Bauer Disc Differentiation Overlay Technique

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<th>PA Strain</th>
<th>MH (mm)</th>
<th>EPBS-Glc (mm)</th>
<th>EPBS-Suc (mm)</th>
<th>EPBS-CA (mm)</th>
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NG = no growth, S = sensitive, R = resistant.

Isolates were somewhat more resistant to imipenem when EPBS-CA was used as the growth and test medium.

**MIC and MBC Values.** The MIC and MBC values were determined by the tube dilution method using EPBS-Glc and EPBS-CA as the test media for PA Strains PA01(PVP), ATCC 27853, 87-05-11-004, and 87-05-15-004. The results in Table 3 show that the MIC values were the same on both media for the wild-type organisms (Strains PA01(PVP) and ATCC 27853), but the MBC was higher for Strain ATCC 27853 on EPBS-Glc as compared to EPBS-CA while Strain PA01(PVP) showed no difference. Strain 87-05-11-004, however, had higher values for both the MIC and MBC in EPBS-CA.
TABLE 3. MIC and MBC Values of Imipenem (µg/ml) for Four Strains of PA on EPBS-Glc and EPBS-CA Media Determined by the Tube Dilution Method

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medium than in EPBS-Glc medium. Strain 87-05-15-004 had a higher MIC value in EPBS-CA than in EPBS-Glc, but the MBC values were the same for both media.

SDS-PAGE of Outer Membrane Proteins. The results of SDS-PAGE of outer membrane proteins from PA Strains PA01(PVP) and 87-05-11-004 when grown on EPBS-Glc, EPBS-Suc, and EPBS-CA are shown in Figure 1. No differences in the profile of outer membrane proteins of PA Strain PA01(PVP) were observed irrespective of the medium in which the organism was grown (Fig 1, Lanes 1-3).

PA Strain 87-05-11-004, on the other hand, had two outer membrane proteins missing when grown on EPBS-CA (Fig 1, Lane 6) as compared to cells grown on EPBS-Glc and EPBS-Suc (Fig 1, Lanes 4 and 5). These outer membrane proteins had molecular weights of approximately 47,000 and 52,000. As mentioned above, these outer membrane proteins were present in cells grown on EPBS-Glc and EPBS-Suc. Interestingly, the 47,000-dalton outer membrane protein separated into two bands, suggesting that it was a heat-modifiable protein. Regrettably, the photograph and its reproduction in Figure 1 is of such low quality that this cannot be clearly seen.

Zones of Inhibition with Aqueous 0.5% Casamino Acids Medium. Although not a planned series of studies for the research problem that was undertaken as described herein, certain results were obtained inadvertently that should be mentioned. In one series of studies, the basal salts were omitted from the media with casamino acids as the carbon and energy sources. Instead, a 0.5% aqueous solution of casamino acids was used, solidified with 1.5% agar-agar as appropriate. The basal salts that normally would have been incorporated into an EPBS-based medium are mono- and dibasic potassium phosphate, ammonium sulfate, and magnesium sulfate.

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FIGURE 1. SDS-PAGE of outer membrane proteins of PA Strain PA01(PVP) grown on EPBS-Glc (Lane 1), EPBS-Suc (Lane 2), and EPBS-CA (Lane 3), and of PA Strain 87-05-11-004 grown on EPBS-Glc (Lane 4), EPBS-Suc (Lane 5), and EPBS-CA (Lane 6). Lane 7 shows molecular weight standards.

When the various strains of PA were grown and tested on 0.5% aqueous casamino acids media, unexpected results were observed as compared to those obtained on EPBS-CA media (Table 4). Notably, the diameters of the zones of inhibition for the various antibiotics seen on 0.5% aqueous casamino acids were greater as compared to those seen on EPBS-CA. In many cases, organisms that would have been judged to have been susceptible to certain antibiotics on EPBS-CA appeared instead to be resistant when 0.5% aqueous casamino acids were used. In other cases, the reverse was observed.

Finally, when these results were compared to those obtained on MH medium, the approved antibiotic test assay medium, it was seen that results obtained from the use of both EPBS-CA and 0.5% aqueous casamino acids differed substantially from those seen when MH medium was used (Table 5).

The results shown in Tables 4 and 5 are too complex for further discussion. Instead, they are presented here to
TABLE 4. Comparison of the Inhibition of PA Strains by Antibiotics on Casamino Acids Media With and Without the Addition of Basal Salts

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( ) = Zones of inhibition when PA strains were grown in an aqueous 0.5% solution of casamino acids and then antibiotic inhibition determined on the same medium solidified with 1.5% agar-agar.
ND = Not done.
NG = No growth.
### TABLE 5. Comparison of the Inhibition of PA Strains by Antibiotics on Mueller-Hinton Media Determined by the Kirby-Bauer Standard Disk Diffusion Overlay Technique

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<td>25 ± 3</td>
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<td>T</td>
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</tbody>
</table>

S = Sensitive, I = Intermediate, R = Resistant, and ND = Not Done.
dramatically illustrate the importance of environmental factors in antibiotic susceptibility testing and to raise questions about the response of infectious microorganisms in the infected tissue environment of the host as compared to that under laboratory conditions. The results shown in Tables 4 and 5, therefore, should be carefully studied by those workers interested in susceptibility testing.

DISCUSSION

Outer membrane protein D1 of PA has been reported to be a glucose-inducible protein and to be repressed when the cells were grown on succinate (7,8). If true, outer membrane protein D1 in PA would be genetically similar to the glucose catabolic enzymes, glucose transport protein(s), and glucose periplasmic binding protein in PA, all of which are glucose-inducible and succinate-repressible (9). We did not find this to be the case. Instead, outer membrane proteins of molecular weights approximating that of outer membrane proteins D1 and D2 were formed in wild-type PA Strain PA01(PVP) irrespective of the carbon and energy sources in which it was grown, i.e., whether glucose, succinate, or casamino acids were the carbon and energy sources. Moreover, there was no perceptible change in imipenem susceptibility of wild-type PA strains PA01(PVP) and ATCC 27853 irrespective of the carbon and energy sources on which they were grown. This is important because, according to current concepts, imipenem is considered to be especially effective against PA because it is able to permeate the outer membrane through outer membrane protein/porin D1 which, as mentioned herein, has been reported to be glucose-inducible and repressed by growth on succinate or casamino acids (5-8).

The imipenem resistant clinical isolate of PA Strain 87-05-11-004 responded differently than the wild-type laboratory strains. Two outer membrane proteins of approximate molecular weights of 47,000 and 52,000 were not observed in cells grown on EPBS-CA medium. This is in agreement with reports by others that outer membrane protein D1 is repressed by growth on casamino acids (5-8). However, in contrast to reports by others (7,8), growth on succinate did not repress the formation of these outer membrane proteins. The clinical imipenem-resistant isolates, however, were more resistant to imipenem when grown on casamino acids, suggesting a role for outer membrane proteins D1/D2 as diffusion channels for imipenem as reported by others (5,6).

The data indicate, therefore, that there was a basic difference between wild-type strains of PA as compared to imipenem-resistant clinical isolates. Namely, outer membrane proteins of molecular weights similar to that of proteins D1/D2 were repressed in the imipenem-resistant isolates by growth on casamino acids, but not in the wild-type strains. However, growth on succinate did not repress these outer membrane
proteins in either an imipenem-resistant isolate or in a wild-type strain.

Several well-defined mutants of PA blocked in glucose transport or in glucose catabolism were not resistant to imipenem. Instead, they showed wild-type susceptibility to imipenem. It would have been expected that these mutants would be lacking in outer membrane protein D1 if the synthesis of this protein was regulated similarly to that of the glucose catabolic enzymes, of the glucose cell membrane transport protein, and of the glucose periplasmic binding protein. Our data strongly suggest, however, that such was not the case.

Finally, the imipenem-resistant clinical isolates of PA used in these studies appeared to differ substantially from the wild-type, prototrophic strains in the synthesis and thus in genetic regulatory factors of the two outer membrane proteins whose molecular weights approximated that of outer membrane proteins D1 and D2 of PA.

PRESENTATIONS/PUBLICATIONS

None.

REFERENCES


RESEARCH COMPLETION REPORT

PROJECT NUMBER: 3M161102BS14-00, BASIC RESEARCH

PROJECT TITLE: ALTERATION OF HOST RESISTANCE IN BURNED SOLDIERS: Therapy with IgG and T4 in Burn Patients

US ARMY INSTITUTE OF SURGICAL RESEARCH
FORT SAM HOUSTON
SAN ANTONIO, TEXAS 78234-5012

1 October 1986 - 30 September 1987

INVESTIGATORS

Anne E. Missavage, MD, Major, MC
George M. Vaughan, MD, Lieutenant Colonel, MC
Albert T. McManus, PhD
David G. Burleson, PhD, Major, MS
William F. McManus, MD, Colonel, MC
Basil A. Pruitt, Jr., MD, Colonel, MC
Arthur D. Mason, Jr., MD
ABSTRACT

PROJECT NUMBER: 3M161102BS14-00, BASIC RESEARCH

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INSTITUTION: US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas 78234-5012

PERIOD COVERED IN THIS REPORT: 1 Oct 86 through 30 Sep 87

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David G. Burleson, PhD, Major, MS
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Basil A. Pruitt, Jr., MD, Colonel, MC
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The possible prophylactic effects of exogenously administered IgG were evaluated in a cohort of 69 seriously burned patients. Treated patients were given 500 mg/kg twice a week starting 2-5 days postburn. Patients were treated for a minimum of 2 wk or until death or wound coverage. Control patients were resuscitated under the same protocol but did not receive IgG. Group assignment was by randomization of admissions having initial predicted survival probabilities between 20 and 80%. The resulting groups were similar in total burn, full-thickness burn, presence of inhalation injury, age, sex, and pretreatment serum IgG levels. Infections and mortality were recorded during the 60 days following injury. At 60 days postburn, 64 infections had occurred in 26 treated patients and 7 in control patients. Final mortality was 11 treated patients and 9 control patients. No significant improvement in mortality or reduction in infections could be attributed to the use of intravenous gamma globulin.
THERAPY WITH IgG AND T4 IN BURN PATIENTS

INTRODUCTION

In thermally injured subjects, relentless sepsis remains a major problem despite the availability of potent antibiotics and major advances in the areas of fluid therapy, nutritional support, and burn wound management. Profound suppression of humoral and cellular immune responsiveness and of neutrophil function represent characteristic defects in the defense mechanisms of burn patients. This state of acquired immune deficiency in burn patients is somewhat akin to the depressed immune status of patients with primary immune deficiency syndromes and manifests itself as deficiency in the cell-mediated and humoral components of immunity; patients in both groups are susceptible to infection (1,2). For two decades, IgG replacement in patients with immune deficiency syndromes has been recognized as the mainstay of prophylaxis and has distinctly reduced the incidence of infection in these individuals. In burn patients, the lack of a protective skin barrier and the use of invasive monitoring enhance the risk of colonization of deeper tissues with a wide variety of organisms. Associated defects in the immune defenses may further aid the evolution of infectious processes. This study proposed immunomodulation of burned patients by IgG replacement during the first 4 wk postburn when concentrations of endogenous IgG in these subjects were subnormal and they were prone to systemic infection.

MATERIALS AND METHODS

This unblinded, randomized study evaluated the efficacy of exogenously administered IgG and T4 for prophylaxis of infection in burn patients. Adult patients were offered admission to the study if, for that particular patient's age, the total burn size was such that the probability of death was between 20 and 80%, based on previous logistic regressions over a large population of burn patients receiving about the same general care as our patients were expected to receive.

Inclusion Criteria. Burn patients of either sex >18 yr of age with a probability of mortality between 20 to 80% admitted to this Institute during the first 5 days postburn were considered eligible for this study.

Exclusion Criteria. Patients under the age of 18, females of childbearing age with a positive pregnancy test, patients with <20% or >80% chance of mortality (based on age and total burn size), and those admitted to this Institute more than 5 days postburn were not eligible for this study.

Design. Each patient was randomized into the IgG treatment group (those who receive IgG) or the IgG control group (those
who do not receive IgG). This protocol originally included a thyroid hormone replacement component for selected randomized hypothyroid patients. However, because of a very low accretion due to a lack of patients meeting the inclusion criteria, this component was discontinued. IgG therapy (500 mg/kg IV infused over 4 hr twice weekly for 2 wk) began between the second and fifth postburn days. IgG administration was advanced to the immediate postoperative period for patients undergoing surgery.

**Hormonal, Biochemical, and Immunological Monitoring.** From the time of initial randomization, blood samples were obtained twice weekly (just before the IgG infusion in the group receiving IgG) for hormonal, biochemical, and immunological monitoring. Indices of cellular and humoral immunity, bacteriologic and virologic surveillance, and hormonal responses were examined twice weekly over a 4-wk period. However, patients with persistently abnormal findings often required further investigation beyond 4 wk.

**Cellular and humoral immunity.** Indices of immune responsiveness included measurements of T-cells and their subsets and B-cells and their subsets by flow cytometry using the immunofluorescence technique, polyclonal lymphocyte activation by standard mitogens, and measurements of total and differential leukocyte count as well as chemiluminescence.

**Bacteriologic and virologic surveillance.** Surveillance for infectious agents included bacteriologic (blood, urine, and sputum cultures) and virologic (urine and throat washings for cytomegalovirus culture and antibody titers) tests. Blood cultures were drawn only when clinically indicated.

**Hormonal responses.** Hormonal response testing included catechols, steroids, renin-angiotensin-aldosterone, vasopressin, and thyroid hormones (T4, T3, rT3' TSH, T3U).

Immunglobulins were analyzed by nephelometry (8). A fluorescence-activated cell sorter was employed to separate various lymphocyte subpopulations (9) and monoclonal cells (10). Chemiluminescence was measured as previously described (11). Bacteriologic and viral isolation was performed by standard methods. Hormones were measured by RIA with standard kits (thyroid hormones, cortisol, aldosterone, PRA) and by procedures developed at this Institute (angiotensin I and II).

**RESULTS**

A comparison of demographic characteristics of treated and control patients is presented in Table 1. The randomization resulted in two comparable groups. Measurement of serum IgG prior to treatment, or within the first 5 days postburn for the control group, showed mean values of 421.2±33.8 and 382.4±38.6 mg/dl, respectively. A value of 800 mg/dl is considered the lower limit of the normal range for adults.
TABLE 1. General Information

<table>
<thead>
<tr>
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<th>Treatment Group</th>
<th>Control Group</th>
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</thead>
<tbody>
<tr>
<td>Number of Patients</td>
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<td>34</td>
</tr>
<tr>
<td>Sex (Male/Female)</td>
<td>27/8</td>
<td>31/3</td>
</tr>
<tr>
<td>Age (Mean Yr)</td>
<td>43</td>
<td>44</td>
</tr>
<tr>
<td>Total Burn Size (Mean %)</td>
<td>45</td>
<td>47</td>
</tr>
<tr>
<td>3° Burn Size (Mean %)</td>
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<td>22</td>
</tr>
<tr>
<td>Inhalation Injury (Cases)</td>
<td>18</td>
<td>20</td>
</tr>
<tr>
<td>Survivors</td>
<td>18</td>
<td>19</td>
</tr>
<tr>
<td>Nonsurvivors</td>
<td>11</td>
<td>9</td>
</tr>
</tbody>
</table>

The distribution of the weekly mean maximum serum IgG values are presented in Figure 1. Treated patients had IgG values within normal limits by the first week postburn and control patients had synthesized IgG to within normal limits by the second week postburn.

A comparison of infections that occurred within the first 30 days is presented in Table 2. A comparison at 60 days is presented in Table 3. No differences in the number of sites or infections were noted. As shown in Figure 2, the types of organisms causing infections were also similar between the two groups.

Mortality data are presented in Table 4. As can be seen, no differences in mortality between the groups were observed. No reduction in infection or mortality could be attributed to the use of intravenous gamma globulin.

DISCUSSION

Overwhelming sepsis significantly contributes to the mortality of patients with extensive burns (12). A multitude of perturbations encountered in the immunologic defenses of burn patients point to the immunosuppressive nature of burn injury. Burn-induced changes in the humoral and cellular responses include hypogammaglobulinemia (13), reduction in the complement levels (14), depressed serum opsonic activity (15), decreased granulocyte chemotaxis (16), anergy to recall antigens (17), prolonged survival of skin allograft (18), depressed response in autologous mixed lymphocyte culture, and decreased OKT4/OKT8 ratio (19). In the past, encouraged by the favorable
FIGURE 1. Maximum weekly 1gG concentration.
TABLE 2. Comparison of Infections Observed in Treatment and Control Patients at the End of 30 Days' Hospitalization (Infections/Patients)

<table>
<thead>
<tr>
<th></th>
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<tr>
<td>Pneumonia</td>
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<td>22/21</td>
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<tr>
<td>Bacteremia</td>
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<td>12/11</td>
</tr>
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<td>Urinary Tract Infection</td>
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<td>4/4</td>
</tr>
<tr>
<td>Cellulitis</td>
<td>7/7</td>
<td>7/7</td>
</tr>
<tr>
<td>Burn Wound Invasion</td>
<td>1/1</td>
<td>3/3</td>
</tr>
<tr>
<td>Other</td>
<td>3/3</td>
<td>4/4</td>
</tr>
</tbody>
</table>

**TOTAL NUMBER OF INFECTIONS** 48 52

TABLE 3. Comparison of Infections Observed in Treatment and Control Patients at the End of 60 Days' Hospitalization (Infections/Patients)

<table>
<thead>
<tr>
<th></th>
<th>Treatment Group</th>
<th>Control Group</th>
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</thead>
<tbody>
<tr>
<td>Pneumonia</td>
<td>27/20</td>
<td>29/31</td>
</tr>
<tr>
<td>Bacteremia</td>
<td>15/12</td>
<td>20/15</td>
</tr>
<tr>
<td>Urinary Tract Infection</td>
<td>8/7</td>
<td>6/5</td>
</tr>
<tr>
<td>Cellulitis</td>
<td>8/7</td>
<td>8/8</td>
</tr>
<tr>
<td>Burn Wound Invasion</td>
<td>3/3</td>
<td>3/3</td>
</tr>
<tr>
<td>Other</td>
<td>3/3</td>
<td>4/4</td>
</tr>
</tbody>
</table>

**TOTAL NUMBER OF INFECTIONS** 64 70

194
FIGURE 2A. Organisms causing infections (control group).

FIGURE 2B. Organisms causing infections (treatment group).
TABLE 4. Comparison of Mortality Between Treated and Control Patients (Dead/Alive)

<table>
<thead>
<tr>
<th>Treatment Group</th>
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<td>30 Days</td>
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<tr>
<td>60 Days</td>
<td>10/25</td>
</tr>
<tr>
<td>TOTAL DEATHS</td>
<td>11/24</td>
</tr>
</tbody>
</table>

experience observed in patients with immune deficiency syndromes (2), two clinical trials in burn patients (20,21) employed IgG for prophylaxis of infection with apparently contradictory results. IgG administered in sufficient amounts in the early postburn period was credited with improved survival in children (20). However, in adult patients, IgG therapy in relatively small doses administered during a variable postburn period failed to show any beneficial effects (21). During the past, clinical trials of IgG in burn patients has been impeded on two counts, by nonexistence of a suitable IgG preparation for intravenous use and by a lack of information about the kinetics of infused IgG in burn patients. Currently available IgG preparations are completely devoid of the vasomotor and other side effects reported with older products. This has been achieved by the modification of IgG through a process of reduction and alkylation (Gammimune, Cutter Biological) and by reduction alone (IGIV, pH 4.25, Cutter Biological). This latter preparation, however, preserves more than 90% of the IgG molecule in its native monomer form. In recent kinetic studies, we have demonstrated that twice weekly IgG infusions given in a dose of 500 mg/kg are sufficient to normalize serum IgG concentration in burn patients (22). Within 48 hours of thermal injury, serum IgG is at its lowest concentration, and thereafter gradually returns to normal over a 3- to 4-wk period (12). Based on this state of knowledge, it was reasonable to test whether immunomodulation of burn victims with IgG would aid in reduction of infection and thus improve survival in these immunocompromised individuals. For therapeutic interventions aimed at prophylaxis, it was imperative that such therapy be instituted during the period of extreme vulnerability which precedes clinical manifestations of established sepsis. For this reason, patients in this study received IgG therapy during the early postresuscitation period when concentrations of their endogenous IgG were subnormal. This study attempted to characterize modifications produced in burn-induced immunologic and endocrine responses and their correlation with subsequent development of sepsis in the study population.
REFERENCES


RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

NAME
US Army Institute of Surgical Research

ADDRESS
Fort Sam Houston
San Antonio, Texas 78234-2012

TELEPHONE NUMBER
512-221-5416

NAME OF RESPONSIBLE INDIVIDUAL
VAUGHAN, J M

NAME OF ASSOCIATE INVESTIGATOR

NAME OF PRINCIPAL INVESTIGATOR

TYPE OF RESEARCH
11. TITLE (Provide with Security Classification Code)
(U) Role of Thyroid Hormones in Burn Pathophysiology

SUBJECT AREAS
06 09 Medicine and Medical Research 06 01 Biochemistry

DATE EFFECTIVE
17 CONTRACT/GRANT NUMBER
61102A 3M616107BS11

ROLE RELEVANCY CERTIFICATION

DATE COMMENCEMENT
86 10 01

DATE COMPLETION
86 10 01

PROJECT NUMBER
BD

SUMMARY
In another rat study (no T4 treatment), serum T4 means in thyroidectomized rats (P < 0.001) was not different between burned and thyroidectomized rats. TSH was markedly lower in burned rats than in thyroidectomized rats (P < 0.001). In another rat study (no T4 treatment), serum T4 was depressed in burned rats (vs. sham-burned rats) by 24 h postburn. Hormone values in sham-burned and normal rats were similar. At
48 h, T4 exhibited a partial return toward normal (though still depressed), only to regress further to very low levels by postburn days 7 and 14. Serum T3 remained depressed in burned rats at all time points. Whereas TSH was able to respond in a qualitatively normal fashion by 24 h, this elevation above normal was lost by 48 h. TSH fell to depressed levels thereafter in a pattern parallel with T4. The major resetting of TSH control in burned rats was evident only after the previously reported time of appearance (by 48 h) of ectopic supraependymal neurons on the floor of the third ventricle seen by scanning electron microscopy of the medial basal hypothalamus. Relative TSH deficiency occurs in burn injury and is associated with augmented negative feedback, allowing even low levels of thyronines to restrict elevation of serum TSH. This may reflect involvement of the central nervous system.
ANNUAL RESEARCH PROGRESS REPORT

PROJECT NUMBER: 3M161102BS14-00, BASIC RESEARCH

PROJECT TITLE: ROLE OF THYROID HORMONES IN BURN PATHOPHYSIOLOGY: Control of Thyrotropin (TSH) After Burn Injury

US ARMY INSTITUTE OF SURGICAL RESEARCH
FORT SAM HOUSTON
SAN ANTONIO, TEXAS 78234-5012

1 October 1986 - 30 September 1987

INVESTIGATORS

George M. Vaughan, MD, Lieutenant Colonel, MC
Khan Z. Shirani, MD, Lieutenant Colonel, MC
Mary K. Vaughan, PhD*
Sandy H. Coggins
David E. Scott, PhD, Colonel, USAR**
Basil A. Pruitt, Jr., MD, Colonel, MC

*Department of Cellular and Structural Biology
The University of Texas Health Science Center
San Antonio, Texas 78284-7877

**Department of Anatomy and Cell Biology
Eastern Virginia Medical School
Norfolk, Virginia 23501
ABSTRACT

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Previous studies in burned humans identified a relative inadequacy of TSH secretion, which might be viewed functionally as an altered control setting for negative feedback. To assess this possibility, male adult Sprague-Dawley rats with a 50% full-thickness scald burn (BURN) received a continuous subcutaneous thyroxine (T4) infusion via osmotic pumps at one of two dose levels (or no T4) for 6 days prior to sampling on postburn day 8. Thyroidectomized (THYREX) rats received the same T4 treatment, which raised the low T4 in BURN and in THYREX to or above normal (dose related). High TSH in THYREX and low normal mean TSH in BURN (without subcutaneous T4) were both lowered by T4 (dose related). Among dose groups, serum T4 means in THYREX encompassed the range of mean T4 in BURN. For a given serum T4 (including that in the normal range where triiodothyronine (T3) was not different between BURN and THYREX), TSH was markedly lower in BURN than in THYREX (P<0.001). In another rat study (no T4 treatment), serum free thyroxine index (FT4I) was depressed in BURN (vs. sham-burn) by 24 h postburn. At 48 h, FT4I exhibited a partial return toward normal (though still depressed), only to regress further to very low levels by days 7 and 14. Serum T3 remained depressed in BURN at all time points. Whereas TSH was able to respond in a qualitatively normal fashion by 24 h, this TSH elevation above normal was lost by 48 h. TSH fell (to depressed levels) thereafter in a pattern parallel with FT4I. The major resetting of TSH control in BURN was evident only after the appearance of ectopic supraependymal neurons on the floor of the third ventricle noted at and after 48 h on scanning electron microscopy of the medial basal hypothalamus.

Relative TSH deficiency occurs in burn injury, associated with augmented negative feedback, allowing even low levels of...
thyronines to restrict elevation of serum TSH. In rats, this correlates in time with morphologic changes in the hypothalamus, suggesting involvement of the central nervous system.
CONTROL OF THYROTROPIN (TSH) AFTER BURN INJURY

INTRODUCTION

Though circulating thyroxine (T4) and triiodothyronine (T3) may be depressed in patients with burns or other nonthyroidal illness, and more severely depressed in nonsurvivors, the behavior of serum TSH is not well characterized. With use of a highly sensitive and specific two-site immunoradiometric assay, we previously were able to identify an abnormality of the serum TSH pattern in severely burned patients (1). Though mean T4 and T3 and their free concentrations were depressed already by postburn days (PBD) 2-3, mean TSH was in the normal range and rose only to the upper normal limit in survivors as T4 and T3 gradually returned to normal. However, all these hormones fell progressively in nonsurvivors. While the data were compatible with augmented peripheral thyronine disposal, a TSH deficiency was also indicated by the falling TSH in nonsurvivors and by the many normal values of TSH in the first week in survivors despite low free T4 and T3. If there were no abnormality of TSH secretion, one would expect a marked rise in serum TSH when thyronines are low and providing less negative feedback. Relative inadequacy of TSH secretion, less marked in survivors, might be viewed functionally as an altered control setting for negative feedback. This hypothesis was tested in the present studies.

MATERIALS AND METHODS

For the first study, adult male Sprague-Dawley rats were given a full-thickness scald burn (BURN) according to a standardized procedure (including hair clipping in the dorsal and ventral areas to be burned) under sodium pentobarbital anesthesia (2). The burn covered 50% of the total body surface area. Since burning is known to produce low serum T4, other rats for comparison were thyroidectomized (THYREX) under similar anesthesia (but not burned) and maintained with 0.9% calcium chloride in the drinking water. All other rats were given tap water. All rats ate rodent chow ad libitum. A group of 5 controls received no treatment (CON) and served to indicate the normal values of thyroid hormones. Both the BURN and THYREX rats were divided into 3 subgroups (5 rats each), receiving, respectively, no infusion (T4 INF = 0), T4 infusion at 1.1 µg/100 g/day (T4 INF = 1), or T4 infusion at 11.0 µg/100 g/day (T4 INF = 2). Rats received T4 as a continuous infusion from osmotic minipumps implanted subcutaneously under sodium pentobarbital anesthesia, which remained in the dorsal neck region for 6 days up to the time of sacrifice and sampling at postburn day 8 or post-thyroidectomy day 12. At this time, rats were anesthetized with sodium pentobarbital, opened ventrally, and exsanguinated by central venipuncture for assay of T4, T3, and TSH.
For the second study, rats were scalded (BURN) as for the first study, except that the burn size was 60% of the total body surface area, and other rats received anesthesia and clipping but no burn (SHAM). For this study, there were no T4 infusions, no thyroidectomized groups, and BURN and SHAM rats were sacrificed by guillotine in groups of 5 rats at 6 h, 24 h, 48 h, 7 days, and 14 days after burn. CON (9 rats) received no procedure except sacrifice at various times along with the groups of BURN and SHAM rats. Trunk serum was saved for analysis of T4, T3, in vitro radiotracer T3 uptake from serum onto charcoal (T3U), and TSH. The medial basal hypothalami were prepared for scanning electron microscopy. Those results, together with thyroid hormone values, were reported previously (3). However, the crucial TSH values were not then available, and herein they are presented along with an estimate of the bioavailable T4. Thus, a cohesive interpretation of central control of the thyroid axis is now possible from this study.

During both studies, the rats were sampled at 0800-0900 h (except for one group 6 h later after burning at 0800 h in the second study). Thyroid hormones and T3U were measured with RIA kits from Diagnostic Products. TSH was measured by RIA, with the antibody and standard supplied by the National Institute of Arthritis and Metabolic Diseases (Bethesda, MD), with sample volumes of 200 μl. The free T4 index (FT4I), the product of T4 and the T3U, is a measure of the circulating T4 available to tissues. Data were analyzed on the VAX 11/780 with the BMDP software package from the University of California (Los Angeles, CA) (4). In the first study, for a given T4 INF, group intercomparisons were made by Bonferroni-corrected t-tests, and a given hormonal variable versus T4 INF was tested for rectilinear correlation within BURN or THYREX. In the second study, an hormonal variable at a given postburn time was compared by Bonferroni-corrected t-test (BURN vs. CON).

RESULTS

Figure 1 shows that infusion of T4 for 6 days can replace low circulating T4 after BURN or THYREX in a dose-related fashion. T3 was not fully normalized. TSH was depressed by T4 infusion in both BURN and THYREX in a dose-related fashion. However, as also shown in Figure 2 with TSH as a function of T4, TSH was much lower in BURN than in THYREX for a given T4 value. Of particular importance, neither T4 nor T3 were statistically different between THYREX after T4 INF = 1 and BURN after T4 INF = 2, and this mean T4 (5 μg/dl) was nearly identical to that of CON. Nevertheless, at this normal T4 level, TSH in BURN was markedly below TSH in CON and in THYREX.

Figure 3 shows that T4 is depressed already by 6 h postburn, and such a rapid fall probably results from accelerated disposal of T4, perhaps due partly to extravascular shifts of circulating constituents diluted by the 30 ml (0.15 M
FIGURE 1. Circulating thyroid axis hormones (mean ± SE) in rats of the first study at 12 days after thyroidectomy (THYREX) or 8 days after a full-thickness burn (BURN) of 50% of the total body surface area (n = 5/group). One group had no treatment and are represented by the box with a vertical extent of the mean ± 2 SD. In THYREX and BURN groups, T4 was infused (INF) in saline from implanted osmotic pumps at one of two concentrations (1 = 1.1 μg/100 g/day; 2 = 11 μg/100 g/day) or pumps were not implanted (T4 INF = 0). Pumps were present for 6 days up to the time of sampling for rats with implanted pumps (T4 INF = 1 or 2). *P<0.05, **P<0.01, ***P<0.001 vs. controls. +++P<0.01, +++P<0.001 vs. BURN. LD, least detectable TSH. T4, T3, and TSH (THYREX) and T4 and TSH (BURN) correlated significantly (P<0.05 or better) with T4 INF (0-2), negatively for TSH and positively for T4 and T3. T3 did not change with T4 INF in BURN.
FIGURE 2. Data from Figure 1 relating TSH and T4 (mean ± SE). The T4 infusion number (0-2) appears next to the symbols. THYREX, thyroidectomized; CON, controls. *P<0.05, **P<0.01, vs. CON for groups with similar T4 means. T3 means did not differ among THYREX and burn groups with T4 INF of 1 or 2, but T3, both in THYREX (INF 1) and in BURN (INF 2), was lower than the mean T3 in CON (see Fig 1). Thus, the comparison between BURN and THYREX indicates that for equivalent levels of T4, TSH is lower in BURN than in THYREX (equivalent T3) or in CON (in spite of higher T3 in CON). Thus, negative feedback of T4 on TSH secretion appears reset at a lower setpoint (stronger effect) for TSH after burn injury than in the normal pituitary-thyroid axis (THYREX).
FIGURE 3. Serum FT4I and TSH (mean ± SE) in controls (CON), sham-burned (SHAM), and 60% total body surface area full-thickness burned (BURN) rats at various postburn days (PBD) in study 2. There were 5 rats/group. Enough serum from only 2 BURN rats was available for TSH determination at PBD 0.25 (6 h). **P<0.01, ***P<0.001 vs. CON. The qualitatively normal TSH elevation in response to low FT4I in burns at PBD 1 is not seen on PBD 2, and TSH becomes depressed at PBD 7 and 14 despite FT4I approaching the level seen on PBD 1. The resetting of TSH secretion to lower levels on PBD 2 in BURN was accompanied by appearance of ectopically placed supraependymal neurons above the medial basal hypothalamus present only on PBD 2, 7, and 14.
sodium chloride) resuscitation injection given intraperitoneally just before the ventral burn plus any imbibed water. Such a mechanism for the initial fall of T4 (and T3) has yet to be documented. The fall of T4 was greater by 24 h, when TSH became elevated, perhaps producing a secondary rise in FT4I noted at 48 h. By 48 h, TSH had fallen to normal and subsequently fell further in parallel with a fall of T4 and FT4I. Ectopic supraependymal neurons were seen in the third cerebral ventricle just above the medial basal hypothalamus only in BURN beginning only at 48 h.

DISCUSSION

The lower TSH concentration in BURN (PBD 8) for a given altered circulating T4 level compared with that for THYREX (Fig 2) suggests that burn injury alters the feedback setting for T4 control of TSH secretion. The range of T4 means in THYREX encompassed that in BURN. The known approximately 35% elevation of the T4 dialyzable fraction in BURN rats at this time point would not have elevated even free T4 concentration of the BURN rats without T4 infusion to that in CON, and FT4I and free T4 are depressed at PBD 8 and 14 (5). Thus, even without T4 infusion, TSH is dramatically lower in BURN than it should be as indicated by TSH in THYREX for an equivalent T4 or for any other THYREX T4 in the range exhibited in BURN groups.

In low-T4-nonthyroidal illness, the FT4I appears to represent a better index of the concentration of circulating T4 available to cells than does the free T4 concentration by dialysis (6). The time course of thyroid hormone changes after a large burn injury (Fig 3) indicates that FT4I (and T4, not shown) fall by 6 h, perhaps as a result of accelerated peripheral disposal, though the precise disposal factors altered are not yet clarified; and that after a secondary small rise of FT4I (and T4, not shown) but still below normal at 48 h, they fall again further over about 2 wk. T3 (not shown) is low at all time points (3). TSH is able to rise in a qualitatively normal fashion by 24 h. However, after this, by 48 h, this elevation of TSH is lost. This indicates that by 48 h, the major resetting of the control of TSH (no longer able to respond to low T4 and T3, as if the negative feedback effect of thyronines is exaggerated) has taken place. The temporal coincidence of this event with the appearance of ectopic supraependymal neurons above the hypothalamus suggests that it is associated with a change in CNS function. The most likely change would seem to be a possible reduction of hypothalamic support of pituitary thyrotrophs normally exerted by secretion of TSH-releasing hormone (TRH). Such a deficiency of TRH could explain what would appear as overly effective negative feedback by thyronines on TSH secretion.
PRESENTATIONS/PUBLICATIONS


REFERENCES


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5. READING: R
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7. LEVEL OF SLM: A
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   b. ADDRESS: Fort Sam Houston
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   f. TELEPHONE NUMBER: 512-221-2705

19. RESPONSIBLE DOD ORGANIZATION:
   a. NAME: US Army Institute of Surgical Research
   b. ADDRESS: Fort Sam Houston
   c. CITY: San Antonio
   d. STATE: Texas
   e. ZIP CODE: 78234-5012
   f. TELEPHONE NUMBER: 512-221-2705

20. PERFORMING ORGANIZATION:
   a. NAME: US Army Institute of Surgical Research
   b. ADDRESS: Fort Sam Houston
   c. CITY: San Antonio
   d. STATE: Texas
   e. ZIP CODE: 78234-5012
   f. TELEPHONE NUMBER: 512-221-2705

21. GENERAL USE:
   a. NAME: FINA
   b. ADDRESS: Fort Sam Houston
   c. CITY: San Antonio
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23. TECHNICAL OBJECTIVE:
   24. APPROACH:
   25. PROGRESS:

22. (U) Lab Animals; (U) Sheep; (U) PAl

23. (U) To evaluate the effect of smoke inhalation on pulmonary ventilation and perfusion. To study the effects of positive end-expiratory pressure and oxygen on pulmonary ventilation-perfusion ratio. To study the role of the coexisting system in the respiratory insufficiency following smoke inhalation. A literature search was conducted.

24. (U) Ventilation-perfusion ratios will be measured utilizing the six-inert gas technique. These pulmonary variables will be correlated with standard cardiopulmonary variables before and after the introduction of inhalation injury and subsequent treatment with different types and volumes of resuscitation fluid. Lung and prefemoral lymph will be collected and a specific gravity method for estimation of extravascular lung water volume will be used to assess the pathophysiologic mechanisms of pulmonary edema formation after smoke inhalation. A platelet-activating factor antagonist will be used to estimate the role of platelet-activating factors in edema formation following smoke inhalation.

25. (U) 8610 - 3709. Ventilation-perfusion alterations following smoke inhalation injury have been established. Techniques for collecting lung and prefemoral lymph have been developed and studies of a specific gravity method for estimating extravascular lung water volume have been initiated.
ANNUAL RESEARCH PROGRESS REPORT

PROJECT NUMBER: 3M161102BS14-00, BASIC RESEARCH

PROJECT TITLE: INEQUALITY OF VA/Q RATIOS FOLLOWING SMOKE INHALATION INJURY AND THE EFFECT OF ANGIOTENSIN ANALOGUES: Effects of Positive End-Expiratory Pressure (PEEP) and Oxygen on Ventilation-Perfusion (VA/Q) Distribution After Smoke Inhalation Injury

US ARMY INSTITUTE OF SURGICAL RESEARCH
FORT SAM HOUSTON
SAN ANTONIO, TEXAS 78234-5012

1 October 1986 - 30 September 1987

INVESTIGATORS

Takeshi Shimazu, MD
Hisashi Ikeuchi, MD
Gene B. Hubbard, DVM, MS, Lieutenant Colonel, VC
Avery A. Johnson, Jr., BS
Arthur D. Mason, Jr., MD
Basil A. Pruitt, Jr., MD, Colonel, MC
ABSTRACT

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INSTITUTION: US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas 78234-5012

PERIOD COVERED IN THIS REPORT: 1 Oct 86 through 30 Sep 87

INVESTIGATORS: Takeshi Shimazu, MD, Hisashi Ikeuchi, MD, Gene B. Hubbard, DVM, Lieutenant Colonel, VC, Avery A. Johnson, Jr., BS, Arthur D. Mason, Jr., MD, Basil A. Pruitt, Jr., MD, Colonel, MC

To evaluate respiratory management after smoke inhalation injury, effects of PEEP alone (Part I) and oxygen with or without PEEP (Part II) on cardiopulmonary function and VA/Q ratio were studied. In Part I, various severities of smoke inhalation injury were produced in 15 sheep and the effects of 10 cmH\textsubscript{2}O PEEP were evaluated under general anesthesia using mechanical ventilation with an Fi\textsubscript{2}O of 0.21 either 12 or 72 h after smoke exposure. There were no significant differences, at 12 or 72 h, in the effects of PEEP; PEEP increased dead space ventilation but did not improve PaO\textsubscript{2} on the whole, and exerted no significant effect on the shunt (VA/Q=0) or low (0<VA/Q<0.1) VA/Q compartments. In Part II, 12 sheep received severe smoke inhalation injury. Baseline measurements were made at 24 h using mechanical ventilation and an FIO\textsubscript{2} of 0.21 without PEEP. Six sheep were then ventilated with 100\% oxygen without PEEP and the remaining six with 100\% oxygen with 10 cmH\textsubscript{2}O PEEP and a second set of measurements was made 30 min later. Oxygen alone converted the low VA/Q compartment into the true shunt and the average PaO\textsubscript{2} rose minimally from 54.3 to 101 torr. This change was not completely prevented by 10 cmH\textsubscript{2}O PEEP; three sheep (responders) showed PaO\textsubscript{2} above 300 torr during treatment while the remaining three (nonresponders) had a PaO\textsubscript{2} below 150 torr. Mechanical obstruction of the major bronchi by pseudomembrane was more prominent in the nonresponders and resulted in diffuse atelectasis.

Part I thus showed unfavorable short-term effects of PEEP after smoke inhalation injury, suggesting that PEEP...
overinflated alveoli that were already open rather than opening collapsed alveoli. Part II showed that PEEP can not totally prevent oxygen atelectasis and suggests that bronchoscopic removal of the pseudomembranes from major bronchi may be crucial to successful oxygen therapy with PEEP after smoke inhalation injury.
EFFECTS OF POSITIVE END-EXPIRATORY PRESSURE (PEEP) AND OXYGEN ON VENTILATION-PERFUSION (VA/Q) DISTRIBUTION AFTER SMOKE INHALATION INJURY

INTRODUCTION

Although smoke inhalation injury is one of the primary determinants of mortality following smoke inhalation injury, the pathophysiologic mechanism of such injury is not yet clear. Treatment of the injury remains controversial, especially in regard to the use of steroids and anti-inflammatory agents. On the other hand, higher concentrations of oxygen and PEEP are well accepted as treatment for hypoxic patients after smoke inhalation, although there have been few studies of the cardiopulmonary effects of oxygen and PEEP in inhalation injury (1-3).

We have developed a dose-responsive model of smoke inhalation injury in which we have investigated pathophysiologic alterations in cardiopulmonary function, including VA/Q ratio, and demonstrated development of a low VA/Q (0<VA/Q<0.1) compartment as hypoxia progressed (4-6). In the present study, we have evaluated respiratory management of smoke inhalation injury in terms of acute effects of PEEP and oxygen on cardiopulmonary indices.

MATERIALS AND METHODS

Animals. Thirty-one neutered male sheep weighing 26-40 kg were used in this study. In Part I, various severities of smoke inhalation injury were produced in 15 sheep; 4 uninjured sheep were studied as controls. In Part II, severe smoke inhalation injury was produced in 12 sheep.

Smoke Exposure. Smoke was generated by burning 10 disposable underpads in a smoke generator. The smoke was equilibrated at room temperature to avoid thermal injury of the airway and contained 10-14% oxygen, 3-8% carbon dioxide, and 0.7-2.2% carbon monoxide and other combustion products, but no cyanide. Smoke inhalation injury was produced under general anesthesia (methohexital sodium, 9 mg/kg) as previously described (4). Severity of injury was controlled by changing the duration of exposure to the smoke.

Study Design. In Part I, 8 sheep were studied at 12 h and 7 at 72 h after smoke exposure. The animals were anesthetized (alpha-chloralose, 0.05 g/kg), paralyzed (pancuronium bromide, 0.03-0.04 mg/kg), and ventilated mechanically with an FIO₂ of 0.21, tidal volume of 15 ml/kg, and respiratory rate of 12/min. Arterial, central venous, peripheral venous, and Swan-Ganz (7F, American Edward Laboratories, Irvine CA) catheters and an esophageal balloon were inserted. After a 2-h stabilization period, initial measurements were made at FIO₂ of 0.21 without
PEEP. Then 10 cmH₂O of PEEP was applied for 30 min and a second set of measurements were made with an FIO₂ of 0.21 and 10 cmH₂O PEEP.

In part II, the sheep were reanesthetized 24 h after smoke exposure, catheterized as in Part I, and baseline cardiopulmonary measurements were made using mechanical ventilation and an FIO₂ of 0.21 without PEEP. Then 6 sheep were ventilated with 100% oxygen without PEEP (Group A) and the remaining 6 with 100% oxygen and 10 cmH₂O PEEP (Group B). A second set of measurements were made after 30 min of treatment.

Monitoring. Central venous pressure (CVP), pulmonary artery pressure, and systemic artery pressure were monitored continuously. Pulmonary capillary wedge pressure (PCWP), cardiac output, arterial and venous blood gasses were measured every 30 min after catheter placement. Respiratory indices were monitored with a pneumotachograph for flow rate and tidal volume and a differential transducer for transpulmonary pressure. VA/Q ratio was measured by the multiple inert gas elimination technique (MIGET) using a Hewlett-Packard Model 5985 GC-MS (5,7-9). All animals were sacrificed at the end of studies and tissues were obtained for histological evaluation.

Statistical Analyses. Comparisons between the two groups before treatment was made by Student's t-test (Table 1). Effects of treatment were evaluated by paired t-test within each group, and differences induced by treatment was compared between the groups by t-test (Tables 1 and 2, Figs 1 and 2). Fractional blood flows to the four major compartments were compared using multivariate analysis (Figs 3 and 4).

RESULTS

Part I. Figure 1 shows the PaO₂ before and after application of 10 cmH₂O PEEP in each group. There were no significant changes in PaO₂ in any group. Other cardiopulmonary changes are summarized in Table 1. Since there were no significant differences in response to PEEP, measurements at 12 and 72 h were combined for the smoke-exposed group. In controls, significant changes included increases of PCWP and MPAP and decreases in LVSW index. In the smoke-exposed group, however, increases in PaCO₂, CVP, PCWP, MPAP, and dead space were significant.

Mean fractional blood flows to the four major compartments, i.e., shunt (VA/Q=0), low VA/Q compartment (0<VA/Q<0.1), normal VA/Q compartment (0.1<VA/Q<10), and high VA/Q compartment (10<VA/Q), are shown in Figure 3. To emphasize the severity-related alterations in VA/Q distribution, the smoke-exposed animals were subdivided into those with PaO₂ above 65 torr and below 65 torr. Before the application of PEEP, blood flow to the normal VA/Q compartment decreased as hypoxia became more severe, while blood flow to the low VA/Q
## TABLE 1. Changes in Cardiopulmonary Indices (Mean ±SE)

<table>
<thead>
<tr>
<th></th>
<th>Oxygen Only (Group A) Pretherapy</th>
<th>Oxygen Only (Group A) Posttherapy</th>
<th>Oxygen + PEEP (Group B) Pretherapy</th>
<th>Oxygen + PEEP (Group B) Posttherapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>PaCO2 (torr)</td>
<td>40.1±5.5</td>
<td>45.2±8.1*</td>
<td>42.9±2.2</td>
<td>48.7±4.8**</td>
</tr>
<tr>
<td>CVP (cmH2O)</td>
<td>1.3±1.5</td>
<td>2.0±2.1</td>
<td>1.4±1.7</td>
<td>6.3±5.4</td>
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<tr>
<td>PCWP (torr)</td>
<td>6.8±3.5</td>
<td>6.0±2.4</td>
<td>8.5±4.2</td>
<td>12.3±4.0**</td>
</tr>
<tr>
<td>MPAP (torr)</td>
<td>17.6±4.7</td>
<td>17.0±2.4*</td>
<td>21.6±3.6</td>
<td>22.3±3.2**</td>
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<tr>
<td>Cardiac index (l/min/m²)</td>
<td>4.7±1.1</td>
<td>4.3±0.9</td>
<td>5.3±1.7</td>
<td>4.8±1.5*</td>
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<tr>
<td>LVSW index (g m/m²²)</td>
<td>36.7±10.5</td>
<td>38.2±8.9</td>
<td>49.8±15.3</td>
<td>49.4±19.1</td>
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<tr>
<td>Compilcance (ml/cmH₂O)</td>
<td>64±24</td>
<td>62±19</td>
<td>75±28</td>
<td>82±27</td>
</tr>
<tr>
<td>Resistance (cmH₂O sec/l)</td>
<td>38.5±16.5</td>
<td>41.3±10.5</td>
<td>27.5±9.1</td>
<td>26.0±5.2</td>
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<tr>
<td>Peak inspiratory pressure (cmH₂O)</td>
<td>13.8±4.4</td>
<td>14.5±3.2</td>
<td>10.5±3.4</td>
<td>15.4±2.8</td>
</tr>
<tr>
<td>Dead space (% minute ventilation)</td>
<td>42.6±8.5</td>
<td>45.7±11.7</td>
<td>39.7±5.2</td>
<td>68.1±12.7**</td>
</tr>
</tbody>
</table>

*P < 0.05, **P < 0.01, paired t-test.
### TABLE 2. Changes in Cardiopulmonary Indices by PEEP (Mean ± SD)

<table>
<thead>
<tr>
<th></th>
<th>Control Group (n=4)</th>
<th>Smoke-Exposed Group (n=15)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-PEEP</td>
<td>Post-PEEP</td>
</tr>
<tr>
<td>PaCO₂ (torr)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30.1±5.2</td>
<td>30.2±5.4</td>
<td>34.9±7.4</td>
</tr>
<tr>
<td>CVP (cmH₂O)</td>
<td>0.3±1.3</td>
<td>2.5±3.4</td>
</tr>
<tr>
<td>PCWP (torr)</td>
<td>4.3±1.7</td>
<td>8.8±1.3*</td>
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<tr>
<td>MPAP (torr)</td>
<td>12.0±4.1</td>
<td>15.8±3.5*</td>
</tr>
<tr>
<td>Cardiac index (l/min/m²)</td>
<td>3.9±0.8</td>
<td>3.1±0.5</td>
</tr>
<tr>
<td>LVSW index (g m/m²)</td>
<td>38.5±6.9</td>
<td>30.9±7.7*</td>
</tr>
<tr>
<td>Compliance (ml/cmH₂O)</td>
<td>135±37</td>
<td>150±56</td>
</tr>
<tr>
<td>Resistance (cmH₂O sec/l)</td>
<td>14.8±2.5</td>
<td>14.2±3.9</td>
</tr>
<tr>
<td>Peak inspiratory pressure (cmH₂O)</td>
<td>6.4±1.2</td>
<td>8.1±1.4</td>
</tr>
<tr>
<td>Dead space (% minute ventilation)</td>
<td>34.2±10.3</td>
<td>33.1±11.5</td>
</tr>
</tbody>
</table>

*P < 0.05, **P < 0.01, paired t-test.
Effect of PEEP

FIGURE 1. PaO₂ changes induced by PEEP in the 3 groups. There was no statistically significant change in any group.

compartment increased reciprocally (Fig 3A). There were no significant changes in the shunt and high VA/Q compartment. Figure 3B represents fractional blood flow after PEEP. A similar severity-related pattern was observed, but no significant change were induced by PEEP in the mean fractional blood flow in any group.

Although there was no significant change as a group in the fractional blood flow, PEEP improved PaO₂ substantially in some animals and worsened in others. Figure 5 illustrates VA/Q changes in a "successful" PEEP. In that case, the typical low VA/Q compartment decreased markedly with PEEP, resulting in improved PaO₂ from 67 to 75 torr. There was also a substantial decrease in cardiac output and increase in dead space. An
FIGURE 2. PaO₂ changes by oxygen with or without PEEP. Because of the diversity in response to PEEP, the change was not statistically significant, although some animals (responders) showed substantial increase in PaO₂ during treatment.

Example of "unsuccessful" PEEP is shown in Figure 6. Before application of PEEP, typical low VA/Q compartment and shunt were observed. The low VA/Q area, however, did not change with the institution of PEEP, while the true shunt increased considerably from 5.1 to 23.5% cardiac output. Consequently, PaO₂ decreased from 66 to 56 torr, but there was minimal change in PaCO₂, dead space, or cardiac output.

PART II. Changes of PaO₂ in the two groups are shown in Figure 2. Before administration of oxygen with (Group B) or
FIGURE 3. Fractional blood flow to the 4 major compartments before (top panel) and after (bottom panel) application of PEEP. Blood flow to the normal VA/Q compartment decreased as the extent of hypoxia increased and blood flow to the low VA/Q compartment increased reciprocally. There was no significant change in the shunt and high VA/Q compartments. PEEP did not exert significant effect as a group in any of the 3 groups. See text for detail.
FIGURE 4. Fractional blood flow to the 4 major compartments before (top panel) and after (bottom panel) treatment by oxygen with or without PEEP. There was no significant difference between the two groups in any fraction. PEEP recruited blood flow from the low VA/Q compartment to the shunt. There was no significant difference in the response to oxygen between the two, although central peak to the normal VA/Q compartment was broadened more in the oxygen alone group.
FIGURE 5. Ventilation-perfusion change of a successful PEEP treatment. Typical low VA/Q compartment was reduced substantially with improvement of PaO2. Decrease in cardiac output and increase in dead space was also noted. See text for detail.

without PEEP (Group A), there were no significant differences in any of the cardiopulmonary indices between the two groups. In Group A, PaO2 rose from 54.3±7.3 torr to 100.5±31.4 torr during treatment; while in Group B, PaO2 changed from 56.8±7.3 torr to 238.9±142.1 torr. These changes as a group were not significantly different because of the large variability within
FIGURE 6. Ventilation-perfusion change of an unsuccessful PEEP treatment. PEEP did not reduce blood flow to the low VA/Q compartment, but blood flow to the shunt increased significantly, leading to a 10-torr fall in PaO₂.

Group B. Changes in other cardiopulmonary indices are summarized in Table 2. In Group B, significant changes were observed in PaCO₂, PCWP, cardiac index, peak inspiratory pressure, and dead space, while only PaCO₂ increased.
significantly in Group A. Differences (post- vs. pre-values) induced by the treatment were compared between the two groups and the increases in PCWP, peak inspiratory pressure, and dead space were significantly greater in Group B. Fractional blood flows to the four major compartments are shown in Figure 4. Before treatment, there was no significant difference between Group A (oxygen alone, open bars) and Group B (oxygen + PEEP, shaded bars) (Fig 4A). After 30 min of treatment, blood flow to the low VA/Q area decreased and blood flow to the shunt increased considerably (Fig 4B). However, there was no significant difference in blood flow to any of the four compartments between the two groups before or after treatment. The relationship between increased shunt flow and decreased blood flow to the low VA/Q compartment is plotted in Figure 7. The abscissa (X) represents change in the blood flow to the low VA/Q segment and the ordinate (Y) represents change in the flow to the shunt. Changes are shown as % cardiac output since only minimal change was induced in cardiac output by treatment. The regression for all the animals (n=12) was Y = 9.7 - 0.74X (r=0.54, P<0.05). Typical VA/Q changes in Group B are illustrated in Figure 8. Low VA/Q units were totally converted into true shunt (oxygen atelectasis) and the normal VA/Q compartment became wider. Such changes were not completely prevented by institution of 10 cmH₂O PEEP.

**DISCUSSION**

Smoke inhalation injury is a sequence of progressive deterioration in cardiopulmonary function, which manifests itself by 6 h after injury and reaches a peak by 72 h, after which superimposed infection alters the pathophysiology (4,10,11). In Part I, we have studied the effects of PEEP at an early phase of inhalation injury without significant infection (12 and 72 h) with FIO₂ maintained at 0.21. There were several adverse effects which, although they are common in other disease conditions as well, we must consider in applying PEEP to smoke inhalation injury (12). First, slight suppression of LVSW was observed in uninjured controls; this was not significant in the smoke-exposed group. Dead space ventilation, and consequently PaCO₂, increased significantly in the smoke-exposed group, but not in the control group. These results suggest that PEEP overinflated alveoli that were already open, rather than opening collapsed alveoli. Previous studies of PEEP in smoke inhalation injury showed improved PaO₂ by PEEP with spontaneous ventilation (CPAP) at 2 h after inhalation and improved survival with PEEP and mechanical ventilation during the first 72 h (2,3). In the present study, we did not observe any consistent effect of PEEP on PaO₂, making improvement in survival unlikely. This absence of effect might be attributable to the fact that we did not optimize PEEP for each animal and applied PEEP for only 30 min. However, 10 cmH₂O PEEP would be a common initial clinical level and optimal PEEP is a controversial and poorly defined concept. The acute effects of PEEP were the focus of this part of the
Blood flow to shunt

![Graph showing relationship between blood flow to low V/Q compartment and increased blood flow to the shunt. Changes in the blood flow to the low VA/Q compartment (abscissa) and to the shunt (ordinate) are expressed as % cardiac output. Regression line for the overall points was $Y = 9.7 - 0.74X$ ($r=0.54$, $P<0.05$). Shaded area represents mean ±SE. There was no significant difference between the two groups.]

study; we did not study long-term effects or the effects of high PEEP with oxygen concentrations.

The effects of PEEP with 100% oxygen were studied in Part II. Smoke inhalation injury produces various degrees of partial alveoli collapse due to lung edema, increased secretion, inflammatory cell infiltration, and sloughing of the
FIGURE 8. Ventilation-perfusion alteration induced by 100% oxygen without PEEP. Low VA/Q area was completely converted to the shunt and the central peak (VA/Q near 1) was broadened by oxygen, which explained very limited increase in PaO₂ by pure oxygen.

Airway mucosa. These hypoventilated avleoli are susceptible to oxygen and easily collapse (oxygen atelectasis) (13). Under such condition, the acute effects of PEEP might be different. In severe smoke inhalation injury, pure oxygen without PEEP converted the low VA/Q compartments into true shunt by 30 min, with broadening of the central peak (VA/Q ratio near one); PaO₂
increased minimally during treatment from 54 to 101 torr (Figs 4 and 8). This result is compatible with the theory of absorption atelectasis (13). PEEP did not prevent such change completely, but was effective in some (responders, Fig 2), raising PaO₂ above 300 torr. There was, however, no statistically significant predictor of responders. Because of the diversity in response to PEEP, there was no significant difference in mean PaO₂ or VA/Q distribution after treatment between the two groups (Fig 4B). At autopsy, mechanical obstruction of the major bronchi by pseudomembranes was more prominent in the nonresponders and resulted in diffuse atelectasis. Although there are reports that oxygen without PEEP does not increase true shunt in ARDS with low VA/Q, oxygen therapy without PEEP in smoke inhalation injury could do harm (14,15). Hart et al have reported treatment of smoke inhalation with hyperbaric oxygen, but they addressed carbon monoxide poisoning or cyanide poisoning associated with smoke inhalation (16). The indication for hyperbaric oxygen may be limited to the early postinjury period, and in using such treatment, one must consider the possibility of detrimental respiratory sequelae.

In conclusion, immediate adverse effects of PEEP, including increased dead space and PaCO₂, must be considered when PEEP is used in smoke inhalation injury. In this study, oxygen converted the low VA/Q compartment into true shunt (alveolar collapse), and this was not completely prevented by PEEP and was more prominent when major bronchi were mechanically obstructed with pseudomembrane. Bronchoscopic removal of such pseudomembranes from major bronchi is probably crucial to successful oxygen therapy with PEEP after smoke inhalation injury.

PRESENTATIONS


PUBLICATIONS


REFERENCES


23. The main objective of this research is to determine the changes in zinc metabolism caused by burn and infection in a murine model. The study is designed to study the effect of injury on physiological control mechanisms at the whole body, tissue, and molecular levels. Information obtained from these studies will provide a better understanding of the primary changes in the metabolism of this important trace element and the role of these changes in systemic complications, ultimately leading to the development of optimal supplementation of zinc in burned humans. A literature search was conducted.

24. A semi-certified, zinc-deficient (0.5 ppm) diet was fed to adult rats. The effects of zinc nutriture were studied by subcutaneously injecting either saline or L-methyl-D-aspartic acid to create a zinc-deficient or zinc-sufficient status, respectively. The zinc-deficient and sufficient groups were further divided into newborn and burn groups. The burn groups received a 33% total body surface area burn.

25. Previous studies with this model have shown that significant increases in endogenous fecal and urine excretion were detected during the first 13 days postburn. Burn injury and burn injury plus zinc deficiency caused a decrease in total peripheral blood T-cells. However, the burn zinc-deficient animals showed an increase in T-suppressor cell populations and an increase in T-suppressor cell populations while the burn zinc-sufficient animals were comparable with the
control/zinc-sufficient and control/zinc-deficient animals. Recent studies have used this model to determine the effect of zinc nutriture and burn injury on antibody response in the rat. This study has shown that there is an increase in antibody response due to burn injury in the zinc-sufficient animals. This response was suppressed in the burn/zinc-deficient rats. This study demonstrated a modulating effect of zinc nutriture on various aspects of the immunological system after burn injury.
RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

AGENCY ACCESSION: 2
DATE OF SUMMARY: 01.11.11
RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. NAME:
   US Army Institute of Surgical Research

2. ADDRESS:
   Fort Sam Houston
   San Antonio, Texas 78234-5812

3. NAME OF PRINCIPAL INVESTIGATOR:
   CARLSON, D.

4. TELEPHONE NUMBER:
   512-221-6532

5. GENERAL USE:
   NAME: STAFF, J.

6. KEYWORDS:
   (1) Nitrogen Balance; (2) Serum Visceral Protein; (3) Albumin; (4) Transferrin; (5) Retinol-Binding Protein.

7. SECURITY CLASSIFICATION:
   CONFIDENTIAL

8. APPROACH:
   (1) Volunteers; (2) RAIR.

9. REVIEWED BY:
   (1) The purpose of this study is to assess whether albumin, pre-albumin, transferrin, or retinol-binding protein can be used indicators of nitrogen balance in normal soldiers. A literature search was conducted.

10. RESULTS:
    - Baseline serum visceral protein levels will be measured at post是你 before 6 following stabilization of the patient's fluid status. Serum levels will be repeated every 3 days until postmenstrual day 10. Changes from baseline level will be correlated with nitrogen balance.

11. CONCLUSION:
    - This project was approved as a minimal risk protocol by the US Army Institute of Surgical Research Human Use Committee on 11 September 1993.
\textbf{Effect of Growth Factors on the Healing of Partial-Thickness Scald Wounds in the Guinea Pig}

\textbf{Objective:}

The purpose of this study is to determine whether these three growth factors can enhance burn epithelization in a partial-thickness burn wound. A literature search was conducted.

\textbf{Methods:}

23. (U) Epidermal growth factor (EGF), initially isolated from the submaxillary gland of mice and subsequently identified in human urine, has shown to increase the rate of endothelial and epithelial proliferation. Fibroblast growth factor (FGF), originally noted for its mitogenic effect on fibroblast, has recently been found to have potent angiogenic properties. Platelet-derived growth factor (PDGF) appears to have a variety of properties, one of which is stimulation of epithelization. The purpose of this study is to determine whether these three growth factors can enhance burn epithelization in a partial-thickness burn wound in guinea pigs. A literature search was conducted.

24. (U) Male guinea pigs weighing 440–500 g will receive a deep partial-thickness burn. The burn wound edges will be tattooed. Four groups of 40 animals each will be studied. Group I will receive EGF, Group II will receive FGF, and Group IV will receive PDGF. Group I animals will receive 0.5 cc of lanolin cream applied to the burn wound twice daily. Groups II, III, and IV will receive 0.5 cc of EGF, FGF, and PDGF, respectively, in a lanolin cream applied twice daily to the burn wound. Wounds will be measured daily for assessment of contraction. On postburn days 5 and 10, 5 animals in each group will be sacrificed and 15 animals in each group will be sacrificed on postburn days...
20 and 30. At the time of sacrifice, the extent of healing by contraction will be assessed utilizing a planimeter and the extent of reepithelization will be assessed histologically.

25. 8610 - 8709. (U) This project was approved by the US Army Institute of Surgical Research Animal Care and Use Committee on 14 January 1987. Progress on this study has been delayed. First, the Amgen Corporation has been unable to supply a stable form of either FGF or PDGF in a lanolin cream. The Amgen Corporation is currently trying to derive a stable formulation of both compounds. Secondly, the animal care facilities at this Institute are currently unable to handle a large number of animals due to renovation. The renovation of the facilities is expected to be completed by 1 October 1987. When these problems are solved, the project will begin.
RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

**Title:** Cellular Host Defense Function After Thermal Injury: Assessment by Flow Cytometry of Peripheral Blood Cells

**Subject Areas:**
- Biochemistry
- Medicine and Medical Research

**Keywords:**
- Flow Cytometry
- Lymphocyte Subpopulations
- Burn Injury
- Infection
- Immunocompetence

**Technical Objective:**

23. (U) The objectives of this study are to develop methods to analyze the complex leukocyte mixtures seen in the blood of burn patients, to quantify the changes in cell morphology and function that occur, and to correlate those findings with the clinical outcome of the patient. The ultimate goal is to develop tests for effective diagnosis of infection susceptibility and evaluation of the effectiveness of new treatment modalities. A literature search was conducted.

24. (U) The development of techniques for assessing immune status of burn patients will focus on the evaluation of lymphocyte subpopulations composition and function. The resolving power of the flow cytometer will be used to differentiate the lymphocyte subpopulations. Five measurements will be made on each cell simultaneously, consisting of two light scatter measurements of physical properties and three subpopulation or functional markers. The multiparameter analysis will be correlated with patient mortality and morbidity and compared to parameters measured on control subjects. Abnormal lymphocyte subset composition or function that correlates with patient outcome will be analyzed in more detail for efficacy as a clinical diagnostic tool and as a probe to determine how the abnormality in related to the defect in host defense.
Continuation of DD Form 1498 for "Cellular Host Defense Function After Thermal Injury: Assessment by Flow Cytometry of Peripheral Blood Cells"

25. (U) 8610 - 8709. Detecting surface markers for relatively small populations has been particularly difficult because of contaminating blood leukocytes. A panel of monoclonal reagents was surveyed to find one marker with specificity to immature granulocytes and monocytes in patient lymphocyte preparations. One reagent was found with a strong specificity against monocyte antigen and a weak affinity for myelocytic cells. A scatter discriminates between the contaminating cells and the cells of interest. Data has been collected on 66 patients and is now undergoing detailed analysis. Preliminary analysis of the data indicates there is a general decrease (10-30%) in the T lymphocyte subpopulations in burn patients as compared to unburned controls. The proportion of B lymphocytes, NK cells, and monocytes were unchanged, indicating there was an increase in the proportion of cells with no distinguishable antigens in the blood of burn patients and this proportion of "null" cells was higher in those patients who did not survive their injury. Further, patients who died had significantly decreased (10-35%) proportions of T lymphocyte subpopulations as compared to those who survived. The proportion of lymphocytes of the "suppressor" subset were depressed (35%) even more than those of the "helper" subset (24%) as compared to the subsets in surviving patients.
ANNUAL RESEARCH PROGRESS REPORT

PROJECT NUMBER: 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

PROJECT TITLE: CELLULAR HOST DEFENSE FUNCTION AFTER THERMAL INJURY: ASSESSMENT BY FLOW CYTOMETRY OF PERIPHERAL BLOOD CELLS

US ARMY INSTITUTE OF SURGICAL RESEARCH
FORT SAM HOUSTON
SAN ANTONIO, TEXAS 78234-5017

1 October 1986 - 30 September 1987

INVESTIGATORS

David G. Burleson, PhD, Lieutenant Colonel, MS
Arthur D. Mason, Jr., MD
Basil A. Pruitt, Jr., MD, Colonel, MC
ABSTRACT

PROJECT NUMBER: 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

PROJECT TITLE: CELLULAR HOST DEFENSE FUNCTION AFTER THERMAL INJURY: ASSESSMENT BY FLOW CYTOMETRY OF PERIPHERAL BLOOD CELLS

INSTITUTION: US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas 78234-5012

PERIOD COVERED IN THIS REPORT: 1 Oct 86 through 30 Sep 87

INVESTIGATORS: David G. Burleson, PhD, Lieutenant Colonel, MS Arthur D. Mason, Jr., MD Basil A. Pruitt, Jr., MD, Colonel, MC

Changes in lymphocyte phenotype that may be related to the defect in host resistance in burned patients were measured by flow cytometry. Purified lymphocyte preparations from 33 burn patients and 33 controls were analyzed for T lymphocyte and non-T lymphocyte subpopulation antigens. The proportion of all subpopulations of T lymphocytes (Leu-4, Leu-2, and Leu-3) were decreased in burn patients as compared to controls. The decrease in T lymphocyte subpopulations caused an increase in the proportion of B cells, but the proportion of all other non-T cell subpopulations was unchanged. Absolute numbers of circulating T lymphocytes were also decreased in burn patients as compared to controls, while absolute numbers of non-T cells remained unchanged. The proportion of T lymphocyte subpopulations in those patients who died were decreased further than in the patients who survived their injury. There were no differences between non-T cell subpopulations in the survivors and nonsurvivors. When viewed as a function of postburn day, subpopulations of helper and suppressor lymphocytes were decreased soon after injury. The subpopulations continued to decrease for those patients who died and gradually returned to normal for those patients who recovered. The ratio of helper lymphocytes to suppressor lymphocytes did not differ for controls, patients who died, or patients who survived.
CELLULAR HOST DEFENSE FUNCTION AFTER THERMAL INJURY: ASSESSMENT BY FLOW CYTOMETRY OF PERIPHERAL BLOOD CELLS

Changes in function and phenotype of circulating leukocytes may be related to the defect in host resistance underlying the increased susceptibility of burn patients to opportunistic infection. Among the changes frequently reported in burn patients are decreased cell-mediated immune reactions, expressed as a reduced capacity to reject allogeneic skin grafts (1-3), and impaired response to delayed hypersensitivity antigens (4). The component of the host defense system responsible for cell-mediated immunity responses are T lymphocytes. The relationship between depression of T cell function and susceptibility to opportunistic infection remains unclear.

We have investigated the phenotype of circulating lymphocyte subpopulations after burn injury and compared them to unburned controls. The proportion of both of the major subpopulations of T lymphocytes are decreased in burn patients soon after injury and remain decreased for several weeks after injury. Absolute numbers of non-T cells remained at levels comparable to unburned controls. Further, patients that died had a greater decrease in the proportion of their T cell subpopulations than those who lived. The absolute number of non-T cell subpopulations was unchanged between the two groups, but the proportion of B cells in the lymphocyte preparations increased as the T lymphocytes decreased.

MATERIALS AND METHODS

Lymphocyte subpopulations were measured in 33 burn patients and 33 unburned controls. All patients in this study were admitted during the first 5 days postinjury and had expected mortalities between 20 and 90%. The average burn size was 44.5% and average age 43.4 yr. The expected mortality for the group, based on previous experience at this Institute, was 50.9%. Peripheral blood samples were obtained twice weekly for up to 8 wk postburn for assessment of cell number, cell subpopulations, and morphology. The controls were sampled at random for comparison.

Fresh whole blood was diluted 1:3 with HBSS (Gibco Laboratories, Life Technologies, Inc.) containing 5 mg of EDTA and 1 g bovine serum albumin (BSA) per liter. Lymphocytes were purified by adding a layer of 8 ml diluted whole blood over 4 ml of Ficoll-Hypaque (Pharmacia Fine Chemicals, Inc.). The gradients were centrifuged for 30 min at 450 g at 15°C. Cells were harvested from the Ficoll-Hypaque interface and washed 3X with HBSS. Slides for differential analysis were made by placing cells (5 x 10^6 in 0.5 ml) in a cytocentrifuge (Shandon), spun at 400 rpm for 4 min, stained with Wright's stain, and evaluated to determine the purity of the cell
suspension. The remaining cells were suspended in 1 ml HBSS and 2 ml of a lysing solution (4.15 g NH₄Cl, 0.5 g KHCO₃, and 0.185 g (NH₄)₂EDTA/L) to lyse any contaminating red blood cells. After a 15-min incubation, the lysed cell suspension was underlaid with a cushion of 0.8 ml of fetal bovine serum and centrifuged for 10 min at 200 g at 15°C.

The cells were resuspended in HBSS at a concentration of 1 X 10⁷ cells/ml and 25 μl of the cell suspension was added to a sample tube for staining with monoclonal antibody. The cells were stained with up to 3 monoclonal antibodies in a sequential fashion. An unstained monoclonal antibody was added, the cells were incubated for 15 min at 5°C and washed with HBSS. Goat anti-mouse IgG (Fab2' fragments, 25 μl diluted 1:40 with HBSS) labelled with Texas Red was added, incubated for 15 min, and the cells washed. Normal mouse serum (5 μl) was added to prevent cross-reaction of primary conjugated monoclonal antibodies with the goat anti-mouse Texas Red. Monoclonal antibodies labelled with phycoerythrin and with fluorescein (Becton-Dickinson, Inc.) were added and the cell suspensions were washed twice and fixed by adding 400 μl of 2% paraformaldehyde and HBSS.

Cells were analyzed on either a FACS 400 flow cytometer modified by the addition of a third analysis channel and a Consort 40 data analysis system (Becton-Dickinson, Inc.) or a Coulter EPICS model 753 flow cytometer (Coulter Electronics, EPICS Division). Data analysis was performed by a student's t-test using program P7D from BMDP(UCLA) on a VAX 11-780 computer (DEC).

RESULTS

The changes in the number and morphology of circulating leukocytes following thermal injury are quite dramatic. Shortly after injury, burn patients become leukocytotic and lymphopenic (5). Most of the cells are granulocytes, many being immature or otherwise abnormal. This combined leukocytosis and lymphopenia make separation of lymphocytes for analysis difficult. Ficoll-Hypaque density gradients separate PMN and RBC from lymphocytes by density, the heavier PMN and RBC settling to the bottom of the tube while the less dense mononuclear cells staying at the blood:gradient interface. In normals, this results in a preparation containing about 90% lymphocytes. In burn patients, however, abnormal PMN with densities similar to lymphocytes significantly contaminate the lymphocyte preparation.

Measurement of immune function or cell phenotype requires a pure cell population on which to make measurements. Obtaining pure lymphocyte populations from burn patients would involve physical or antibody panning separation techniques in addition to density gradient separation. Every purification procedure has the potential to introduce artifacts in subsequent
measurements. To avoid this problem and the artifacts that might be introduced by extensive physical separation techniques, we used flow cytometry to measure lymphocyte subpopulations. Flow cytometry examines cells individually in a sequential fashion so that each cell can be analyzed independently of all the other cells in the preparation. Lymphocytes can be distinguished from monocytes and PMN by measuring light scatter intensity at forward and 90° angles. Lymphocyte subpopulations were resolved by the addition of subset specific monoclonal antibodies bound to fluorescent dyes and determining the proportion of the lymphocytes binding the monoclonal antibody marker.

A summary of the results of the proportions of T lymphocyte subpopulations determined in peripheral blood from burn patients and controls is shown in Figure 1. The proportion of T cells (as measured by the Pan-T cell markers, Leu-1, Leu-4 and Leu-5) as well as the helper and suppressor/cytotoxic subsets (Leu-3 and Leu-2, respectively) were reduced in burn patients. The proportion of positive lymphocytes in the samples does not relate directly to the number of positive cells in the circulation since the lymphocytes are concentrated in the Ficoll-Hypaque purification. To represent these subpopulations as the absolute numbers of cells per unit of whole blood, it is assumed that the cells isolated from the gradient are a true representation of those in the circulation. The peripheral blood concentration of a subpopulation of cells is then given by:

\[
\text{Positive Cells per nl Whole Blood} = \text{WBC} \times \frac{\% \text{ Lymphs}}{100} \times \frac{\% \text{ Positive}}{100}
\]

where WBC is the whole WBC in cells/nl and % lymph is the percentage of lymphocytes determined from the whole blood differential and % positive is the percent positive cells passing through the "lymphocyte" light scatter gate. The comparison between control and burn patients after this transformation is shown in Figure 2. The difference in T cell subpopulations between the two groups was even greater than when compared as percent positive. The concentration of all the T lymphocyte subpopulations measured in whole blood were significantly decreased in the burn patients as compared to controls.

Several other lymphocyte markers were also tested, Leu-12 (B cells) and Leu-M3 (monocytes), which mark exclusively non-T cells, and Leu-11 (NK cells), Leu-7 (large granular lymphocytes), and Leu-15 (complement III receptor), which have a limited cross-reactivity with some T cells. As shown in Figure 3, there was a decrease only in the proportion of B lymphocytes. All the other non-T subpopulations were unchanged from the percent positive levels found in controls. There was no difference in any of the non-T cell subpopulations when the absolute number of positive cells per nl of whole blood were
FIGURE 1. T lymphocyte subpopulations from the blood of burned patients and controls. Values shown are mean % positive cells ±SEM of controls and burn patients. *P<0.05, **P<0.01, ***P<0.001.
FIGURE 2. T lymphocyte subpopulations from the blood of burned patients and controls. Values shown are mean ± positive cells/μl of whole blood ±SEM. *P<0.05, **P<0.01, ***P<0.001.
FIGURE 3. Non-T cell subpopulations from the blood of burned patients and controls. Values shown are mean % positive cells ±SEM of controls and burn patients. *P<0.05, **P<0.01, ***P<0.001.
compared for patients and controls (Fig 4). Since the proportion of T lymphocytes decreased sharply and the proportion of non T cells remained relatively constant, there must have been an increase in "null" cells having none of the common antigens that were tested.

The patients were further divided into two groups based on mortality. Patients who died had decreased T cell subpopulations as compared to those who lived as shown in Figure 5. Survivors had decreased T lymphocytes as compared to unburned controls, but the nonsurvivors had greater decreases in these subpopulations. There was no difference in non-T cell subpopulations between survivors and nonsurvivors (Fig 6).

The data were analyzed with respect to the change in T lymphocyte subpopulations over time. Figure 7 shows the data depicted as percent Leu-2 positive cells with respect to postburn day for survivors and nonsurvivors. The mean for the surviving patients is plotted along with the mean for the nonsurvivors up to postburn day 30. Both survivors and nonsurvivors had decreased suppressor/cytotoxic T subpopulations soon after burn injury. The mean for nonsurvivors was less than that for survivors after the first few days postburn. The mean for nonsurviving patients continued to decrease with increasing postburn day, while that for survivors increased after the third wk postburn. A similar pattern is depicted in Figure 8 for the helper (Leu-3 positive) T cell subpopulation, although the differences between survivors and nonsurvivors was not as great as for the suppressor/cytotoxic subpopulation.

The ratio of the percent positive helper T lymphocytes (Leu-3 positives) and the percent suppressor/cytotoxic T lymphocytes (Leu-2) positives was also determined and the ratios for survivors, nonsurvivors, and unburned controls are compared in Table 1. There was no significant difference between the helper/suppressor ratio for any of the three groups. Although the helper subpopulations were decreased in

| Table 1. Helper/Suppressor Ratios (Mean ± SD) |
|-------------------------------|------------------|------------------|---|
| **Comparison** | **Control Patients** | **Burn Patients** | **P Value** |
| Number | Average | Number | Average |  |
| Control vs. Burn | 289 | 2.07±1.69 | 286 | 2.09±1.59 | NS |
| Survivors | 251 | 1.97±1.54 | 35 | 2.92±1.77 | .0043 |

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FIGURE 4. Non-T cell subpopulations from the blood of burned patients and controls. Values shown are mean positive cells/μl of whole blood ±SEM of controls and burn patients. *P<0.05, **P<0.01, ***P<0.001.
FIGURE 5. Comparison of T lymphocyte subpopulations for survivors and nonsurvivors. Values shown are mean % positive cells ±SEM of each group. *P<0.05, **P<0.01, ***P<0.001.
FIGURE 6. Comparison of non-T cell subpopulations for survivors and nonsurvivors. Values shown are mean % positive cells ± SEM of each group. *P<0.05, **P<0.01, ***P<0.001.
FIGURE 7. Comparison of Leu-2 \% positive cells for survivors and nonsurvivors as a function of postburn day. Values shown are moving mean \% positive values for 7 days past the postburn day indicated ±SEM. The control mean values SEM for Leu-2 \% positive are shown as a background bar.
FIGURE 8. Comparison of Leu-3 % positive cells for survivors and nonsurvivors as a function of postburn day. Values shown are moving mean % positive values for 7 days past the postburn day indicated ±SEM. The control mean values SEM for Leu-3 % positive are shown as a background bar.
the burned groups, the suppressor subpopulations were decreased by a similar amount and the mean ratios did not change.

**DISCUSSION**

The relative proportion of helper to suppressor cells (termed the helper/suppressor ratio) has been used as a measure of immunosuppression. Antonacci and coworkers at Cornell (6) as well as McIrvine and coworkers in Boston (7) have reported a decreased helper/suppressor ratio in burn patients as compared to normal controls, and the ratio could be related to mortality in one study (7). We have demonstrated no difference in the helper suppressor ratio between burn patients and unburned controls. Further, there was no difference in ratio between surviving and nonsurviving patients. The reasons for the difference between the results of those studies and the present study are not known. One of the studies was done by fluorescence microscopy rather than flow cytometry. Both studies were done on substantially smaller patient populations than the present study. The reason for the change in the ratio in the other two studies was a selective decrease in helper cells as compared to the cells of the suppressor/cytotoxic subpopulation, whereas in our study there was a substantial decrease in both subpopulations.

We observed a selective decrease in all major T lymphocyte subpopulations in burn patients and a greater decrease in nonsurviving than in surviving patients. As a result, there was an increased proportion of cells of unknown phenotype or "null" cells. It is possible that the decreased cell-mediated immunity and increased infection susceptibility of the burn patients are related to the decreased numbers of T cells in the circulation. The decrease in the number of T cells may decrease the number of functional effectors (helper, helper/inducer, or cytotoxic lymphocytes) able to respond to an infection challenge.

The nature of the "null" cells is unknown but several possibilities exist, i.e., they could be "activated" cells that have shed their normal antigens, nonlymphoid cells contaminating the sample because they were not eliminated from analysis by the light scatter "lymphocyte window," immature cells that have not developed detectable markers, or cells of currently undefined specificity and function. Whatever their phenotype, they may represent active "suppressors" of the host response or merely cells of limited function that cannot respond appropriately. Although it is important to enumerate the cells of each subset, information about cell function may be more critical to evaluating host defense. Our future efforts will endeavour to measure cell function as well as cell phenotype.

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PRESENTATIONS/PUBLICATIONS

Burleson DG: A system for batch processing positive calculations from multiparameter flow cytometry list mode data. Presented at the Second Annual Coulter Users Meeting and Immunology Symposium, San Francisco, California, 2-5 December 1986.


REFERENCES


**RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY**

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**SUBJECT AREAS**

- Acute and Physiological
- Partial-Thickness Rat Burn Wound

**START DATE**

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**FUNDING ORGANIZATION**

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<td>U.S. Army Institute of Surgical Research</td>
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**TECHNICAL OBJECTIVE**

The purpose of this study is to determine biochemical and molecular changes that occur in the in vivo partial-thickness rat burn wound during the early postburn period which might be deleterious to recovery of function in cells in which thermal injury was potentially reversible. Cellular injury due to short episodes of ischemia in heart, liver, muscle, and brain is reversible but becomes irreversible when ischemia and local tissue acidosis is prolonged. If this same sequence of events is found to occur in the in vivo rat burn wound, it may be possible to work to reverse the metabolic changes and limit the progression in the size and degree of injury in wounds of burned soldiers. A literature search was conducted.

**PROGRESS APPROACH**

- Microelectrodes will be used to measure changes in extracellular pH and ion content and in pH and/or carbon dioxide partial pressure at various sites in the in vivo burn wound. Samples from sites adjacent to the microelectrodes will be taken to measure selected metabolites and enzymatic methods. Cells and subcellular organelles will be isolated by centrifugation in self-generating gradients or Percoll for measurement of changes in cellular function with time postburn.

**PERFORMANCE ORGANIZATION**

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<td>Fort Sam Houston</td>
<td>San Antonio, Texas 78234-5012</td>
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**NAME OF INVESTIGATOR**

- MASON, A. D. (Name of Associate Investigator, if different from above)

**NAME OF ASSOCIATE INVESTIGATOR**

- MASON, A. D. (Name of Associate Investigator, if different from above)
CONTINUATION OF DD FORM 1498 FOR "A STUDY OF BIOCHEMICAL CHANGES IN THE CELLULAR ENVIRONMENT OF TISSUE OF THE in vivo PARTIAL-THICKNESS RAT BURN WOUND"

previously been observed in full-thickness 20% total body surface area scald burn wounds during the first 6 to 9 h postburn. Afterwards, however, during the period in which the full-thickness burns exhibited a further increase in the rate of water accumulation, the partial-thickness burn wound water content gradually decreased from a peak of 6.5 to 7 ml excess water to approximately 1 ml excess water at 48 h postburn. Sham wound water content was approximately 5 g throughout the 48-h period.

Tissue pH was measured in sham and burn wounds at times from 1 to 48 h postburn. Needle pH microelectrodes and glass reference microelectrodes were inserted through a slit in the wound tissue to the depth of the panniculus carnosus. Tissue pH was recorded when the pH meter indicated stable electrode readings. The pH of the sham wound tissue ranged from 7.44 to 7.13 over the 48-h postburn period. The pH of burn wounds decreased from 7.41 at 1 h postburn to a low of 6.76 at 12 h postburn; from 18 to 48 h postburn, the burn wound pH was approximately 6.9.

Some measurements of tissue carbon dioxide partial pressure were made at the same sites using a combination carbon dioxide microelectrode. In the occluded artery ischemic heart model, it has been shown that the decrease in tissue pH and the increase in tissue carbon dioxide partial pressure correlate very well. However, in the burn wounds in which pH had decreased, measured carbon dioxide partial pressure was lower than in sham wounds, indicating that the decrease in pH was due to the presence of acidic compounds other than carbon dioxide. More analyses will have to be performed to determine if these changes are statistically significant. When that has been completed, samples will be taken from wounds to measure selected metabolites using enzymatic methods. Subsequently, if indicated, cells will be isolated to determine whether changes in cellular function occur with time postburn.
ANNUAL RESEARCH PROGRESS REPORT

PROJECT NUMBER: 3A161101A91C-Co, IN-HOUSE LABORATORY
INDependent research

PROJECT TITLE: A study of biochemical changes in the cellular environment of tissue of the in vivo partial-thickness rat burn wound

US ARMY INSTITUTE OF SURGICAL RESEARCH
FORT SAM HOUSTON
SAN ANTONIO, TEXAS 78234-5012

1 October 1986 – 30 September 1987

INVESTIGATORS

Wanda L. Brown, MS
Arthur D. Mason, Jr., MD
Basil A. Pruitt, Jr., MD, Colonel, MC
ABSTRACT

PROJECT NUMBER: 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

PROJECT TITLE: A STUDY OF BIOCHEMICAL CHANGES IN THE CELLULAR ENVIRONMENT OF TISSUE OF THE in vivo PARTIAL-THICKNESS RAT BURN WOUND

INSTITUTION: US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas 78234-5012

PERIOD COVERED IN THIS REPORT: 1 Oct 86 through 30 Sep 87

INVESTIGATORS: Wanda L. Brown, MS
 Arthur D. Mason, Jr., MD
 Basil A. Pruitt, Jr., MD, Colonel, MC

Sprague-Dawley rats weighing 180-200 g were anesthetized and subjected to a 20% total body surface area sham or partial-thickness dorsal scald burns. Microelectrodes were used to measure the pH, and in some cases, the pCO₂ of the wounds. Burn wound pH was within control limits at 1 h postburn (PB) but then decreased to its lowest value of 6.76 at 6 h PB. It did not return to the sham control range until 24 h PB. Burn wound pCO₂ was lower than that of sham wounds during the period of decreased pH, in contrast to findings in ischemic heart. Mean water content of the partial-thickness burn wound increased rapidly and attained its maximum value at 18 h PB when burn wounds contained 7.8 ml more water than sham wounds. The water content then decreased slowly but the wounds still contained 3.6 ml excess water at 72 h PB. Burn wound dry weights were greater than sham wound dry weights at each time measured.
A STUDY OF BIOCHEMICAL CHANGES IN THE CELLULAR ENVIRONMENT OF TISSUE OF THE in vivo PARTIAL-THICKNESS RAT BURN WOUND

INTRODUCTION

The progressive increase in the depth of irreversibly injured tissue of burn wounds during the first 24-48 h postburn (PB) has been attributed to ischemia (1), but the sequence of metabolic and biochemical changes which occur in vivo in the burn wound have not been well characterized. However, the changes that occur in other ischemic organs have been well documented (2-4). These include a rapid depletion of glycogen and high energy phosphate compounds, an accumulation of lactate, carbon dioxide, NADH, and protons from other metabolic processes, and, ultimately, a cessation of all energy-dependent functions. Since the changes that occur are similar to heart, brain, liver, kidney, and muscle, it is possible that they may also occur in vivo in the burn wound during the early PB period, although they may differ in time and extent because of the relative contribution of different pathways for energy production in a particular organ. Our purpose in this study is to determine the changes that occur in vivo in the burn wound during the early PB period using the information about changes which occur in other ischemic organs as a guide.

MATERIALS AND METHODS

Young Sprague-Dawley rats weighing from 180-200 g were anesthetized with alpha-chloralose (5.5 mg/100 g, IP). The hair on the dorsum was clipped and the rats were placed in a protective mold which limited the area to be burned to 20% of the total body surface area. Immersion of the exposed area in water at 80°C for 8 sec produced a partial-thickness burn. Sham control rats were anesthetized, clipped, and placed in the same protective mold and an area on the dorsum equal to that of the burn area was outlined in ink. Rats were housed in individual cages and permitted access to food and water ad libitum. No parenteral fluids were administered. The rats were reanesthetized prior to measurements.

Microelectrode Measurements. The pH of sham and partial-thickness burn wounds of rats were measured at intervals from 1-72 h PB using an MI-408 needle pH microelectrode and an MI-401 microreference electrode with glass barrel inserted through a slit in the skin into the wound tissue. The microelectrode pairs were connected through an Orion Model 607 Electrode Switchbox to an Orion Model ZA 9040 microprocessor-controlled IonAnalyzer which was equipped with a Model GLP printer. In some cases, the pCO₂ of the wound tissue was measured using an MI-720 microcarbon dioxide electrode. Microelectrodes were obtained from Microelectrodes, Inc. (Londonderry, NH). Tissue pH and pCO₂ were recorded when the IonAnalyzer indicated stable electrode readings.
Measurement of Water Content and Dry Weight of Wounds. The total area of the 20% total body surface area sham or partial-thickness burn wound was rapidly excised to the depth of the fascia, immediately weighed, and dried to constant weight at 70°C. Water content was determined from the difference in the wet and dry weight of the wound tissue.

RESULTS

The pH of sham wounds ranged from 7.07 to 7.17 from 1-72 h PB (Fig. 1). The pH of the partial-thickness burn wound was within the sham control range at 1 h PB but decreased to its lowest value of 6.76 at 6 h PB. The burn wound pH increased slowly to 6.92 at 18 h PB and was within control range from 24-72 h PB.

In contrast to findings reported for ischemic heart (5), pCO$_2$ of the burn wounds decreased rather than increasing during the period in which wound pH was lower than control values, indicating that the decrease in pH of burn wounds was due to other acidic compounds.

FIGURE 1. pH of Sham Control and Partial-Thickness Burn Wounds from 1-72 h PB.
The mean water content of the sham wounds was 4.6 ml (Fig. 2). At 1 h PB, the burn wounds contained 3.6 ml more water than sham wounds. At 3 h PB, they contained 4.6 ml more water. From 6-12 h PB, burn wounds contained approximately 7 ml more water than sham wounds and increased to a maximum of 7.8 ml excess water at 18 h PB before decreasing slowly to 3.6 ml excess water at 72 h PB.

![Graph showing the total water content of sham control and partial-thickness burn wounds from 1-72 h PB.](image)

**FIGURE 2.** Total Water Content of Sham Control and Partial-Thickness Burn Wounds from 1-72 h PB.

The dry weights of the burn wounds were greater than that of the equivalent sham wounds at each time measured (Fig. 3).

We are currently testing methods for separating epidermal and dermal cells of tissue from sham control and partial-thickness burn wounds to be used in studies to determine changes in cellular function.

**PRESENTATIONS/PUBLICATIONS**

None.
FIGURE 3. Dry Weights of Sham Control and Partial-Thickness Burn Wounds from 1-72 h PB.
REFERENCES


23. (U) Following a severe thermal injury, alterations in the host immune system develop which lead to depression of the immune response. Defects in cellular and humoral systems have been reported. These defects are manifested by increased susceptibility to sepsis, impaired delayed hypersensitivity reaction, and prolongation of allograft rejection. The role of interleukin-2 administration following thermal injury in soldiers remains unclear. Therefore, the effects of exogenous interleukin-2 administration following burn wound infection will be studied in a rodent model. A literature search was conducted.

24. (U) Two phases of this study will be performed to determine the optimal dose of interleukin-2 and the optimal postburn day for administration. During the first phase, varied doses of interleukin-2, ranging from low therapeutic levels to toxic doses, will be administered beginning on postburn day 3. Each dosing schedule will be continued 3X daily for 7 days. If an improvement in survival is seen during the first phase of the study, another phase will be performed to determine the optimum day postburn for administration of interleukin-2.

25. (U) 28610 - 8709. This project was approved by the US Army Institute of Surgical Research Animal Care and Use Committee on 4 September 1987.
### RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

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#### PRIMARY
- **PROJECT NUMBER**: 2515A
- **TASK AREA NUMBER**: 00
- **WORK UNIT NUMBER**: 072

#### TITLE
- **Derivative Spectroscopy Chemiluminescent Probe of Granulocyte Redox Function in Healthy Controls and Burn Patients**

#### SUBJECT AREAS
- **Area**
  - Medical Science
  - Biophysics

#### OBSERVATION
1. **The two major objectives of this study are the development of techniques for quantifying the redox enzyme content and function of granulocyte leukocytes, and the evaluation of the sensitivity and specificity of these techniques for assessing the status of antimicrobial defense in burn patients.**

2. **Chemiluminescent probe methods for differential assessment of oxidase and peroxidase activities will be further refined and new techniques based on difference and derivative ultraviolet-visible spectroscopy will be developed for quantifying oxidase and peroxidase enzyme content.**

3. **The strong relationship between granulocyte function and humoral immune status in the burn patient may also require assessment of serum complement. A literature search was conducted.**

4. **Ultrasensitive and differential assessment of granulocyte function can be achieved by measuring the luminescence resulting from oxygenation of high quantum yield substrates.**

5. **The recent availability of diode array ultraviolet-visible spectrophotometers allows for rapid spectral analysis over a broad range.**

6. **Moreover, the digital nature of the data is ideal for microprocessor-assisted derivative spectral analysis of the enzymes responsible for granulocyte redox activity.**

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**DD FORM 1498**

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**EPA NO. 003-005/0029**

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allow analysis of oxidase and peroxidase structure-function relationships. Such information may explain the derangement in granulocyte function responsible for increased susceptibility to infection.

25. (U) 8610 - 8709. Equipment was received during this reporting period. However, this study will be terminated effective 1 October 1987 due the primary investigator's resignation from the service.
23. (U) To evaluate cultured keratinocytes as grafts for epithelial closure of burn wounds. To identify technical and immunological requirements to establish frozen banks of histocompatible keratinocytes for wound coverage in burned soldiers. A literature search was conducted.

24. (U) The possible utility of cultured keratinocytes will be established initially with cultured autologous keratinocytes. Keratinocytes will be cultured from biopsies taken early after admission of patients with large burns and limited unburned donor sites for standard partial thickness autografts. If such grafts are deemed cleanly useful, efforts will expand into investigations of allogeneic skin cultures.

25. (U) 8610 - 8709. The preliminary arrangements are almost complete. The commencement of the clinical use of the cultivated keratinocyte grafts is expected to begin soon.
**RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY**

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**TITLE:** The Effect of High Frequency Ventilation on VA/Q in Sheep with Inhalation Injury

**SUBJECT AREAS:**
- Medicine and Medical Research
- Anatomy and Physiology

**START DATE:** 87-01

**DATE EFFECTIVE:**

**CONTRACT GRANT NUMBER:**

**KIND OF AWARD:**

**NAME OF RESPONSIBLE INDIVIDUAL:**

**ADDRESS:**
- San Antonio, Texas 78234-5012

**TELEPHONE NUMBER:**

**NAME OF PRINCIPAL INVESTIGATOR:**

**NAME OF ASSOCIATE INVESTIGATOR:**

**KEYWORDS:** Inhalation Injury; High Frequency Ventilation; Ventilation-Perfusion Ratio; Cardiac Output

22. (Continued) (U) Animals: (U) Sheep; (U) ILIR; (U) RAIL

23. (U) To compare volumetricdiffusive ventilation with conventional ventilation in effecting changes in the pulmonary and hemodynamic parameters which are altered in an ovine inhalation injury model. A literature search was conducted.

24. (U) Inhalation injury will be induced using the standard ovine smoke inhalation model developed at this Institute. Animals will be then be placed on conventional mechanical ventilation with a volume-limited ventilator. Ventilator settings will be altered to maintain a pH between 7.35 and 7.40 and a partial oxygen pressure between 80 and 100 mmHg. A lactated Ringer's solution will be constantly infused. Central venous pressure, pulmonary artery pressure, transpulmonary pressure, inspiratory and expiratory gas concentrations, percutaneous oxygen saturation, and partial carbon dioxide pressure will be continuously monitored. Once the ventilator settings are maximized, yielding a partial oxygen pressure between 80 and 100 mmHg and a pH between 7.35 and 7.40, the animal will be allowed to stabilize for 2 h. VA/Q distributions will then be measured utilizing the multiple inert gas elimination technique. After stabilization, the lactated Ringer's infusion will be replaced with a lactated Ringer's solution containing six inert gases. Blood and expired gas samples will be analyzed immediately by a GCMS. The animals will then be disconnected from the conventional ventilator and switched to a...
CONTINUATION OF DD FORM 1498 FOR "THE EFFECT OF HIGH FREQUENCY VENTILATION ON VA/Q IN SHEEP WITH INHALATION INJURY"

...metric-diffusive ventilator. After the 2-h stabilization period, VA/Q distribution will again be measured. Data following the stabilization periods will be compared utilizing the student's t-test.

25. Re10 - 8709. (U) This project was approved by the US Army Institute of Surgical Research Animal Care and Use Committee on 14 January 1987. Six animals are to be studied, with the first animal being studied in September 1987.
### Technical Objective

22. (Continued) (U) Crystalloids; (U) Lab Animals; (U) Sheep; (U) ILIR; (U) RAII

23. (U) To determine the hemodynamic consequences of controlled pure plasma loss in sheep using a method to simulate the acute burn. Subsequently, response to therapy will be assessed in burned soldiers. A literature search was conducted.

24. (U) A plasmapheresis filter will be used to create the model of intravascular plasma loss unique to the burn injury. This device selectively removes plasma while leaving the formed elements of blood in the vascular system.

5. (U) 8701 – 8709. This project was approved by the US Army Institute of Surgical Research Animal Care and Use Committee on 14 January 1987. Equipment and animals have been procured and seven animals have been studied. The study is promising and will be continued.
PRESENTATIONS


Pruitt BA Jr: Diagnosis and treatment of inhalation injury and other pulmonary complications. Presented at the Buffalo Burn Symposium, Department of Surgery, State University of New York at Buffalo, Buffalo, New York, 10-11 October 1986.


McCoy KF: Care of the thermally injured patient. Presented to the Physical Therapy Specialist Course (91J), Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 23 October 1986.


McCoy KF: Physical therapy in burn care. Presented to students from Southwest Texas State University, Fort Sam Houston, San Antonio, Texas, 3 November 1986.


Pruitt BA Jr: Infection and burn problems. Presented to the Physician's Assistant Course (C4A), Academy of Health
Summers TM: Introduction to the hospital ministry course. Presented at Brooke Army Medical Center, Fort Sam Houston, San Antonio, Texas, 10 November 1986.


Pruitt BA Jr: Fluid resuscitation and wound care. Presented to the Department of Surgery, University of Oklahoma College of Medicine, Oklahoma City, Oklahoma, 21-22 November 1986.


Carlson D: Combat field feeding system - force development and experimentation. Presented to Dietetic Interns, Nutrition Care Division, Brooke Army Medical Center, Fort Sam Houston, San Antonio, Texas, 23 January 1987.


Reilly DA: Nutritional care. Presented at the OT/PT Conference on Management of Burns in the Theater of Operations,


McManus WF: Innovations in burn care. Presented to the Department of Medicine, Brackenridge Hospital, Austin, Texas, 12 March 1987.


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Carlson D: The role of the dietitian in the critical care setting: critical knowledge for dietitians. Presented to the Nutritional Assessment Postgraduate Short Course, Brooke Army Medical Center, Fort Sam Houston, San Antonio, Texas, 8 June 1987.


Pruitt BA Jr: Management of patients with extensive burns. Presented to the German Army Burn Unit, Koblenz, Germany, 27 June 1987.

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Carlson D: Panel Discussion: Use of the clinical nutrition specialist ASI. Presented to the Nutritional Assessment Postgraduate Short Course, Brooke Army Medical Center, Fort Sam Houston, San Antonio, Texas, 26 June 1987.

Summers TM: Introduction to the hospital ministry. Presented to the Chaplain Candidate Hospital Ministry Course, Brooke Army Medical Center, Fort Sam Houston, San Antonio, Texas, 6 July 1987.


Pruitt BA Jr: Diagnosis and treatment of opportunistic infections in man. Presented at the Martinez Veterans Administration Medical Center, Martinez, California, 22-23 July 1987.


Luster SH: Predicting daily occupational staff requirements in a burn center. Presented to the Mary Lipscomb Hamrick Army Medical Specialist Corps Research Course, Xerox International Training Center, Leesburg, Virginia, 13 August 1987.


Pruitt BA Jr: Surgical treatment of patients with massive burns. Presented at the Royal Childrens Hospital, Melbourne, Australia, 30 September 1987.
PUBLICATIONS


Shiazu T, Yukioka T, Hubbard GB, Langlinais PC, Mason AD Jr, and Pruitt BA Jr: A dose-responsive model of smoke


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<tr>
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Commander
US Army Research Institute
of Environmental Medicine
Building 42
Natick, Massachusetts 01760-5007

1
Defense Technical Information Center
ATTN: DTIC-DDA
Alexandria, Virginia 22304-9990

1
Commandant
Academy of Health Sciences
ATTN: AHS-CDM
Fort Sam Houston, Texas 78234

1
Director
Biological and Medical Sciences Division
Office of Naval Research
800 North Quincy Street
Arlington, Virginia 22217

1
Commanding Officer
Naval Medical Research and Development Command
National Naval Medical Center
Bethesda, Maryland 20014

1
Headquarters
United States Air Force Medical Service Center
ATTN: Chief, Aerospace Medical Consultant
Division
Building 150 (SGPA)
Brooks Air Force Base, Texas 78255

1
Director of Defense Research and Engineering
ATTN: Assistant Director
Environmental and Life Sciences
Washington, DC 20301
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