(1)

US ARMY INSTITUTE OF SURGICAL RESEARCH



# ANNUAL RESEARCH PROGRESS REPORT

## FOR FISCAL YEAR 1990

1 OCTOBER 1989 - 30 SEPTEMBER 1990

**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
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1 October 1990

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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 studies 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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**DEPARTMENT OF THE ARMY**
**U.S. ARMY INSTITUTE OF SURGICAL RESEARCH**
**FORT SAM HOUSTON, TEXAS 78234-5012**

REPLY TO
ATTENTION OF

SGRD-USZ

1 October 1991

MEMORANDUM FOR SEE DISTRIBUTION

SUBJECT: US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Year 1990.

The US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Year 1990 is forwarded under the provisions of OTSG Regulation 70-31 dated 2 April 1969.

Basil A. Pruitt, Jr.

Encl

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

## FOREWORD

The clinical activities and research projects reported in this volume and its predecessors have advanced our understanding of the pathophysiologic responses of injured soldiers and have increased the survival of burn patients to unprecedented levels. The expertise derived from caring for severely burned patients during the past 43 years has been applied to minimize the morbidity and mortality associated with burns sustained as a result of military action in Korea, Vietnam, Lebanon, Granada, and Panama, as well as those injuries sustained mass casualty incidents involving both military personnel and civilians. Studies such as those of the biochemical alterations in burn injured tissue have led to further refinements in fluid resuscitation. The use of newly developed mechanical ventilators has significantly reduced the occurrence of pneumonia and the mortality associated with inhalation injury. Similarly, increased understanding of the changing epidemiology of infection in burn patients, the effect of burn injury on both serologic and cellular limbs of host resistance, and the complex metabolic and nutritional interactions induced by thermal injury have led to improvements in the diagnosis and treatment of infection, the prevention of sepsis, and metabolic support.

The teaching activities of the multidisciplinary members of the burn team have increased the burn care capability of the physicians, nurses, and allied health professionals either in residence at the Institute, or in attendance at the Institute's continuing medical education courses and lectures. In addition, 42 ABLS courses have been conducted by Institute personnel to increase the burn care skills of 752 military health care personnel, both active duty and members of the reserve components. Retention of the instructional information in useable form should enhance the overall burn readiness posture of the medical departments.

The burn disaster assistance mission in which this Institute deployed a multidisciplinary burn team to Ufa, Russia documented the Institute's readiness level in terms of the rapidity of response and effectiveness of action. The swift integration of U.S. and Soviet medical personnel exemplified the importance of using Institute physicians to supervise and train indigenous personnel. That mode of amplification of burn center expertise easily applicable in a Soviet city hospital environment should be even more readily achievable in the U.S. military medical framework. The Ufa mission also identified burn specific equipment and supplies that will be required for full function of a burn center in the theater of operations.

The need to stage the aeromedical transfer of burn patients who have to be moved over long distances, was amply validated during the Vietnam conflict. The system of burn patient transfer that the Institute established in collaboration with the U.S. Air Force effected the intercontinental transfer of 824 burn patients during the period April 1967 to December 1972 with only one inflight death (a patient with acute fulminant melioidosis). The

staging of long distance aeromedical transfer limits the out-of-hospital time to intervals that are physiologically tolerated by even severely burned patients.

The expertise and experience indigenous in the personnel of this Institute will permit establishment of a theater of operations burn center in support of Operation Desert Shield in which care is directed and supervised by an ISR burn team, establishment of a burn staging unit in Germany where ISR personnel will continue care and carry out definitive triage for further treatment, and establishment, in collaboration with the Air Force, of an aeromedical transfer system utilizing ISR flight teams. This system of care and movement will ensure continuity of quality care from the time of injury through convalescence to minimize complications, reduce mortality, effect early wound closure, optimize functional recovery, and accelerate return to duty. The readiness of this Institute and its record of continuous improvement of combat casualty care fully justify the Institute's position within the military and validate the merit of the staff members whose work is reported herein.

Basil A. Pruitt, Jr.

**BASIL A. PRUITT, JR., MD, FACS**
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The opinions or assertions contained herein are the private views of the author and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.

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23/24. (U) The objectives of this work are to provide specialized care for thermally injured patients, investigate diagnostic and therapeutic technics to improve the survival and function of thermally injured patients, and to promulgate scientific medical information to health professionals. Thermally injured patients from the Continential United States and throughout the world are transported to this Institute for intensive, specialized treatment. Carefully controlled evaluations of new treatment technics are conducted by professional staff.

25. (U) 8710 - 8809. Two hundred and twenty-one seriously burned patients were admitted and treated at this Institute during calendar year 1987.

(U) 8810 - 8909. Two hundred and twenty-three seriously burned patients were admitted and treated at this Institute during calendar year 1988.

(U) 8910 - 9009. Two hundred and sixteen seriously burned patients were admitted and treated at this Institute during calendar year 1989. Current clinical research activities include studies of host resistance, endocrine changes following injury, nutritional support regimens, skin substitutes, and postinjury infection control.

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**ANNUAL RESEARCH PROGRESS REPORT**

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**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 1989 - 31 December 1989**

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**PERIOD COVERED IN THIS REPORT:** 1 January 1989 - 31 December 1989

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Two hundred and sixteen patients were admitted to this Institute during calendar year 1989. 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. Areas of research included 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, a clinical evaluation of the use of high frequency ventilation in patients with inhalation injury, quantification of dynamic splint forces on metacarpophalangeal function recovery, evaluation of *in vitro* cultivated keratinocytes as epithelial autografts for the closure of burn wounds, evaluation of imipenem-cilastatin sodium for prophylactic activity against bacterial pneumonias in burned patients with inhalation injury, a study of medium-chain triglyceride utilization, determination of the caloric requirements of thermally injured children, a study of salt and water balance in

the thermally injured patient, assessment by flow cytometry of peripheral blood cells, a study of the effect of recombinant human growth hormone treatment on the rate of healing of burn patients who require skin grafting, a study of human granulocyte-macrophage colony-stimulating factor in patients with thermal injury, an investigation of the importance of alterations in tumor necrosis factor in burn patients, a study of interleukin-1 activity in the serum of thermally injured patients, and a project to characterize certain biochemical indicators of infection in the thermally injured.

## CLINICAL OPERATION
## CENTER FOR TREATMENT OF BURNED SOLDIERS

During calendar year 1989, 216 patients were admitted to this Institute. The average total burn size of the entire population was 22.8% of the total body surface area, with a 10.7% average extent of full-thickness injury. The average hospital stay of all patients, excluding the convalescent leave of active duty military patients, was 40 days.

During calendar year 1989, 494 operative procedures were performed on 160 patients, an average of 3.1 operative procedures per patient.

### ADMISSION DATA

The Clinical Division of this Institute admitted 216 soldiers and other authorized patients with thermal, chemical, or electric injury during calendar year 1989. Aeromedical teams from the Institute conducted 78 missions to transfer 98 (45.4%) of the admitted patients. Seventy-five missions were within the continental United States and three were to areas overseas. Thirty missions (38.5%) were carried out by rotary-wing aircraft and 45 (57.8%) by fixed-wing aircraft. One hundred and forty of the 216 patients (67%) were admitted within 24 h of injury and 151 (70%) were admitted within 48 h of injury. One hundred and seventy-four patients (80.6%) were male and 42 (19.4%) were female.

### DISPOSITION DATA

The following statistics are based on 216 patient admissions during calendar year 1989. The ages of these patients ranged from 2 months to 93 yr, with an average age of 28.1 yr. Burn sizes averaged 22.8% of the total body surface area, with an average full-thickness component of 10.7%. Fifty-one patients were in the pediatric age group (< 15 yr), with an average age of 4.5 yr and an average burn size of 16.1% of the total body surface area. The average hospital stay of all admissions was 40.4 days when convalescent leave for active duty military patients was included in the calculation and 40 days when convalescent leave was excluded. There were 15 patients with high voltage electric injury and 7 patients with chemical injury. The sources of admission are identified in Table 1 and the causes of burn injury are detailed in Table 2.

**TABLE 1.** Sources of Admissions (1989)

| AREA | A | AD | AF | AFD | N/MC | ND | VAB | OTHER | TOTAL |
|---|---|---|---|---|---|---|---|---|---|
| First Army | 2 | 1 | 2 | 1 | 1 | 0 | 1 | 0 | **8** |
| Third Army | 6 | 1 | 1 | 1 | 2 | 0 | 3 | 0 | **14** |
| Fifth Army | 9 | 20 | 6 | 9 | 4 | 2 | 9 | 93 | **152** |
| Sixth Army | 1 | 0 | 1 | 2 | 1 | 0 | 0 | 1 | **6** |
| Brazil | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | **1** |
| Italy | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | **1** |
| Korea | 2 | 1 | 1 | 1 | 13 | 0 | 0 | 0 | **18** |
| Nicaragua | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | **1** |
| Panama | 9 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | **11** |
| Philippines | 0 | 1 | 0 | 0 | 2 | 0 | 0 | 0 | **3** |
| San Salvador | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | **1** |
| **TOTAL** | **29** | **24** | **11** | **14** | **25** | **2** | **13** | **98** | **216** |

**A** = Army, **AF** = Air Force, **N** = Navy, **M** = Marine Corps, **D** = Dependent, **VAB** = Veterans Administration Beneficiary, and **OTHER** = Civilian Emergency, US Public Health Service Beneficiary, and Bureau of Employees Compensation Beneficiary.

**TABLE 2.** Burn Etiology (1989)

| Causes | Number of Patients | Disposition (%) | Deaths | Mortality (%) |
|---|---|---|---|---|
| Gasoline, diesel, and kerosene | 43 | 19.9 | 5 | 25.0 |
| Hot liquids | 39 | 18.1 | – | – |
| Structural fires | 26 | 12.0 | 2 | 10.0 |
| Butane, propane, or natural/sewer gas explosions | 19 | 8.8 | 4 | 20.0 |
| Electrical | 15 | 6.9 | 1 | 5.0 |
| Aircraft accidents | 14 | 6.5 | – | – |
| Open flames | 14 | 6.5 | 4 | 20.0 |
| Bomb, shell, simulator grenade, and gunpowder explosions | 13 | 6.0 | 1 | 5.0 |
| Contact | 10 | 4.6 | – | – |
| Chemical | 7 | 3.2 | – | – |
| Motor vehicle accidents | 5 | 2.3 | 1 | 5.0 |
| Smoking, clothes ignited | 5 | 2.3 | 2 | 10.0 |
| Self-Inflicted | 4 | 1.9 | 1 | – |
| Welding | 2 | 0.9 | – | – |
| **TOTAL** | **216** | **100.0** | **20** | **9.3** |

Inhalation injury was identified in 62 patients (28.7% of admissions). Ninety-eight patients (45.4%) had some associated injury (includes 62 patients with inhalation injury) which included fractures of dislocations in 13 patients, lacerations in 8 patients, and head injuries in 5 patients.

**Morbidity and Mortality.** Twenty of the 216 admissions (9.3%) died during calendar year 1989. Autopsies were performed in 11 (55%) of these hospital deaths. The average burn size of patients who died was 52.9% of the total body surface area and the full-thickness burn was 35% of the total body surface area. Age ranged from 2 to 93 yr. Seventeen of these patients (85%) had inhalation injury as a primary or contributing cause of death. Eighteen patients (90%) had burn injuries exceeding 30% of the total body surface area, 16 (80%) exceeding 40%, and 11 (55%) exceeding 50% of the total body surface area. Two of the 20 deaths (10%) occurred in pediatric patients. These children had an average total body surface area burn of 68% and an average full-thickness burn of 38.8%. The average age of children who died was 6 yr. No autopsies were performed in these children.

Infection was once again the most common complication following thermal injury, with pneumonia occurring in 31 patients. The most common organism isolated in patients with bacterial pneumonia was *Staphylococcus aureus* in 23 patients. Gram-negative organisms were responsible for the pneumonias in the remaining 8 patients. No patients had bacterial invasion of the burn wound; however, 9 had burn wound invasion by fungi or yeast. Aspergillus was the organism identified in viable tissue in 6 of these patients, Candida in 2, and Mucor in 1. No patients had suppurative thrombophlebitis during this reporting period.

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 1985-8. Table 5 summarizes the survival of patients with extensive burns from 1963-89. 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 1989.

## EDUCATIONAL ACTIVITIES

During calendar year 1989, the professional staff of the Clinical Division continued to provide education to professional an paraprofessional groups at the local, national, and international levels. A total of 38 resident physicians were attached for periods of 1-2 months, including 8 each from Wilford Hall USAF Medical Center and Pensacola Naval Air Station, 7 from the University of Texas Health Science Center (San Antonio TX), 4 each from Brooke Army Medical Center and Letterman Army Medical Center, 2 each from Travis Air Force Base Medical Center, Providence Hospital (Southfield MI), and Fitzsimons Army Medical Center, and

**TABLE 3.** Age, Body Surface Involvement, and Mortality (1989)

| Age (Yr) | Total Body Surface Area Burn (%) | | | | | | | | | | Cases | Deaths | Mortality (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0-9 | 10-19 | 20-29 | 30-39 | 40-49 | 50-59 | 60-69 | 70-79 | 80-89 | 90-100 | | | |
| 0 | 2 | 3 | - | 1 | - | - | - | - | - | - | 6 | - | - |
| 1 | 10 | 2 | - | 1 | - | - | - | - | - | - | 13 | - | - |
| 2 | 3 | 2 | 1 | - | - | - | - | - | 1 | - | 8 | 1 | 12.5 |
| 3 | - | 1 | 1 | - | 1 | 1 | - | - | - | - | 4 | - | - |
| 4 | 1 | 2 | - | - | - | - | - | - | - | - | 3 | - | - |
| 5 - 9 | 3 | 1 | 1 | - | - | - | - | - | - | - | 5 | - | - |
| 10 - 14 | 4 | 4 | 1 | 2 | - | 1 | - | - | - | - | 12 | 1 | 8.3 |
| 15 - 19 | 9 | 3 | 2 | 3 | 1 | 1 | - | - | - | - | 19 | - | - |
| 20 - 29 | 23 | 12 | 6 | 7 | 8 | 2 | 4 | 1 | 2 | - | 65 | 3 | 4.6 |
| 30 - 39 | 9 | 6 | 4 | 5 | 2 | 3 | - | 1 | 2 | - | 32 | 2 | 6.3 |
| 40 - 49 | 1 | 7 | 2 | 2 | 2 | 2 | 1 | - | - | - | 17 | 4 | 23.5 |
| 50 - 59 | 3 | 3 | 2 | 2 | 1 | - | - | - | - | - | 11 | - | - |
| 60 - 69 | 4 | 2 | 1 | 1 | 1 | - | - | - | - | - | 9 | 1 | 11.1 |
| 70 - 79 | 1 | 1 | 2 | 1 | 1 | 1 | - | - | - | - | 7 | 3 | 42.9 |
| 80 - 89 | - | - | - | 1 | 3 | - | - | - | - | - | 4 | 4 | 100.0 |
| 90 - 100 | - | - | - | - | - | 1 | - | - | - | - | 1 | 1 | 100.0 |
| Total Cases | 73 | 49 | 23 | 26 | 21 | 12 | 5 | 2 | 5 | - | 216 | | |
| Total Deaths | - | 1 | - | 3 | 5 | 7 | - | - | 4 | - | | 20 | |
| Mortality (%) | - | 2.0 | - | 11.5 | 23.8 | 58.3 | - | - | 80.0 | - | | | 9.3 |

**TABLE 4.** Percent Body Surface Area Burn Involvement and Mortality (1985-9)

| | 0-9 | 10-19 | 20-29 | 30-39 | 40-49 | 50-59 | 60-69 | 70-79 | 80-89 | 91-100 | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1989 | | | | | | | | | | | |
| Number of Patients | 73 | 49 | 23 | 26 | 21 | 12 | 5 | 2 | 5 | - | 216 |
| Number of Deaths | - | 1 | - | 3 | 5 | 7 | - | - | 4 | - | 20 |
| Mortality (%) | - | 2.0 | - | 11.5 | 23.8 | 58.3 | - | - | 80.0 | - | 9.3 |
| 1988 | | | | | | | | | | | |
| Number of Patients | 80 | 55 | 21 | 21 | 16 | 8 | 6 | 6 | 5 | 2 | 220 |
| Number of Deaths | - | 2 | 1 | 2 | 2 | 2 | 3 | 1 | 3 | 2 | 18 |
| Mortality (%) | - | 3.6 | 4.8 | 9.5 | 12.5 | 25.0 | 50.0 | 16.7 | 60.0 | 100.00 | 8.2 |
| 1987 | | | | | | | | | | | |
| Number of Patients | 67 | 52 | 36 | 20 | 19 | 12 | 10 | - | 2 | 3 | 221 |
| Number of Deaths | - | - | 1 | 2 | 3 | 4 | 6 | - | 2 | 3 | 21 |
| Mortality (%) | - | - | 2.8 | 10.0 | 15.8 | 33.3 | 60.0 | - | 100.0 | 100.00 | 9.5 |
| 1986 | | | | | | | | | | | |
| Number of Patients | 61 | 40 | 32 | 21 | 19 | 7 | 11 | 7 | 4 | 5 | 207 |
| Number of Deaths | 1 | 2 | 2 | 2 | 5 | 2 | 4 | 2 | 4 | 5 | 29 |
| Mortality (%) | 1.6 | 5.0 | 6.3 | 9.5 | 26.3 | 28.6 | 36.4 | 28.6 | 100.0 | 100.00 | 14.0 |
| 1985 | | | | | | | | | | | |
| Number of Patients | 41 | 46 | 28 | 28 | 19 | 11 | 9 | 6 | 5 | 4 | 197 |
| Number of Deaths | 2 | 3 | 5 | 3 | 7 | 3 | 5 | 5 | 5 | 4 | 42 |
| Mortality (%) | 4.9 | 6.5 | 17.9 | 10.7 | 36.8 | 27.3 | 55.6 | 83.3 | 100.0 | 100.00 | 21.3 |

**TABLE 5.** Survival and Nonsurvival by Year for Patients with Burns > 30% of the Total Body Surface Area (1963-89)

| Year | SURVIVORS | | | NONSURVIVORS | | |
|---|---|---|---|---|---|---|
| | Number of Cases | Average Burn (%) Total | 3° | Number of Cases | Average Burn (%) Total | 3° |
| 1989 | 52 | 44.3 | 23.7 | 19 | 54.8 | 36.4 |
| 1988 | 56 | 40.9 | 20.6 | 16 | 58.8 | 46.4 |
| 1987 | 46 | 43.7 | 17.2 | 20 | 63.0 | 44.9 |
| 1986 | 51 | 45.9 | 17.2 | 29 | 59.8 | 41.4 |
| 1985 | 48 | 43.6 | 21.7 | 42 | 54.3 | 37.1 |
| 1984 | 43 | 46.4 | 24.8 | 32 | 59.5 | 38.7 |
| 1983 | 37 | 43.5 | 17.5 | 30 | 62.8 | 50.7 |
| 1982 | 53 | 43.7 | 24.8 | 54 | 53.9 | 38.3 |
| 1981 | 54 | 42.7 | 17.5 | 43 | 62.2 | 39.8 |
| 1980 | 62 | 42.7 | 15.1 | 66 | 64.3 | 41.8 |
| 1979 | 61 | 45.4 | 13.4 | 74 | 65.0 | 37.0 |
| 1978 | 67 | 45.7 | 14.8 | 69 | 55.2 | 33.0 |
| 1977 | 66 | 42.2 | 14.4 | 70 | 56.9 | 29.0 |
| 1976 | 69 | 45.5 | 15.0 | 79 | 64.2 | 31.1 |
| 1975 | 80 | 46.1 | 14.7 | 94 | 61.3 | 32.8 |
| 1974 | 55 | 43.9 | 12.2 | 97 | 60.8 | 35.9 |
| 1973 | 47 | 43.7 | 19.6 | 113 | 60.3 | 36.2 |
| 1972 | 62 | 42.0 | 17.2 | 103 | 56.7 | 35.9 |
| 1971 | 63 | 41.9 | 14.0 | 68 | 60.8 | 38.0 |
| 1970 | 92 | 39.4 | 10.7 | 70 | 51.9 | 32.6 |
| 1969 | 113 | 43.2 | 11.1 | 70 | 58.7 | 26.4 |
| 1968 | 143 | 44.2 | 12.6 | 38 | 54.6 | 24.6 |
| 1967 | 103 | 42.7 | 13.3 | 51 | 59.9 | 32.3 |
| 1966 | 68 | 41.5 | 14.9 | 59 | 59.9 | 31.3 |
| 1965 | 47 | 43.8 | 21.0 | 33 | 66.0 | 33.4 |
| 1964 | 40 | 41.8 | 14.8 | 35 | 67.7 | 44.8 |
| 1963 | 28 | 45.8 | 19.6 | 53 | 58.6 | 42.6 |

**TABLE 6.** Comparison of Burn Mortality Rates (1962-3 and 1964-89)

| YEARS | TOTAL BODY SURFACE AREA BURN (%) | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0-29 | | | 30-39 | | | 40-49 | | | 50-59 | | | 60-100 | | |
| | Number of Patients | Number of Deaths | Mortality (%) | Number of Patients | Number of Deaths | Mortality (%) | Number of Patients | Number of Deaths | Mortality (%) | Number of Patients | Number of Deaths | Mortality (%) | Number of Patients | Number of Deaths | Mortality (%) |
| 1962-3 | 140 | 6 | 4.3 | 36 | 16 | 44.4 | 36 | 22 | 61.1 | 23 | 18 | 78.3 | 55 | 49 | 89.1 |
| 1964-88 | 3,485 | 126 | 3.6 | 900 | 162 | 18.0 | 717 | 216 | 30.1 | 504 | 234 | 46.4 | 920 | 752 | 81.8 |
| 1989 | 135 | 5 | 3.7 | 32 | 2 | 6.3 | 17 | 4 | 23.5 | 11 | - | - | 20 | 8 | 40.0 |

**TABLE 7.** Causes of Death (1989)

| Patient | Age | Sex | BURN SIZE (%) Total | 3° | Postburn Day | Cause of Death |
|---|---|---|---|---|---|---|
| 1 | 26 | M | 89 | 73 | 17 | 89% total body surface area burn with inhalation injury and pneumonia. |
| 2 | 29 | M | 88 | 82 | 44 | *88% total body surface area burn with fungal burn wound infection. |
| 3 | 32 | M | 85 | 67 | 1 | 85% total body surface area burn with inhalation injury. |
| 4 | 2 | M | 81 | 62 | 3 | *81% total body surface area burn. |
| 5 | 93 | M | 60 | 37 | 9 | 60% total body surface area burn with inhalation injury. |
| 6 | 45 | M | 58 | 45 | 60 | *58% total body surface area burn with inhalation injury and pneumonia. |
| 7 | 39 | M | 57 | 37 | 8 | *57% total body surface area burn with inhalation injury and pneumonia. |
| 8 | 10 | M | 56 | 16 | 4 | *56% total body surface area burn with inhalation injury and cerebral edema. |
| 9 | 22 | M | 54 | 44 | 29 | 54% total body surface area burn with inhalation injury and pneumonia. |
| 10 | 70 | M | 53 | 45 | 4 | *53% total body surface area burn with inhalation injury. |
| 11 | 41 | M | 53 | 6 | 24 | *53% total body surface area burn with acute myocardial infarction. |

**TABLE 7** (Continued)

| Patient | Age | Sex | BURN SIZE (%) Total | 3° | Postburn Day | Cause of Death |
|---|---|---|---|---|---|---|
| 12 | 81 | F | 47 | 46 | 43 | 47% total body surface area burn with CMV infection - intestinal necrosis - septic shock. |
| 13 | 63 | M | 44 | 30 | 17 | 44% total body surface area burn with acute myocardial infarction. |
| 14 | 74 | M | 42 | 31 | 19 | 42% total body surface area burn with inhalation injury and pneumonia. |
| 15 | 84 | F | 42 | 5 | 8 | 42% total body surface area burn with cardiomyopathy. |
| 16 | 83 | M | 41 | 29 | 27 | 41% total body surface area burn with acute myocardial infarction. |
| 17 | 77 | M | 32 | 11 | 70 | 32% total body surface area burn with acute myocardial infarction. |
| 18 | 88 | F | 30 | 26 | 45 | 30% total body surface area burn with acute bacterial endocarditis and pneumonia. |
| 19 | 44 | F | 32 | 7 | 11 | 32% total body surface area burn with inhalation injury and pneumonia. |
| 20 | 49 | F | 15 | 7 | 50 | 15% total body surface area burn with bronchopneumonia and septicemia. |

*Autopsy not performed.

1 from William Beaumont Hospital (Royal Oak, MI). Five interns from Brooke Army Medical Center rotated through this Institute. A total of 8 medical students rotated through the Institute, including 2 students each from the Uniformed Services University of Health Sciences and the University of North Carolina and 1 each from Tufts University, the University of Indiana, the University of Pittsburgh, and the Medical College of Pennsylvania. In addition, 1 critical care fellow from Brooke Army Medical Center rotated through the Institute. A total of 20 physicians visited from foreign countries for periods ranging from 1 day to 3 months, which included 5 from Pakistan, 4 from Russia, 2 from Norway, and 1 each from Indonesia, Egypt, Spain, Canada, Finland, Nepal, the Philippines, Tunisia, and Turkey. One foreign medical student visited the Institute for a period of 2 months. The Respiratory Therapy Branch had 107 trainees; the Physical Therapy Branch had 148 trainees; and the Occupational Therapy Branch had 143 trainees. Twenty-one scientific publications appeared in refereed medical journals and 271 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 Combat Casualty Care Courses of the United States Army. In addition, weekly professional staff conferences were conducted for and by Institute personnel.

**PRESENTATIONS**

**Stetz CK**: What is a nurse? Presented at Career Day, Nulsen Preschool Program, Fort Sam Houston, San Antonio, Texas, 4 January 1989.

**Luster SH**: Occupational Therapy in Burn Care. Presented as part of the Occupational Therapy Assistant Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 5 January 1989.

**Pruitt BA Jr**: Treatment of patients with extensive burns. Presented to the Department of Surgery, Rush-Presbyterian-St. Luke's Medical Center, Chicago, Illinois, 6 January 1989.

**Pruitt BA Jr**: Fluid therapy of injured patients. Presented to the Department of Surgery, Rush-Presbyterian-St. Luke's Medical Center, Chicago, Illinois, 7 January 1989.

**Duncan DJ**: Initial management of the burn victim. Presented to the Nursing Service Branch, United States Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 10 January 1989.

**DePew CL**: Standards of care for the large burn victim in the initial 48 hours. Presented to the Nursing Service Branch, United States Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 10 January 1989.

**Bergstrom RJ**: Nursing documentation in critical care. Presented to the Nursing Service Branch, United States Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 10 January 1989.

**Driscoll DM**: Burn wound management. Presented to the Nursing Service Branch, United States Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 10 January 1989.

**Jennings JL**: Aeromedical transport of the burn victim. Presented to the Nursing Service Branch, United States Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 10 January 1989.

**Summers TM**: Communicating effectively. Presented to the Nursing Service Branch, United States Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 10 January 1989.

**Hollan E**: Infection control in the burn unit. Presented to the Nursing Service Branch, United States Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 11 January 1989.

**Summers TM**: Psychosocial aspects of burn care. Presented to the Nursing Service Branch, United States Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 11 January 1989.

**Wright ML**: Perioperative care of the burn patient. Presented to the Nursing Service Branch, United States Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 11 January 1989.

**Pruitt BA Jr**: Past and projected combat casualty care. Presented at the United States Army Medical Research and Development Command Combat Casualty Care Review, Denver, Colorado, 16 January 1989.

**Summers TM**: United States Army Institute of Surgical Research. Presented to the Organization of Retired Registered Nurses, United States Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 17 January 1989.

**Driscoll DM**: United States Army Institute of Surgical Research. Presented at the 410th Evacuation Hospital, Fort Sam Houston, San Antonio, Texas, 18 January 1989.

**Duncan DJ**: Burn injuries. Presented at the Southwest Middle School, San Antonio, Texas, 18 January 1989.

**Pruitt BA Jr**: Clinical and laboratory studies of inhalation injury. Presented to the North American Burn Society, Colorado, 23 January 1989.

**Pruitt BA Jr**: Infection surveillance in burn patients. Presented to the North American Burn Society, Colorado, 24 January 1989.

**Duncan DJ**: Environmental emergencies. Presented at the Emergency Medical Technician Certification Program, Fort Sam Houston, San Antonio, Texas, 25 January 1989.

**Duncan DJ**: Hazardous materials. Presented as part of the Emergency Medical Technician Certification Program, Fort Sam Houston, San Antonio, Texas, 25 January 1989.

**Floyd JR**: Wear and apparel of the military uniform. Presented as part of the NCO Development Course, Fort Sam Houston, San Antonio, Texas, 26 January 1989.

**DePew CL**: Acid base balance. Presented as part of the Critical Care Course, Brooke Army Medical Center, Fort Sam Houston, San Antonio, Texas, 30 January 1989.

**DePew CL**: Fluid and electrolytes. Presented as part of the Critical Care Course, Brooke Army Medical Center, Fort Sam Houston, San Antonio, Texas, 30 January 1989.

**DePew CL**: Initial management of the burn victim. Presented as part of the Critical Care Course, Brooke Army Medical Center, Fort Sam Houston, San Antonio, Texas, 30 January 1989.

**Duncan DJ**: Initial management of the burn victim. Presented as part of the Critical Care Course, Brooke Army Medical Center, Fort Sam Houston, San Antonio, Texas, 30 January 1989.

**Selzer RA**: Management of burn injuries in the theater of operation. Presented as part of the United States Air Force Battlefield Nursing Course, Brooks Air Force Base, San Antonio, Texas, 7 February 1989.

**Cioffi WG Jr**: Resuscitation of the injured patient. Presented as part of the Advanced Trauma Life Support Course, University of Texas, San Antonio, Texas, 11 February 1989.

**McManus WF**: Advances in burn care. Presented to the Department of Surgery, University of Nebraska College of Medicine, Omaha, Nebraska, 11 February 1989.

**McManus WF**: Advances in burn care: research for clinical care. Presented at the Midwest Student Medical Research Forum XX, Omaha, Nebraska, 11 February 1989.

**Pruitt BA Jr**: Ethics in biomedical research. Presented at the Noon Conference Lecture Series, Brooke Army Medical Center, Fort Sam Houston, San Antonio, Texas, 15 February 1989.

**Burleson DG**: Variation of T lymphocyte antigen expression. Presented at the Becton Dickinson Biannual User's Conference, San Francisco, California, 16 February 1989.

**Pruitt BA Jr**: Clinical and laboratory research at the United States Army Institute of Surgical Research. Presented at the Noon Conference Lecture Series, Brooke Army Medical Center, Fort Sam Houston, San Antonio, Texas, 22 February 1989.

**Cioffi WG Jr**: Initial care of the thermally injured patient. Presented as part of the United States Air Force Physical Therapy Course, Brooke Army Medical Center, Fort Sam Houston, San Antonio, Texas, 1 March 1989.

**Duncan DJ**: Environmental emergencies. Presented as part of the Emergency Medical Technician Certification Program, Fort Sam Houston, San Antonio, Texas, 1 March 1989.

**Duncan DJ**: Hazardous materials. Presented as part of the Emergency Medical Technician Certification Program, Fort Sam Houston, San Antonio, Texas, 1 March 1989.

**Pruitt BA Jr**: Current management of extensive burns. Presented to the Department of Surgery, WC MacKenzie Health Sciences Center, University of Alberta, Edmonton, Canada, 5 March 1989.

**Pruitt BA Jr**: Role of environment in nosocomial infection. Presented to the Department of Surgery, WC Mackenzie Health Sciences Center, University of Alberta, Edmonton, Canada, 6 March 1989.

**Pruitt BA Jr**: Diagnosis and treatment of infections in surgical patients. Presented to the Edmonton Surgical Society, Edmonton, Canada, 7 March 1989.

**Pinkston GD**: Army medical MOS training. Presented at the South San Antonio High School, San Antonio, Texas, 10 March 1989.

**Burgess MC**: Acute burn trauma: burn wound management. Presented to the 57th Air Evacuation Squadron, Scott Air Force Base, Belleville, Illinois, 13 March 1989.

**Burgess MC**: Acute burn trauma: standards of care for the large burn victim during the initial 48 hours. Presented to the 57th Air Evacuation Squadron, Scott Air Force Base, Belleville, Illinois, 13 March 1989.

**Duncan DJ**: Acute burn trauma: initial management of burn victims. Presented at the Baptist Memorial Hospital School of Nursing, San Antonio, Texas, 13 March 1989.

**Duncan DJ**: Acute burn trauma: standards of care for the large burn victim during the initial 72 hours. Presented to the 57th Air Evacuation Squadron, Scott Air Force Base, Belleville, Illinois, 13 March 1989.

**Selzer RA**: Acute burn trauma: aeromedical evacuation of burn victims. Presented to the 57th Air Evacuation Squadron, Scott Air Force Base, Belleville, Illinois, 13 March 1989.

**Selzer RA**: Acute burn trauma: initial management of burn victims. Presented to the 57th Air Evacuation Squadron, Scott Air Force Base, Belleville, Illinois, 13 March 1989.

**Pruitt BA Jr**: Diagnosis and treatment of opportunistic infections in injured man. Presented to the Department of Surgery, Lankenau Hospital, Philadelphia, Pennsylvania, 14 March 1989.

**Pruitt BA Jr**: Care of the burn wound. Presented at the Crozier-Chester Hospital, Philadelphia, Pennsylvania, 14 March 1989.

**DePew CL**: Acid base balance. Presented as part of the Critical Care Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 14 March 1989.

**DePew CL**: Fluid and electrolytes. Presented as part of the Critical Care Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 14 March 1989.

**Summers TM**: Crisis and families. Presented as part of the Critical Care Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 14 March 1989.

**Molter NC**: Nursing at the United States Army Institute of Surgical Research. Presented at the FORESCOM Conference, Atlanta, Georgia, 16 March 1989.

**Driscoll DM**: Initial management of burn victims in the theater of operation. Presented at the 4th Annual United States Naval Hospital Nursing Symposium, Guantanamo Bay, Cuba, 18 March 1989.

**Molter NC**: Nursing at the United States Army Institute of Surgical Research. Presented as part of the Recruiting Tour, Fort Sam Houston, San Antonio, Texas, 20 March 1989.

**Selzer RA**: Initial management of burn victims in the theater of operation. Presented as part of the United States Air Force Battlefield Nursing Course, Brooks Air Force Base, San Antonio, Texas, 21 March 1989.

**Driscoll DM**: Aeromedical evacuation. Presented at Fort Campbell, Kentucky, 22 March 1989.

**Driscoll DM**: Burn wound care. Presented at Fort Campbell, Kentucky, 22 March 1989.

**DePew CL**: Pacemakers. Presented as part of the United States Army Critical Care Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 28 March 1989.

**Summers TM**: Stress and crisis management. Presented as part of the United States Army Critical Care Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 28 March 1989.

**Jordan BS**: Nursing research in burn care. Presented at the 21st Annual Meeting of the American Burn Association, New Orleans, Louisiana, 29 March 1989.

**Pruitt BA Jr**: Burns in the high risk patient: the burn patient with multiple trauma. Presented at the 21st Annual Meeting of the American Burn Association, New Orleans, Louisiana, 29 March 1989.

**Chu C-S**: Accelerating split-thickness graft healing on tangentially excised deep second degree burn wounds by weak direct current application. Presented at the 21st Annual Meeting of the American Burn Association, New Orleans, Louisiana, 30 March 1989.

**Luster SH**: An evaluation device for quantifying joint stiffness in the burned hand. Presented at the 21st Annual Meeting of the American Burn Association, New Orleans, Louisiana, 31 March 1989.

**Molter NC**: Workload management. Presented at the 21st Annual Meeting of the American Burn Association, New Orleans, Louisiana, 31 March 1989.

**DePew CL**: Acute burn trauma: aeromedical transport of the burn victim. Presented at the 349th Combat Support Hospital, St. Petersburg, Florida, 1 April 1989.

**Chu C-S**: Multiple graft harvesting from donor wounds healed under the influence of weak direct current. Presented at the 21st Annual Meeting of the American Burn Association, New Orleans, Louisiana, 1 April 1989.

**DePew CL**: Acute burn trauma: standards of care for the large burn victim during the initial 72 hours. Presented at the 349th Combat Support Hospital, St. Petersburg, Florida, 1 April 1989.

**Selzer RA**: Acute burn trauma: burn wound care. Presented at the 349th Combat Support Hospital, St. Petersburg, Florida, 1 April 1989.

**Selzer RA**: Acute burn trauma: initial management of the burn victim. Presented at the 349th Combat Support Hospital, St. Petersburg, Florida, 1 April 1989.

**Duncan DJ**: Initial management of the burn victim. Presented at the Glens Falls Hospital, Glens Falls, New York, 3 April 1989.

**Duncan DJ**: Initial management of the burn victim. Presented at the State University of New York College of Arts and Sciences, Plattsburgh, New York, 3 April 1989.

**Driscoll DM**: Environmental emergencies. Presented as part of the Emergency Medical Technician Certification Program, Fort Sam Houston, San Antonio, Texas, 4 April 1989.

**Carlson DE**: Nutritional needs of the burn patient. Presented at the Patients' Family Group Meeting, United States Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 4 April 1989.

**Duncan DJ**: Initial management of the burn victim. Presented at the Rutland Regional Medical Center, Rutland, Vermont, 5 April 1989.

**Duncan DJ**: Initial management of the burn victim. Presented at the University of Vermont, Burlington, Vermont, 4 April 1989.

**Duncan DJ**: Initial management of the burn victim. Presented at the Vermont College of Norwich University, Montpelier, Vermont, 5 April 1989.

**Pruitt BA Jr**: The surgical basic sciences examination history, content, and curriculum. Presented at the annual meeting of the Association for Surgical Education, Tampa, Florida, 7 April 1989.

**DePew CL**: Fluid and electrolytes. Presented at the United States Army Institute of Surgical Research, San Antonio, Texas, 11 April 1989.

**Beverly E**: Anatomy and physiology of the respiratory system. Presented to the 91A Section, Combat Medical Support Division, Fort Sam Houston, San Antonio, Texas, 11 April 1989.

**Summers TM**: Psychosocial aspects of critical care. Presented as part of the Introduction to Hospital Ministry Course, Brooke Army Medical Center, Fort Sam Houston, San Antonio, Texas, 12 April 1989.

**McManus WF**: The burn patient as a trauma model. Presented at the Gary P. Wratten Surgical Symposium, Tacoma, Washington, 13 April 1989.

**Anderson SE**: Aeromedical transport of the burn victim. Presented at the United States Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 12 April 1989.

**Driscoll DM**: Burn wound management. Presented at the United States Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 12 April 1989.

**Hollan E**: Infection control in the burn unit. Presented at the United States Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 12 April 1989.

**Wright ML**: Perioperative management of burn victims. Presented at the United States Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 12 April 1989.

**Bergstrom RJ**: Nursing documentation at the United States Army Institute of Surgical Research. Presented at the United States Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 13 April 1989.

**Duncan DJ**: Initial management of the burn victim. Presented at the United States Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 13 April 1989.

**Summers TM**: Communicating effectively. Presented at the United States Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 13 April 1989.

**Summers TM**: Psychosocial aspects of thermal injuries. Presented at the United States Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 13 April 1989.

**McManus AT**: What's in a name? Is that which we call MRSA just another *Staphylococcus aureus* when treated with vancomycin? Presented at the 9th Annual Meeting of the Surgical Infection Society, Denver, Colorado, 14 April 1989.

**Waymack JP**: Effect of prostaglandin E on resistance to endotoxin and tumor necrosis factor shock. Presented at the 9th Annual Meeting of the Surgical Infection Society, Denver, Colorado, 13 April 1989.

**Pruitt BA Jr**: Infection and use of antibiotics in trauma patients. Presented at the 3rd Congress of Latin American Chapters of the American College of Surgeons, Santiago, Chile, 17 April 1989.

**Pruitt BA Jr**: The pathophysiology of posttraumatic metabolic changes. Presented at the 3rd Congress of Latin American Chapters of the American College of Surgeons, Santiago, Chile, 17 April 1989.

**Pruitt BA Jr**: Management of the severely burned patient. Presented at the 3rd Congress of Latin American Chapters of the American College of Surgeons, Santiago, Chile, 18 April 1989.

**Pruitt BA Jr**: The latest developments in prognostic indices. Presented at the 3rd Congress of Latin American Chapters of the American College of Surgeons, Santiago, Chile, 19 April 1989.

**Pruitt BA Jr**: Replacement of skin in the seriously burned patient. Presented at the 3rd Congress of Latin American Chapters of the American College of Surgeons, Santiago, Chile, 19 April 1989.

**Pruitt BA Jr**: Management of airway injury. Presented at the 3rd Congress of Latin American Chapters of the American College of Surgeons, Santiago, Chile, 20 April 1989.

**Pruitt BA Jr**: The training and role of the surgeon in intensive care. Presented at the 3rd Congress of Latin American Chapters of the American College of Surgeons, Santiago, Chile, 20 April 1989.

**Beverly E**: Anatomy and physiology of the musculoskeletal system. Presented to the 91A Section, Combat Medical Support Division, Fort Sam Houston, San Antonio, Texas, 24 April 1989.

**Luster SH**: Occupational therapy in burn care. Presented as part of the Occupational Therapy Assistant Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 25 April 1989.

**Duncan DJ**: Burns and hazardous materials. Presented as part of the Emergency Medical Technician Course, Fort Sam Houston, San Antonio, Texas, 2 May 1989.

**Gutierrez RT**: AMSC role in mobile medical training teams. Presented as part of the United States Army Medical Specialist Corps Clinical Management Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 3 May 1989.

**Molter NC**: Families in crisis. Presented at the Medical Intensive Care Unit, Humana Metropolitan Hospital, San Antonio, Texas, 9 May 1989.

**Jennings JL**: Burn wound management. Presented at the Aerospace Medical Association Flight Nurse Symposium, Washington, DC, 11 May 1989.

**Duncan DJ**: Acute burn trauma – the first 72 hours: standards of care for the burn victim. Presented at the Pre-Conference Workshop, American Association of Critical Care Nurses National Teaching Institute, Atlanta, Georgia, 14 May 1989.

**Summers TM**: Acute burn trauma – the first 72 hours: psychosocial aspects of thermal injuries and pain management. Presented at the Pre-Conference Workshop, American Association of Critical Care Nurses National Teaching Institute, Atlanta, Georgia, 14 May 1989.

**Selzer RA**: Initial management of burn victims in the theater of operation. Presented as part of the United States Air Force Battlefield Nursing Course, Brooks Air Force Base, San Antonio, Texas, 16 May 1989.

**Anderson SE**: Functioning in an intensive care environment. Presented as part of the OT/PT Management of Burns in the Theater of Operation Course, United States Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 16 May 1989.

**Carlson DE**: Nutritional management of the burn patient. Presented as part of the OT/PT Management of Burns in the Theater of Operation Course, United States Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 16 May 1989.

**Hollan E**: Infection control in burn care. Presented as part of the OT/PT Management of Burns in the Theater of Operation Course, United States Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 16 May 1989.

**Jordan BS**: Research activities at the United States Army Institute of Surgical Research. Presented as part of the OT/PT Management of Burns in the Theater of Operation Course, United States Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 16 May 1989.

**Molter NC**: Pain management in burn care. Presented as part of the OT/PT Management of Burns in the Theater of Operation Course, United States Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 16 May 1989.

**Summers TM**: Psychosocial considerations in the care of the burn patient. Presented as part of the OT/PT Management of Burns in the Theater of Operation Course, United States Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 16 May 1989.

**Carlson DE**: Nutritional management of the burn patient. Presented as part of the OT/PT Management of Burns in the Theater of Operation Course, United States Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 16 May 1989.

**Gutierrez RT**: Physical therapy and thermal injuries. Presented as part of the Advanced Clinical Competencies Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 22 May 1989.

**Pruitt BA Jr**: Diagnosis and treatment of opportunistic infection in severely injured patients. Presented at the Southwestern Hospital, Third Military Medical College, Chongqing, Sichuan, China, 22 May 1989.

**Pruitt BA Jr**: Fluid therapy of injured man. Presented at the 2nd Sino-American Burn Conference, Beijing, China, 22 May 1989.

**Pruitt BA Jr**: Organization and delivery of burn care. Presented at the 2nd Sino-American Burn Conference, Beijing, China, 22 May 1989.

**Cioffi WG Jr**: Failure of recombinant interleukin-2 to improve survival in a rat model of Pseudomonas burn wound sepsis. Presented at the 2nd Sino-American Burn Conference, Beijing, China, 23 May 1989.

**Cioffi WG Jr**: Relationship of transfusion and infection in a burn population. Presented at the 2nd Sino-American Burn Conference, Beijing, China, 24 May 1989.

**Cioffi WG**: Smoke inhalation: laboratory and human studies. Second Military Hospital, Shanghai, China, 24 May 1989.

**Chu C-S**: Wound healing and direct current. Presented at the Burn Injury and Trauma Symposium, Chongqing, China, 29 May 1989.

**McManus WF**: Burns in combat. Presented at the 3rd Annual Acute Combat Trauma Symposium, Tidewater Chapter of the Association of Military Surgeons of the United States, Norfolk, Virginia, 1 June 1989.

**Molter NC**: Bridging the wall - critical care nursing in China. Presented at the Baptist Memorial Hospital School of Nursing, San Antonio, Texas, 2 June 1989.

**Driscoll DM**: Care of the burn trauma patient. Presented at the 9th Annual Military Medical Symposium, New York, New York, 3 June 1989.

**Cioffi WG Jr**: The use of high frequency ventilation in the treatment of patients with inhalation injury. Presented to the John H. Davis Society, Burlington, Vermont, 2 June 1989.

**Cioffi WG Jr**: Chemical injury. Presented as part of the Advanced Burn Life Support Instructor Course, Randolph Air Force Base, San Antonio, Texas, 3 June 1989.

**Cioffi WG Jr**: Wound care. Presented as part of the Advanced Burn Life Support Instructor Course, Randolph Air Force Base, San Antonio, Texas, 3 June 1989.

**Cioffi WG Jr**: Initial care of the thermally injured patient. Presented as part of the United States Air Force Physical Therapy Course, Brooke Army Medical Center, Fort Sam Houston, San Antonio, Texas, 4 June 1989.

**DePew CL**: Acid base balance. Presented as part of the Critical Care Course, Fort Sam Houston, San Antonio, Texas, 5 June 1989.

**DePew CL**: Fluid and electrolytes. Presented as part of the Critical Care Course, Fort Sam Houston, San Antonio, Texas, 5 June 1989.

**Keenan JR**: Initial management of the burn victim. Presented as part of the Critical Care Course, Fort Sam Houston, San Antonio, Texas, 5 June 1989.

**Gutierrez RT**: Physical therapy and burns. Presented as part of the United States Army-Baylor University Physical Therapy Program, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 7 June 1989.

**Gutierrez RT**: Physical therapy and burns. Presented as part of the United States Army-Baylor University Physical Therapy Program, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 8 June 1989.

**Chapman TH**: Environmental emergencies. Presented as part of the Emergency Medical Technician Course, Brooke Army Medical Center, Fort Sam Houston, San Antonio, Texas, 13 June 1989.

**Duncan DJ**: Initial management of the burn victim in the theater of operation. Presented to the 11th United States Air Force Contingency Hospital Casualty Care and Skills Training Course, Lackland Air Force Base, San Antonio, Texas, 13 June 1989.

**Pruitt BA Jr**: Treatment of the burned child. Presented at the 26th Annual Teaching Conference, Pediatrics for the Practitioner, University of Texas Health Science Center, San Antonio, Texas, 16 June 1989.

**Vaughan GM**: The thyroid axis in the rat burn model of non-thyroidal illness (NTI): serum binding defect and altered control of TSH. Presented at the 71st Annual Meeting of the Endocrine Society, Seattle, Washington, 21 June 1989.

**Selzer RA**: Overview of burn care. Presented to the CMSD Cadre, Fort Sam Houston, San Antonio, Texas, 26 June 1989.

**Jordan BS**: Review of current research at the United States Army Institute of Surgical Research. Presented as part of the OT/PT Management of Burns in the Theater of Operations Course, United States Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 26 June 1989.

**Molter NC**: Meeting the needs of families of critically ill patients. Presented at the University of Texas Health Science Center, San Antonio, Texas, 6 July 1989.

**Cioffi WG Jr**: Advanced Trauma Life Support Course, Department of Surgery, The University of Texas at San Antonio, Texas, 9 July 1989.

**Vaughan GM**: A burn model of non-thyroidal illness: the thyroid axis. Presented at the 21st International Congress of Physiological Sciences, Helsinki, Finland, 9 July 1989.

**Pruitt BA Jr**: Management of infections in burn patients. Presented to the Department of Infectious Diseases, Stanford University Medical Center, Stanford, California, 11 July 1989.

**Gutierrez RT**: Thermal injuries. Presented as part of the Physical Therapy Specialist Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 11 July 1989.

**Burgess MC**: Making the critical difference - nursing at the United States Army Institute of Surgical Research. Presented at the United States Army Institute of Surgical Research, 13 July 1989.

**Vaughan GM**: A burn model of nonthyroidal illness: the thyroid axis. Presented at the 31st International Congress of Physiological Sciences, Helsinki, Finland, 14 July 1989.

**DePew CL**: Acid base balance. Presented as part of the United States Army Critical Care Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 18 July 1989.

**DePew CL**: Fluid and electrolytes. Presented as part of the United States Army Critical Care Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 18 July 1989.

**Molter NC**: Making the critical difference - nursing at the United States Army Institute of Surgical Research. Presented at the United States Army Medical Research and Development Command Nursing Research Conference, Baltimore, MD, 19 July 1989.

**Summers TM**: Stress and crisis management. Presented as part of the United States Army Critical Care Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 20 July 1989.

**Driscoll DM**: Environmental emergencies. Presented as part of the Emergency Medical Technician Course, Brooke Army Medical Center, Fort Sam Houston, San Antonio, Texas, 25 July 1989.

**Luster SH**: A device to evaluate stiffness in burned hands. Presented at the United States Army Medical Specialist Corps Research Symposium, Leesburg, Virginia, 30 July 1989.

**Selzer RA**: Initial management of burn victims in the theater of operation. Presented as part of the United States Air Force Battlefield Nursing Course, Brooks Air Force Base, San Antonio, Texas, 1 August 1989.

**DePew CL**: Pacemakers. Presented as part of the United States Army Critical Care Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 1 August 1989.

**Driscoll DM**: Initial management of burn injuries. Presented at the 324th General Hospital, Fort Sam Houston, San Antonio, Texas, 3 August 1989.

**Hickey BD**: Initial management of burn victims. Presented as part of the Specialty Care Course, Brooke Army Medical Center, Fort Sam Houston, San Antonio, Texas, 11 August 1989.

**Vaughan GM**: Diabetes. Presented as part of the Sophomore Endocrine Pathophysiology Course, Division of Endocrinology, Department of Medicine, University of Texas Health Science Center, San Antonio, Texas, 11 August 1989.

**Vaughan GM**: Diabetes. Presented as part of the Sophomore Endocrine Pathophysiology Course, Division of Endocrinology, Department of Medicine, University of Texas Health Science Center, San Antonio, Texas, 11 August 1989.

**Duncan DJ**: Initial management of the burn victim. Presented to the United States Navy Association of Physicians Assistants, Corpus Christi, Texas, 12 August 1989.

**Loresch DC**: Initial management and wound care for the burn victim. Presented at the Clinical Skills for the LPN Conference, El Paso, Texas, 14 August 1989.

**Duncan DJ**: Initial management of burn victims. Presented as part of the United States Army Critical Care Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 18 August 1989.

**Duncan DJ**: Standards of care for the large burn victim in the resuscitative phase. Presented as part of the United States Army Critical Care Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 18 August 1989.

**Driscoll DM**: Sleep. Is it necessary? Presented at the United States Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 28 August 1989.

**Molter NC**: Meeting the needs of families of critically ill patients. Presented at the Audie L. Murphy Veterans Administration Medical Center, San Antonio, Texas, 6 September 1989.

**Pruitt BA Jr**: The changing epidemiology of infections in burn patients. Presented to the International Surgical Group, Edinburgh, Scotland, 7 September 1989.

**Driscoll DM**: Transport of patients with highly infectious diseases. Presented at the Scientific Assembly, Emergency Nurses Association, Washington, DC, 9 September 1989.

**Cannon DJ**: Perioperative burn update. Presented to the Association of Operating Room Nurses, San Antonio, Texas, 9 September 1989.

**Pruitt BA Jr**: Monitoring and diagnosis of infections in burn patients. Presented at the International Society for Burn Injuries Panel Session, Toronto, Canada, 13 September 1989.

**Selzer RA**: Initial management of burn victims. Presented at the Indiana University, Indianapolis, Indiana, 11 September 1989.

**Duncan DJ**: Initial management of the burn victim. Presented at the Wilford Hall United States Air Force Medical Center, Lackland Air Force Base, San Antonio, Texas, 12 September 1989.

**Duncan DJ**: Standards of care for the large burn victim during the resuscitative phase. Presented at the Wilford Hall United States Air Force Medical Center, Lackland Air Force Base, San Antonio, Texas, 12 September 1989.

**Carlson DE**: Nutritional needs of burn patients. Presented at the Patients' Family Group Meeting, United States Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 12 September 1989.

**Driscoll DM**: Initial management of the burn victim. Presented to the Emergency Medical System, Concord, New Hampshire, 25 September 1989.

**Harden NG**: Thermal injuries. Presented as part of the Physical Therapy Specialist Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 29 September 1989.

**Duncan DJ**: Burn trauma: initial management and standards of care. Presented at the Baptist Memorial Hospital School of Nursing, San Antonio, Texas, 2 October 1989.

**Duncan DJ**: Initial management of the burn victim. Presented at the Emergency Nursing Conference, Fort Sam Houston, Texas, 4 October 1989.

**Pruitt BA Jr**: Status of the American Trauma Society. Presented at the 49th Annual Meeting of the American Association for the Surgery of Trauma, Chicago, Illinois, 5 October 1989.

**Pruitt BA Jr**: Review of the Ufa, Russia train/fire disaster. Presented at the 49th Annual Meeting of the American Association for the Surgery of Trauma, Chicago, Illinois, 6 October 1989.

**Loresch D**: Prevention of burn injuries. Presented as part of Fire Safety Week, Kelly Air Force Base, San Antonio, Texas, 8 October 1989.

**Molter NC**: Needs of families of critically ill patients. Presented at St. Elizabeth Hospital, Beaumont Texas, 11 October 1989.

**Burleson DG**: Immune function and infection in burned patients. Presented at the Annual Meeting of the Society for Leukocyte Biology, Marco Island, Florida, 12 October 1989.

**Shippee RL**: Primary and secondary humoral response to sheep red blood cells in a rat model. Presented at the Annual Meeting of the Society for Leukocyte Biology, Marco Island, Florida, 12 October 1989.

**Becker WK**: Medical care in the Soviet Union. Presented at the Armed Forces Medical Intelligence Center, Fort Detrick, Frederick, Maryland, 12 October 1989.

**Duncan DJ**: Initial management of the burn victim. Presented at the Wilford Hall USAF Medical Center, Lackland Air Force Base, San Antonio, Texas, 16 October 1989.

**Duncan DJ**: Standards of care for the large burn victim in the resuscitative phase. Presented at the Wilford Hall USAF Medical Center, Lackland Air Force Base, Texas, 16 October 1989.

**Chapman T**: Initial management of burn victims in the theater of operation, Bergstrom Air Force Base, Texas, 24 October 1989.

**Burgess M**: Developing clinical expertise. Presented at the Drusilla Poole Nursing Education and Staff Development Conference, San Antonio, Texas, 25 October 1989.

**Duncan D**: Developing clinical expertise. Presented at the Drusilla Poole Nursing Education and Staff Development Conference, San Antonio, Texas, 25 October 1989.

**Molter N**: Developing clinical expertise. Presented at the Drusilla Poole Nursing Education and Staff Development Conference, San Antonio, Texas, 25 October 1989.

**DePew CL**: Toxic epidermal necrolysis syndrome. Presented at the AACN Spotlights on Critical Care Conference, San Antonio, Texas, 26 October 1989.

**Okerberg CV**: Mammary gland and integument system of laboratory animals. Presented at the International Life Sciences Institute Histopathology Seminary, Baltimore, Maryland, 29 October 1989.

**Kim SH**: Viral infection in severely burned patients: a review of seven years (1981–1987). Presented at the ASCP/CAP Fall Meeting and Exhibit, Washington, DC, 28 October 1989.

**Keenan J**: Initial management of burn victims in the theater of operation. Presented as part of the United States Air Force Battlefield Nursing Course, Brooks Air Force Base, San Antonio, Texas, 31 October 1989.

**Duncan DJ**: Emergency treatment of thermal and chemical burns. Presented at the Pfizer Safety and Health Conference, San Antonio, Texas, 2 November 1989.

**Burgess M**: Initial management of the burn victim. Presented at the New Rochelle, New Rochelle, New York, 2 November 1989.

**Burgess M**: Initial management of the burn victim. Presented at Pace University, Pleasantville, New York, 3 November 1989.

**Pruitt BA Jr**: Fluid resuscitation following injury. Presented to the Department of Surgery, University of South Florida College of Medicine, Tampa, Florida, 3 November 1989.

**Pruitt BA Jr**: Diagnosis and treatment of opportunistic infections in injured man. Presented to the Department of Surgery, University of South Florida College of Medicine, Tampa, Florida, 4 November 1989.

**Kim SH**: Pitfalls in the evaluation of burn wound biopsies. Presented at the 83rd Annual Scientific Assembly of the Southern Medical Association, Washington, DC, 5 November 1989.

**Duncan DJ**: Initial management of the burn victim. Presented at the Wilford Hall USAF Medical Center, Lackland Air Force Base, San Antonio, Texas, 7 November 1989.

**Duncan DJ**: Standards of care for the large burn victim in the resuscitative phase. Presented at the Wilford Hall USAF Medical Center, Lackland Air Force Base, San Antonio, Texas, 7 November 1989.

**Cioffi WG Jr**: High-frequency percussive ventilation in patients with inhalation injury. Presented to the VDR User's Forum at the 21st Annual Dr. Douglas Davis Pulmonary Symposium, Louisville, Kentucky, 9 November 1989.

**McManus WF**: Septic shock. Presented at the Critical Minutes Symposium, Oklahoma City, Oklahoma, 9 November 1989.

**Pruitt BA Jr**: Clinical and research activities of the United States Army Institute of Surgical Research. Presented at the Lab 21 Review, 14 November 1989.

**DePew CL**: Acid base balance. Presented as part of the United States Army Critical Care Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 22 November 1989.

**Becker WK**: Medical experience in the Soviet Union. Presented at the Officers Club, Randolph Air Force Base, San Antonio, Texas, 21 November 1989.

**DePew CL**: Fluid and electrolytes. Presented as part of the United States Army Critical Care Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 22 November 1989.

**Summers TM**: Families and crisis. Presented as part of the United States Army Critical Care Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 30 November 1989.

**Pruitt BA Jr**: Early burn wound excision and closure. Presented at the 14th Annual Meeting of the International Society of Burn Injuries, Denver, Colorado, 1 December 1989.

**Pruitt BA Jr**: Infections in burn patients. Presented at the 14th Annual Meeting of the International Society of Burn Injuries, Denver, Colorado, 1 December 1989.

**Pruitt BA Jr**: Diagnosis and treatment of infection in injured man. Presented at the University of Colorado Health Sciences Center, Denver, Colorado, 2 December 1989.

**Pruitt BA Jr**: Inhalation injury. Presented at the 14th Annual Meeting of the International Society of Burn Injuries, Denver, Colorado, 2 December 1989.

**Pruitt BA Jr**: Unsolved problems in burn care. Presented at the 14th Annual Meeting of the International Society of Burn Injuries, Denver, Colorado, 2 December 1989.

**Summers TM**: Stress management. Presented as part of the United States Army Critical Care Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 6 December 1989.

**McManus WF**: Elderly, high risk burn patients. Presented at the Comprehensive Care of the Burn Patient Conference, Kansas City, Missouri, 8 December 1989.

**DePew CL**: Pacemakers. Presented as part of the United States Army Critical Care Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 8 December 1989.

**Pruitt BA Jr**: Management of the pregnant burn patient. Presented as part of the Ninth Annual Comprehensive Care of the Burn Patient Course, Kansas City, Missouri, 8 December 1989.

**Pruitt BA Jr**: Management of the pregnant burn patient. Presented as part of the Ninth Annual Comprehensive Care of the Burn Patient Course, Kansas City, Missouri, 9 December 1989.

**Selzer R**: Initial management of burn victims in the theater of operation. Presented as part of the United States Air Force Battlefield Nursing Course, Brooks Air Force Base, San Antonio, Texas, 12 December 1989.

**Becker WK**: USSR train disaster intervention. Presented at the Seventh Users' Stress Workshop on Training for Psychic Trauma, San Antonio, Texas, 14 December 1989.

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**ANNUAL RESEARCH PROGRESS REPORT**

**PROJECT NUMBER:** 3M162787A874-00, APPLIED RESEARCH AND EXPLORATORY DEVELOPMENT

**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 1989 - 31 December 1989**

**INVESTIGATORS**

Roger L. Wesley, MD, Captain, MC
Thomas B. Dougherty, MD, Ph.D., Major, MC

## ABSTRACT

**PROJECT NUMBER:** 3M162787A874-00, APPLIED RESEARCH AND EXPLORATORY DEVELOPMENT

**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 89 through 31 Dec 89

**INVESTIGATORS:** Roger L. Wesley, MD, Captain, MC
Thomas B. Dougherty, MD, Ph.D., Major, MC

In the period covered by this report, 493 anesthetics were administered to 172 patients, an average of 2.87 anesthetics per patient. The most commonly used anesthetic agents were narcotics (54.56%), followed by isoflurane (33.87%), ketamine (7.30%), halothane (2.43%), and enflurane (0.62%). Due to the nature and combinations of procedures now preformed, regional anesthesia was infrequently used.

## ANESTHESIOLOGY

### PREOPERATIVE PROCEDURES

**Evaluation.** Most burned patients are several days post-injury when first seen by the anesthesiologist. Time in the immediate postburn period is used to stabilize the patients by utilizing various physiological data gained from routine and invasive monitoring of several indices: hematologic (hematocrit, electrolytes, liver and renal function tests); pulmonary (arterial blood gases, pulse oximetry, respiratory rates, daily chest roentgenogram, pulmonary function tests); cardiovascular (arterial blood pressure, central venous pressure, pulmonary arterial and wedge pressures, cardiac output measured by Swan-Ganz catheters); and renal (urine output, urine chemistries). In addition, the anesthesiologist conducts the usual preoperative chart review, patient interview, and physical exam.

All patients who have electrical injuries have a preoperative electrocardiogram performed and serum cardiac enzyme levels measured to rule out possible myocardial damage.

**Preparation.** Patients are kept NPO after 2400 hours the day prior to surgery except in the following situations. Children may receive clear liquids up to five hours prior to surgery, and infants up to three hours prior to surgery. Any patient with an enteral feeding tube, the distal end of which is shown to be beyond the Ligament of Treitz, may have tube feedings continued perioperatively.

**Premedication.** Routine medications such as cimetidine, sucralfate, or cardiovascular medications are continued up to the time of surgery. Benzodiazepines such as valium, along with oral metoclopramide are routinely given as premedicants for patients taking a p.o. diet. Morphine is frequently given as premedication for ICU patients. Atropine, 20 mcg/kg IV is given routinely to pediatric patients under the age of two immediately prior to induction of anesthesia. Glycopyrrolate, 0.005 mg/kg, to a maximum dose of 0.2 mg, is given IV immediately prior to induction with ketamine.

**Intraoperative Fluids.** All fluids except hyperalimentation solutions are changed to Ringer's Lactate or Ringer's Lactate with 5% dextrose on arrival in the operating room. Normal saline is used as a packed red blood cell diluent, and its use is kept to a minimum to avoid sodium loading.

### TYPES OF ANESTHESIA

At the US Army Institute of Surgical Research, narcotics including fentanyl and sufentanil are the most frequently used anesthetic agent, having replaced isoflurane which was the primary

anesthetic during recent years. Isoflurane, enflurane, ketamine, and halothane are also used, but to a lesser extent (Table 1).

**Narcotics.** The opioids fentanyl and sufentanil are the narcotic anesthetics most often utilized, with morphine and alfentanil less often utilized. These compounds produce analgesia, drowsiness, mood alterations, respiratory depression, euphoria, sedation, miosis, dysphoria, and vasomotor stimulation via stimulation of various opioid receptors. The opioids are used as the primary anesthetic, alone with an adjunct such as nitrous oxide, or low dose volatile agent. Narcotics decrease the hypermetabolic and hyperdynamic tendencies of the burned patient, and provide postoperative pain relief. As a general rule, burned patients require larger doses of narcotic anesthetics than unburned patients. The opioids are the most frequently used primary anesthetic agent at the USAISR.

**Isoflurane.** Isoflurane is the most recently introduced halogenated ether anesthetic agent to be introduced to the USAISR. Biotransformation amounts to only 0.25% of an inhaled dose, and no toxic reactions to the metabolic products have been reported to date. Although it has a rather pungent odor that tends to limit its use as a sole mask induction agent, its use in combination with sodium pentothal, ketamine, or etomidate provides a smooth anesthetic induction that is significantly more rapid than that with enflurane. Isoflurane has been the most commonly used anesthetic agent at the USAISR until the opioids supplanted it this year.

**Ketamine.** This agent is used both IV and IM to produce its characteristic dissociative anesthetic state. Basal functions and laryngeal reflexes tend to be preserved, and the cardiovascular system is supported as well. Unfortunately, ketamine shares with its parent compound, phencyclidine, the production of a significant incidence of unpleasant side effects. However, proper patient preparation and premedication with a benzodiazepine appear to have reduced the unpleasant emergence reactions to a level where they currently are of little consideration in the carefully-selected patient. Laryngospasm, airway obstruction, and regurgitation can occur with ketamine. All ketamine anesthetics, other than in children, are preceded by IV diazepam (0.15–0.2 mg/kg) or midazolam (0.05 mg/kg).

**Enflurane.** Enflurane is an isomer of isoflurane which provides a relatively smooth anesthetic induction and good muscle relaxation. Biotransformation only amounts to 2 - 2.5% of an inhaled dose which, perhaps, accounts for the few clinical toxic effects observed. Plasma fluoride levels in hypermetabolic burned patients during and after enflurane administration have been measured and found not to be in the toxic range.

**Halothane.** Halothane is a halogenated alkane that has met with limited use at the USAISR over recent years. 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 isoflurane and enflurane, few indications exist for halothane's use in this patient population that may be predisposed to hepatitis from multiple transfusions with blood products. Halothane is much less pungent and causes a more rapid anesthetic induction than isoflurane or enflurane. As a result, halothane's use is indicated primarily in the burned pediatric patient who requires that his/her airway be secured by an endotracheal tube following a smooth, rapid induction of anesthesia.

**Regional Anesthesia.** Although regional anesthesia is generally considered one of the safest methods available, its use in the thermally injured patient is limited for the following reasons. Infection over or near the site of injection and sepsis are contraindications for its use. Also, multiple-site operations limit the practicality of this approach.

**Nitrous Oxide.** This agent is used in concentrations of 50-70% with oxygen. It is used to supplement other analgesic or anesthetic agents.

**Muscle Relaxants.** Succinylcholine has not been used for any purpose in this unit for more than a decade. On the other hand, nondepolarizing muscle relaxants (vecuronium, pancuronium, or atracurium) were used in 91.08% of the operative cases over the past year.

## MONITORING TECHNIQUES

The following monitoring techniques were used intraoperatively to ensure:

1. Adequate oxygenation of the patient

    a. Inspired and expired oxygen concentration (in-circuit oxygen analyzer and mass spectrometer

    b. Arterial hemoglobin oxygen saturation (pulse oximeter)

    c. Patient's color

2. Adequate ventilation of the patient

    a. Respiratory rate

    b. Chest excursions

c. Auscultation of breath sounds (esophageal and precordial stethoscopes)

d. End-tidal CO2 concentration (continuous capnometer and mass spectrometer)

e. Indices of pulmonary function (Siemens ventilator)

f. Arterial blood gases (if indicated)

The noninvasive measurements of the end-tidal carbon dioxide, arterial hemoglobin oxygen saturation by pulse oximetry, and indices of pulmonary function (e.g. tidal volume, peak inspiratory pressure) all represent no risk to the patient, are easily obtainable, and are accurate. These monitors are standard in our anesthetic care. Table 2 show the frequencies of use of selected intraoperative monitors over the past three years.

3. Hemodynamic stability of the patient

a. Continuous EKG

b. Auscultation of heart sounds (precordial and esophageal stethoscopes)

c. Peripheral pulse

d. Arterial blood pressure

e. Central venous and wedge pressures, cardiac output, systemic vascular resistance (if indicated)

f. Serial hematocrits

g. Urine output

Direct arterial lines are used when indicated. The Dinamap automated blood pressure cuff is routinely used for intraoperative blood pressure monitoring. Since it can be used over dressings and is noninvasive, it is the most practical method of monitoring blood pressure in our patient population.

Efforts continue toward a safe reduction in the usage of blood products in our patients. Patient are now routinely returned from the operating room with hematocrits in the range of 22 - 30%.

4. Adequate body temperature of the patient

a. Continuous monitoring of skin, rectal, nasopharyngeal, or esophageal temperatures

**TABLE 1. Primary Agents**

| AGENT | 1986 NUMBER | 1986 % | 1987 NUMBER | 1987 % | 1988 NUMBER | 1988 % | 1989 NUMBER | 1989 % |
|---|---|---|---|---|---|---|---|---|
| Narcotics | 7 | 1.71 | 39 | 8.61 | 114 | 24.46 | 269 | 54.56 |
| Isoflurane | 23 | 5.61 | 196 | 43.27 | 278 | 59.66 | 167 | 33.87 |
| Ketamine | 63 | 15.37 | 57 | 12.58 | 35 | 7.51 | 36 | 7.30 |
| Enflurane | 272 | 66.34 | 129 | 28.48 | 23 | 4.94 | 3 | 0.62 |
| Halothane | 35 | 8.54 | 29 | 6.40 | 7 | 1.50 | 12 | 2.43 |
| Local | 10 | 2.44 | 9 | 1.99 | 9 | 1.93 | 6 | 1.22 |
| Nitrous oxide | 0 | 0 | 4 | 0.88 | 1 | 0.21 | 1 | 0.22 |
| TOTAL | 410 | 100 | 453 | 100 | 466 | 100 | 493 | 100 |

**TABLE 2. Frequencies of Use of Selected Intraoperative Monitors**

| MONITOR PARAMETER | 1987 No. of Uses | 1987 %Total Cases | 1988 No. of Uses | 1988 %Total Cases | 1989 No. of Uses | 1989 %Total Cases |
|---|---|---|---|---|---|---|
| Blood Pressure | 453 | 100 | 466 | 100 | 493 | 100 |
| Heart Tones | 453 | 100 | 466 | 100 | 493 | 100 |
| End-tidal CO2 | 441 | 97.35 | 459 | 98.50 | 473 | 95.94 |
| Inspired CO2 Conc. | 442 | 97.57 | 461 | 98.93 | 493 | 100 |
| Pulse Oximeter | 444 | 98.01 | 465 | 99.79 | 488 | 98.98 |
| PFT's | 391 | 86.31 | 423 | 90.77 | 461 | 93.51 |
| Arterial Line | 75 | 16.56 | 138 | 29.61 | 124 | 25.15 |
| CVP | 29 | 6.40 | 14 | 3.00 | 21 | 4.26 |
| Swan Ganz | 26 | 5.74 | 29 | 6.22 | 42 | 8.52 |
| Temperature | 443 | 97.79 | 460 | 98.71 | 485 | 98.38 |
| TOTAL CASES | 453 | | 466 | | 493 | |

b. Because of the greatly increased evaporative losses in burned patients, hypothermia can be a serious problem. Several methods are employed to maintain adequate body temperature during anesthesia:

(1) The temperature of the operating room is maintained between 85 and 90 degrees F

(2) Anesthetic gases are heated and humidified

(3) Radiant heat lamps are utilized as needed

(4) Disposable K-thermia heating blankets are used when indicated

(5) Scrub solutions, IV fluids, and blood products are warmed

## COMPLICATIONS

There were no anesthetic complications noted during 1989.

## PATIENT DATA AND OVERALL PROCEDURES

Table 3 illustrates the overall patient anesthetic data for the year 1979 through 1989. Table 4 shows the recent trends in operative procedures at the USAISR.

**TABLE 3. Overall Patient Anesthetic Data, USAISR (1979-1989)**

| Year | Total No. of Patients | No. of Patients Anesth.* | % of Patient Anesth.* | Total Anesthetics Given | No. of Anesthetics Per Patient |
|---|---|---|---|---|---|
| 1979 | 267 | 161 | 60.30 | 554 | 3.44 |
| 1980 | 243 | 148 | 60.91 | 531 | 3.59 |
| 1981 | 208 | 127 | 61.16 | 404 | 3.18 |
| 1982 | 231 | 151 | 65.37 | 532 | 3.52 |
| 1983 | 179 | 98 | 54.75 | 291 | 2.97 |
| 1984 | 190 | 139 | 73.16 | 461 | 3.32 |
| 1985 | 197 | 133 | 67.51 | 388 | 2.92 |
| 1986 | 207 | 143 | 69.08 | 410 | 2.87 |
| 1987 | 221 | 179 | 81.00 | 453 | 2.53 |
| 1988 | 219 | 161 | 73.52 | 466 | 2.89 |
| 1989 | 218 | 172 | 78.89 | 493 | 2.87 |

*Abbreviation: Anesth.= Anesthetized

**TABLE 4. Nature of Surgery, USAISR (1985-1989)**

| PROCEDURE | 1985 No. | 1985 % | 1986 No. | 1986 % | 1987 No. | 1987 % | 1988 No. | 1988 % | 1989 No. | 1989 % |
|---|---|---|---|---|---|---|---|---|---|---|
| Excision | 304 | 43.4 | 303 | 38.3 | 397 | 44.7 | 421 | 45.6 | 453 | 46.6 |
| Autograft | 304 | 43.4 | 372 | 47.0 | 389 | 43.8 | 395 | 42.7 | 424 | 43.6 |
| Orthopedic | 19 | 2.7 | 29 | 3.7 | 27 | 3.0 | 36 | 3.9 | 25 | 2.6 |
| Chondrectomy | 0 | 0 | 1 | 0.1 | 5 | 0.6 | 2 | 0.2 | 1 | 0.1 |
| Eye & Lid | 9 | 1.3 | 19 | 2.4 | 9 | 1.0 | 11 | 1.2 | 17 | 1.7 |
| Intra-abdominal | 12 | 1.7 | 4 | 0.5 | 7 | 0.8 | 6 | 0.6 | 6 | 0.6 |
| Plastic | 9 | 1.3 | 5 | 0.6 | 5 | 0.6 | 12 | 1.3 | 11 | 1.1 |
| Other | 44 | 6.3 | 58 | 7.3 | 50 | 5.6 | 41 | 4.4 | 36 | 3.7 |
| **TOTAL** | **701** | **100** | **791** | **100** | **889** | **100** | **924** | **100** | **973** | **100** |

**RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY**

| 1 AGENCY ACCESSION | 2 DATE OF SUMMARY | REPORT CONTROL SYMBOL |
|---|---|---|
| DAOG6971 | 01 Oct 90 | DD-DR&E(AR) 636 |

| 3 DATE PREV SUM'RY | 4 KIND OF SUMMARY | 5 SUMMARY SCTY | 6 WORK SECURITY | 7 REGRADING | 8 DISB'N INSTR'N | 9 LEVEL OF SUM A WORK UNIT |
|---|---|---|---|---|---|---|
| 01 Oct 89 | D. Chg | U | U | | CX | |

| 10 NO. CODES | PROGRAM ELEMENT | PROJECT NUMBER | TASK AREA NUMBER | WORK UNIT NUMBER |
|---|---|---|---|---|
| a. PRIMARY | 62787A | 3M162787A874 | FB | 162 |
| b. CONTRIBUTING | | | | |
| c. CONTRIBUTING | | | | |

11. TITLE (Precede with Security Classification Code) (U) 5% Aqueous Sulfamylon Soaks Used in Topical Treatment of Burned Patients

12 SUBJECT AREAS
0605 Medicine and Medical Research 0613 Microbiology 0615 Pharmacology

| 13. START DATE | 14. ESTIMATED COMPLETION DATE | 15. FUNDING ORGANIZATION | 16 PERFORMANCE METHOD |
|---|---|---|---|
| 7610 | 9909 | DA | C. In-House |

17. CONTRACT/GRANT
a. DATE EFFECTIVE
b. CONTRACT/GRANT NUMBER
c. TYPE
d. AMOUNT
e. KIND OF AWARD
f. CUM TOTAL

MILITARY RELEVANCY CERTIFIED
APPROVED BY

18. RESOURCES ESTIMATE

| a FISCAL YEARS | a PROFESSIONAL WORK YEARS | b FUNDS (In thousands) |
|---|---|---|
| 90 | 2.0 | 120 |
| 91 | 2.0 | 126 |

| 19. RESPONSIBLE DOD ORGANIZATION | 20 PERFORMING ORGANIZATION |
|---|---|
| a. NAME: US Army Institute of Surgical Research | a NAME: US Army Institute of Surgical Research |
| b. ADDRESS (include zip code): Fort Sam Houston, San Antonio, Texas 78234-5012 | b ADDRESS: Fort Sam Houston, San Antonio, Texas 78234-5012 |
| c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A | c. NAME OF PRINCIPAL INVESTIGATOR: MC MANUS, W F |
| d. TELEPHONE NUMBER (include area code): 512-221-2720 | d TELEPHONE NUMBER (include area code): 512-221-3301 |
| 21. GENERAL USE: FINA<br>MILITARY/CIVILIAN APPLICATION: M | f. NAME OF ASSOCIATE INVESTIGATOR (if available)<br>g. NAME OF ASSOCIATE INVESTIGATOR (if available) |

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Burns (Injuries); (U) Skin Grafts; (U) Therapy; (U) Wounds and Injuries

23 TECHNICAL OBJECTIVE 24 APPROACH 25 PROGRESS (Precede text of each with Security Classification Code)

23/24. (U) The objective of this work is 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. Patients admitted to this Institute for care following thermal, chemical, or electric injury will be treated with 5% aqueous mafenide acetate soaks daily.

25. (U) 8710 - 8809. One hundred and seventy-two patients were treated with 5% aqueous mafenide acetate soaks. Sixteen of these patients exhibited mild cutaneous atopy.

(U) 8810 - 8909. One hundred and sixty-eight patients were treated with 5% aqueous mafenide acetate soaks. Twenty-two of these patients exhibited mild cutaneous atopy.

(U) 8910 - 9009. One hundred and sixty-six patients were treated with 5% aqueous mafenide acetate soaks. Eleven of these 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.

DD FORM 83 MAR 1498 EDITION OF MAR 68 IS OBSOLETE * U.S.G.P.O. 1988-491-003/50329

**SUPPLEMENTAL WORK UNIT INFORMATION FOR THE PROJECT ENTITLED "5% AQUEOUS SULFAMYLON SOAKS USED IN TOPICAL TREATMENT OF BURNED PATIENTS"**

**Subrecord/Linking Accession Number:** Not applicable.

**Search Control Data:** W6R17I/W6323L, 19 October 1989

**Product Identification:** For technical reports, refer to the US Army Institute of Surgical Research Annual Research Progress Report for fiscal years 1977-90.

**Unclassified Special Categories:** Volunteers: Adults; Children; RA II

**ANNUAL PROGRESS REPORT**

**PROJECT NUMBER:** 3M162787A874-00, APPLIED RESEARCH AND EXPLORATORY DEVELOPMENT

**PROJECT TITLE** 5% Aqueous Sulfamylon Soaks Used in Topical Treatment of Burned Patients

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

**1 October 1989 - 30 September 1990**

**INVESTIGATORS**

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

## ABSTRACT

**PROJECT NUMBER:** 3M162787A874-00, APPLIED RESEARCH AND EXPLORATORY DEVELOPMENT

**PROJECT TITLE:** 5% Aqueous Sulfamylon Soaks Used in Topical Treatment of Burned Patients

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

**PERIOD COVERED IN THIS REPORT:** 1 Oct 89 through 30 Sep 90

**INVESTIGATORS:** William F. McManus, M.D., Colonel, MC
Basil A. Pruitt, Jr., M.D., Colonel, MC

During this report period, 5% aqueous Sulfamylon dressings have continued to be an efficacious treatment modality in the care of the burn wound. One hundred and sixty-six patients were treated with 5% aqueous Sulfamylon dressings employed either for final debridement of a wound or following application of meshed cutaneous autograft to prevent desiccation of tissue exposed in the interstices of such grafts. A 7% incidence of skin rash (atopy) was noted as the only adverse reaction. The clinical results achieved by the use of 5% aqueous Sulfamylon solution strongly support its continued use.

**5% AQUEOUS SULFAMYLON SOAKS USED IN TOPICAL TREATMENT OF BURNED SOLDIERS**

During this reporting period of 1 October 1989 through 30 September 1990, the evaluation of 5% Sulfamylon acetate solution for topical treatment of the burn wound has continued at this Institute and was used in 166 patients (71%) of the 234 patients admitted to the U.S. Army Institute of Surgical Research. The 5% Sulfamylon acetate soaked dressings are used as wet to dry dressings to debride nonviable tissue 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% Sulfamylon acetate to decrease the rate of bacterial growth and to prevent desiccation of tissue exposed in the interstices of such grafts.

Eleven patients (7%) demonstrated allergic reactions (atopy) with the use of 5% aqueous Sulfamylon solution and these nineteen patients demonstrated rapid resolution of the atopic reaction discontinuation of the 5% aqueous Sulfamylon soaked dressings. Saline or other aqueous topical antimicrobial agents were utilized thereafter and no other adverse reactions were noted in this group of patients.

The use of 5% aqueous Sulfamylon acetate 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 Sulfamylon acetate solution is most helpful when meshed cutaneous autografts are applied so that desiccation or uncontrolled bacterial colonization does not occur, thus permitting the dressings over such meshed autografted skin to remain in place for an average of three days allowing good adherence prior to the first dressing change. The efficacy and the low incidence of adverse side effects speak for continued use of this solution.

| RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY | | | | 1. AGENCY ACCESSION | 2. DATE OF SUMMARY | REPORT CONTROL SYMBOL |
|---|---|---|---|---|---|---|
| | | | | DAOG6970 | 01 Oct 90 | DD-DR&E(AR) 636 |

| 3. DATE PREV SUM'RY | 4. KIND OF SUMMARY | 5. SUMMARY SCTY | 6. WORK SECURITY | 7. REGRADING | 8. DISP'N INSTR'N | 9. LEVEL OF SUM / A. WORK UNIT |
|---|---|---|---|---|---|---|
| 01 Oct 89 | D. Chg | U | U | | CX | |

| 10. NO./CODES: | PROGRAM ELEMENT | PROJECT NUMBER | TASK AREA NUMBER | WORK UNIT NUMBER |
|---|---|---|---|---|
| a. PRIMARY | 62787A | 3M162787A874 | CJ | 163 |
| b. CONTRIBUTING | | | | |
| c. CONTRIBUTING | | | | |

11. TITLE *(Precede with Security Classification Code)*
(U) Studies of the Neuroendocrine Abnormalities in Burn Injury

12. SUBJECT AREAS
0605 Medicine and Medical Research 0615 Pharmacology

| 13. START DATE | 14. ESTIMATED COMPLETION DATE | 15. FUNDING ORGANIZATION | 16. PERFORMANCE METHOD |
|---|---|---|---|
| 7910 | 9909 | DA | C. In-House |

17. CONTRACT/GRANT [illegible] MILITARY RELEVANCY CERTIFIED APPROVED BY [illegible]

| a. DATE EFFECTIVE | | c. RESOURCES ESTIMATE | | |
|---|---|---|---|---|
| b. CONTRACT/GRANT NUMBER | | FISCAL YEARS | a. PROFESSIONAL WORKYEARS | b. FUNDS *(In thousands)* |
| c. TYPE | d. AMOUNT | 90 | 0.1 | 51 |
| e. KIND OF AWARD | f. CUM/TOTAL | 91 | 0.1 | 54 |

| 19. RESPONSIBLE DOD ORGANIZATION | 20. PERFORMING ORGANIZATION |
|---|---|
| a. NAME: US Army Institute of Surgical Research | a. NAME: US Army Institute of Surgical Research |
| b. ADDRESS *(include zip code)*: Fort Sam Houston, San Antonio, Texas 78234-5012 | b. ADDRESS: Fort Sam Houston, San Antonio, Texas 78234-5012 |
| c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A | c. NAME OF PRINCIPAL INVESTIGATOR: VAUGHAN, G M |
| d. TELEPHONE NUMBER *(include area code)*: 512-221-2720 | d. TELEPHONE NUMBER *(include area code)*: 512-221-5416 |
| 21. GENERAL USE: FINA | f. NAME OF ASSOCIATE INVESTIGATOR *(if available)* |
| MILITARY/CIVILIAN APPLICATION: M | g. NAME OF ASSOCIATE INVESTIGATOR *(if available)* |

22. KEYWORDS *(Precede EACH with Security Classification Code)* (U) Burns (Injuries); (U) Pineal Gland; (U) Indoles; (U) Catecholamines

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS *(Precede text of each with Security Classification Code)*

23/24. (U) The objective of this work is to characterize alterations of neuroendocrine function in burned patients in order to improve survival. Photic control of the melatonin rhythm and the daytime loss of sympathetic responsiveness of the pineal will be studied in murine models and the findings related to the sympathetic unresponsiveness of critical injury.

25. (U) 8710 - 8809. Actions of the pineal hormone, melatonin, appear to depend partly on the timing of its rhythmic surge. The pineal has been shown to control several hormonal and metabolic functions in nontrauma murine models. Though some of these functions are altered also in severe injury, the interaction of the pineal in injury can only be assessed properly after a better understanding of the control of the pineal itself is obtained. Analysis of pineal N-acetyltransferase and melatonin and serum melatonin in Sprague-Dawley rats adapted to 10 or 14 h of dark per light cycle showed that the nocturnal rise, fall, and acrophase of melatonin synthesis and secretion are controlled by entrainment to lights-on rather than lights-off. The surge depends on beta-adrenergic input to the pineal, with daytime pineal unresponsiveness to catecholamine (particularly in humans and Syrian hamsters) beginning just after lights-on and not due to a daytime fall in beta-receptors. In hamster pineals, activities of two lysosomal enzymes fell abruptly after lights on, indicating a possible role of lysosomes in permitting catecholamine responsiveness. Evaluation of melatonin's role in normal and burned organisms will depend on its accurate measurement. Progress in refining the assay technique (especially difficult in hamster serum) has been made.

DD FORM 1498 83 MAR — EDITION OF MAR 68 IS OBSOLETE — * USGPO 1986 -491-003/50329

**CONTINUATION OF DD FORM 1498 FOR THE PROJECT ENTITLED "STUDIES OF THE NEUROENDOCRINE ABNORMALITIES IN BURN INJURY"**

(U) 8810 - 8909. In Syrian hamsters (the model for human pineal control), pineal adrenergic responsiveness (AR) is absent during most of the day. Early morning hamster pineal AR was assessed with a standard bolus injection of isoproterenol that raises pineal melatonin content in the dark phase. Injection at 50 min after the end of the usual 10-h dark phase, either in light or extension of darkness, raised daytime pineal melatonin almost to the same level as seen after injection during the nocturnal "sensitive" period. Extension of the sensitive period into the first part of the light phase (but not acutely affected by light) indicates that AR is controlled very differently from endogenous melatonin production which is acutely lowered by light at night to daytime values. A new antibody, iodinated melatonin, and a new technique that achieves detectability near the low daytime serum level and an ED50 near the peak nocturnal level will permit further investigation of AR mechanisms.

(U) 8910 - 9009. In order to investigate the normal day-night difference in pineal sympathetic responsiveness and the role of melatonin in the neuroendocrine changes seen after burn injury, it is necessary to be able to measure changes of serum melatonin in the low daytime range. We found that this was not possible with current assays for melatonin. In addition, we found that one system (chloroform extraction), single antibody, 125I-melatonin tracer, ammonium sulfate precipitation increases specificity and sensitivity to near the required levels. However, incomplete recovery was still a problem and was worsened by ethanol precipitation. Studies with 3H-MEL have begun in order to identify the site of loss. A double antibody system will be evaluated as a means of increasing sensitivity and recovery.

**SUPPLEMENTAL WORK UNIT INFORMATION FOR THE PROJECT ENTITLED "STUDIES OF THE NEUROENDOCRINE ABNORMALITIES IN BURN INJURY"**

**Subrecord/Linking Accession Number:** Not applicable.

**Search Control Data:** W6R11I/W6R13L, 19 October 1989

**Product Identification:** For technical reports, refer to the US Army Institute of Surgical Research Annual Research Progress Report for fiscal years 1980-90.

**Unclassified Special Categories:** Volunteers: Adults; Lab Animals: Rats; RA II

**ANNUAL RESEARCH PROGRESS REPORT**

**PROJECT NUMBER:** 3M162787A874-00, APPLIED RESEARCH AND EXPLORATORY DEVELOPMENT

**PROJECT TITLE:** STUDIES OF THE NEUROENDOCRINE ABNORMALITIES IN BURN INJURY: Refinement of Melatonin Measurement

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

**1 October 1989 - 30 September 1990**

**INVESTIGATORS**

George M. Vaughan, M.D., Colonel, MC

**ABSTRACT**

**PROJECT NUMBER:** 3M162787A874-00, APPLIED RESEARCH AND EXPLORATORY DEVELOPMENT

**PROJECT TITLE:** STUDIES OF THE NEUROENDOCRINE ABNORMALITIES IN BURN INJURY: Refinement of Melatonin Measurement

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

**PERIOD COVERED IN THIS REPORT:** 1 Oct 89 through 30 Sep 90

**INVESTIGATORS:** George M. Vaughan, MD, Colonel, MC

The study of sympathetic responsiveness in burn injury in vivo would be enhanced by a reliable technique to measure serum melatonin. We obtained an antibody whose relatively high specificity has already been demonstrated by its previous use directly in unextracted serum (though with considerable limitation in the low daytime range) and tested several major modifications in its use that improved its utility. These modifications include: preparation of standards in buffer instead of melatonin-stripped serum, the addition of chloroform extraction and petroleum ether washing of the buffer eluate, the substitution of a radioiodinated tracer for the tritiated one, and use of ammonium sulfate precipitation of the bound tracer instead of charcoal separation. Daytime serum values are at the least detectable of 5 pg/ml with use of a sample volume on only 0.25 ml, representing improvements upon previous assays.

## REFINEMENT OF MELATONIN MEASUREMENT

### INTRODUCTION

Sympathetic unresponsiveness, which occurs terminally in the cardiovascular system of nonsurviving critically ill or injured patients, occurs normally during the daytime in the pineals of humans and Syrian hamsters (1). This pineal model of sympathetic responsiveness may be studied by measuring pineal melatonin content or melatonin delivery into the medium of incubated pineals (1-5). For such studies, radioimmunoassay (RIA) of melatonin in pineal tissue homogenate or in incubation medium can be carried out with little if any significant difficulty related to sensitivity or specificity with a number of readily available antibodies. This is because of the large amount of melatonin in these materials in which nonspecific cross-reacting substances can be diluted beyond effect. However, such studies are limited to animal models and, within these models, to observations of sympathetic effects on melatonin production in pineal glands.

It would be advantageous to monitor pineal function in vivo by measurement of serum levels of melatonin, since such levels appear to index at least relatively large changes in pineal function as influenced by the gland's sympathetic innervation in several species, including the hamster, rat, and human (6-8). However, the precision and accuracy of these measurements are limited by the low levels of circulating melatonin (particularly during the light phase of the light/dark cycle), the inadequate sensitivities of RIA's and other methods for melatonin in the lower ranges of serum melatonin, and difficulty in removing nonspecific cross-reacting substances from serum which may produce falsely high values and contribute to variation in the signal that is interpreted as melatonin. It is still not yet known what normal daytime values are, except that they appear to be near or below the least detectable value for some of the assays used to date.

It has not yet been possible to identify and eliminate completely the influence of cross-reacting substances. However, with the appearance of different extraction and washing procedures and somewhat more specific melatonin antibodies, it has become clear that an index of the optimization of serum melatonin determination is a lowering of apparent melatonin values in normal samples, wherein continued recovery of added melatonin in the procedure is insured. In previous observations with use of extraction into chloroform, aqueous NaOH washing, and the Rollag antibody (9), we observed daytime human values of 40-100 pg/ml (6). These were reduced to approximately 10-20 pg/ml with petroleum ether washing of the aqueous eluate obtained after evaporation of the chloroform and with transfer of a portion of the eluate to fresh assay tubes (10). However, this level was still above the least detectable (4 pg/ml) determined in buffer. These comparisons are for a sample size of 0.5 ml serum, and this antibody cannot be

used directly in unextracted serum. This antibody has the advantage of reacting with a melatonin analogue which could be radioiodinated, used as the RIA tracer, and counted in a gamma scintillation counter (obviating the lower specific activity and inconveniences of tritiated melatonin tracer and its beta-counting).

Considerably greater specificity was evident for an antibody developed subsequently by J. Arendt in England (11). With preparation of the assay standards in melatonin-free serum, direct addition of the antibody to the unextracted samples and standards (0.5 ml), use of tritiated melatonin tracer, and separation of the free and antibody-bound tracer with activated charcoal, this procedure has given a least detectable value of 10 pg/ml. Normal human daytime values have been mostly below this level, though not in all cases. "Blocking" out a cross-reactivity element with melatonin-free serum does not account for variations in this element. Use of a tritiated tracer and charcoal separation provide further complicating aspects. However, because the antibody was specific enough to be used with unextracted serum and to become fairly widely used in this way, we decided to test it with modifications of the procedure, including sample extraction in particular.

## METHODS

Sheep anti-melatonin serum 704/8483 was obtained from Stockgrand LTD, Guildford, Surrey, England, and diluted 1:3000 in assay buffer solution. The latter consists of 0.1 M tricine buffer, NaCl 9 g/L, and gelatin 1 g/L. The original assay specified a buffer pH of 5.3. However, this involved a final reaction mixture including 0.5 ml serum, 0.2 ml antibody in buffer, and 0.1 ml of tracer in buffer, and the final pH of such a solution (with the buffer originally at pH 5.3) was found actually to be about 7.0. Since we planned ultimately to use chloroform extraction of the samples, after evaporation of the chloroform, the eluting assay buffer would need to be at the optimal pH of the incubation reaction.

We had been aware that melatonin itself (rather than an analogue) could be directly radioiodinated (12, 13) and $^{125}$I-melatonin (IMEL) could be obtained commercially from Amersham Corporation, Arlington Heights, IL (product no. IM.215). If IMEL (specific activity approximately 2000 Ci/mmole) could be used as the RIA tracer instead of tritiated melatonin (approximately 85 Ci/mmole), the mass of tracer that could be used would be smaller and the required amount of antibody less, with a possible resultant improvement in sensitivity. We diluted IMEL to 8000 cpm/0.05 ml in buffer, and incubated 0.4 ml buffer, 0.05 ml of 1% sheep gamma globulin in buffer, 0.05 ml (1/4 the volume specified in the original procedure) of antibody, and 0.05 ml IMEL at 4°C overnight. We varied the pH of the buffer (reaction mixture) and explored

several methods of precipitation of antibody-bound IMEL. Precipitates (bound fraction) were counted in a gamma scintillation counter.

Further, we investigated a number of factors potentially influencing the reaction and additional elements of procedure that might allow its use for a melatonin assay, as outlined under "Results and Discussion".

We used tritiated melatonin (Amersham, product no. TRK.795) to assess recovery from extraction into organic solvent and elution in buffer. Liquid scintillation (beta activity) counting in Beckman Ready Solv® HP fluor, with standard quench correction, was performed on aqueous buffer eluates and unextracted samples, as indicated in "Results and Discussion".

## RESULTS AND DISCUSSION

Trials of incubation at various Ph followed by addition of saturated ammonium sulfate in water (the latter as the initial attempt to separate protein-bound IMEL) showed that the maximal radioactivity was precipitated after an incubation pH of 7.0 was used. Further trials of various preparations of ammonium sulfate indicated that it was most effective at a concentration of 35 g/100 ml, in a volume of 2 ml, added without vortexing at 4°C, and left to stand for 30 min at 4°C. Centrifugation at 4°C, 2000 G yielded precipitation of 30-40% of the IMEL (bound) with antibody present and 5-6% without antibody. Trials of separation with second antibody (donkey and rabbit anti-sheep gamma globulin), polyethylene glycol, and/or trichloroacetic acid under a number of conditions were unsuccessful. Furthermore, use of ethanol (which precipitates antibody-bound melatonin analogue in the Rollag assay) was also ineffective.

This apparent ability of the antibody to bind IMEL specifically, as well as the utility of the ammonium sulfate precipitation of antibody-bound IMEL, could only be shown if the presence of nonradioactive melatonin would reduce the binding. It was found that the binding was reduced from that (approximately 35%) without melatonin, progressively down to near the nonspecific binding level with concentrations of melatonin from 6.25 to 1000 pg/ml in the buffer. This suggested the utility of the procedure for melatonin assay.

To test recovery of melatonin in an extraction procedure previously used in the Rollag assay (10), samples of buffer and human (normal and postburn), hamster, and rat serum were prepared with trace amounts of tritiated melatonin, and 0.25 ml aliquots (n=6 per sample source) were extracted with 2 ml chloroform in 12 x 75 mm glass tubes. The chloroform was washed in succession with 0.25 ml of 0.1 N NaOH and twice with 0.25 ml $H_2O$, and evaporated in a vacuum centrifuge. The samples were eluted overnight in 0.35 ml

buffer, and aliquots of extracted and unextracted samples were counted for tritium. Analysis of variance indicated no differences in the percent recovery among sample sources. Mean recovery was 74%, indicating that the extraction from serum and elution yields recovery that is 100% of that from buffer, and that standards prepared in buffer for an RIA would be acceptable for comparison with serum samples. No improvement of recovery was obtained by addition of saturated NaCl to the samples prior to extraction or by use of methylene chloride instead of chloroform as the organic solvent.

Melatonin standards (zero, 6.25 pg/ml, and two-fold increments to 1000 pg/ml) were prepared in buffer. Standards and samples of human, rat, or hamster serum (0.25 ml) were extracted and processed identically as described above in 12 x 75 mm glass tubes. Nonzero standards were extracted in triplicate and samples in quadruplicate. The zero-standard (buffer) was extracted in nine aliquotes in each trial. After extraction, washing of the chloroform with 0.25 ml 0.1 N NaOH and $H_2O$, vacuum evaporation, elution in 0.2 - 0.5 ml buffer overnight at 4°C, with or without washing the eluate with 2 ml petroleum ether and transfer of the eluate to a fresh assay tube, and with addition of 0.05 ml 1% sheep gammaglobulin, 0.05 ml antibody and 0.05 ml IMEL, tubes were incubated at 4°C overnight. Ammonium sulfate (2 ml 35 g/dl) was added and tubes were kept at 4°C for 30 min prior to centrifugation at 4°C for 30 min at 2000 G. The unbound IMEL was decanted, and the tubes (containing the bound IMEL) were counted in a gamma counter for $^{125}I$, usually with no more than a 2% error. The counts (cpm) for the standards (including zero-melatonin) were compared in a four-parameter logistic regression with the pre-extraction original melatonin concentration [BMDP software, PAR subroutine (14)], with resultant determination of parameters: the maximal expected cpm (A) at zero melatonin, the minimal expected cpm (NSB) at infinite melatonin, the 50% effective concentration (E) at which cpm were inhibited to a level half-way between A and NSB, and a slope index (S). The relationship between cpm and pre-extraction standard concentration (C) was logistic: predicted cpm = $((A-NSB)/(1 + (C/E)^S)) + NSB$. Observed cpm in replicates of a standard C almost always did not overlap with cpm of an adjacent standard C.

The proportion (B) of the IMEL specifically bound for any given tube was also estimated from its cpm, the parameter NSB used as the nonspecific binding, and the total cpm (T, determined in tubes with IMEL but not decanted): $B = (cpm-NSB) / T$. $B_o$, the B for zero-melatonin standard (buffer), was usually approximately 35%, E was about 30 pg/ml, and NSB approximately 7-9% of T. Another measure of nonspecific binding, the cpm in tubes without antibody, was usually 5-6% of T. For nonstandard samples, the predicted C was calculated from the cpm by solving the logistic equation above for C. The mean cpm minus two standard deviations for the zero-standard tubes was used to determine the least detectable pre-extraction C (LD). The LD was usually about 5 pg/ml.

After extraction of standards and samples into and washing of the chloroform as above, and after overnight elution of the evaporated chloroform residue by 0.5 ml buffer followed by washing of the eluate with 2 ml petroleum ether and transfer of 0.4 ml eluate into new tubes, the overnight RIA incubation (0.05 ml each of 1% sheep gammaglobulin, antibody, and IMEL) in a 4°C water bath was followed by precipitation in 2 ml cold 35 g/100 ml ammonium sulfate for 30 min prior to centrifugation at 4°C for 30 min at 2000 G. Daytime sera from humans, rats, and hamsters gave melatonin values at or below the least detectable. Assay recovery of added melatonin (50 or 100 pg/ml) in serum sample was 95-105%, and within-assay between-extraction (four extractions per sample) coefficients of variation were 3-15%. A pool of hamster serum taken at the expected time of the peak nocturnal serum melatonin (0300-0400 h, lights on 0600 h) gave values ranging 17-22 pg/ml in different runs. Pooled human or rat night serum undiluted and diluted 1:2 and 1:4 gave binding parallel to the standard curve. However, pooled hamster night serum gave slightly enhanced inhibition of binding at 1:2 and 1:4 dilution, resulting in apparent enhancement of the dilution-corrected recovery to 110-130%. Omitting the petroleum ether/transfer step worsened this problem in hamster serum, the mechanism of which has not yet been determined.

So far, it appears that with use of an antibody having relatively high specificity, preparation of standards in buffer instead of melatonin-stripped serum, the addition of chloroform extraction and petroleum ether washing, the substitution of a radioiodinated melatonin tracer for a tritiated one, and use of ammonium sulfate precipitation instead of charcoal separation has reduced the LD to about 5 pg/ml with sampling of 0.25 ml, compared to 10 pg/ml with sampling 0.5 ml reported for this antibody without these modifications. This allows assay of smaller samples with use of 1/4 the amount of antibody and a little greater sensitivity. In that daytime serum gives values at or below this lower LD without the presence of melatonin-free serum in the standards to "blank out" some of the nonspecific signal (noise) present in serum without use of the new procedure, the latter is apparently more specific. However, even this procedure is not yet sensitive and specific enough to characterize the low daytime levels, and some question remains about its use in hamster serum. Further characterization of the assay by correlating serum with pineal melatonin over nocturnal time intervals and under different in vivo conditions of sympathetic stimulation and inhibition will be necessary to determine its usefulness in studying pineal sympathetic responsiveness in vivo.

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| 1. AGENCY ACCESSION | 2. DATE OF SUMMARY | REPORT CONTROL SYMBOL DD-DR&E(AR) 636 |
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| DA314707 | 01 Oct 90 | |

| 4. CLASSIFICATION/DOWNGRADING | 5. SPECIAL HANDLING DESCRIPTION | 6. GOVT SECURITY CLASSIFICATION | 7. REGRADING | 8. DISPOSITION INSTRUCTION | 9. LEVEL OF SUMMARY |
|---|---|---|---|---|---|
| Chg | U | U | | CX | A WORK UNIT |

| | 3. PROGRAM ELEMENT | 3. PROJECT NUMBER | 3. TASK AREA NUMBER | 3. WORK UNIT NUMBER |
|---|---|---|---|---|
| a. PRIMARY | 62787A | 3M162787A874 | EA | 165 |
| b. CONTRIBUTING | | | | |
| c. CONTRIBUTING | | | | |

11. TITLE (Precede with Security Classification Code) (U) The Effect of Recombinant Human Growth Hormone Treatment on the Rate of Healing on Burn Patients Who Require Skin Grafting

12. SUBJECT AREAS
0605 Medicine and Medical Research 0615 Pharmacology

| 13. START DATE | 14. ESTIMATED COMPLETION DATE | 15. FUNDING ORGANIZATION | 16 PERFORMANCE METHOD |
|---|---|---|---|
| 8805 | 9109 | DA | C. In-House |

17. CONTRACT/GRANT NUMBER [redacted] MILITARY RELEVANCY CERTIFIED APPROVED BY [redacted]
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d. AMOUNT
e. KIND OF AWARD
f. CUM/TOTAL

18. RESOURCES ESTIMATE

| FISCAL YEARS | a PROFESSIONAL WORKYEARS | b FUNDS (In thousands) |
|---|---|---|
| 90 | 0.1 | 48 |
| 91 | 0.1 | 51 |

19. RESPONSIBLE DOD ORGANIZATION
a. NAME: US Army Institute of Surgical Research
b. ADDRESS (Include zip code): Fort Sam Houston, San Antonio, Texas 78234-5012
c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A
d. TELEPHONE NUMBER (Include area code): 512-221-2720

20 PERFORMING ORGANIZATION
a. NAME: US Army Institute of Surgical Research
b. ADDRESS: Fort Sam Houston, San Antonio, Texas 78234-5012
c. NAME OF PRINCIPAL INVESTIGATOR: CIOFFI, W G
d. TELEPHONE NUMBER (Include area code): 512-221-4440
e. NAME OF ASSOCIATE INVESTIGATOR (If available): MC MANUS, W F
g. NAME OF ASSOCIATE INVESTIGATOR (If available): VAUGHAN, G M

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MILITARY/CIVILIAN APPLICATION: M

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Burns (Injuries); (U) Healing; (U) Pharmacology; (U) Hormones

23. TECHNICAL OBJECTIVE 24 APPROACH 25 PROGRESS (Precede text of each with Security Classification Code)

23/24. (U) The objective of this work is to evaluate the effects and safety of recombinant human growth hormone when given to accelerate the rate of wound healing in burned patients. Analysis of preliminary data from this multicenter study failed to reveal the expected increases in somatomedin-C levels in response to the exogenous administration of growth hormone. It was felt that this might reflect inadequate dosing. An addendum has been approved to increase the dose of growth hormone. In addition, growth hormone is to be administrated immediately following resuscitation instead of on the day of the first operation. The remainder of the study remains the same. The rate of healing of donor sites is measured by daily inspection and computerized planimetry of photographs taken every other day.

25. (U) 8710 - 8809. This project was approved by the USAISR Research Council, the US Army Institute of Surgical Research Human Use Committee, and The Surgeon General's Human Subjects Research Review Board during the third quarter of fiscal year 1988. Two patients were enrolled in the study during this reporting period.

(U) 8810 - 8909. Eight patients were enrolled in the study during this reporting period. Addenda were submitted and approved.

(U) 8910 - 9009. A total of 12 patients have been enrolled in this study to date, with 2 patients being enrolled during this reporting period after approval of the addenda which increased the growth hormone dosage.

DD FORM 83[illegible] *98 EDITION OF MAR 68 IS OBSOLETE * USGPO 1988-491-003/50329

**CONTINUATION OF DD FORM 1498 FOR THE PROJECT ENTITLED "THE EFFECT OF RECOMBINANT HUMAN GROWTH HORMONE TREATMENT ON THE RATE OF HEALING ON BURN PATIENTS WHO REQUIRE SKIN GRAFTING"**

The enrollment of patients has now been completed. Data will be analyzed in conjunction with the other investigators during the next fiscal year.

**SUPPLEMENTAL WORK UNIT INFORMATION FOR THE PROJECT ENTITLED "THE EFFECT OF RECOMBINANT HUMAN GROWTH HORMONE TREATMENT ON THE RATE OF HEALING ON BURN PATIENTS WHO REQUIRE SKIN GRAFTING"**

**Subrecord/Linking Accession Number:** Not applicable.

**Search Control Data:** W6R00J/W6R03I, 19 October 1989

**Product Identification:** For technical reports, refer to the US Army Institute of Surgical Research Annual Research Progress Report for fiscal years 1988-90.

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**ANNUAL RESEARCH PROGRESS REPORT**

**PROJECT NUMBER:** 3M162787A874-00, APPLIED RESEARCH AND EXPLORATORY DEVELOPMENT

**PROJECT TITLE:** The Effect of Recombinant Human Growth Hormone Treatment on the Rate of Healing on Burn Patients who Require Skin Grafting

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

**1 October 1989 - 30 September 1990**

**INVESTIGATORS**

William G. Cioffi, Jr., MD, Major, MC
William F. McManus, MD, Colonel, MC
George M. Vaughan, MD, Colonel, MC
Basil A. Pruitt, Jr., MD, Colonel, MC
Barry M. Sherman, MD*
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*Genentech, Inc.
South San Francisco, California 94080

**Harvard Medical School and
Brigham and Woman's Hospital
Boston, Massachusetts 02115

## ABSTRACT

**PROJECT NUMBER:** 3M162787A874-00, APPLIED RESEARCH AND EXPLORATORY DEVELOPMENT

**PROJECT TITLE:** The Effect of Recombinant Human Growth Hormone Treatment on the Rate of Healing on Burn Patients who Require Skin Grafting

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

**PERIOD COVERED IN THIS REPORT:** 1 Oct 89 through 30 Sep 90

**INVESTIGATORS:** William G. Cioffi, Jr., MD, Major, MC
William F. McManus, MD, Colonel, MC
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Basil A. Pruitt, Jr., MD, Colonel, MC
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South San Francisco, California 94080

**Harvard Medical School and
Brigham and Woman's Hospital
Boston, Massachusetts 02115

This double-blinded, randomized, placebo-controlled, multi-institution study was designed to determine whether the administration of recombinant human growth hormone could accelerate wound healing in burn patients. Preliminary data have suggested that the administration of growth hormone can promote anabolism in surgical patients. The clinical impression of these investigators was that the increased nitrogen retention associated with growth hormone can promote anabolism in surgical patients and that the increased nitrogen retention associated with growth hormone administration was accompanied by accelerated wound healing. Burn patients in this study were randomized to receive either 5 or 10 milligrams a day of recombinant human growth hormone or a placebo with administration commencing on the day of their first surgery. The rate of donor site healing was used as an index of the effect of growth hormone on wound healing in these patients.

Ten patients from this Institute and 34 patients overall were enrolled in this study. In the patients receiving growth hormone, the administration of either 5 or 10 milligrams per day of growth hormone resulted in a dose range of 0.05 to 0.15 milligrams per kilogram of ideal body weight. Analysis of the IGF-1 response in these patients indicated the lack of a consistent response as well as IGF levels not significantly elevated over controls until postburn day 14. Since IGF-1 response is an indicator of growth

hormone action, it is hypothesized that this lack of response may be secondary to inadequate drug dosing. There was a wide range of donor site healing times amongst all patients, ranging from 4 to 19 days in the treated group and 8 to 14 days in the control group. Again, this lack of response may be secondary to inadequate drug dosing or the fact that therapy was initiated at various times following resuscitation. No untoward effects secondary to administration of recombinant growth hormone was noted in this group of patients.

Because of the inconsistent and variable response to growth hormone administration as indexed by donor site healing and IGF-1 response, the design of the study was changed. In an attempt to ensure adequate dosing of recombinant hormone, the dose was changed so it was indexed to body weight (0.2 mg/kg). In addition, treatment was started as early as possible following resuscitation and stabilization, with treatment continuing for the duration of hospitalization. During this fiscal year, two patients were enrolled in the study. The total number of patients required by Genentech for completion of this multi-center study were enrolled during the reporting period. Data analysis and interpretation has begun.

| RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY | | | | 1. AGENCY ACCESSION DA312333 | 2. DATE OF SUMMARY 01 Oct 90 | REPORT CONTROL SYMBOL DD-DR&E(AR) 636 |
|---|---|---|---|---|---|---|
| 3. DATE PREV SUM'RY 01 Oct 89 | 4. KIND OF SUMMARY D. Chg | 5. SUMMARY SCTY U | 6. WORK SECURITY U | 7. REGRADING | 8. DISP'N INSTR'N CX | 9 LEVEL OF SUM A WORK UNIT |

| 10. NO./CODES | PROGRAM ELEMENT | PROJECT NUMBER | TASK AREA NUMBER | WORK UNIT NUMBER |
|---|---|---|---|---|
| a. PRIMARY | 62787A | 3M162787A874 | CA | 166 |
| b. CONTRIBUTING | | | | |
| c. CONTRIBUTING | | | | |

11. TITLE *(Precede with Security Classification Code)*
(U) High Frequency Ventilation in Patients with Inhalation Injury

12. SUBJECT AREAS 0605 Medicine and Medical Research
0612 Medical Facilities, Equipment, and Supplies

| 13. START DATE | 14. ESTIMATED COMPLETION DATE | 15. FUNDING ORGANIZATION | 16 PERFORMANCE METHOD |
|---|---|---|---|
| 8703 | 9109 | DA | C. In-House |

17. CONTRACT/GRANT I. MILITARY RELEVANCY CERTIFIED
a. DATE EFFECTIVE APPROVED BY [illegible]
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18. RESOURCES ESTIMATE

| FISCAL YEARS | a. PROFESSIONAL WORKYEARS | b FUNDS *(In thousands)* |
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| 90 | 0.1 | 47 |
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PRUITT, B A

d. TELEPHONE NUMBER *(include area code)*
512-221-2720

21. GENERAL USE
FINA

MILITARY/CIVILIAN APPLICATION M

20. PERFORMING ORGANIZATION

a. NAME
US Army Institute of Surgical Research

b. ADDRESS
Fort Sam Houston
San Antonio, Texas 78234-5012

c. NAME OF PRINCIPAL INVESTIGATOR
CIOFFI, W G

d. TELEPHONE NUMBER *(include area code)*
512-221-4440

f. NAME OF ASSOCIATE INVESTIGATOR *(if available)*
MC MANUS, W F

g. NAME OF ASSOCIATE INVESTIGATOR *(if available)*

22. KEYWORDS *(Precede EACH with Security Classification Code)* (U) Burns (Injuries); (U) Pulmonary Function; (U) Inhalation; (U) Pneumonia; (U) Morbidity; (U) Therapy;

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS *(Precede text of each with Security Classification Code)*

23/24. (U) The objective of this work is to evaluate the use of high frequency ventilation in the treatment of respiratory failure in patients with inhalation injury with respect to incidence of pneumonia, time of initial onset and duration of pneumonitis, total duration of ventilatory support, and the age- and burn size-adjusted mortality. Initial high frequency ventilation support will be established by adjustment of the driving pressure, rate, and inspiratory-expiratory ratio. A chest roentgenogram will be obtained and sputum or endotracheal secretions will be examined by culture and Gram stain. It will then be determined how high frequency ventilation affects outcome after inhalation injury with respect to several well defined factors as compared to an historical cohort using conventional ventilation. The incidence of pneumonia will be related to burn size by appropriate burn-size stratification within the study group.

25. (U) 8710 - 8809. Fourteen patients were enrolled in the study during this reporting period.

(U) 8810 - 8909. Fourteen patients were enrolled in the study during this reporting period.

(U) 8910 - 9009. Forty-seven patients have been enrolled in the study to date, 18 during this reporting period. Upon enrollment of 50 patients,

DD FORM 83 MAR 1498 EDITION OF MAR 68 IS OBSOLETE * USGPO 1988 -491-003/50329

**CONTINUATION OF DD FORM 1498 FOR THE PROTOCOL ENTITLED "HIGH FREQUENCY VENTILATION IN PATIENTS WITH INHALATION INJURY"**

the effects of high frequency ventilation on the outcome after inhalation injury, i.e., mortality and pulmonary morbidity, will be compared to an historical cohort. Preliminary review of the data indicates a significant decrease in the incidence of pneumonia and mortality for this group of patients when compared to an historical cohort treated during 1980-4.

**SUPPLEMENTAL WORK UNIT INFORMATION FOR THE PROJECT ENTITLED "HIGH FREQUENCY VENTILATION IN PATIENTS WITH INHALATION INJURY"**

**Subrecord/Linking Accession Number:** Not applicable.

**Search Control Data:** W6Q15K/W6Q18M, 19 October 1989

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**Unclassified Special Categories:** Volunteers: Adults; RA II

**ANNUAL RESEARCH PROGRESS REPORT**

**PROJECT NUMBER:** 3M162787A874-00, APPLIED RESEARCH AND EXPLORATORY DEVELOPMENT

**PROJECT TITLE:** High Frequency Ventilation in Patients with Inhalation Injury

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

**1 October 1989 - 30 September 1990**

**INVESTIGATORS**

William G. Cioffi, Jr., MD, Major, MC
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Theresa A. Graves, MD
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Arthur D. Mason, Jr., MD
Basil A. Pruitt, Jr., MD, Colonel, MC

## ABSTRACT

**PROJECT NUMBER:** 3M162767A874-00, APPLIED RESEARCH AND EXPLORATORY DEVELOPMENT

**PROJECT TITLE:** High Frequency Ventilation in Patients with Inhalation Injury

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

**PERIOD COVERED IN THIS REPORT:** 1 Oct 89 through 30 Sep 90

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Mortality and the incidence of pneumonia are significantly increased in burn patients with inhalation injury, despite application of conventional ventilatory support techniques. We have studied the effect of high frequency percussive ventilation (HFPV) on mortality, incidence of pulmonary infection, and barotrauma in 54 burn patients with documented inhalation injury admitted between March 1987 and September 1990 as compared to an historic cohort treated between 1980 and 1984. All patients satisfied clinical criteria for mechanical ventilation. HFPV was initiated within 24 hours of intubation. The patient's mean age and burn size were 32.2 years and 47.8% respectively (range 15 to 88 years, 0-90%). The mean number of ventilator days was 15.3 ± 16.7 (range 1 to 150) with 26% of patients ventilated for more than two weeks. Fourteen patients (25.9%) developed pneumonia vs an historic frequency of 45.8% ($p<.005$). Mortality was 18.5% (ten patients) with an expected historic mortality of twenty-three (95% C.L. 17 to 28). The documented improvement in survival and decrease in the incidence of pneumonia in patients treated with prophylactic HFV, as compared to a cohort of patients treated in the seven years prior to the trial, indicates the importance of small airway patency in the pathogenesis of inhalation injury sequelae and supports further use and evaluation of HFV.

# HIGH FREQUENCY VENTILATION IN PATIENTS WITH INHALATION INJURY

## INTRODUCTION

During the past three decades, improvements in burn wound management, infection control, and metabolic support have increased the survival of thermally injured patients. Inhalation injury, however, continues to be a significant comorbid factor in such patients, and its treatment has been little improved by the use of conventional means of pulmonary support. Bacterial pneumonia, which has historically occurred in 38% of all patients with inhalation injury but only 8% of those without such injury, continues to be the leading cause of morbidity and mortality. The combination of inhalation injury and pneumonia exert independent but additive effects on the age related mortality attributable to burn size (1).

Current treatment for inhalation injury is supportive and includes meticulous pulmonary toilet, mechanical ventilatory support when indicated, and prompt treatment of pneumonia when diagnosed. In an ovine model, we have shown that the major insult following smoke injury (as indexed by early post injury VA/Q mismatching and histopathologic findings) is the obstruction and collapse of small airways leading to distal atelectasis and subsequent pneumonia (2). Experimental and clinical data suggest that high frequency ventilation may be beneficial in recruiting, and stabilizing such collapsed diseased lung segments (3-12). In addition, some investigators have reported improved clearance of secretions from the tracheobronchial tree with the use of high frequency ventilation (13). These observations support the hypothesis that high frequency ventilation, by preventing alveolar collapse and improving secretion clearance, may be beneficial in patients with inhalation injury.

We previously reported a small cohort of ten patients with inhalation injury requiring mechanical ventilatory support in whom the prophylactic use of high frequency percussive ventilation appeared to reduce the incidence of pneumonia (14). This report extends our observations to 54 patients in whom high frequency percussive ventilation was used in a prophylactic manner in an attempt to decrease the incidence of pneumonia and improve survival.

## METHODS

### PATIENT POPULATION

All adult patients admitted to the United States Army Institute of Surgical Research between March, 1987 and September, 1990 with a diagnosis of inhalation injury were eligible for enrollment in this study. Inhalation injury was confirmed in each patient by bronchoscopy and/or $^{133}$Xenon ventilation-perfusion lung

scan. The presence of carbonaceous debris beneath the true vocal cords, mucosal erythema, and ulceration were used to define moderate to severe inhalation injury. Patients with a positive $^{133}$Xenon scan and negative bronchoscopy were defined as having mild inhalation injury. These criteria were established in our earlier review (1). After meeting the entrance requirements listed in Table 1, and meeting the requirements for intubation and mechanical ventilatory support listed in Table 2, informed consent was obtained from each patient and high frequency percussive ventilation initiated for pneumonia prophylaxis.

## HIGH FREQUENCY PERCUSSIVE VENTILATION

Description of the high frequency percussive ventilator used in this study has been published (14). Briefly, high frequency percussive ventilation was delivered by a high frequency pulse generator with gas from the high frequency pulse generator delivered through a non-gated sliding venturi to a standard endotracheal tube. The venturi entrains humidified gas from a fresh bias gas flow provided from the ventilator. The system combines serial high frequency sub-dead space volume breaths with a variable I:E ratio. Periodic interruption of the high frequency pulsatile flow is programmed to allow return of airway pressure to baseline CPAP. The duration of the percussive phase and of the return to baseline phase are adjusted to manipulate oxygenation and $CO_2$ elimination. Peak airway pressure can also be independently varied to maintain $CO_2$ clearance. The frequency of the sub-dead space breaths can range between 1.5 and 15 hertz. $FIO_2$, and PEEP are adjusted to maintain $O_2$ saturation greater than 90%.

All patients were initially placed on a conventional mechanical ventilator. In those patients intubated elsewhere, such support was of less than 24 hour duration, and all patients were converted to HFPV within an hour of admission. The patients intubated at this Institute received conventional ventilation during admission processing but were converted to HFPV within one hour. After placing the patient on high frequency percussive ventilation, standard ventilator settings were used as a baseline, and then altered as indicated by arterial blood gas determinations, pulse oximetry, and end tidal $CO_2$ monitoring. The duration of the percussive phase was set at two seconds, with a rate of return to baseline approximately 2 less than the IMV setting required to maintain normal acid-base balance on conventional mechanical ventilation. Peak airway pressures were set at 5 cm $H_2O$ less than those developed when a conventional volume limited ventilator was set to deliver a tidal volume of 12 - 15 ml/kg. The $FIO_2$ and PEEP were initially maintained at the same levels as on conventional mechanical ventilation. The frequency of the sub-dead space tidal breaths was initially set at 10 hertz. After stabilization for approximately 30 minutes, arterial blood gas measurements were obtained and adjustments made as indicated. The goal of ventilator therapy was to maintain oxygenation and ventilation at the lowest

possible peak airway pressure and fractional inspired oxygen concentration. Patients were weaned and extubated according to standard criteria.

## DIAGNOSIS Of PNEUMONIA

The diagnosis of pneumonia was based upon standard criteria used in this Institute for the past decade. Patients with sputum leukocytosis (greater than 25 white blood cells per high power field), lack of oropharyngeal contamination (less than 10 squamous cells/high power field), a predominant organism on culture, and an infiltrate on chest roentgenograms were diagnosed as having pneumonia.

## DATA ANALYSIS

The incidence of pneumonia and death in the study patients was compared with predicted values based on two previous studies. The first predictor used relates burn size and age to mortality for all patients admitted to the Institute of Surgical Research between January 1980 and December 1986. The second predictor used as a basis for comparison relates burn size, age, the presence of inhalation injury and the occurrence of pneumonia to mortality in patients admitted between 1980 and 1984. The incidence of pneumonia in this latter patient population was also used for comparison purposes. Solution of the logistic equations listed in Table 3 provides the terms for use in calculating the two values for predicted mortality.

# RESULTS

## PATIENT POPULATION

Fifty-four patients meeting the entrance criteria were enrolled in the study. Routine demographic data are included in Table 4. Ten patients died, a mortality of 18.5%. The distribution of patients by burn size demonstrates that 50% of the patients had burns ranging between 30-60% of the body surface, which is the group of patients in which inhalation injury has been reported to have its greatest impact on mortality (Fig 1). Segregation of the patients by outcome revealed the expected differences between the two groups (Table 5); non-survivors were older, and had larger burns, and a greater incidence of pneumonia. Fifty-two of the 54 patients were diagnosed as having inhalation injury by bronchoscopy. The two patients with negative bronchoscopy but positive $^{133}$Xenon scans developed severe ARDS in the first postburn week, necessitating mechanical ventilatory support.

Historically, 45.8% of patients with positive bronchoscopy, and 19.5% of patients with negative bronchoscopy but a positive

**TABLE 1. Study Entrance Criteria**

- Inhalation injury documented by bronchoscopy or xenon lung scan
- Clinical requirement for ventilatory support
- Admission within 48 hours of injury
- Greater than 15 years of age

**TABLE 2. Requirements for Mechanical Ventilatory Support**

1. Respiratory rate > 35/min
2. Vital capacity < 15 ml/kg
3. Inspiratory force < 25 Cm $H_2O$
4. $PAO_2/FIO_2$ < 200
5. $PCO_2$ > 50 mm Hg
6. Vd/Vt > .6
7. Upper Airway Edema
8. $PCO_2$ < 50 mm Hg but progressively increasing
9. Increased work of breathing

**TABLE 3. Burn Mortality Predictors**

$$\text{Predicted Mortality (PM)} = \frac{eY}{1 + e^{Y}}$$

I. Logistic equation relating burn size and age to mortality:

1980 - 1986

$$Y = -4.8216 + 0.10299\ (PCTB) - 0.18879\ (Age) + 0.50873\ (Age^2/100) - 0.27915\ (Age^3/10,000)$$

II. Logistic equation relating burn size, age, inhalation injury, and pneumonia to mortality: 1980 - 1984

$$Y = -3.4953 + 0.09589\ (PCTB) - 0.1988\ (Age) + 0.4478\ (Age^2/100) - 0.20314\ (Age^3/10,000) + 0.59056\ (II) + 0.92530\ (PNEU)$$

PCTB = Percent of total body surface burned

II = - 1.0 if inhalation injury absent
+ 1.0 if inhalation injury present

PNEU = - 1.0 if pneumonia absent
+ 1.0 if pneumonia present

**TABLE 4. Demographic Data**

| | |
|---|---|
| Age | 32.2 ± 1.8 (15 - 88)* |
| TBSB** | 47.8 ± 3.1 (0 - 90) |
| Sex | 40 male, 14 female |
| Days on ventilator | 15.3 ± 2.2 (1-150) |
| Bronchoscopy positive | 96.3% |
| Incidence of pneumonia | 25.9% |
| Mortality | 10/54 (18.5%) |

* X ± SEM (RANGE)
** TOTAL BODY SURFACE BURN

**TABLE 5. Comparison of Survivors and Nonsurvivors**

| | SURVIVORS | NONSURVIVORS | |
|---|---|---|---|
| Age | 29.6 ± 1.5* | 43.3 ± 6.5 | P <.05 |
| TBSB | 43.7 ± 3.2 | 65.3 ± 7.1 | P <.01 |
| Incidence of pneumonia | 20.5% | 50% | P <.05 |

* mean ± SEM

**TABLE 6. Actual Versus Predicted Outcome**

| PREDICTOR | PREDICTED DEATHS | 95% C.L. | OBSERVED |
|---|---|---|---|
| #1 (1980 - 1986) | 19 | 13-25 | 10 |
| #2 (1980 - 1984)* | 23 | 17-28 | 10 |

*This predictor includes the impact that inhalation injury and pneumonia have on outcome.

**TABLE 7. Cause of Death**

| TBSB | AGE | PBD | CAUSE DEATH |
|---|---|---|---|
| 90% | 32 | 01 | Resuscitation failure |
| 85% | 25 | 03 | Resuscitation failure |
| 59% | 40 | 07 | Accidental extubation |
| 36% | 59 | 40 | Removed from study |
| 47% | 29 | 50 | SBE, CVA, 30 days following extubation |
| 65% | 60 | 80 | CVA, 45 days following extubation |
| 89% | 25 | 12 | Pulmonary failure |
| 30% | 88 | 43 | Pneumonia (*Staph. aureus*), pulmonary failure |
| 64% | 49 | 01 | Unable to ventilate |
| 86% | 29 | 50 | Pneumonia, Aspergillus wound infection |

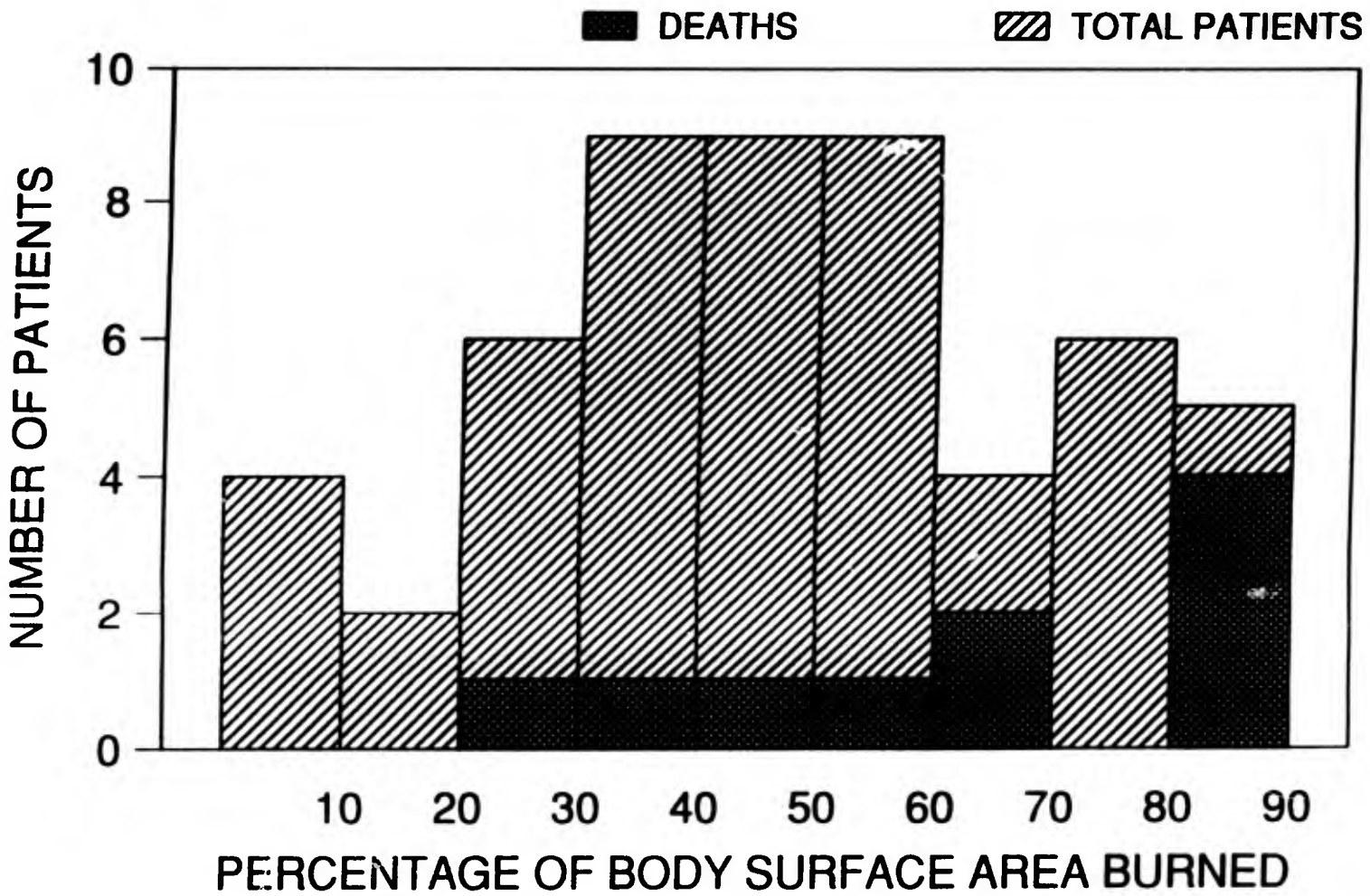


**FIGURE 1**: Distribution of burn size for the fifty-four patients in the study. Of note is that 50% of the patient burn sizes are between 30-60% of the body surface area, which is the group of patients on which inhalation injury exerts its greatest influence on mortality. Survivors are reported by the crossed bars, and nonsurvivors by the crosshatched bars.

$^{133}$Xenon lung scan have developed pneumonia. Based upon that experience, 25 of the study patients would have been expected to develop pneumonia during hospitalization. Pneumonia was diagnosed in only 14 (26%) of the patients in this study, an incidence differing significantly from that of the comparison cohort ($P < .003$).

## ACTUAL VERSUS PREDICTED MORTALITY

Ten deaths occurred in this group of patients, an observed mortality of 18.5%. To determine whether high frequency percussive ventilation influenced outcome in this group of patients, we compared this observed mortality with two mortality predictions generated from patient data from this institution, as noted above. The first, based upon burn size and age related mortality in all patients admitted to this Institute between January 1980 and December 1986, predicts the deaths of 19 patients (35%) in the study population, with a 95% confidence interval of 13 to 25 deaths. The second, based upon burn size and age related mortality in conjunction with the additive effects of inhalation injury and pneumonia and generated from patient data between January 1980 and December 1984, predicts 23 deaths (42.6%) with a 95% confidence interval of 17 to 28 deaths. Thus, mortality in this cohort of patients was significantly less than that predicted by either technique ($P < .05$). (Table 6)

The causes of death in those patients who expired are listed in Table 7. Of the ten deaths, four were from pulmonary failure. One patient could not be ventilated and oxygenated, and was crossed over to conventional ventilatory support with the same result. Three patients developed progressive pulmonary failure and died on postburn day 12, 43, and 50 respectively. Of the remaining six patients, two were resuscitation failures who died with severe inhalation injury, one patient extubated himself on postburn day 7 and died of cardiopulmonary arrest despite an emergency tracheostomy, and one patient was removed from the study by his attending surgeon. Two patients died from cerebrovascular accidents after they had been extubated for 30 and 45 days respectively.

Ventilator complications were rare. Two patients developed severe necrotizing tracheobronchitis. It could not be ascertained whether this was secondary to the ventilator or the disease process itself. Barotrauma occurred in three patients. Two developed significant subcutaneous emphysema, and one patient developed bilateral pneumothoraces requiring tube thoracostomies.

## DISCUSSION

The combination of cutaneous thermal injury and inhalation injury results in a significantly higher mortality rate than that attributable to cutaneous thermal injury alone. This additive effect of inhalation injury on mortality is most apparent in

patients in whom predicted mortality attributable to age and burn size ranges from 40 to 60%. Inhalation injury also results in a marked increase in the incidence of bacterial pneumonia. As previously stated, only 8.8% of patients with thermal injury but without inhalation injury develop pneumonia during their course of treatment. The presence of inhalation injury, whether diagnosed by bronchoscopy or $^{133}$Xenon scan, has historically resulted in a 38% incidence of pneumonia, and the combination of inhalation injury and pneumonia has an even more drastic effect on outcome, increasing mortality by as much as 60% (1).

Ideally, the optimal treatment of any disease should reverse the pathophysiologic process without causing further injury. When inhalation injury is severe enough to require conventional mechanical ventilatory support, such an outcome is not achieved. The pathophysiologic response to inhalation injury includes extensive tracheobronchial injury which results in sloughing of the mucosal lining of the respiratory tract and leads to obstruction of small and moderate sized airways. In addition, the mucociliary transport mechanism is impaired, resulting in impaired clearance of secretions and the sloughed debris. Distal airway obstruction results in atelectasis, and in conjunction with the disruption of the endothelial and epithelial integrity of the alveolus, produces foci for the development of bacterial overgrowth and subsequent pneumonia. The combination of atelectasis, pneumonia, and airway obstruction produces significant derangement of ventilation-perfusion relationships.

Conventional mechanical ventilatory support does not reverse these processes, is not characterized by improved clearance of secretions, and may actually compound the existing injury (15). Conventional volume-limited ventilation in patients with inhalation injury is normally instituted at a tidal volume of 12 to 15 ml/kg. With such a ventilatory setting, peak inspiratory pressures are often elevated during the resuscitative and fluid mobilization phase of care. Recently, Tsuno has reported adverse pulmonary effects of volume limited mechanical ventilation when peak inspiratory pressures exceed 30 centimeters of water in paralyzed, anesthetized healthy sheep (16). Animals ventilated with an $FIO_2$ of 40% and a tidal volume of 10 ml/kg, with peak inspiratory pressure less than 18 centimeters of water, showed no measurable deleterious changes in lung function or histopathology after 48 hours of support. Animals ventilated with larger tidal volumes, resulting in peak inspiratory pressures greater than 30 cm $H_2O$, demonstrated progressive deterioration in static lung compliance, functional residual capacity, and arterial blood gases. Severe pulmonary atelectasis, increased wet lung weight, and an increase in the minimum surface tension of saline lung lavage fluid were noted at autopsy. These data indicate that even in normal healthy lungs, prolonged elevation of inspiratory pressures may result in injury.

Following resuscitation if pneumonia develops, the requirement for increased inspired oxygen concentrations to achieve normoxia may result in increased pulmonary damage when infection is present. Coalson, et al. have recently reported a synergistic effect of hyperoxia and infection resulting in significant pulmonary dysfunction and damage (17). In a primate model, the combination of 80% $O_2$ and Pseudomonas pneumonia was as injurious as 100% oxygen over an 11 day period, while 80% $O_2$ or pneumonia alone resulted in minimal dysfunction.

The reported beneficial effects of high frequency ventilation (ventilator frequency greater than 60 breaths/minute and tidal volumes of less than anatomic dead space), include lower peak airway pressures than those generated by conventional ventilation, positive endotracheal pressure throughout the ventilatory cycle, increased FRC, and more efficient pulmonary gas distribution (18). Unfortunately, each of the advantages claimed for specific high frequency ventilators has been refuted in various reports (9-10,12). If, however, a form of high frequency ventilation could achieve some of these advantages, and maintain oxygenation and $CO_2$ clearance at lower inspiratory pressures and fractional inspired concentrations of oxygen, it might be possible to provide ventilatory support and avoid the deleterious side effects of conventional support.

In evaluating clinical reports of high frequency ventilation one must recognize that there are several types of high frequency ventilators, all with different characteristics and different potentially adverse effects. Moreover, one must differentiate between prophylactic use of the ventilator as in this study and therapeutic or salvage use of the high frequency device for patients in whom conventional mechanical ventilatory support has failed. Many reports have documented the effectiveness of short-term salvage use of high frequency ventilation in patients with ARDS (7-8). Our own previously reported experience demonstrated that the ventilator used in these studies could oxygenate and ventilate patients at lower airway pressures and inspired oxygen concentrations, but all the patients expired despite improved pulmonary performance (14). Other reports have also failed to identify a survival advantage with the use of high frequency ventilation as a salvage mode of ventilatory support.

In this study, we employed high frequency percussive ventilation prophylactically in an attempt to avoid the adverse effects of mechanical ventilatory support while reversing or minimizing some of the pathophysiologic changes which occur following inhalation injury. Our data indicate that, as compared to a recent historical cohort, the use of high frequency percussive ventilation resulted in a significant decrease in the incidence of pneumonia, and a decrease in mortality.

There are several problems inherent to the use of historical controls. The development of more sensitive diagnostic techniques resulting in the diagnosis of less severe injury could favorably bias the results of recent studies but the diagnostic modalities and criteria have remained constant since 1976. It is generally accepted that over the past three decades, survival of all patients with thermal injury has improved. Even so, the effects of inhalation injury and pneumonia on outcome have remained refractory to standard treatment as indicated by the mortality predictor used at this Institute. Moreover, the predictors used in this study introduce some bias against finding an improvement in outcome in the current study population as compared to the populations upon which the predictors were based. The predictor which takes into account the effects of both burn size and age as well as pneumonia and inhalation injury on mortality was based upon all patients with inhalation injury admitted during the years 1980-1984 irrespective of whether they required mechanical ventilatory support. The present study population includes only the sickest patients with the most significant injuries, all requiring ventilatory support. Demonstration of a survival advantage in this group of patients compared to a group that included patients with less severe injury supports the hypothesis that high frequency percussive ventilation has a significant, beneficial effect. In short, it seems reasonable to assign a major portion of the decrease in incidence of pneumonia and improvement in outcome of the study patients to the ventilatory support employed.

Only two other published studies in the literature have evaluated the prophylactic use of high frequency ventilation in patients requiring ventilatory support as prophylaxis against ARDS. In 1986, Carlon reported a study of 309 patients who were randomized to high frequency jet ventilation or conventional ventilatory support (18). All patients who were admitted to the intensive care unit and who were at risk for the development of pulmonary failure were entered into the study. The use of high frequency jet ventilation resulted in lower peak airway pressures, but did not decrease the 4% incidence of barotrauma or improve the overall outcome as compared to conventional support. In 1990, Hurst, et al. reported a study of 113 patients at risk for the development of Adult Respiratory Distress Syndrome who were randomized to receive ventilatory support with high frequency percussive ventilation or conventional mechanical ventilation prior to the onset of ARDS (19). Changes in ventilator settings were made to achieve the same therapeutic endpoints in both groups of patients. There was no difference in the percentage of patients who developed ARDS in either group. In the patients who developed ARDS, high frequency ventilation achieved therapeutic endpoints at lower peak airway pressures, lower positive end expiratory pressures, and an increased inspiratory time as compared to the conventional group. There was, however, no difference in the incidence of barotrauma or outcome in those patients. Both of these studies involved heterogeneous patient populations, in which

the etiology of respiratory failure was diverse, usually a consequence of a systemic insult which resulted in diffuse parenchymal disease and dysfunction. This type of insult is quite distinct from that seen following smoke inhalation in both humans and animal models, in which edema resolves rapidly following resuscitation and repair of the airway mucosa typically occurs within 14-21 days.

The exact mechanism by which high frequency percussive ventilation achieved the results reported in this study is not known. We hypothesize that the ability to maintain ventilation and oxygenation at lower peak airway pressures and inspired oxygen concentrations may decrease the iatrogenic injury which occurs with conventional mechanical ventilatory support. Extrapolation of the data reported by Tsuno to humans would indicate that ventilation at lower peak airway pressures offers significant advantage, especially in lungs which have already been injured. In addition, several studies now suggest that asymmetric high frequency breaths improve clearance of secretions, a result obtained with high frequency jet ventilators and high frequency oscillators, both *in vitro* and *in vivo* (13,20-22). Our clinical experience supports this finding. Patients with severe inhalation injury treated prophylactically with high frequency percussive ventilation are typically found, by bronchoscopic examination, to have large deposits of secretions at the tip of the endotracheal tube. After removal of these secretions, the main stem bronchi and distal airways are often patent and free of pathologic secretions. The documented improvement in survival and the decrease in the incidence of pneumonia in patients treated with prophylactic high frequency percussive ventilation, as compared to the recent historic cohort, indicate the importance of maintaining small airway patency in reducing the sequela of inhalation injury. The beneficial effects reported here, and the paucity of ventilator complications, support continued use and further evaluation of high frequency ventilation in patients with inhalation injury.

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| RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY | 1 AGENCY ACCESSION DA313318 | 2 DATE OF SUMMARY 25 Aug 90 | REPORT CONTROL SYMBOL DD-DR&E(AR) 636 |
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| 3. DATE PREV SUM'RY | 4. KIND OF SUMMARY | 5. SUMMARY SCTY | 6. WORK SECURITY | 7 REGRADING | 8. DISP'N INSTR'N | 9 LEVEL OF SUM A WORK UNIT |
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| 1 Oct 89 | H. Term | U | U | | CX | |

| 10. NO./CODES | PROGRAM ELEMENT | PROJECT NUMBER | TASK AREA NUMBER | WORK UNIT NUMBER |
|---|---|---|---|---|
| a. PRIMARY | 62787A | 3M162787A874 | EC | 167 |
| b. CONTRIBUTING | | | | |
| c. CONTRIBUTING | | | | |

11. TITLE *(Precede with Security Classification Code)* (U) Quantification of Dynamic Splint Forces on Metacarpophalangeal Function Recovery

12. SUBJECT AREAS
0605 Medicine and Medical Research

| 13. START DATE | 14. ESTIMATED COMPLETION DATE | 15. FUNDING ORGANIZATION | 16. PERFORMANCE METHOD |
|---|---|---|---|
| 8710 | 9109 | DA | C. In-House |

17. CONTRACT/GRANT MILITARY RELEVANCY CERTIFIED
a. DATE EFFECTIVE APPROVED BY Susan L. Bunting
b. CONTRACT/GRANT NUMBER
c. TYPE d. AMOUNT
e. KIND OF AWARD f. CUM/TOTAL

18. RESOURCES ESTIMATE

| FISCAL YEARS | a. PROFESSIONAL WORKYEARS | b. FUNDS *(In thousands)* |
|---|---|---|
| 89 | 1.0 | 25 |
| 90 | 1.0 | 26 |

| 19. RESPONSIBLE DOD ORGANIZATION | 20 PERFORMING ORGANIZATION |
|---|---|
| a. NAME<br>US Army Institute of Surgical Research | a. NAME<br>US Army Institute of Surgical Research |
| b. ADDRESS *(include zip code)*<br>Fort Sam Houston<br>San Antonio, Texas 78234-5012 | b. ADDRESS<br>Fort Sam Houston<br>San Antonio, Texas 78234-5012 |
| c. NAME OF RESPONSIBLE INDIVIDUAL<br>PRUITT, B A | c. NAME OF PRINCIPAL INVESTIGATOR<br>LUSTER, S H |
| d. TELEPHONE NUMBER *(include area code)*<br>512-221-2720 | d. TELEPHONE NUMBER *(include area code)*<br>512-221-5957 |
| 21. GENERAL USE<br>FINA<br>MILITARY/CIVILIAN APPLICATION<br>M | f. NAME OF ASSOCIATE INVESTIGATOR *(if available)*<br>PATTERSON, P E<br>g. NAME OF ASSOCIATE INVESTIGATOR *(if available)*<br>CIOFFI, W G |

22. KEYWORDS *(Precede EACH with Security Classification Code)* (U) Burns (Injuries); (U) Joints (Anatomy); (U) Fingers; (U) Contraction; (U) Volunteers: (U) Adults; (U) RA II

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS *(Precede text of each with Security Classification Code)*

23. (U) A DTIC literature search was conducted under DTIC request number W6Q08N dated 19 October 1989 for the technical report database and request number W6Q11K dated 19 October 1989 for the work unit database. The proposed effort will not result in the duplication of effort. The objective of this work is to develop instrumentation to quantify initial stiffness in metacarpophalangeal joints and, using this instrumentation, to compare the effects of different splinting regimens in the rehabilitation of burned hands.

24. (U) Joint stiffness will be quantified with an electronic device that will duplicate the effect of a traction splint while recording the reactive force being generated by the joint. A range of force measurements for normal finger joints will be established as a basis for defining joint stiffness in injured joints. Serial measurements of injured joints will be used to document the joint stiffness-reducing effects of various hand rehabilitation techniques.

25. (U) 8910 - 9008. A prototype stiffness monitoring device capable of recording changes in reactive force and joint angle was designed and tested. The device was then redesigned to facilitate data collection in the clinical setting. A second prototype was received and tested in June 1989, but it failed to meet contract specifications. The third prototype was not delivered until 15 August 1990. It was tested and again failed to meet contract specifications. Given the extended delays, inability of the contractor to solve the technical problems, and transfer of the principal investigator, this project was terminated. For technical reports, refer to the US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Year 1990.

DD FORM 1498 83 MAR EDITION OF MAR 68 IS OBSOLETE

**ANNUAL RESEARCH PROGRESS REPORT**

**PROJECT NUMBER:** 3M162787A874-00, APPLIED RESEARCH AND EXPLORATORY DEVELOPMENT

**PROJECT TITLE:** Quantification of Dynamic Splint Forces on Metacarpophalangeal Function Recovery

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

**1 October 1989 - 30 September 1990**

**INVESTIGATORS**

Stephen H. Luster, OTR, MS, Major, SP
*Patrick E. Patterson, Ph.D.
William G. Cioffi, Jr., MD, Major, MC
William F. McManus, MD, Colonel, MC
Arthur D. Mason, Jr., MD
Basil A. Pruitt, Jr., MD, Colonel, MC

*Iowa State University

## ABSTRACT

**PROJECT NUMBER:** 3M162787A874-00, APPLIED RESEARCH AND EXPLORATORY DEVELOPMENT

**PROJECT TITLE:** Quantification of Dynamic Splint Forces on Metacarpophalangeal Function Recovery

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

*Iowa State University

**PERIOD COVERED IN THIS REPORT:** 1 Oct 89 through 30 Sep 90

**INVESTIGATORS:** Stephen H. Luster, OTR, MS, Major, SP
*Patrick E. Patterson, Ph.D.
William G. Cioffi, Jr., MD, Major, MC
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Basil A. Pruitt, Jr., MD, Colonel, MC

The objective of this study was to develop a device to quantify joint stiffness in burned hands. The study was planned in two phases. The first phase consisted of a pilot study using an experimental device to quantify finger joint stiffness. This study was completed and the results reported. Phase two of the project was to use a clinical version of the finger stiffness measurement device in a large series of burned hands. Technical problems in the development of the second finger stiffness measurement device have prevented the start of this phase of the project. Without the finger stiffness measurement device, the project cannot be completed. The project has, therefore, been terminated.

## REFERENCES

1. Luster SH, Patterson PE, Cioffi WG: An evaluation device for quantifying joint stiffness in the burned hand. **J Burn Care Rehab**, 11(4):312-317, 1990.

**RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY**

| 1 AGENCY ACCESSION | 2 DATE OF SUMMARY | REPORT CONTROL SYMBOL (DD-DR&E(AR) 636) |
|---|---|---|
| DA314853 | 4 Jun 90 | |

| 3. DATE PREV SUM'RY | 4. KIND OF SUMMARY | 5. SUMMARY SCTY | 6. WORK SECURITY | 7 REGRADING | 8 DISP'N INSTR'N | 9 LEVEL OF SUM A WORK UNIT |
|---|---|---|---|---|---|---|
| 1 Oct 89 | K. Comp | U | U | | CX | |

| 10. NO./CODES | PROGRAM ELEMENT | PROJECT NUMBER | TASK AREA NUMBER | WORK UNIT NUMBER |
|---|---|---|---|---|
| a. PRIMARY | 62787A | 3S162787A874 | CH | 169 |
| b. CONTRIBUTING | | | | |
| c. CONTRIBUTING | | | | |

11. TITLE *(Precede with Security Classification Code)* (U) Phase II Study of Human Recombinant Granulocyte Macrophage Colony-Stimulating Factor in Patients with Thermal Injury

12. SUBJECT AREAS
0605 Medicine and Medical Research 0615 Pharmacology

| 13 START DATE | 14 ESTIMATED COMPLETION DATE | 15 FUNDING ORGANIZATION | 16 PERFORMANCE METHOD |
|---|---|---|---|
| 8806 | 9006 | DA | C. In-House |

17 CONTRACT/GRANT MILITARY RELEVANCY CERTIFIED APPROVED BY [signature]

| a. DATE EFFECTIVE | | 18 RESOURCES ESTIMATE | | |
|---|---|---|---|---|
| b. CONTRACT/GRANT NUMBER | | a FISCAL YEARS | a PROFESSIONAL WORKYEARS | b FUNDS *(In thousands)* |
| c. TYPE | d. AMOUNT | 89 | 0.8 | 32 |
| e. KIND OF AWARD | f. CUM TOTAL | 90 | 0.8 | 34 |

| 19. RESPONSIBLE DOD ORGANIZATION | 20 PERFORMING ORGANIZATION |
|---|---|
| a. NAME<br>US Army Institute of Surgical Research | a NAME<br>US Army Institute of Surgical Research |
| b. ADDRESS *(include zip code)*<br>Fort Sam Houston<br>San Antonio, Texas 78234-5012 | b ADDRESS<br>Fort Sam Houston<br>San Antonio, Texas 78234-5012 |
| c. NAME OF RESPONSIBLE INDIVIDUAL<br>PRUITT, B A | c NAME OF PRINCIPAL INVESTIGATOR<br>CIOFFI, W G |
| d. TELEPHONE NUMBER *(include area code)*<br>512-221-2720 | d. TELEPHONE NUMBER *(include area code)*<br>512-221-4440 |
| 21. GENERAL USE<br>FINA<br>MILITARY/CIVILIAN APPLICATION<br>M | e. NAME OF ASSOCIATE INVESTIGATOR *(if available)*<br>BURLESON, D G<br>g NAME OF ASSOCIATE INVESTIGATOR *(if available)*<br>MC MANUS, W F |

22. KEYWORDS *(Precede EACH with Security Classification Code)* (U) Burns (Injuries); (U) Immunosuppression; (U) Colony-Stimulating Factor; (U) Granulocytes; (U) Macrophages;

23 TECHNICAL OBJECTIVE 24 APPROACH 25 PROGRESS *(Precede text of each with Security Classification Code)*

22. (Continued) (U) Lymphokines; (U) Pharmacology; (U) Volunteers; (U) Adults; (U) RA II

23. (U) A DTIC literature search was conducted under DTIC request number W6044A dated 18 October 1989 for the technical report database and request number W6045D dated 18 October 1989 for the work unit database. The proposed effort will not result in the duplication of effort. The objective of this work was to determine the safety and activity of human recombinant granulocyte macrophage colony-stimulating factor in patients with thermal injury.

24. (U) A maximum of 20 patients with 20-30% burns will be entered into this study. The occurrence of grade 3 or 4 toxicity or a white blood cell count >50,000/cubic mm of blood in at least 60% of the patients at any dose level will be used as evidence of toxicity. Treatment will continue for as long as the patient is hospitalized but will not exceed 2 months. Within a few days of stopping treatment, the patient will undergo a final examination. At this time, the patient will be evaluated with respect to complications of therapy, dose-limiting toxicity (if any), and adverse experiences and responses (if any).

25. (U) 8806 - 9009. Ten patients were enrolled in this study. Patients receiving the drug had the anticipated increase in peripheral white blood cell counts. Mild adverse reactions such as hyperthermia, mild bone pain, and pruritus were experienced. One patient developed a suppurative parotid abscess while undergoing treatment. One patient had his dose decreased secondary to an elevation of his white count above 50,000. There is not seem to be any significant risks associated with the use of this drug in thermally injured patients without innalation injury. For technical reports, refer to the US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Year 1990.

DD FORM 1498 83 MAR EDITION OF MAR 68 IS OBSOLETE * U.S.G.P.O. 1988-491-003/50329

**ANNUAL RESEARCH PROGRESS REPORT**

**PROJECT NUMBER:** 3S162787A874-00, APPLIED RESEARCH AND EXPLORATORY DEVELOPMENT

**PROJECT TITLE:** Phase II Study of Human Recombinant Granulocyte Macrophage Colony-Stimulating Factor in Patients with Thermal Injury

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

**1 October 1989 - 4 June 1990**

**INTESTIGATORS**

William G. Cioffi, Jr., MD, Major, MC
David G. Burleson, PhD, Lieutenant Colonel, MS
William F. McManus, MD, Colonel, MC

**ABSTRACT**

**PROJECT NUMBER:** 3S162787A874-00, APPLIED RESEARCH AND EXPLORATORY DEVELOPMENT

**PROJECT TITLE:** Phase II Study of Human Recombinant Granulocyte Macrophage Colony-Stimulating Factor in Patients with Thermal Injury

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

**PERIOD COVERED IN THIS REPORT:** 1 Oct 89 through 4 Jun 90

**INVESTIGATORS:** William G. Cioffi, Jr., MD, Major, MC
David G. Burleson, PhD, Lieutenant Colonel, MS
William F. McManus, MD, Colonel, MC

We studied the effects of granulocyte-macrophage colony-stimulating factor in burn patients. Serial measurements of granulocyte oxidative function were obtained in treated patients and in a group of controls matched for age and total burn size. The administration of granulocyte-macrophage colony-stimulating factor resulted in a 50% increase in mean leukocyte counts. Both groups showed significant baseline increases in granulocytic cytosolic oxidative function. Treated patients showed normal stimulated cytosolic oxidative function, which was significantly depressed compared with that of untreated patients. Myeloperoxidase activity was increased in treated patients during the first postburn week but then declined to normal levels. Untreated patients had a significant increase in myeloperoxidase activity for the first three weeks following injury. Untreated patients exhibited a significant decrease in superoxide activity during the second three weeks following injury. Treated patients demonstrated normal superoxide activity.

This study was completed 4 June 1990. No patients were entered into the study during this reporting period.

## PHASE II STUDY OF HUMAN RECOMBINANT GRANULOCYTE MACROPHAGE COLONY-STIMULATING FACTOR IN PATIENTS WITH THERMAL INJURY

Improvements in fluid management, wound care, and nutritional support have markedly reduced early mortality from thermal injury, but significant late mortality persists. Burn-induced defects of the immune system appear to contribute to late mortality, which is primarily due to infection and sepsis.

Although the specific cause of the immune dysfunction following thermal injury is unknown, both qualitative and quantitative defects have been noted in all limbs of the immune system (1-13). Defective migration, phagocytosis, and degranulation have been described as manifestations of granulocyte dysfunction. In addition, burn serum contains an inhibitor of complement conversion that may cause opsonization failure that further inhibits neutrophil function (14). Such granulocyte dysfunction may contribute significantly to the marked predisposition to infection (15).

Granulocyte-macrophage colony-stimulating factor (GM-CSF) is a lymphokine that was first described nearly 20 years ago. Not only does GM-CSF stimulate the proliferative potential of granulocyte and macrophage progenitor cells in the bone marrow, but it also stimulates various functional activities of mature cells. In the presence of GM-CSF, macrophages are stimulated to secrete plasminogen-activating factor (16) and also exhibit increased phagocytic and cytocidal activity for bacteria, yeast, (17) and malignant cell lines (18). Granulocytes increase RNA and protein synthesis and exhibit increased antibody-dependent cytotoxic killing of tumor cells and enhanced oxidative metabolism in the presence of GM-CSF in vitro (17,19-22). Recombinant GM-CSF stimulates mature neutrophils to augment cell surface antigenic expression as well as increase their phagocytic activity, synthesis of biologically active molecules, and expression of various cell surface markers (23).

In a group of patients with thermal injury, a comparison of the serum levels of hematopoietic colony-stimulating factors (CSFs) has revealed distinct differences between survivors and non-survivors (24). The nonsurviving patients demonstrated an inappropriate lag in the generation of CSF early in the course of burn injury and inappropriately low levels of the factor even in the presence of documented sepsis. This aberrant response was associated with a relative failure of granulopoiesis. Further studies have demonstrated that serum from patients with thermal injury inhibits the in vitro production of CSFs by mononuclear cells (25).

The multiple defects in granulocytic function and the decreased levels of CSFs following lethal thermal injury suggest that a beneficial effect on granulocyte count and function might result

from the administration of CSFs. Our study was designed to determine the safety of the administration of human recombinant GM-CSF (hr-GM-CSF) in patients with thermal injury.

## PATIENTS AND METHODS

**Patient Population.** Patients with burns over 20% to 70% of the total body surface area were eligible for enrollment in the study. Patients with inhalation injury diagnosed by xenon 133 lung scanning but with no bronchoscopic evidence of upper airway injury were also eligible for enrollment. Bronchoscopic evidence of inhalation injury resulted in exclusion from the study. All eligible patients were admitted within 48 hours of injury and underwent uneventful resuscitation. Routine care was not altered. In all patients, sulfadiazine silver was applied once daily. Patients treated with other lymphokines, prophylactic antibiotics, or corticosteroids were excluded from the study. Data from patients with thermal injury admitted during the same period but not enrolled in the study and a group of healthy laboratory controls were obtained for comparison with the treated patients.

**hr-GM-CSF.** Nonglycosylated hr-GM-CSF was obtained from the bacterial fermentation of a strain of *Escherichia coli* bearing a genetically engineered plasmid containing the human GM-CSF gene. The product is a highly purified, sterile, stable, water-soluble protein with a molecular weight of 14 477 d. The GM-CSF was shown to be biologically active in the KG-1 cell proliferation assay and a colony-stimulating assay that employed bone marrow cells.

**Drug Administration.** Patients were administered 3 or 10 μg/kg daily of hr-GM-CSF intravenously during a 4-hour period. Treatment began within 5 days of injury and continued for a minimum of 2 weeks or until a grade 3 or 4 toxic reaction developed. All potential adverse effects were recorded and graded on the following scale: 1, mild; 2, moderate; 3, severe; and 4, life-threatening. Any patient who experienced a grade 3 or 4 toxic reaction that was deemed attributable to the hr-GM-CSF received no further treatment until the adverse reaction resolved. The patient was then re-treated at a dosage not more than 50% of the original. Recurrence of the same toxic reaction necessitated withdrawal from the study. In patients who exhibited a white blood cell count greater than $50.0 \times 10^9$/L, subsequent doses of the lymphokine were withheld until the white blood cell count decreased to less than $30.0 \times 10^9$/L. Administration was then resumed at a dose of 30% to 50% of the original dose.

***In Vitro* Testing.** Complete blood cell counts were obtained daily from each patient. In vitro granulocyte function tests were performed twice weekly during treatment and for up to 3 weeks following cessation of lymphokine administration. Granulocytes were isolated from heparinized whole blood by Ficoll-Hypaque gradients. Cells passing through the gradient were recovered from

the cell pellet. Contaminating red blood cells were removed by hypotonic lysis. The cell pellet from the Ficoll-Hypaque gradient was resuspended in 50 mL of Hanks' balanced salt solution (HBSS), spun at 2250g for 10 minutes, and 3 mL of the buffy coat was removed and placed in a 50-mL conical centrifuge tube. Distilled water (20 mL) was added during agitation of the sample on vortex mixer. After 20 seconds, 20 mL of hypertonic (2x) HBSS was added, the cells were centrifuged at 200 g for 10 minutes, and the supernatant was removed. The cells were suspended in 2 mL of HBSS and transferred to a 15-mL conical centrifuge tube. A second lysis was performed with the addition of 4 mL of distilled water for 20 seconds, after which 4 mL of 2x hypertonic HBSS was added to restore isotonicity. The cells were suspended at a concentration of 1 x $10^6$ cells/mL in 1 mL of barbital buffer (pH 7.25) (26). 2',7'-dichlorofluorescein diacetate (DCF-DA, at a final concentration of 5 µmol/L) was added to each sample and incubated for 20 minutes at 37°C to allow DCF-DA to enter the cells. Whereas DCF-DA easily permeates the cells where the acetyl groups are hydrolyzed to 2',7,-dichlorofluorescein (DCF), the DCF is too polar to pass through the plasma membrane and is effectively trapped within the cell. When oxidized by peroxide, DCF becomes highly fluorescent and the measurement of this fluorescence serves as an index of cytosolic peroxidative activity. Cell fluorescence was measured by flow cytometry. The mean fluorescence of 10,000 cells was calculated for each data point. After an initial fluorescent measurement, cells were incubated for 20 minutes with and without phorbol myristate acetate (PMA, 700 ng/mL) as stimulant. Measurements were recorded as log fluorescence and were compared with values obtained from granulocytes from healthy volunteers.

Additional studies of granulocyte oxidative metabolism were performed with the use of two chemilumigenic probes, luminol, and dimethyl biacridinium dinitrate (DBA) (26). Heparinized whole blood was diluted 1:10 in HBSS (pH 7.2). Aliquots (20 µL) of diluted whole blood were added to 2 mL of barbital buffer solution in siliconized glass vials. The appropriate chemilumigenic probe was then added to each sample, and three prestimulation background measurements were performed. All measurements were made at 25°C in a liquid scintillation counter set for photon counting. Saline, PMA (350 n/mL), or zymosan (6.25 mg/L), preopsonified with guinea pig serum, was added to the vial, and luminescence was measured at 13-minute intervals for 2 hours. The total luminescence produced in each sample was calculated from the light-intensity measurements by trapezoidal approximation. The values obtained for luminol correspond to the total oxygenation events produced primarily by myeloperoxidase. The values obtained when DBA was used as a probe corresponded to the total oxygenation events produced by extracellular superoxide anion and other oxidative species.

**Statistical Analysis.** Differences between groups were analyzed with use of the *t* test and analysis of variance, with post

hoc testing, when appropriate, with use of the BMDP statistical package.

## RESULTS

**Patient Population.** Ten patients with a mean age of 28.6 years and a mean burn size of 37% were enrolled in the study. Individual patient data, including the dose of hr-GM-CSF, and the duration of treatment, are outlined in Table 1. Two patients, both with inhalation injury, died, for a mortality rate of 20%. Fourteen patients with thermal injury with a mean age of 30.5 years and mean burn size of 36%, admitted during the same period, were used as nonrandomized controls for comparison of oxidative metabolism data. There was no statistical difference between the two groups of patients with respect to age, burn size, and mortality rate, although a greater proportion of untreated patients had inhalation injury (Table 2). Grades 1 and 2 adverse effects were common. Seven patients complained of pruritus, four exhibited pyrexia during administration of hr-GM-CSF, two complained of back pain, and one experienced pleuritic chest pain. Acute parotitis and a subcutaneous abscess occurred in one patient, each requiring incision and drainage.

**Blood Count Data.** Patients receiving GM-CSF demonstrated a significant increase in total white blood cell count during the second postburn week compared with the first, third, fourth, fifth, sixth, and seventh postburn weeks (Fig 1). One patient, who received 10 μg/kg of GM-CSF, had a white blood cell count greater than 50.0 x $10^9$/L during the second postburn week. Lymphokine administration was discontinued for 2 days, during which time the white blood cell count decreased to 26.0 x $10^9$/L, and treatment was then resumed at 3 μg/kg per day. The majority of treated patients demonstrated a relative decrease in their white blood cell counts during the third postburn week despite continued administration of GM-CSF. Compared with the untreated burn patients, the patients receiving GM-CSF exhibited a significant elevation in their white blood cell counts only during the second postburn week. The percentage of granulocytes was not different between treated and untreated burn patients during the first 3 weeks. However, on cessation of GM-CSF administration, a significant decrease in the percentage of granulocytes was noted in the treated patients compared with the untreated burn patients (63.5% vs 80.9%) (Fig 2). The percentage of polymorphonuclear cells was not different during treatment but decreased significantly during the fourth postburn week compared with untreated patients (43.3% vs 71.2%), accounting for the difference in the granulocyte percentages (Fig 2). No statistically significant differences between treated and untreated patients were noted in the percentage of monocytes, lymphocytes, myelocytes, or band forms either during or after treatment, although patients receiving the cytokine tended to have an increased percentage of band forms and myelocytes during treatment.

**TABLE 1. Patient Demographics**

| Patient | Age | Sex | %TBSB | % FULL | DOSE | DAYS |
|---|---|---|---|---|---|---|
| 1 | 24 | M | 36 | 32 | 3 | 17 |
| 2 | 45 | M | 39 | 5 | 3 | 17 |
| 3 | 24 | M | 24 | 18 | 10 | 17 |
| 4 | 27 | M | 20 | 17 | 10->3 | 12 |
| 5 | 24 | M | 35 | 0 | 3 | 12 |
| 6 | 35 | F | 23 | 23 | 3 | 11 |
| 7 | 23 | M | 45 | 42 | 3 | 17 |
| 8 | 22 | M | 54 | 44 | 3 | 5 |
| 9 | 21 | F | 42 | 8 | 3 | 29 |
| 10 | 41 | M | 52 | 10 | 3 | 2 |

Factor administration was stopped on days 5 and 2 in patients 8 and 10, respectively, because of worsening pulmonary status. Both patients had abnormal Xenon 133 bone scans but normal bronchoscopic findings. The degradation in pulmonary function was not temporally related to the administration of factor.

**TABLE 2. Comparison of Treated and Untreated Groups**

| | Treated | Untreated |
|---|---|---|
| Age | 28.6 ± 2.7 | 30.6 ± 3.0 |
| % TBSB | 37.1 ± 3.8 | 36.2 ± 3.0 |
| Inhalation Injury | 2/10 (20%) | 6/14 (43%) |
| Mortality | 2/10 (20%) | 0/14 (0%) |

Values are mean±SD or number affected/total number (%). No difference in age or percent total burn surface area was noted between the two patient groups, but the untreated patients had a higher incidence of inhalation injury.

**Dichlorofluorescein Oxidation.** No significant difference in baseline unstimulated cytosolic oxidative activity was noted between the two patient groups, although both were significantly higher than values for unburned controls (Table 3). Patients receiving hr-GM-CSF exhibited a significant decrease in maximal

cytosolic oxidative activity compared with untreated burn patients (92.3% vs 114.7% of control values; P<.01) during the 3 weeks of treatment. On cessation of cytokine administration, the peak cytosolic oxidative activity of treated patients increased slightly to 97% of that for controls whereas untreated patients remained elevated at 113% of control values (Table 4).

**TABLE 3. Cytosolic Peroxidase Activity for Postburn Days 0-21**

| | PTUR | PTSR |
|---|---|---|
| Treated | 0.249 ± .01 | 0.929 ± .06 |
| Untreated | 0.243 ± .01 | 1.150 ± .05 |

Values are mean±SD. PTUR indicates the ratio of the mean log fluorescence for unstimulated patients' cells to stimulated control subjects' cells (normal, 0.16) (both patient groups were significantly different from controls [P<.05]); PTSR, the ratio of the mean log fluorescence for stimulated patients' cells to control subjects' cells (normal, 1.0) (untreated patients were significantly different from treated patients and controls [P<.05]).

**TABLE 4. Cytosolic Peroxidase Activity for Postburn Days 22-42**

| | PTUR | PTSR |
|---|---|---|
| Treated | 0.223 ± .02 | 0.972 ± .05 |
| Untreated | 0.227 ± .01 | 1.140 ± .04 |

Values are mean±SD and are for the 3 weeks after cessation of factor administration. See Table 3 for explanation of PTUR and PTSR. The PTUR for both patient groups remained significantly elevated compared with controls (P<.05); PTSR for untreated patients remained significantly different from treated patients and controls (P<.05).

**Chemiluminescence**. During the first 7 days following injury, both treated and untreated burn patients exhibited a significant increase in luminol chemiluminescence compared with healthy controls. This increase was independent of the type of stimulation employed to activate the granulocytes, as the response to opsonified zymosan and PMA were essentially identical (Table 5). After 1 week, oxidation of luminol following stimulation by opsonified zymosan and PMA decreased to control values for the treated patients but remained elevated for the untreated patients. During the third postburn week (the last week of therapy with GM-CSF), luminol chemiluminescence remained unchanged in the untreated patients and increased significantly in the treated patients in response to PMA but not opsonified zymosan administration. After 21 days, luminol chemiluminescence began to decrease in untreated patients but remained significantly elevated in treated patients in response to PMA administration.

**TABLE 5. Chemiluminescence Data**

| | LOZ | LPMA | DPMA | n |
|---|---|---|---|---|
| | | PBD 0-7 | | |
| Control | 2344 ± 179 | 2482 ± 158 | 14371 ± 944 | 137 |
| Untreated | 4060 ± 686 | 6635 ± 784 | 11076 ± 1803 | 14 |
| Treated | 6355 ± 3047(a) | 7523 ± 4513(a) | 10932 ± 4956 | 11 |
| | | PBD 8-14 | | |
| Control | 2344 ± 179 | 2482 ± 158 | 14371 ± 944 | 137 |
| Untreated | 4298 ± 912(a) | 5330 ± 763(a,b) | 12036 ± 1670 | 24 |
| Treated | 2302 ± 750 | 2989 ± 861 | 14248 ± 4934 | 15 |
| | | PBD 15-21 | | |
| Control | 2344 ± 179 | 2482 ± 158 | 14371 ± 944 | 137 |
| Untreated | 4779 ± 987(a) | 4919 ± 678(a) | 13110 ± 2170 | 22 |
| Treated | 3649 ± 593 | 3943 ± 632(C) | 19812 ± 4904 | 14 |
| | | PBD >21 | | |
| Control | 2344 ± 179 | 2482 ± 158 | 14371 ± 944 | 137 |
| Untreated | 3120 ± 319 | 4009 ± 897 | 8781 ± 961(a,b) | 74 |
| Treated | 3217 ± 469 | 5141 ± 1166(c) | 16791 ± 2422 | 44 |

LOZ: Opsonified zymosan stimulated luminol chemiluminescence, LPMA: PMA stimulated luminol chemiluminescence, and DPMA: PMA stimulated DBA chemiluminescence. (a): Significant difference when compared to control subjects ($P<.01$), (b): Significant difference when compared to untreated patients ($P<.01$), and (c): Significant difference when compared to controls ($P<.05$).

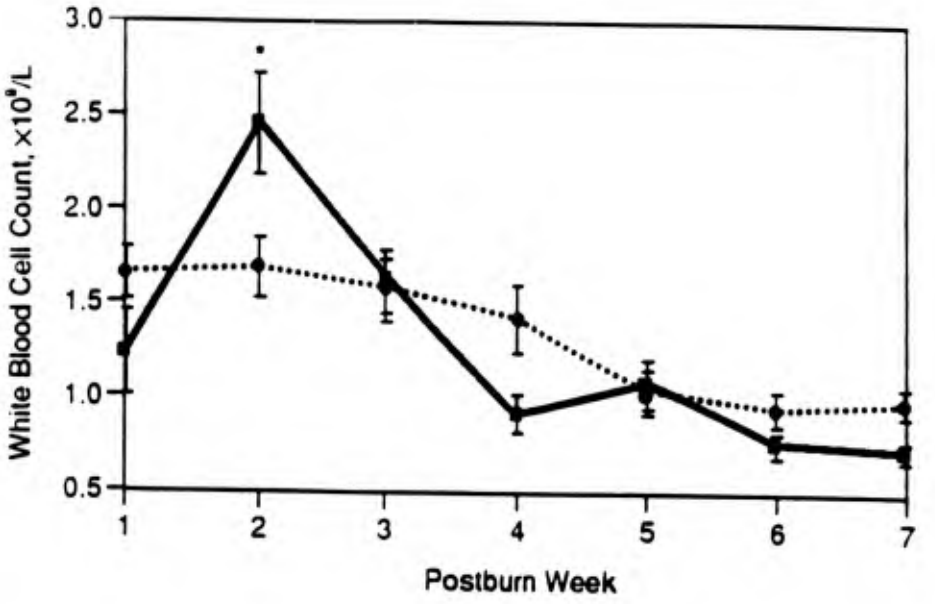


**FIGURE 1.** White blood cell counts (thousands) are graphically displayed for the first 7 postburn weeks for the treated and untreated patients. The only significant difference was during the 2nd postburn week ($P<.05$).

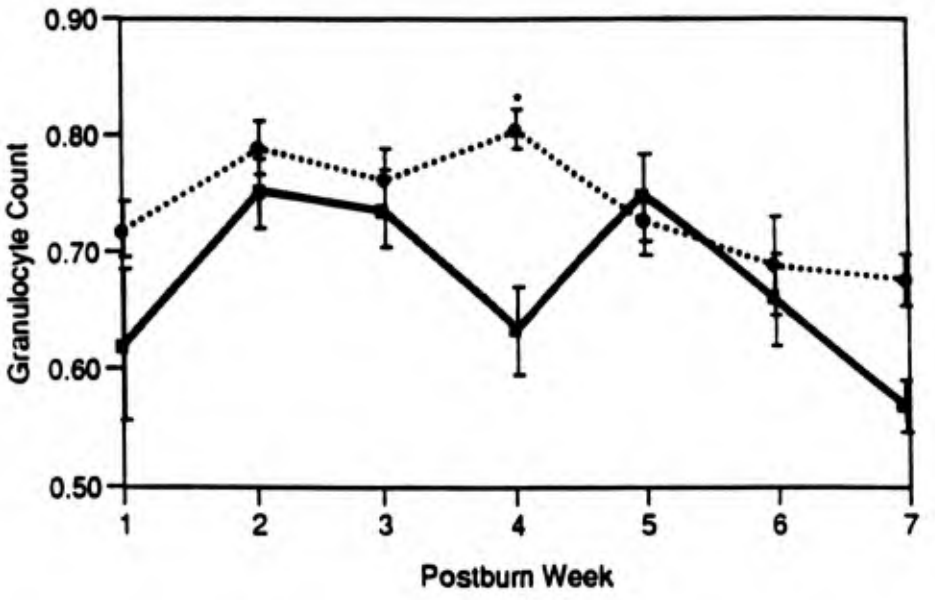


**FIGURE 2.** The percent of granulocytes for both groups of patients is displayed for the first 7 postburn weeks. No differences were evident except during postburn week 4 when treated patients had significantly fewer granulocytes than the untreated patients. The percentage of lymphocytes increased in the treated patients but the difference between groups was not significant.

The oxidation of DBA, an indicator of extracellular superoxide production, normally declines as time after injury progresses. In this group of untreated burn patients, the mean luminescence value was 87% of the control patients' mean value during the first 3 weeks and 61% of the control value ($P<.01$) during the second 3-week period following injury (Table 5). Patients treated with GM-CSF failed to show a decrement in oxidation of DBA, with luminescence values similar to those for controls during the first 2 weeks following injury and 137% of those for controls during the third and final week of treatment. The DBA chemiluminescence remained significantly elevated at 117% of control values when administration of GM-CSF was discontinued.

**COMMENT.** Adequate numbers of properly functioning granulocytes may be one of the most important factors in a patient's defense against infection. Thermal injury induces a variety of abnormalities in granulocyte production and function. Peterson et al. (27) have reported decreased numbers of circulating granulocyte stem cells in nonsurviving patients with large burns, which was thought to reflect a reduction of the bone marrow progenitor cell pool. This decrease in circulating colony-forming units was associated with a higher incidence of fatal septicemia. Defects in chemotaxis, random migration, phagocytosis, bactericidal capacity, superoxide production, and in vitro oxygen consumption have all been described, but a relationship between these defects and the propensity for infection has not been shown.

Granulocyte-macrophage CSF is a cytokine produced by activated T cells and macrophages as well as by certain fibroblasts and endothelial cells (28). It is a potent stimulus of bone marrow progenitor cell production of neutrophils, monocytes, and eosinophils. Significant increases in numbers of circulating granulocytes have been documented in both healthy primates and humans following parenteral administration of GM-CSF (17,21,24-25,29-31). Clinical trials in patients with leukopenia secondary to aplastic anemia, (32) acquired immunodeficiency syndrome, (33) chronic idiopathic neutropenia, (34) and chemotherapy-induced neutropenia (35-37) have all shown the ability of GM-CSF to increase circulating levels of mature granulocytes. Parenteral administration of GM-CSF to our cohort of patients with thermal injuries resulted in a similar response. After a lag time of approximately 1 week, white blood cell counts increased significantly compared with untreated burn patients. After cessation of GM-CSF administration, counts quickly decreased to expected normal levels. Eosinophilia, commonly seen in primate studies following the parenteral administration of GM-CSF, was not observed in our treated patients.

The *in vitro* effect of GM-CSF on white blood cells isolated from healthy volunteers has been well documented. Although GM-CSF has little effect on white blood cell function alone, it appears to

"prime" the cell for increased oxidative function when activated *in vitro* by physiologic chemoattractants, such as PMA, FMLP (F Met-Leu-Phe), C5a, leukotriene $B_4$, and opsonified zymosan (38). Chemotaxis, cytotoxic and phagocytic activity, superoxide production, and degranulation are all increased by prior incubation with GM-C SF (39).

Few data exist concerning the effect of parenteral GM-CSF on various white blood cell functions in patients with documented functional defects. Defects in granulocyte phagocytosis and bactericidal capacity in two patients with acquired immunodeficiency syndrome were resolved with the parenteral administration of GM-CSF (38). Reductions in phagocytic capacity, nitroblue tetrazolium reduction, and migration were restored to normal by the administration of GM-CSF in one patient with chronic idiopathic neutropenia (35).

The parenteral administration of GM-CSF to our group of patients with thermal injury did not affect the baseline (non-stimulated) increase in *in vitro* cytosolic oxidative activity previously described (40). When the oxidation of DCF is expressed as a percentage of the mean fluorescence of stimulated white blood cells from healthy control subjects, unstimulated cells from healthy controls demonstrate approximately 16% activity. Both the treated and untreated burn patients' cells had significantly higher baseline activity compared with normal controls (24.9% and 24.3%, respectively). This increase in unstimulated oxidative capacity persisted even after discontinuation of the GM-CSF. Patients receiving GM-CSF had normal stimulated DCF oxidation values (92.9%) that were significantly lower than the 115% activity seen in white blood cells from untreated patients. Thus, it appears that GM-CSF decreases the capacity of granulocytes to oxidize DCF, presumably due to the lower production of intracellular hydrogen peroxide.

Myeloperoxidase activity, as indexed by luminol chemiluminescence following stimulation by opsonified zymosan and PMA, was markedly elevated in untreated patients for the first 3 weeks after injury. Treated patients showed a significant increase in luminol chemiluminescence during the first few days of treatment, which subsequently declined to normal control values during the second week of treatment. During the third week of treatment and on discontinuation of GM-CSF administration, opsonified zymosan-stimulated chemiluminescence remained normal. In contrast, PMA-stimulated luminol chemiluminescence rose to supranormal levels.

The level of DBA chemiluminescence, which primarily indexes superoxide anion production, was significantly affected by the administration of GM-CSF. During the first 3 weeks after injury, granulocytes from untreated patients showed normal to slightly decreased PMA-stimulated chemiluminescence when DBA was used as a probe. During the subsequent 3 weeks, this defect was exaggerated. In patients receiving GM-CSF, DBA chemiluminescence was only

slightly depressed during the first postburn week, normal during the second postburn week, and supranormal during the third week of drug administration. After discontinuation of GM-CSF administration, DBA chemiluminescence remained normal and did not decrease in contrast to that in the untreated patients. The maintenance of DBA chemiluminescence following cessation of GM-CSF administration indicates that the effect of the cytokine is not direct, because the half-life of circulating neutrophils is substantially less than 1 day.

The administration of parenteral GM-CSF to patients with thermal injury but without inhalation injury appears to be safe and resulted in the expected increase in circulating numbers of granulocytes. Whether this compound can be safely administered to patients with inhalation injury cannot be answered from our study. Although both patients with inhalation injury who received GM-CSF died, deterioration in the patients' status was not temporally related to its administration. A more complex question concerns whether the effect of parenteral administration of GM-CSF on white blood cell function is beneficial. Restoration of superoxide production by stimulated cells has the potential for both beneficial and adverse effects. An increase in extracellular superoxide may lead to an increase in capillary permeability due to endothelial injury from adherent white blood cells. The reduction in myeloperoxidase activity might also be viewed as detrimental to the patient, as this enzyme plays an important role in the bactericidal capabilities of the phagocyte. The effect of these changes on morbidity and mortality cannot be determined from our nonrandomized trial of GM-CSF administration in patients with limited thermal injury. Our results caution against the extrapolation of data obtained through the *in vitro* incubation of normal cells with GM-CSF. Future studies concerning the effect of parenteral administration of GM-CSF on white blood cell function in healthy subjects as well as its effect on pulmonary function in lung injury in animal models will be important to define the *in vivo* effects and the potential beneficial or detrimental effects when administered to injured patients.

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**RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY**

| 1. AGENCY ACCESSION | 2. DATE OF SUMMARY | REPORT CONTROL SYMBOL DD-DR&E(AR) 636 |
|---|---|---|
| DA315359 | 01 Oct 90 | |

| 3. DATE PREV SUM'RY | 4. KIND OF SUMMARY | 5 SUMMARY SCTY | 6. WORK SECURITY | 7. REGRADING | 8. DISB'N INSTR'N | 9 LEVEL OF SUM A WORK UNIT |
|---|---|---|---|---|---|---|
| 01 Oct 89 | D. Chg | U | U | | CX | |

| 10. NO./CODES | PROGRAM ELEMENT | PROJECT NUMBER | TASK AREA NUMBER | WORK UNIT NUMBER |
|---|---|---|---|---|
| a. PRIMARY | 62787A | 3M162787A874 | EA | 170 |
| b. CONTRIBUTING | | | | |
| c. CONTRIBUTING | | | | |

11. TITLE (Precede with Security Classification Code) (U) Evaluation of in vitro Cultivated Keratinocytes as Epithelial Autografts for the Closure of Burn Wounds

12. SUBJECT AREAS
0605 Medicine and Medical Research

| 13. START DATE | 14. ESTIMATED COMPLETION DATE | 15. FUNDING ORGANIZATION | 16 PERFORMANCE METHOD |
|---|---|---|---|
| 8610 | 9109 | DA | C. In-House |

17. CONTRACT/GRANT MILITARY RELEVANCY CERTIFIED
a. DATE EFFECTIVE APPROVED BY Paul Smith
b. CONTRACT/GRANT NUMBER
c. TYPE
d. AMOUNT
e. KIND OF AWARD
f. CUM/TOTAL

18. RESOURCES ESTIMATE

| FISCAL YEARS | a PROFESSIONAL WORKYEARS | b FUNDS (In thousands) |
|---|---|---|
| 90 | 0.1 | 871 |
| 91 | 0.1 | 2500 |

19. RESPONSIBLE DOD ORGANIZATION

a. NAME
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20 PERFORMING ORGANIZATION

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US Army Institute of Surgical Research

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Fort Sam Houston
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512-221-4440

21. GENERAL USE
FINA

MILITARY/CIVILIAN APPLICATION
M

f. NAME OF ASSOCIATE INVESTIGATOR (if available)
CIOFFI, W G

g. NAME OF ASSOCIATE INVESTIGATOR (if available)
DRISCOLL, D M

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Burns (Injuries); (U) Healing; (U) Skin Grafts; (U) Biopsy

23 TECHNICAL OBJECTIVE 24 APPROACH 25 PROGRESS (Precede text of each with Security Classification Code)

23/24. (U) The objective of this work is to evaluate cultured keratinocytes as grafts for epithelial closure of burn wounds and identify technical and immunological requirements to establish banks of frozen histocompatible keratinocytes for wound coverage of burned soldiers. The possible utility of cultured keratinocytes will be established initially with cultured autologous keratinocytes. Keratinocytes will be cultured from biopsies of unburned skin taken early after admission of patients with large burns and limited unburned donor sites. If such grafts are deemed clinically useful, efforts will expand into investigations of allogeneic skin cultures.

25. (U) 8710 - 8809. Two patients were enrolled in the study during this reporting period.

(U) 8810 - 8909. Nine applications were performed on seven patients.

(U) 8910 - 9009. Due to poor results with cultured keratinocytes obtained from the contractor, an addendum was developed to change to a different contractor for the growth of the keratinocyte sheets. Thirteen applications have been performed on 10 patients. As a consequence of graft take varying between 30 and 80%, work is underway to develop the optimal technics for managing the wound sites after application of the cultured cells.

DD FORM 1498 83 MAR EDITION OF MAR 68 IS OBSOLETE * USGPO 1988-491-003/50324

**SUPPLEMENTAL WORK UNIT INFORMATION FOR THE PROJECT ENTITLED "EVALUATION OF *in vitro* CULTIVATED KERATINOCYTES AS EPITHELIAL AUTOGRAFTS FOR THE CLOSURE OF BURN WOUNDS"**

**Subrecord/Linking Accession Number:** Not applicable.

**Search Control Data:** W6K05C and W6J57D/W6K07C, 20 October 1989

**Product Identification:** For technical reports, refer to the US Army Institute of Surgical Research Annual Research Progress Report for fiscal years 1986-90.

**Unclassified Special Categories:** Volunteers: Adults; RA II

**ANNUAL RESEARCH PROGRESS REPORT**

**PROJECT NUMBER:** 3M162787A874-00, APPLIED RESEARCH AND EXPLORATORY DEVELOPMENT

**PROJECT TITLE:** Evaluation of In Vitro Cultivated Keratinocytes as Epithelial Autografts for the Closure of Burn Wounds

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

**1 October 1989 - 30 September 1990**

**INVESTIGATORS**

Loring W. Rue, III, MD, Major, MC
William G. Cioffi, Jr., MD, Major, MC
Dennis M. Driscoll, Captain, AN

**ABSTRACT**

**PROJECT NUMBER:** 3M162787A874-00, APPLIED RESEARCH AND EXPLORATORY DEVELOPMENT

**PROJECT TITLE:** Evaluation of In Vitro Cultivated Keratinocytes as Epithelial Autografts for the Closure of Burn Wounds

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

**PERIOD COVERED IN THIS REPORT:** 1 Oct 89 through 30 Sep 90

**INVESTIGATORS:** Loring W. Rue, III, MD, Major, MC
William G. Cioffi, Jr., MD, Major, MC
Dennis M. Driscoll, Captain, AN

Burns cause more than 2 million injuries annually in the United States. More than 10,000 deaths each year are a result of serious burn injury. The ultimate outcome of burn patients is determined by wound coverage. Until recently, the only means by which to achieve permanent closure of burn wounds was to use autologous split-thickness skin grafts harvested under general anesthesia from the patient's uninjured body surfaces. This technique presents treatment limitations due to the limited availability of donor sites. Recent ability to culture autologous epidermal cells *in vitro* with subsequent placement on excised wound beds has been recognized as a potential solution to the disparity between available donor sites and burn wound in patients with extensive burn injuries. The objective of this study is to determine the suitability of cultured autologous epithelium for the closure of burn wounds as compared to similar wounds covered with fresh autograft. Investigation will also be undertaken to determine a more efficient means of applying the cultured epithelium to wound beds and to reduce graft loss due to bacterial colonization of the recipient bed.

**EVALUATION OF IN VITRO CULTIVATED KERATINOCYTES AS EPITHELIAL AUTOGRAFTS FOR THE CLOSURE OF BURN WOUNDS**

## INTRODUCTION

The ultimate goal in burn wound care is to achieve timely, permanent closure of the open wound. Currently, the only adequate permanent coverage is autograft, since all other biological membranes are temporary wound covers and wounds covered with artificial skin substitutes ultimately require autografting. Often, the surface area and depth of burn are so extensive the patient's available donor sites are insufficient to provide adequate wound coverage. Consequently, a new source of autograft would be most desirable.

Human keratinocytes can now be cultured *in vitro* to produce confluent epithelial sheets (1). These cells can be grown from relatively small initial samples of the patients' unburned skin, and can be expanded over a period of weeks to months to a size sufficient to cover the entire body surface area. The use of cultured autologous epithelium in burn patients has been reported by several institutions, and is becoming a more well recognized therapeutic modality for the extensively burned patient (2-5). Due to poor results with the use of cultured keratinocytes provided from the original source (refer to Annual Report FY 89), a subsequent addendum to this protocol was introduced to obtain cultured keratinocytes from BioSurface Technology, Inc., of Cambridge, Massachusetts. As well, the number of patients to be entered in the protocol was increased from 10 to 30 patients. This study has been implemented to evaluate the quality of autologous cultured keratinocytes in the closure of burn wounds as compared to the gold standard of autografting. As well, methods to optimize the wound take and preoperative wound bed are being investigated.

## MATERIALS AND METHODS

A total of 30 patients will be enrolled into the study after properly signed and witnessed volunteer agreement affidavits have been obtained. All patients hospitalized for burn injury with burn sizes between 40 to 80% of the total body surface area will be considered for inclusion into the protocol. Within 48 hours of admission to this Institute, two full-thickness skin biopsies from unburned areas will be obtained under local anesthesia after alcohol skin preparations. These will be approximately 2 to 5 square centimeters in size. The biopsies are then placed in a transport medium and transported to the tissue culture facilities at BioSurface Technology via Overnight Express Mail for their standard processing. Approximately three weeks following burn injury and submission of the biopsy, the patient will be returned to the operating room for excision of the proposed site of application of the available cultured keratinocytes. The wound bed will be selected for excision, following which the cultured

epithelial autograft will be applied according to standard operating procedure. Surgical staples will be used to affix the grafts, approximately 25 square centimeters in size, to the wound bed. Both the excised wound bed and the placement of the grafts will be recorded photographically. Protective dressings will then be applied and wet down intermittently with quadruple antibiotic solutions of bacitracin, neomycin, polymyxin and vancomycin as prophylactic treatment against bacterial colonization. The dressings will then be changed on an every other day basis or as clinically indicated beginning on postoperative day three. On postoperative day 10 the vaseline gauze backings of the cultured keratinocytes will be removed, and initial take will be photographically documented. The patients will be left immobile for the initial 14 days postoperatively, and protective dressings applied on the engrafted areas. Antibiotic solutions will be discontinued at about postoperative day 21 and again photographic documentation of percentage of engraftment will be obtained. At the time of discharge a final photographic record of the cultured epithelial autograft take will be made. The fresh split-thickness autograft used in comparison will be treated in a standard fashion per this Institute, will be inspected on approximately postoperative day number five, and intermittently as clinically indicated. A topical antimicrobial agent of 0.5% sulfamylon solution will be applied to the graft sites, and intermittent wet downs continued until interstitial closure is achieved.

## RESULTS

A total of 11 patients have been entered into the protocol, with 14 applications of cultured epithelial autografts. The mean age of the entered patients was 28.2 years with a range of 18 to 48 years, and the mean total body surface area burned was 64.2%, with a range of 40 to 80% of the body surface area burned. Five of the 11 patients underwent primarily fascial wound excision, whereas six of the 11 patients underwent predominantly a dermal excision of their burn wound.

Table 1 summarizes the data with respect to cultured autograft engraftment. Overall, average take of the 14 applications was 56%. Of the six patients who underwent an excision to predominantly a dermal level, the percentage of engraftment was a mean of 62%, with a range of 0 to 85%. With the exception of one patient who had no take of the cultured epithelial autografts, the majority of patient engraftment was between 50 to 85% of applied keratinocytes. Of the five patients who underwent a predominantly fascial bed excision, the mean engraftment was 50%, with a range of 0 to 80%. One patient, initially noted to have approximately 60% engraftment, ultimately had 20% of the cultured cells remaining as a consequence of an Aspergillus wound bed infection. Another patient had a total of four applications of cultured keratinocytes on a predominantly

**TABLE 1**

| APPLICATION | PATIENT | AGE | % BURN | PREDOMINANT WOUND BED | % ULTIMATE ENGRAFTMENT |
|---|---|---|---|---|---|
| 1 | TD | 25 | 58% | Dermis | 70% |
| 2 | GL | 23 | 76% | Fascia | 80% |
| 3 | JR | 26 | 48% | Fascia | 70% |
| 4 | DK | 46 | 61% | Dermis | 50% |
| 5 | RM | 18 | 64% | Dermis | 85% |
| 6 | SK | 23 | 42% | Dermis | 85% |
| 7 | JG | 48 | 51% | Fascia | 80% |
| 8 | LV | 19 | 77% | Dermis | 0% |
| 9 | AG | 29 | 77% | Dermis | 80% |
| 10 | LT | 35 | 80% | Fascia | 20% |
| 11-14 | DP | 18 | 75% | Fascia | 0%,0%,0%,0% |

fascial wound bed, and each application resulted in no engraftment. When the data were analyzed with respect to burn size, patients with wounds grater than 70% TBSA had a mean engraftment of 36%, those with burn sizes of 50-69% TBSA had a mean take of 71%, and those with burns involving less than 50% TBSA had a mean engraftment of 78%.

## DISCUSSION

Though barely one-third of the total number of patients have been entered into this protocol in 1990, the results are somewhat encouraging. With the exception of two patients who ultimately had no engraftment of the cultured epithelial cells, and the patient who ultimately had only a 20% take due to fungal infection, the vast majority of patients for whom this treatment modality was employed had at least 50% engraftment of the cultured keratinocytes. Unfortunately, the patients with larger burn sizes, those in whom the surgery would have its greatest impact, tended to have lower percentages of engraftment. It remains to be seen, however, as to the durability of these epithelial autografts.

Consideration is currently being given to amending the protocol to evaluate the maturation and differentiation of the applied epidermal cells with respect to the formation of basement membranes and anchoring fibrils by employing histologic biopsies of the sites of engraftment. Also, investigation as to the cause of delayed graft loss would be pursued, again using histologic biopsies and evaluating for the possibility of auto-antibody formation to the applied cultured epithelial autografts.

## PRESENTATIONS/PUBLICATIONS

None.

## REFERENCES


1. Pittelkow MR and Scott RE: New Techniques for the *in vitro* culture of human skin keratinocytes and perspectives on their use for grafting of patients with extensive burns. **Mayo Clinic Proceedings** 61:771-777, 1986.

2. Cuono C, Langdon R, and McGuire J: Use of cultured Epidermal autografts as skin replacement after burn injury. **Lancet** 1:1123-1124, 1986.

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5. Munster AM, Wiener SH and Spence RJ: Cultured epidermis for the coverage of massive burn wounds. **Ann Surg** 211:676-680, 1990.

| RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY | | | | 1. AGENCY ACCESSION | 2. DATE OF SUMMARY | REPORT CONTROL SYMBOL |
|---|---|---|---|---|---|---|
| | | | | DA346158 | 01 Oct 90 | DD-DR&E(AR) 636 |
| 3. DATE PREV SUM'RY | 4. KIND OF SUMMARY | 5. SUMMARY SCTY | 6. WORK SECURITY | 7. REGRADING | 8. DISB'N INSTR'N | 9. LEVEL OF SUM A. WORK UNIT |
| 22 Mar 90 | D. Chg | U | U | | CX | |

| 10. NO./CODES | PROGRAM ELEMENT | PROJECT NUMBER | TASK AREA NUMBER | WORK UNIT NUMBER |
|---|---|---|---|---|
| a. PRIMARY | 62787A | 3M162787A874 | EA | 171 |
| b. CONTRIBUTING | | | | |
| c. CONTRIBUTING | | | | |

11. TITLE *(Precede with Security Classification Code)* (U) A Clinical Study of the Efficacy of Eurothane in the Treatment of Skin Graft Donor Sites

12. SUBJECT AREAS 0605 Medicine and Medical Research
0612 Medical Facilities, Equipment, and Supplies

| 13. START DATE | 14. ESTIMATED COMPLETION DATE | 15. FUNDING ORGANIZATION | 16. PERFORMANCE METHOD |
|---|---|---|---|
| 9003 | 9309 | DA | C. In-House |

17. CONTRACT/GRANT MILITARY RELEVANCY CERTIFIED
a. DATE EFFECTIVE APPROVED BY
b. CONTRACT/GRANT NUMBER
c. TYPE
d. AMOUNT
e. KIND OF AWARD
f. CUM/TOTAL

18. RESOURCES ESTIMATE

| FISCAL YEARS | a. PROFESSIONAL WORKYEARS | b. FUNDS *(In thousands)* |
|---|---|---|
| 90 | 0.1 | 46 |
| 91 | 0.1 | 48 |

| 19. RESPONSIBLE DOD ORGANIZATION | 20. PERFORMING ORGANIZATION |
|---|---|
| a. NAME: US Army Institute of Surgical Research | a. NAME: US Army Institute of Surgical Research |
| b. ADDRESS *(include zip code)*: Fort Sam Houston, San Antonio, Texas 78234-5012 | b. ADDRESS: Fort Sam Houston, San Antonio, Texas 78234-5012 |
| c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A | c. NAME OF PRINCIPAL INVESTIGATOR: WAGUESPACK, R L |
| d. TELEPHONE NUMBER *(include area code)*: 512-221-2720 | d. TELEPHONE NUMBER *(include area code)*: 512-221-4440 |
| 21. GENERAL USE: FINA<br>MILITARY/CIVILIAN APPLICATION: M | f. NAME OF ASSOCIATE INVESTIGATOR *(if available)*: RUE, L W<br>g. NAME OF ASSOCIATE INVESTIGATOR *(if available)*: CIOFFI, W G |

22. KEYWORDS *(Precede EACH with Security Classification Code)* (U) Burns (Injuries); (U) Dressings; (U) Skin Grafts; (U) Excision

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS *(Precede text of each with Security Classification Code)*

23/24. (U) The objective of this work is to evaluate the efficacy of Eurothane in the treatment of skin graft donor sites in comparison with fine-mesh gauze. Forty-two burned patients will undergo split-thickness skin graft harvesting from an anterior thigh. One-half of this donor site will be treated with Eurothane and the other half will be covered with fine-mesh gauze. A record will be maintained comparing the time of complete epithelization and the time at which reharvesting of the donor site is considered feasible. Data analysis will be performed using the paired t test.

25. (U) 8710 - 8809. Not applicable.

(U) 8810 - 8909. Not applicable.

(U) 8910 - 9009. This project was approved by the USAISR Research Council and US Army Institute of Surgical Research Human Use Committee during the second quarter of fiscal year 1990. Six patients were enrolled in the study during this reporting period. Upon enrollment of 42 patients, data will be analyzed.

DD FORM 83 MAR 1498 EDITION OF MAR 68 IS OBSOLETE

**SUPPLEMENTAL WORK UNIT INFORMATION FOR THE PROJECT ENTITLED "A CLINICAL STUDY OF THE EFFICACY OF EUROTHANE IN THE TREATMENT OF SKIN GRAFT DONOR SITES"**

**Subrecord/Linking Accession Number:** Not applicable.

**Search Control Data:** W6O10C/W6O07E, 6 March 1990

**Product Identification:** For technical reports, refer to the US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Year 1990.

**Unclassified Special Categories:** Volunteers: Adults; RA II

**ANNUAL RESEARCH PROGRESS REPORT**

**PROJECT NUMBER:** 3M162787A874-00, APPLIED RESEARCH AND EXPLORATORY DEVELOPMENT

**PROJECT TITLE:** A Clinical Study of the Efficacy of a Polyetherurethane Membrane Dressing (Eurothane) in the Treatment of Skin Graft Donor Sites

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

**1 October 1989 - 30 September 1990**

**INVESTIGATORS**

Robert L. Waguespack, MD, Captain, MC
Loring W. Rue, III, MD, Major, MC

## ABSTRACT

**PROJECT NUMBER:** 3M162787A874-00, APPLIED RESEARCH AND EXPLORATORY DEVELOPMENT

**PROJECT TITLE:** A Clinical Study of the Efficacy of a Polyetherurethane Membrane Dressing (Eurothane) in the Treatment of Skin Graft Donor Sites

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

**PERIOD COVERED IN THIS REPORT:** 1 Oct 89 through 30 Sep 90

**INVESTIGATORS:** Robert L. Waguespack, MD, Captain, MC
Loring W. Rue, III, MD, Major, MC

The availability of skin graft donor sites is a limitation in definitive burn wound closure. Methods to hasten donor site healing and consequently burn wound closure will have a positive effect on the care of thermally injured patients. The goal of this study is to evaluate the efficacy of Eurothane in the treatment of skin graft donor sites in comparison with fine mesh gauze, the standard method of treatment at this Institute.

# A CLINICAL STUDY OF THE EFFICACY OF A POLYETHERURETHANE MEMBRANE DRESSING (EUROTHANE) IN THE TREATMENT OF SKIN GRAFT DONOR SITES

## INTRODUCTION

Eurothane is a polyetherurethane dressing assembled with membrane structural properties. The dressing is elastic, and 0.5 millimeters thick. It has open pores at the contact surface of the wound, and an ultraporous outer surface which promotes gas and water vapor transmission. The dressing adhesive is impregnated such that 65% of the dressing surface area is available for exudate absorption.

Eurothane's self-adhesive membrane properties provide for thermal insulation and create an environment for optimal wound healing by controlling the exudate uptake and moisture vapor transmission. Maximum exudate absorption is four times the original weight of the dressing. Water removal from the wound is achieved by absorption into the open pores of the dressing, followed by moisture vapor transmission at the surface. The outer surface composition of the dressing appears to prevent ingress of bacteria.

The current method of treating skin graft donor sites at this Institute is the application of fine mesh gauze and postoperative heat lamps to facilitate drying. This study is designed to compare the synthetic material polyetherurethane to fine mesh gauze with respect to rate of wound healing and wound complications.

## METHODS AND MATERIALS

This study will utilize 42 consecutive patients with burn injuries of less than 70% of the total body surface area undergoing an initial split-thickness skin graft harvest from an anterior thigh. One surgeon will harvest the skin grafts utilizing the same dermatome at the same thickness - 0.01 inches. One-half of the donor site will be treated with the Eurothane after obtaining hemostasis with warm saline laparotomy pads. The dressing will extend at least one centimeter beyond the wound margin for optimal adherence. The other half of the donor site will be covered with fine mesh gauze and hemostasis then achieved with warm saline laparotomy pads. The fine mesh gauze will be applied to the wound edges without overlap.

The Eurothane treated donor site areas will be inspected after removal of the dressing on the seventh postoperative day, or at the time of spontaneous separation of the dressing. If the wound is completely re-epithelialized, it will be exposed to air. If spontaneous separation occurs prior to the seventh postoperative day, and provided no contraindications exist, additional Eurothane will be applied.

Photographs will be taken immediately after graft harvest, after removal of the Eurothane, and at the time of separation of the fine mesh gauze as a comparison. Donor sites will be examined daily for signs of infection or adverse reaction to the dressing. Donor sites from which the dressing prematurely separates will be examined for signs of infection or tissue reaction. Adverse reactions and premature separation of the dressing will be recorded. A record will be maintained comparing the two donor sites, with respect to time of re-epithelialization and the time at which reharvesting of the donor site is felt to be possible.

Patients eligible for the study will be at least 18 years of age, and any female patients must have been surgically sterilized, be postmenopausal (over 45 years of age and the lack of menstrual periods for at least one year), or have a negative pregnancy test prior to initiation into the study. Patient burn size will be confined to those less than 70% of the total body surface area.

## RESULTS

A total of nine patients were entered into this study during fiscal year 1990. Table 1 indicates the postoperative date of Eurothane removal or fine mesh gauze separation, and the time at which each donor site was felt to be reharvestable. Only one patient had problems with premature separation of the Eurothane with signs of erythema. This wound site required the application of topical Silvadene cream to achieve wound healing.

Several patients have had serosanguinous fluid collections beneath the synthetic dressing. No evidence of associated infection was noted.

**TABLE 1**

| PATIENT# | EUROTHANE REMOVED | REHARVESTABLE | POD | FINE MESH GAUZE SEPARATED | REHARVESTABLE |
|---|---|---|---|---|---|
| 1 | 7 | 10 | | 12 | 12 |
| 2 | 7 | 18 | | 16 | 16 |
| 3 | 8 | 14 | | 11 | 11 |
| 4 | 9 | 10 | | 11 | 11 |
| 5 | 9 | 10 | | 11 | 11 |
| 6 | 9 | 9 | | 11 | 11 |
| 7 | 9 | 16 | | 14 | 16 |
| 8 | 4 | 8 | | 8 | 8 |
| 9 | 9 | 11 | | 8 | 10 |
| Mean | 7.8 | 11.7 | | 11.3 | 11.7 |

## DISCUSSION

Because of the very early phase of this study, it is difficult to come to any conclusions with respect to the efficacy of this dressing. Following entry of the total number of patients, data analysis will be performed using a paired T-test. Each patient, therefore, will serve as his or her own control.

## PRESENTATIONS/PUBLICATIONS

None.

## REFERENCES

None.

| RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY | | | | 1. AGENCY ACCESSION | 2. DATE OF SUMMARY | REPORT CONTROL SYMBOL |
|---|---|---|---|---|---|---|
| | | | | DA318017 | 01 Oct 90 | DD-DR&E(AR) 636 |
| 3. DATE PREV SUM'RY | 4. KIND OF SUMMARY | 5. SUMMARY SCTY | 6. WORK SECURITY | 7. REGRADING | 8. DISB'N INSTR'N | 9. LEVEL OF SUM A. WORK UNIT |
| 01 Oct 89 | D. Chg | U | U | | CX | |

| 10. NO./CODES: | PROGRAM ELEMENT | PROJECT NUMBER | TASK AREA NUMBER | WORK UNIT NUMBER |
|---|---|---|---|---|
| a. PRIMARY | 62787A | 3M162787A874 | FA | 142 |
| b. CONTRIBUTING | | | | |
| c. CONTRIBUTING | | | | |

11. TITLE *(Precede with Security Classification Code)* (U) Investigation of the Importance of Alterations in Tumor Necrosis Factor (TNF) in Burn Patients

12. SUBJECT AREAS
0605 Medicine and Medical Research

| 13. START DATE | 14. ESTIMATED COMPLETION DATE | 15. FUNDING ORGANIZATION | 16 PERFORMANCE METHOD |
|---|---|---|---|
| 8902 | 9109 | DA | C. In-House |

17. CONTRACT/GRANT MILITARY RELEVANCY CERTIFIED
a. DATE EFFECTIVE APPROVED BY
b. CONTRACT/GRANT NUMBER
c. TYPE d. AMOUNT
e. KIND OF AWARD f. CUM/TOTAL

18. RESOURCES ESTIMATE

| FISCAL YEARS | a. PROFESSIONAL WORK YEARS | b. FUNDS *(In thousands)* |
|---|---|---|
| 90 | 0.1 | 50 |
| 91 | 0.1 | 52 |

| 19. RESPONSIBLE DOD ORGANIZATION | 20. PERFORMING ORGANIZATION |
|---|---|
| a. NAME<br>US Army Institute of Surgical Research | a. NAME<br>US Army Institute of Surgical Research |
| b. ADDRESS *(include zip code)*<br>Fort Sam Houston<br>San Antonio, Texas 78234-5012 | b. ADDRESS<br>Fort Sam Houston<br>San Antonio, Texas 78234-5012 |
| c. NAME OF RESPONSIBLE INDIVIDUAL<br>PRUITT, B A | c. NAME OF PRINCIPAL INVESTIGATOR<br>MC MANUS, A T |
| d. TELEPHONE NUMBER *(include area code)*<br>512-221-2720 | d. TELEPHONE NUMBER *(include area code)*<br>512-221-3411 |
| 21. GENERAL USE<br>FINA<br>MILITARY/CIVILIAN APPLICATION: M | f. NAME OF ASSOCIATE INVESTIGATOR *(if available)*<br>BURLESON, D G<br>g. NAME OF ASSOCIATE INVESTIGATOR *(if available)*<br>MC MANUS, W F |

22. KEYWORDS *(Precede EACH with Security Classification Code)* (U) Burns (Injuries); (U) Immunosuppression; (U) Blood

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS *(Precede text of each with Security Classification Code)*

23/24. (U) The objective of this work is to determine the significance of tumor necrosis factor levels in burn patients and whether or not elevations in these no reliable blood tests for identifying the early stages of burn wound infection. Such tests would be of prognostic usefulness and might serve as a guide to therapy. A 5-ml sample of whole blood will be drawn on a twice weekly basis from 100 consecutive burn patients with burns exceeding 20% of the total body surface area. Patients will be monitored on a prospective basis for the development of infections and the clinical course will be correlated with the results of tumor necrosis factor assays.

25. (U) 8710 - 9103. Not applicable.

(U) 8810 - 8909. This project was approved by the USAISR Research Council and US Army Institute of Surgical Research Human Use Committee durings the second quarter of fiscal year 1989. Six patients with major burn injuries were enrolled in the study during this reporting period.

(U) 8910 - 9009. Twenty-three patients were enrolled in the study during this reporting period. Serial plasma samples were drawn and are currently being stored at -70°C for later analysis by ELISA. Upon completion of enrollment, the data will be analyzed.

DD FORM 1498 83 MAR EDITION OF MAR 68 IS OBSOLETE

**SUPPLEMENTAL WORK UNIT INFORMATION FOR THE PROJECT ENTITLED "INVESTIGATION OF THE IMPORTANCE OF ALTERATIONS IN TUMOR NECROSIS FACTOR (TNF) IN BURN PATIENTS"**

**Subrecord/Linking Accession Number:** Not applicable.

**Search Control Data:** W6K33C/W6K34C, 20 October 1989

**Product Identification:** For technical reports, refer to the US Army Institute of Surgical Research Annual Research Progress Report for fiscal years 1989-90.

**Unclassified Special Categories:** Volunteers: Adults; RA II

**ANNUAL RESEARCH PROGRESS REPORT**

**PROJECT NUMBER:** 3M162787A874-00, APPLIED RESEARCH AND EXPLORATORY DEVELOPMENT

**PROJECT TITLE:** Investigation of the Importance of Alterations in Tumor Necrosis Factor (TNF) in Burn Patients

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

**1 October 1989 - 30 September 1990**

**INVESTIGATORS**

Albert T. McManus, PhD
Arthur D. Mason, Jr., MD
David G. Burleson, PhD, Lieutenant Colonel, MS
William F. McManus, MD, Colonel, MC
Rey F. Guzman, BS, Sergeant
Basil A. Pruitt, Jr., MD, Colonel, MC

## ABSTRACT

**PROJECT NUMBER:** 3M162787A874-00, APPLIED RESEARCH AND EXPLORATORY DEVELOPMENT

**PROJECT TITLE:** Investigatior of the Importance of Alterations in Tumor Necrosis Factor (TNF) in Burn Patients

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

**PERIOD COVERED IN THIS REPORT:** 1 Oct 89 through 30 Sep 90

**INVESTIGATORS:** Albert T. McManus, PhD
Arthur D. Mason, Jr., MD
David G. Burleson, PhD, Lieutenant Colonel, MS
William F. McManus, MD, Colonel, MC
Rey F. Guzman, BS, Sergeant
Basil A. Pruitt, Jr., MD, Colonel, MC

Burn patients have a significant infection diathesis due in part to the loss of the normal epithelial skin barrier and to postburn immunosuppression. Burn patient wounds are therefore frequently contaminated with various microorganisms. Control of such contamination is attempted through the use of topical antimicrobial agents. Despite the attempts with topical wound care, invasive burn wound infection nonetheless occurs in a significant percentage of patients with major thermal injuries. Currently, the clinical indications of a developing infection are derived from observation of the patient's wounds and a review of the patient's vital signs. When such evidence indicates that a burn wound invasive infection may be developing, appropriate burn wound biopsies are obtained and sent for histopathological sectioning to check for the presence of invasive infection. Blood cultures are also obtained. There are currently no reliable blood tests for indicating the early development of burn wound infections. The development of positive blood cultures indicates that the infection has progressed to a very significant degree and is life-threatening to the patient. It would, therefore, be desirable to have a blood test available which would indicate early burn wound invasion before the patient has become critically and possibly irreversibly septic.

TNF levels are now measurable through the use of an ELISA kit. These assays determine the level of the TNF protein in the serum of patients. Elevations in the serum level of TNF can be expected in patients who are experiencing significant systemic endotoxemia, as would result from a developing infection. The exact levels of TNF expected in burn patients and burned septic patients has not as yet been determined. Therefore, the objective of this study is to

measure TNF levels in all burn patients and to correlate these levels with their clinical course. An attempt will be made to determine if changes in TNF levels are indicative of impending infection and whether they can be used to monitor successful treatment of an infective process.

## INVESTIGATION OF THE IMPORTANCE OF ALTERATIONS IN TUMOR NECROSIS FACTOR (TNF) IN BURN PATIENTS

Infection remains a primary cause of morbidity and mortality in severely burned patients (1). This infection diathesis is due in part to the immunosuppression seen following major thermal injuries. The exact etiology of the postburn immunosuppression is as yet undetermined. Investigators are therefore studying multiple components of the immune system in order to delineate the immunosuppression and determine which patients are at greater risk of developing infections.

One component of the immune system which has not been greatly investigated in burn patients is the production of TNF. TNF is produced by monocytes/macrophages in response to exposure to endotoxin (2). TNF was initially demonstrated to cause significant tumor regression in animal models and to enhance resistance to bacterial infections (3). TNF can also alter thermoregulation and produce multiphasic febrile responses (4). TNF results in an elevation of plasma hematocrit levels due to loss of intravascular fluid into the third space (2). TNF affects metabolism in a manner similar to sepsis as reflected by simultaneous decrease in plasma glucose levels and increase in plasma lactate levels and stimulation of hepatic acute-phase protein synthesis (5,6). Chronic elevation of TNF results in cachexia and muscle wasting (7). TNF also exerts predominantly stimulatory effects on WBCs. At extremely high concentrations, it can induce fatal hemodynamic instability (6).

Despite these findings in animal models, very little investigation has been performed in human burn patients. Therefore, this study will attempt to delineate the significance of TNF levels in burn patients, with special reference toward determining whether or not elevations in TNF levels predict impending sepsis or recovery from infections.

### MATERIALS AND METHODS

**Number of Patients.** One hundred burn patients will be enrolled in this study based upon eligibility criteria and informed consent.

**Criteria for Admission.** Patients admitted to the US Army Institute of Surgical Research are offered the opportunity to participate in this study.

**Patient Inclusion.** Patients meeting the following criteria are considered eligible for entry into the study:

1. Male or female patients ≥ 18 yr old.

2. Patients admitted to the Institute within the first 7 days postburn.

3. Patients with burns > 20% of the total body surface area (the presence of an inhalation injury not being exclusionary).

**Patient Exclusion.** Patients with the following characteristics are excluded from enrollment in the study:

1. Patients < 18 yr old.

2. Patients admitted to the Institute > 7 days postburn.

3. Patients with burns < 20% of the total body surface area.

4. Patients with toxic epidermal necrolysis syndrome.

**Study Design.** A 5-ml sample of whole blood is obtained on a twice weekly basis. These samples are drawn in a tube containing EDTA as an anticoagulant (blue top tube) on Mondays and Thursdays at the time of routine blood drawings until the patient has < 5% of his burn uncovered with autograft. The blood is immediately taken to the Biochemistry Branch for centrifugation. Serum is aspirated and stored at -70°C until the ELISA assay is performed on the sample. The ELISA test will then be performed using a standard ELISA plate containing antibodies of TNF coded on the bottom of the wells of the plate (T-Cell Sciences, Inc., 840 Memorial Drive, Cambridge, MS 02139). The assays will be performed by the Biochemistry Branch utilizing the ELISA machine currently in use. Patients are monitored prospectively on a daily basis for the development of infections as defined by Institute criteria and the clinical course will be correlated with the results of the TNF assays. Test results will be coded for identification purposes only and the key to the code will be available only to the principal investigator.

**Determination of the Number of Subjects Required.** It is estimated that if the average burn size of these patients is ≥ 30% of the total body surface area, they will be hospitalized ≥ 5 weeks, based on patients requiring ≥ 1 days hospitalization per percent total body surface area burned. Therefore, each patient would have ≥ 10 blood samples drawn for TNF assay. This would yield a total number of ≥ 1,000 samples for the study. This number of assays should permit correlation of TNF levels with infection.

**Data Collection.** Infection data will be collected from the Institute's monthly infection report. Additional data will be collected as indicated in Figures 1 and 2.

1. Patient Name: _____ 2. Chart Number: _____
3. Date of Birth: _____ 4. Date of Burn: _____
5. Total Burn Size: _____ 6. Total 3° Burn Size: _____
7. Inhalation Injury: _____ 8. Associated Injuries: _____
9. Preexisting Medical Conditions:
10. Preburn Medications:
11. Burn Wound Infections
    a. Dates of Documentation:
    b. Methods of Documentation:
    c. Organisms Involved:
    d. Treatments Instituted:
12. Pneumonias
    a. Dates of Documentation:
    b. Methods of Documentation:
    c. Organisms Involved:
    d. Treatments Instituted:
13. Urinary Tract Infections
    a. Dates of Documentation:
    b. Methods of Documentation:
    c. Organisms Involved:
    d. Treatments Instituted:
14. Other Infections
    a. Dates of Documentation:
    b. Methods of Documentation:
    c. Organisms Involved:
    d. Treatments Instituted:
15. Bacteremias
    a. Dates of Documentation:
    b. Methods of Documentation:
    c. Organisms Involved:
    d. Treatments Instituted:
16. Operations
    a. Dates:
    b. Area Not Covered by Autograft After Each Operation:
17. Other Significant Clinical Events:
18. Patient Outcome: _____ 19. Date of Discharge/Death: _____
20. Autopsy Findings (if applicable):

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**FIGURE 1.** Data collection scheme.

| Postburn Day | TNF Level | WBC Count | Existing Infection | Maximum Temperature |
|---|---|---|---|---|

**FIGURE 2.** Data collection sheet.

**Data Analysis Plan.** Data will be analyzed comparing TNF levels in infected versus noninfected patients. This will include comparisons between noninfected and bacteremic patients plus non-infected patients and patients with infections of the burn wound, lung (pneumonia), or urinary tract. ANOVA will be utilized for these comparisons. Comparisons will also be made of serum TNF levels in patients during the week prior to the clinical diagnosis of infection to determine if elevations of TNF levels are predictive of impending sepsis. Comparison of TNF levels to WBC counts will be made using linear regression to determine for any correlations. Levels of TNF will also be related to extent of burn, and time postburn as well as to extent of open wound.

## RESULTS

Twenty-three patients were enrolled in the study during this reporting period. Serial plasma samples were drawn and are currently being stored at -70°C for later analysis by ELISA.

## DISCUSSION

Upon completion of the enrollment of 100 patients, the data will be analyzed and presented for publication.

## PRESENTATIONS/PUBLICATIONS

None.

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| RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY | | | | 1. AGENCY ACCESSION DA346159 | 2. DATE OF SUMMARY 01 Oct 90 | REPORT CONTROL SYMBOL DD-DR&E(AR) 636 |
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| 3. DATE PREV SUM'RY 12 Mar 90 | 4. KIND OF SUMMARY D. Chg | 5. SUMMARY SCTY U | 6. WORK SECURITY U | 7. REGRADING | 8. DISB'N INSTR'N CX | 9. LEVEL OF SUM A. WORK UNIT |

| 10. NO./CODES | PROGRAM ELEMENT | PROJECT NUMBER | TASK AREA NUMBER | WORK UNIT NUMBER |
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| a. PRIMARY | 62787A | 3M162787A874 | DA | 173 |
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11. TITLE *(Precede with Security Classification Code)* (U) A Comprehensive Analysis of the Perceived Needs of Families of Critically Injured Burned Patients

12. SUBJECT AREAS
0605 Medicine and Medical Research

| 13. START DATE | 14. ESTIMATED COMPLETION DATE | 15. FUNDING ORGANIZATION | 16. PERFORMANCE METHOD |
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17. CONTRACT/GRANT MILITARY RELEVANCY CERTIFIED APPROVED BY [illegible]

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18. RESOURCES ESTIMATE

| a. FISCAL YEARS | a. PROFESSIONAL WORKYEARS | b. FUNDS (In thousands) |
|---|---|---|
| 90 | 0.1 | 46 |
| 91 | 0.1 | 48 |

| 19. RESPONSIBLE DOD ORGANIZATION | 20. PERFORMING ORGANIZATION |
|---|---|
| a. NAME: US Army Institute of Surgical Research | a. NAME: US Army Institute of Surgical Research |
| b. ADDRESS (Include zip code): Fort Sam Houston, San Antonio, Texas 78234-5012 | b. ADDRESS: Fort Sam Houston, San Antonio, Texas 78234-5012 |
| c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A | c. NAME OF PRINCIPAL INVESTIGATOR: MOLTER, N C |
| d. TELEPHONE NUMBER (Include area code): 512-221-2720 | d. TELEPHONE NUMBER (Include area code): 512-221-2024 |
| 21. GENERAL USE: FINA<br>MILITARY/CIVILIAN APPLICATION: M | f. NAME OF ASSOCIATE INVESTIGATOR (If available): SUMMERS, T M<br>g. NAME OF ASSOCIATE INVESTIGATOR (If available): LESKE, J |

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23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS *(Precede text of each with Security Classification Code)*

23/24. (U) The objective of this work is to describe the psychometric properties of the Critical Care Family Needs Inventory when used with a burn unit population. This descriptive/comparative study will establish a comprehensive data base related to the perceived needs of families of critically burned patients.

25. (U) 8710 - 8809. Not applicable.

(U) 8810 - 8909. Not applicable.

(U) 8910 - 9009. This project was approved by the USAISR Research Council and US Army Institute of Surgical Research Human Use Committee during the second quarter of fiscal year 1990. Data collection began on 19 March 1990. Twelve patients have been enrolled in the study to date, with 32 family responses and 22 staff responses obtained.

DD FORM 83 MAR 1498 EDITION OF MAR 88 IS OBSOLETE. * USGPO: 1988-491-003/50329

**SUPPLEMENTAL WORK UNIT INFORMATION FOR THE PROJECT ENTITLED "A COMPREHENSIVE ANALYSIS OF THE PERCEIVED NEEDS OF FAMILIES OF CRITICALLY INJURED BURNED PATIENTS"**

**Subrecord/Linking Accession Number:** Not applicable.

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**ANNUAL RESEARCH PROGRESS REPORT**

**PROJECT NUMBER:** 3M162787A874-00, APPLIED RESEARCH AND EXPLORATORY DEVELOPMENT

**PROJECT TITLE:** A Comprehensive Analysis of the Perceived Needs of Families of Critically Injured Burned Patients

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

**1 October 1989 - 30 September 1990**

**INVESTIGATORS**

Nancy C. Molter, RN, Colonel, AN
Thomas M. Summers, RN, Lieutenant Colonel, AN
Jane Leske, RN, MSN, PhD

## ABSTRACT

**PROJECT NUMBER**: 3M162787A874-00, APPLIED RESEARCH AND EXPLORATORY DEVELOPMENT

**PROJECT TITLE**: A Comprehensive Analysis of the Perceived Needs of Families of Critically Injured Burned Patients

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

**PERIOD COVERED IN THIS REPORT**: 1 Oct 89 through 30 Sep 90

**INVESTIGATORS**: Nancy C. Molter, RN, Colonel, AN
Thomas M. Summers, RN, Lieutenant Colonel, AN
Jane Leske, RN, MSN, PhD

Hospitalization for a critical illness is frequently viewed as a crisis situation for both the patient and the family. Even though the importance of family support and assistance during critical illness has been recognized from as early as 1945, hospital care often has remained patient-centered only. When anxiety is reduced and healthy coping mechanisms are promoted to facilitate dealing with the crisis, the family is better able to provide the crucial support necessary for the patient to cope with severe illness or injury.

Several studies have described the needs of families of critically ill patients as perceived by the family members. No studies have described such needs of families of critically injured burned patients. The objectives of this study are: 1) to describe the needs of family members of critically injured burn patients as they perceive them across a span of time; 2) to compare perceptions of needs by individual family members with those of nurses who interact with them in the burn unit; and 3) to describe the psychometric properties of the Critical Care Family Needs Inventory (CCFNI) when used with the burn unit population.

An initial six months data collection pilot has been completed resulting in minor revisions of the protocol hypotheses and refinement of data collection methodology. Requests for participation for multisite data collection were sent to 154 burn units listed in the ABA directory.

Results as of 30 September, 1990:

Data collection for the pilot began on 19 March 1990. Twelve patients have been enrolled in the study at ISR with 32 family responses and 22 staff responses. Based on the data collected, the decision was made not to request information concerning who met the family needs. In addition, clarification as to the time frame of

when to administer the CCFNI to the staff was delineated in the protocol. All data collection tools were reviewed by an expert for ease of entry into a computer data base. Requests for participation in the study were sent to 154 burn units listed in the ABA directory.

# A COMPREHENSIVE ANALYSIS OF THE PERCEIVED NEEDS OF FAMILIES OF CRITICALLY INJURED BURNED PATIENTS

## INTRODUCTION

**Purpose.** The major objectives of the study are: 1) to describe the needs of family members of critically injured burned patients as they perceive them across a span of time; 2) to compare perceptions of needs by individual family members with those of nurses who interact with them in the burn unit; and 3) to describe the psychometric properties of the Critical Care Family Needs Inventory (CCFNI) when used with the burn unit population.

**Medical Application.** Hospitalization for a critical illness is frequently viewed as a crisis situation for both the patient and the family. Even though the importance of family support and assistance during critical illness has been recognized from as early as 1945 (1), hospital care often has remained patient-centered only. The move away from patient-centered care to a family-centered care focus became more evident beginning in 1970 as the benefits were evaluated (2-9). When anxiety is reduced and healthy coping mechanisms are promoted to facilitate dealing with the crisis, the family is better able to provide the crucial support necessary for the patient to cope with severe illness or injury.

As nurses became more involved with the families of their patients, the families frequently became a source of stress for the staff (10-13). There have been four main factors identified as a source of this staff stress: 1) the limited amount of time available for the nurse to deal with families; 2) the amount of stress in nurses from other sources; 3) the nurses' knowledge about psychological aspects of dealing with families in crisis; and 4) the role security of the nurse (13). As a result, staff responses to families during their brief periods of visiting often become routinized. Interventions such as orientation to the unit, providing information concerning treatment modalities, and visiting policies are often generalized based on staff perceptions only. Frequently, energy may be spent by the nurses in trying to cope with nonexistent family needs or needs already met by others.

Becoming more aware of the importance of certain needs to families will assist nurses in developing strategies to assist families with stress. An essential component in this process is to determine the self-perceived needs of family members of burn patients and how they correlate with health care providers' perceptions. Any discrepancy can serve to explain why previous strategies may not have assisted families and, therefore, led to further frustration for both families and nurses. The knowledge gained may also serve to focus the staff's time and energy on the family needs that are most appropriate for them to manage.

Health care providers' role security related to family interventions may then increase as they learn specific strategies to deal with a narrowed scope of needs.

When the needs of families of burn injured patients are described, specific interventions need to be evaluated for effectiveness. Once the psychometric properties of the CCFNI are determined in the burn patient population, selected dimensions of the tool may be used more effectively to study relationships between specific interventions and observed outcomes.

**Status**. Several exploratory descriptive studies have been done using the Critical Care Family Needs Inventory (CCFNI), or a variation of it, to identify family needs of critically ill patients. These studies have been conducted in different types of critical care units, with different types of patient diagnoses, and in different geographic locations.

The original study (14) in which the CCFNI was developed was conducted by Molter in a variety of intensive care units (MICU, SICU, CCU, Thoracic ICU), and was based on crisis and human needs theories (15,16). The importance of the family needs were measured within 48 hours after the patient had been transferred from the intensive care unit. Families were asked if their needs were met, and if so by whom. Of interest was the finding that many needs were met by support personnel other then nurses and physicians. Also noteworthy was that the family did not expect the role of health care providers to be family centered.

Leske (17) replicated part of Molter's study (14) using a heterogeneous sample from different institutions. Using a self-report questionnaire format versus the interview format used by Molter, families were surveyed within the first 72 hours of the patient's admission to the intensive care unit. The top nine needs identified in Leske's study were among the top ten needs reported by Molter.

Rodgers (18) used the CCFNI with families of cardiac surgical patients who had uneventful surgery. This was the first study in which the tool was used as a self-report questionnaire. The important needs identified were similar to those reported in other studies.

In a study with families of SICU and MICU patients, Bouman (19) determined that family needs differed across a time span. At 36 hours post admission the family needs were primarily cognitive in nature versus an emphasis on emotional needs at 96 hours post admission. The fact that needs appear to change over a time span becomes important in determining the timing of interventions. Bouman used the results to develop a comprehensive plan of care.

While investigating the needs of families using a modified version of the CCFNI, Daley (20) also evaluated categories of persons (health care providers, clergy, family members, friends, etc.) perceived as most likely to meet the needs. Like Molter, (14) she found a variety of resources used by family members to meet their needs. The Daley study was significant in that it was the first time the needs were placed into broad categories. Unfortunately, the categories were arbitrarily developed rather than psychometrically derived.

In a study that compared needs of brain injured patients versus those without brain injury, Mathis (21), using the CCFNI, found differences and similarities in family needs among varying patient populations. Although the small sample size raises questions about statistical conclusion validity, determining if differences exist among varying patient populations is of interest to all specialties, including burn nursing.

Based on the premise that as stress decreased families could better offer their support to patients, Spatt, et al. (22) surveyed families to determine which needs were perceived as being unmet. The family members completed the CCFNI after the patient was in the intensive care unit at least 48 hours. The most frequently cited unmet needs were daily contact with the physician and consistency among the nursing staff; ability to distinguish various types of hospital staff members; and unclear explanations concerning prognosis and patient condition. A patient information pamphlet was developed and tested to meet these needs.

Norris and Grove (23) used a variation of the CCFNI to compare needs reported by family members to those reported by critical care nurses. Findings indicated that there was a statistical difference between the perceptions of needs of the two groups on four items. This study served to sensitize nurses to the importance of assessing the family's perception of needs. In a similar study, (24) the level of need satisfaction as perceived by 52 family members was compared to how accurately 92 nurses identified those areas of relatively high and low family satisfaction. The nurses were accurate (Spearman's at .50) at identifying the level at which family members perceived needs as being met. A limitation of the study was that there was no attempt to match the family's perceptions with those of a primary nurse for the patient/family unit. In addition, the importance of the needs to the family member was not evaluated.

A review of the literature over the past 15 years related to family needs of burn patients, indicates that most studies/case reports are concerned with informational needs, usually at the time of discharge (25-28). A retrospective survey of 68 family members (29) did evaluate family needs related to receiving information prior to the initial visit. The most important topics identified were the patient's condition, chance of recovery, and a description

of the injury. This study also identified aspects of the first visit that were disturbing to the family (i.e., the appearance of the patient and the environment in the unit). Families also identified that the physician (91.1%) and the nurses (42.6%) were the primary personnel who should meet these needs. No studies were reported that used the CCFNI to identify a more comprehensive data base of family needs in the burn unit population.

There is a comprehensive data base related to family needs in the critical care unit environment, however, very little research has been conducted describing the needs of burned patients' families. Due to the often devastating sequelae of burn injuries, plus the extended periods of hospitalization, it is postulated by the investigators that the family needs will be different in the burn family population than those in the general critical care population. Therefore, a comprehensive data base of family needs of burn victims is necessary to ensure appropriateness and effectiveness of interventions.

**Hypotheses.**

1. The needs of families change across a time span from 72 hours post admission until six weeks post admission and/or transfer to the acute care ward.

2. Family needs differ with respect to age, sex, relationship to the patient, perception of severity of injury, socioeconomic status, and self-reported importance of religion in their lives.

3. Burn nurses have a different perception of importance of family needs than do family members of burn patients.

4. The majority of needs perceived by family are being met.

A significance level of $p = 0.05$ will be used to accept or reject the hypotheses.

**Sample.** A convenience sample will be used of family members who meet the following criteria: 1) Age 18 years or older; 2) able to read English; 3) have a family relationship or are a significant other* to a burn patient admitted to a critical care burn unit. There is no limitation to the number of family members per patient who may enter the study to complete the CCFNI at the first time interval. However, one family member will be designated by the family present at the beginning of the study as the primary visitor for the patient during the hospitalization. (The primary visitor is not necessarily the legal next of kin, but rather the person the family feels will be able to visit the most consistently.) This individual will complete subsequent versions of the CCFNI across the appropriate time frames. A nurse caring for the patient will

be asked to complete a CCFNI within 48 hours of having contact with the primary visitor after he/she has completed a CCFNI. The nurses' CCFNIs will reflect the nurses' perceptions of the needs of the primary visitor only.

* "Significant other" includes common law spouses, housemates, legal guardians, or a close friend designated by the patient if there is no family relative.

**Plan.**

a. **Design**: This will be a descriptive/comparative study to establish a comprehensive data base related to the perceived needs of family of critically burned patients. A letter will be sent to all burn units in the United States currently listed in the ABA Directory inviting participation in the study. The primary benefit to participating units will be the analysis of their unit specific data that can be compared with the total study sample analysis. The unit specific analysis may also serve as baseline data for potential studies of specific interventions in their unit. All units participating will be listed in any published results. Once the unit has obtained permission via their internal review process for research to conduct the study, data will be collected for a six month period. The unit data collection coordinator will code all data forms and maintain the key to the data at the facility. Only coded forms will be forward to the US Army Institute of Surgical Research (USAISR) for analysis. The principal investigators at the USAISR will have the specific unit code key for participating units and each unit will receive their unit code number, study instruments and specific data collection instructions on admission to the study.

b. **Description of Procedures/Data Collection**:

(1) After the family member has visited the patient at least once in the first 72 hours post admission to the critical care burn unit, and after verbal or written consent is obtained to participate, the unit data collection coordinator will have the family member complete the following data tools and instruments:

Family Member data form

Critical Care Family Needs Inventory (CCFNI)

(2) Any number of family members for a specific patient can participate in completing the first version of the CCFNI. One member of the family is designated by the family as the primary visitor".

(3) The family member designated as the primary visitor will be asked to complete the CCFNI again at two, four and six

weeks intervals as appropriate and at the time of patient transfer from the intensive care unit. The following chart summarizes the CCFNI template for administration:

| Administration code | Time Period |
|---|---|
| 1 | Within 72 hours of admission, after first visit |
| 2 | Two weeks post admission |
| 3 | Four weeks post admission |
| 4 | Six weeks post admission |
| 5 | Transfer from unit (does not include death) |

(4) The data coordinator identifies a nurse who has interacted with the primary visitor within 48 hours of their completing the CCFNI and asks that nurse to complete a staff CCFNI and Burn Unit Personnel Data Form. Nurses may participate more than once in the study with the same primary visitor or with different primary visitors for different patients. An individual nurse only completes one Burn Unit Personnel Data Form.

(5) The coordinator will collect the data about the unit once using the Unit Demographic Data Form and data about the patient using the Patient Data Form. Using both the Patient and Family Member Data forms, a code key sheet will be completed for each patient. Only coded forms of data tools will be sent to USAISR.

(6) The primary investigators at ISR will maintain a code key sheet for the unit code numbers only.

**Data Analysis Plan**. In order to pilot proper data collection techniques, six months data from the USAISR will be collected prior to soliciting participation from other burn units in the US. Descriptive and inferential statistical procedures will be used for analysis. Selected psychometric properties of the CCFNI will be determined based on data obtained within the first 72 hours post admission from all burn units participating. Jane Leske will be on contract to determine the psychometric properties. It is anticipated that 400-600 family members will be involved in the study as well as 250-500 health care personnel.

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**RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY**

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|---|---|---|
| DA346179 | 01 Oct 90 | |

| 3. DATE PREV SUM'RY | 4. KIND OF SUMMARY | 5. SUMMARY SCTY | 6. WORK SECURITY | 7. REGRADING | 8. DISP'N INSTR'N | 9. LEVEL OF SUM A. WORK UNIT |
|---|---|---|---|---|---|---|
| 26 Jan 90 | D. Chg | U | U | | CX | |

| 10. NO./CODES | PROGRAM ELEMENT | PROJECT NUMBER | TASK AREA NUMBER | WORK UNIT NUMBER |
|---|---|---|---|---|
| a. PRIMARY | 62787A | 3M162787A874 | CG | 174 |
| b. CONTRIBUTING | | | | |
| c. CONTRIBUTING | | | | |

11. TITLE (Precede with Security Classification Code) (U) Effect of Sucralfate on Prevention of Stress Ulcers and Nosocomial Pneumonia in Thermally Injured Patients

12. SUBJECT AREAS
0605 Medicine and Medical Research

| 13. START DATE | 14. ESTIMATED COMPLETION DATE | 15. FUNDING ORGANIZATION | 16. PERFORMANCE METHOD |
|---|---|---|---|
| 9001 | 9509 | DA | C. In-House |

17. CONTRACT/GRANT MILITARY RELEVANCY CERTIFIED
a. DATE EFFECTIVE APPROVED BY
b. CONTRACT/GRANT NUMBER
c. TYPE
d. AMOUNT
e. KIND OF AWARD
f. CUM/TOTAL

18. RESOURCES ESTIMATE

| FISCAL YEARS | a. PROFESSIONAL WORKYEARS | b. FUNDS (In thousands) |
|---|---|---|
| 90 | 0.1 | 48 |
| 91 | 0.1 | 50 |

19. RESPONSIBLE DOD ORGANIZATION

a. NAME: US Army Institute of Surgical Research
b. ADDRESS (Include zip code): Fort Sam Houston, San Antonio, Texas 78234-5012
c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A
d. TELEPHONE NUMBER (Include area code): 512-221-2720

20. PERFORMING ORGANIZATION

a. NAME: US Army Institute of Surgical Research
b. ADDRESS: Fort Sam Houston, San Antonio, Texas 78234-5012
c. NAME OF PRINCIPAL INVESTIGATOR: CIOFFI, W G
d. TELEPHONE NUMBER (Include area code): 512-221-4440
f. NAME OF ASSOCIATE INVESTIGATOR (If available): MC MANUS, A T
g. NAME OF ASSOCIATE INVESTIGATOR (If available): MC MANUS, W F

21. GENERAL USE: FINA
MILITARY/CIVILIAN APPLICATION: M

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Burns (Injuries); (U) Ulcers; (U) Pneumonia; (U) Therapy

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

23/24. (U) The objective of this work is to verify that sucralfate is effective in the prevention of stress ulcer-induced gastrointestinal bleeding and reduces the incidence of nosocomial pneumonia. Three hundred burn patients will be randomized to receive either standard prophylaxis (cimetidine and antacids) or sucralfate. Differences between treatment groups in the rate of occurrence of pneumonia and clinically evident gastrointestinal bleeding will be evaluated for significance using the Chi square technique.

25. (U) 8710 - 8809. Not applicable.

(U) 8810 - 8909. Not applicable.

(U) 8910 - 9009. This project was approved by the USAISR Research Council, the US Army Institute of Surgical Research Human Use Committee, and The Surgeon General's Human Subjects Research Review Board during the second quarter of fiscal year 1990. Twenty patients were enrolled in the study during this reporting period. Upon completion of enrollment, the data will be analyzed as indicated.

DD FORM 1498 83 MAR — EDITION OF MAR 68 IS OBSOLETE — * USGPO: 1988-491-003/50329

**SUPPLEMENTAL WORK UNIT INFORMATION FOR THE PROJECT ENTITLED "EFFECT OF SUCRALFATE ON PREVENTION OF STRESS ULCERS AND NOSOCOMIAL PNEUMONIA IN THERMALLY INJURED PATIENTS"**

**Subrecord/Linking Accession Number:** Not applicable.

**Search Control Data:** W6N47E/W6N40C, 30 May 1990

**Product Identification:** For technical reports, refer to the US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Year 1990.

**Unclassified Special Categories:** Volunteers: Adults; RA II

**ANNUAL RESEARCH PROGRESS REPORT**

**PROJECT NUMBER:** 3M162787A874-00, APPLIED RESEARCH AND EXPLORATORY DEVELOPMENT

**PROJECT TITLE:** Effect of Sucralfate on Prevention of Stress Ulcers and Nosocomial Pneumonia in Thermally Injured Patients

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

**1 October 1989 - 30 September 1990**

**INVESTIGATORS**

William G. Cioffi, Jr., MD, Major, MC
Albert T. McManus, PhD
William F. McManus, MD, Colonel, MC

## ABSTRACT

**PROJECT NUMBER:** 3M162787A874-00, APPLIED RESEARCH AND EXPLORATORY DEVELOPMENT

**PROJECT TITLE:** Effect of Sucralfate on Prevention of Stress Ulcers and Nosocomial Pneumonia in Thermally Injured Patients

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

**PERIOD COVERED IN THIS REPORT:** 1 Oct 89 through 30 Sep 90

**INVESTIGATORS:** William G. Cioffi, Jr., MD, Major, MC
Albert T. McManus, PhD
William F. McManus, MD, Colonel, MC

This project was approved by the USAISR Research Council, the US Army Institute of Surgical Research Human Use Committee, and the Surgeon General's Human Subjects Research Review Board during the second quarter of FY1990. Twenty patients were enrolled in the study during this reporting period. Upon completion of enrollment, the data will be analyzed.

**EFFECT OF SUCRALFATE ON PREVENTION OF STRESS ULCERS AND NOSOCOMIAL PNEUMONIA IN THERMALLY INJURED PATIENTS**

## INTRODUCTION

Prior to adoption of measures to neutralize gastric acid, acute gastrointestinal (GI) bleeding was a lethal complication in thermally injured patients. Mucosal ulcerations could be endoscopically identified in almost all seriously injured patients (1). Current prophylaxis, aimed at maintaining gastric Ph above 4.5, has virtually eliminated this complication (2).

In 1978, Atherton and White (3) proposed that the stomach might serve as a reservoir for bacteria that then colonize the respiratory tract of mechanically ventilated patients. It is now accepted that when the pH of the gastric contents rises above 4, the stomach becomes rapidly colonized with bacteria. Some authors have suggested that in patients with gram-negative nosocomial pneumonia, the bacteria appear to be of gastric origin. Since the incidence of pulmonary aspiration of gastric contents may be as high as 74% in mechanically ventilated patients (4), a method of stress ulcer prophylaxis which does not allow bacterial colonization of the stomach may be beneficial.

A recently reported prospective randomized trial documented that sucralfate was as effective as antacids or $H_2$ blockers in preventing stress-induced bleeding. The incidence of nosocomial pneumonia was lower in patients receiving sucralfate than those receiving antacids or $H_2$ blockers (5).

Sucralfate, a chemical complex of sucrose octasulfate and aluminum hydroxide, appears to protect against stress ulcer bleeding through pepsin absorption, mucosal protein binding, and cytoprotection without significantly altering gastric pH (6). It has been suggested recently that sucralfate may also have intrinsic antibacterial activity (7).

Previous trials investigating the use of sucralfate as prophylaxis for stress ulcer-induced bleeding have all suffered from the same flaw. The population of patients studies was usually not at significant risk for the development of stress ulcers. Patients with significant thermal injury represent a population at significant risk for the development of stress ulcers. With preliminary work suggesting that sucralfate is adequate for prophylaxis in populations at less risk, the next logical step is to attempt its use in patients at higher risk.

Therefore, the purpose of this study is twofold. First, the efficacy of sucralfate in the prevention of stress ulcer-induced GI bleeding will be investigated. Second, the incidence of pneumonia in patients randomized to receive either standard therapy with

antacids and $H_2$ blockers vs. those who receive sucralfate will be recorded.

## MATERIALS AND METHODS

a. **Design.** Three hundred patients will be randomized to receive either standard prophylaxis or sucralfate. The gastric pH of all patients will be checked and recorded every hour. The incidence of GI bleeding, pneumonia, and tracheobronchitis will be recorded for each patient. Any patient demonstrating clinically evident bleeding will undergo upper gastrointestinal endoscopy to verify the source of the bleeding. Sputum Gram stain and cultures and gastric aspirate cultures will be obtained every Monday, Wednesday, and Friday and as clinically directed. Isolates from each source will be typed and compared. The timing of colonization for each source will be recorded. Differences between treatment groups in the rate of occurrence of pneumonia and clinically evident GI bleeding will be evaluated, with the patients stratified for the presence of inhalation injury.

b. **Description of Procedures.** Three hundred patients meeting entry criteria will be randomized to receive either standard prophylaxis or sucralfate. Standard anti-ulcer prophylaxis will consist of the administration of cimetidine and antacids. Cimetidine (300 mg) will be administered intravenously every 6 hrs. The dose of cimetidine will be adjusted depending upon the patient's renal function and gastric pH. Antacids will be administered as a 30-cc bolus via the nasogastric tube every 2 hrs. Any evidence of clinically significant upper GI bleeding will result in withdrawal of the patient from the study. Withdrawal from the study for any reason will result in the classification of the patient as a treatment failure. Gastric pH will be checked every hour and if the pH is $<4.5$, the dose of antacids will be doubled and administered on an hourly basis until the pH is $\geq 4.5$. Sucralfate (1 g suspended in 20 cc water) will be administered via the nasogastric tube every 6 hrs. The tube will be clamped for 1 hr following administration. The gastric pH of these patients will be checked and recorded every hour. The incidence of clinically evident GI bleeding will be recorded. Any patient who demonstrates clinically evident bleeding will undergo upper endoscopy to verify the source of the bleeding. The diagnosis of pneumonia will be based upon roentgenographic findings consistent with pneumonia, sputum leukocytes >25 WBC/hpf, and less than 25 sqamous epithelial cells and growth of a predominant organism on sputum culture. Diagnosis of tracheobronchitis will be made based upon an elevated sputum culture leukocytosis (>25 WBC/hpf) and a predominant organism in the sputum culture in the absence of roentgenographic changes. The incidence of pneumonia and tracheobronchitis will be recorded for each patient. Sputum Gram stain and cultures and gastric aspirate cultures will be obtained every Monday, Wednesday, and Friday and as clinically directed. Isolates from each source

will be typed and compared. The timing of colonization for each source will be recorded.

(1) **Patient Inclusion:**

(a) Male or female patients 18 years of age and older. Female patients must have been surgically sterilized, be post-menopausal (> 45 yrs of age and the lack of menstrual periods for at least 1 yr), or have a negative pregnancy test immediately prior to entry into the study.

(b) Patients admitted to the United States Army Institute of Surgical Research within 48 hrs postburn.

(c) Patients with burns >20% of the total body surface area (the presence of an inhalation injury not being exclusionary).

(2) **Patient Exclusion:**

(a) Patients under 18 years of age.

(b) Patients who are pregnant or nursing.

(c) Patients admitted to the United States Army Institute of Surgical Research more than 48 hrs postburn.

(d) Patients with burns <20% of the total body surface area.

(e) Patients with a previous history of peptic ulcer disease.

(f) Patients who are presently receiving $H_2$ antagonists.

(g) Patients with a diagnosis of pneumonia at the time of admission to the United States Army Institute of Surgical Research.

c. **Determination of the Number of Subjects Required:** Between 1983 and May 1985, there were 220 patients who developed pneumonia out of a total of 1,300 admissions for an incidence of 17%. For patients with burns exceeding 20% of the total body surface area, this incidence is estimated to be 25-30%. Assuming an incidence of 25% and a 50% reduction in the incidence of sucralfate-treated patients as suggested in the literature, then 150 patients per arm will be required to prove the hypothesis with a type I error <0.05 and a type II error <0.2.

d. **Data Collection:** The gastric pH of all patients will be checked and recorded every hour. The incidence of clinically evident GI bleeding, pneumonia, and tracheobronchitis will be recorded for each patient and the timing of colonization for each source will be recorded.

e. **Data Analysis Plan:** Differences between treatment groups in the rate of occurrence of pneumonia and clinically evident GI bleeding will be evaluated for significance using the Chi-square technique.

## RESULTS

During this fiscal year, 20 patients were enrolled in the protocol. Upon enrollment of 100 patients, the data will be analyzed for the effect of sucralfate on the prevention of stress ulcers and nosocomial pneumonia, and if a trend is noted the trial will be continued for enrollment of the full three hundred patients.

## PRESENTATIONS/PUBLICATIONS

None.

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**RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY**

| 1. AGENCY ACCESSION | 2. DATE OF SUMMARY | REPORT CONTROL SYMBOL DD-DR&E(AR) 636 |
|---|---|---|
| DA346178 | 01 Oct 90 | |

| 3. DATE PREV SUM'RY | 4. KIND OF SUMMARY | 5. SUMMARY SCTY | 6. WORK SECURITY | 7. REGRADING | 8. DISP'N INSTR'N | 9. LEVEL OF SUM A. WORK UNIT |
|---|---|---|---|---|---|---|
| 31 Jan 90 | D. Chg | U | U | | CX | |

| 10. NO./CODES: | PROGRAM ELEMENT | PROJECT NUMBER | TASK AREA NUMBER | WORK UNIT NUMBER |
|---|---|---|---|---|
| a. PRIMARY | 62787A | 3M162787A874 | CA | 175 |
| b. CONTRIBUTING | | | | |
| c. CONTRIBUTING | | | | |

11. TITLE (Precede with Security Classification Code) (U) Study of Effect of Intermittent vs. Continuous Administration of EXOSURF in Patients with ARDS Induced by Thermal Injury

12. SUBJECT AREAS
0605 Medicine and Medical Research

| 13. START DATE | 14. ESTIMATED COMPLETION DATE | 15. FUNDING ORGANIZATION | 16. PERFORMANCE METHOD |
|---|---|---|---|
| 9001 | 9309 | DA | C. In-House |

17. CONTRACT/GRANT MILITARY RELEVANCY CERTIFIED
a. DATE EFFECTIVE APPROVED BY
b. CONTRACT/GRANT NUMBER
c. TYPE
d. AMOUNT
e. KIND OF AWARD
f. CUM/TOTAL

18. RESOURCES ESTIMATE

| FISCAL YEARS | a. PROFESSIONAL WORKYEARS | b. FUNDS (In thousands) |
|---|---|---|
| 90 | 0.1 | 46 |
| 91 | 0.1 | 48 |

19. RESPONSIBLE DOD ORGANIZATION
a. NAME: US Army Institute of Surgical Research
b. ADDRESS (Include zip code): Fort Sam Houston, San Antonio, Texas 78234-5012
c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A
d. TELEPHONE NUMBER (Include area code): 512-221-2720

20. PERFORMING ORGANIZATION
a. NAME: US Army Institute of Surgical Research
b. ADDRESS: Fort Sam Houston, San Antonio, Texas 78234-5012
c. NAME OF PRINCIPAL INVESTIGATOR: CIOFFI, W G
d. TELEPHONE NUMBER (Include area code): 512-221-4440
f. NAME OF ASSOCIATE INVESTIGATOR (If available): RUE, L W
g. NAME OF ASSOCIATE INVESTIGATOR (If available)

21. GENERAL USE: FINA
MILITARY/CIVILIAN APPLICATION: M

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Burns (Injuries); (U) Morbidity; (U) Inhalation; (U) Therapy

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

23/24. (U) The objectives of this work are to determine whether a two-fold increment in EXOSURF dose alters the effects of aeroslized EXOSURF on shunt fraction and other physiologic indices of pulmonary function and to assess patient tolerance of continuous aerosolized EXOSURF for 5 days. Twenty-four patients with early onset ARDS secondary to thermal and smoke inhalation injury will be randomized to one of four treatment groups. Patients in each group will receive continuous aerosol administration of the appropriate agent for 5 days. A variety of ventilatory, hemodynamic, and blood gas data will be collected.

25. (U) 8710 - 8809. Not applicable.

(U) 8810 - 8909. Not applicable.

(U) 8910 - 9009. This project was approved by the USAISR Research Council, the US Army Institute of Surgical Research Human Use Committee, and The Surgeon General's Human Subjects Research Review Board during the second quarter of fiscal year 1990. Enrollment of patients will begin during fiscal year 1991.

DD FORM 83 MAR 1498 EDITION OF MAR 88 IS OBSOLETE. * USGPO 1988-491-003/50329

**SUPPLEMENTAL WORK UNIT INFORMATION FOR THE PROJECT ENTITLED "STUDY OF EFFECT OF INTERMITTENT VS. CONTINUOUS ADMINISTRATION OF EXOSURF IN PATIENTS WITH ARDS INDUCED BY THERMAL INJURY"**

**Subrecord/Linking Accession Number:** Not applicable.

**Search Control Data:** W6N55B/W6N57E, 30 May 1990

**Product Identification:** For technical reports, refer to the US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Year 1990.

**Unclassified Special Categories:** Volunteers: Adults; Children; RA I

**ANNUAL RESEARCH PROGRESS REPORT**

**PROJECT NUMBER:** 3M162787A74-00, APPLIED RESEARCH AND EXPLORATORY DEVELOPMENT

**PROJECT TITLE:** Study of Effect of Intermittent vs. Continuous Administration of EXOSURF in Patients with ARDS Induced Thermal Injury

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

**1 October - 30 September 1990**

**INVESTIGATORS**

William G. Cioffi, Jr., MD, Major, MC
Loring W. Rue, III, MD, Major, MC

## ABSTRACT

**PROJECT NUMBER:** 3M162787A874-00, APPLIED RESEARCH AND EXPLORATORY DEVELOPMENT

**PROJECT TITLE:** Study of Effect of Intermittent vs. Continuous Administration of EXOSURF in Patients with ARDS Induced by Thermal Injury

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

**PERIOD COVERED IN THIS REPORT:** 1 Oct 89 through 30 Sep 90

**INVESTIGATORS:** William G. Cioffi, Jr., MD, Major, MC
Loring W. Rue, III, MD, Major, MC

This project was approved by the USAISR Research Council, The US Army Institute of Surgical Research Human Use Committee, and the Surgeon General's Human Subjects Research Review Board during the second quarter of FY1990. Enrollment of patients will begin during FY1991.

**STUDY OF EFFECT OF INTERMITTENT VS. CONTINUOUS ADMINISTRATION OF EXOSURF IN PATIENTS WITH ARDS INDUCED BY THERMAL INJURY**

## INTRODUCTION

Acute severe pulmonary insufficiency afflicts patients of all ages, ranging from the newborn to geriatric age groups. A common, often fatal form of acute pulmonary insufficiency in adults is termed adult respiratory distress syndrome (ARDS) (1,2).

ARDS develops as a result of various and diverse etiologies (1,3-7). ARDS may occur after direct lung injuries such as chest contusion, aspiration, near drowning, or inhalation of smoke or oxidant gases (1,3-7). ARDS may also develop after various nonpulmonary disorders including sepsis, trauma, shock, burns, fractures, transfusions, and pancreatitis (1,3-7).

Inhalation injury is common in patients with thermal injury. An estimated 10-40% of thermal injury patients concurrently have an inhalation injury which frequently causes pulmonary complications (9,10). Mortality rates of individuals with smoke inhalation injury can increase significantly when compared to similar burn patients without such injury (9). The presence of smoke inhalation injury may increase mortality in every age group and burn size (9). The number of burn patients with inhalation injury who develop respiratory compromise each year remains uncertain. However, pulmonary complications including ARDS are now the leading causes of thermal and inhalation injury mortality.

ARDS, often rapid in onset and requiring mechanically assisted ventilation, results in a mortality which exceeds 65% (1-5). The clinical picture is the consequence of increased pulmonary endothelial and epithelial permeability, resulting in exudation of protein rich fluid in the interstitial and alveolar spaces, severe hypoxia secondary to right to left shunting of blood, micro-atelectasis, and a decreased amount and/or inactivation of surfactant (1-7,12-17).

Surfactant is a lipid protein complex which lines the alveolar surface of the lung (18). Surfactant reduces surface tension at the air liquid interface, lowers end expiratory volumes of the lung, increases lung compliance, and aids in keeping the small alveoli as dry as possible (19,20). Surfactant is a mixture of several phospholipids and numerous proteins; (18) however, the main constituent is dipalmitoyl phosphatidylcholine (DPPC), comprising approximately 50% of the complex. DPPC is the component responsible for the surface tension lowering properties of the complex. Another component of surfactant is phosphatidylglycerol (PG). While a surface activant in DPPC, the role of PG in surfactant is unclear since PG may be unnecessary for good surfactant function (21,22). However, PG is a good marker for mature lung and surfactant in the neonate (23). Most of the

proteins in the surfactant are of serum origin, although there are several surfactant specific proteins with molecular weights in the range of 34-36 kilodaltons and 5-14 kilodaltons (18,24-27). The surfactant specific proteins aid in the spreading of surfactant on the alveolar surface. The lung lavage fluid content of the lower molecular weight protein is decreased in several animal models of lung injury (28). Analyses of bronchoalveolar lavage from ARDS patients and from animal models of acute lung injury demonstrate that the alveolar phospholipids have a reduced content of DPPC and PG and a decrease of the lecithin-sphingomyelin ratio similar to the situation in the neonate (15,17). The ARDS patient has a decreased pulmonary compliance which is one of the physiologic correlates of increased alveolar surface tension (1,2). Although it is believed that the initial lung injury precipitating ARDS is a breakdown of the alveolar endothelial-epithelial permeability barrier, a consequence of the alveolar injury is a marked disturbance of the surfactant system (12-17). However, the etiology of ARDS is complex, and numerous substances are released into the lung which may also cause cellular damage and surfactant inactivation (6).

The present treatment for patients with ARDS secondary to smoke inhalation consists of mechanical ventilation with positive end expiratory pressure and supplemental oxygen and vigorous pulmonary toilet, all of which are supportive attempts to maintain arterial oxygenation rather than specific disease treatment. In fact, high inspired oxygen concentrations administered to patients receiving mechanical ventilation may in and of themselves cause further lung damage. Such supportive therapeutic approaches are often unsuccessful and may result in significant morbidity.

The administration of exogenous surfactant along with mechanical ventilation and positive end expiratory pressure significantly improves the survival of rabbits in a model of ARDS induced by lavage depletion of surfactant and in mice infected with influenza (30-33). In an oxygen toxicity model of ARDS, the administration of a natural surfactant to oxygen-exposed rabbits improved lung compliance, decreased lung edema, and appeared to mitigate the lung injury (34). Similarly, administration of exogenous surfactant to ARDS patients should improve compliance of the lung and increase the functional residual capacity, allowing the patient to be ventilated with lower peak pressures and lower inspired oxygen concentrations. By virtue of the surface tension lowering and antiedema properties, exogenous surfactants may prevent, or reverse alveolar edema (35). Thus, exogenous surfactant administration early in the course of ARDS may help stabilize the lungs, and thereby decrease the need for mechanical ventilation.

Preliminary experiments in patients in whom exogenous surfactant was administered within 72 hours of onset of severe ARDS showed transient improvement in gas exchange. Four grams of

porcine-derived surfactant were administered as 50 ml endotracheal boluses. This large dose of surfactant was well tolerated (36). Natural surfactant equivalent to 110 ml/kg of DPPC has also been tracheally instilled in the terminally ill child with ARDS. Within 4 hours their arterial oxygen tension rose from 19 to 200 mm Hg and there was significant clearing of the chest film. Thus, the initial experience suggests that surfactant administration may be useful in the treatment of ARDS.

Natural surfactant, a combination of lipids and proteins, exhibits not only surface tension reducing properties but also rapid spreading and absorption. Although DPPC by itself markedly reduces surface tension, alone it is ineffective in respiratory distress syndromes because DPPC spreads and absorbs poorly (39-41). The rapid spreading and absorption necessary for normal natural surfactant function is conferred by the apoproteins. The compound to be used in this experiment, EXOSURF, is a totally synthetic surfactant patented by John A. Clemens, M.D., in 1982. Since alcohol spread rapidly on the surface of water, Dr. Clemens postulated that adding alcohol to DPPC would create an effective synthetic surfactant. In this sense, the alcohol constituent of EXOSURF serves the same function of the apoprotein moieties of natural surfactant. EXOSURF is a 13.5:1.5:5.8:1 mixture of DPPC, hexadyconal alcohol, sodium chloride and tyloxapol.

Toxicology studies of EXOSURF administered endotracheally as a liquid bolus were performed elsewhere. Fifty-two newborn rabbit pups which were dosed on the first day of life and subsequently sacrificed at 14 days received daily doses of EXOSURF 1,2, and 3 times the recommended neonatal dose as a single endotracheal bolus. Several pups in both the controls and EXOSURF treated groups died acutely. Postmortem examination showed no significant changes attributable to EXOSURF. A second study using 215 rabbit pups which received EXOSURF on a four-times-a-day basis in doses ranging from 1 to 3 times the recommended dose were sacrificed two weeks later. Again, no significant pathology attributable to EXOSURF was noted (42,43).

To date, four toxicity studies of aerosolized EXOSURF in adult animals have been performed. Pilot studies with rats and monkeys have been performed for five days (44,45). Aerosolized EXOSURF resulted in no gross or microscopic signs of toxicity. Two other studies (46,47) using larger groups of rats and monkeys also demonstrated no toxicity secondary to aerosolized EXOSURF administration.

To date, there have been 15 clinical trials with EXOSURF used in the pediatric age groups. All studies were randomized, parallel, placebo-controlled trials of liquid bolus administration of EXOSURF. Preliminary analysis of these studies indicate significant reductions in deaths in the EXOSURF treated groups with no difference in the incidence of bronchopulmonary dysplasia or

intraventricular hemorrhages between the treated and control subjects.

Pilot studies utilizing aerosolized EXOSURF in adult patients with ARDS have been initiated. Thirteen patients have been treated with aerosolized EXOSURF without adverse reactions. Patients responded with a decreased oxygen requirement, increased $PaO_2/FIO_2$ ratio, and decreased shunt fraction.

Pulmonary complications are now the major determinant of mortality in patients with significant thermal injury and smoke inhalation (58). Data suggest that surfactant depletion and inactivation may be partially responsible for pulmonary dysfunction following smoke irhalation.

The purpose of this study is to determine whether administration of exogenous surfactant will result in an improvement in pulmonary function in patients with thermal and inhalation injury induced ARDS. Additionally, this study will attempt to ascertain whether continuous or intermittent administration of aerosolized EXOSURF are equally effective. Continuous (24 hours per day) versus intermittent (12 hours per day) administration will result in the administration of 30 or 60 ml/kg of DPPC per day.

**Design.** This study is designed as a multicenter, double-blind, randomized, parallel, pilot investigation of the effect of EXOSURF or saline inhalation on pulmonary function in patients with early pulmonary insufficiency secondary to smoke inhalation. Patients meeting entrance criteria will be enrolled in the study and randomly assigned to one of four treatment groups: Group A - EXOSURF administered for 12 hours per day; Group B - 0.1 normal saline administered for 12 hours per day; Group C - EXOSURF administered for 24 hours per day; and Group D - 0.1 normal saline administered for 24 hours per day.

Patients in each treatment group will receive the appropriately aerosolized agent for five days. Randomization will be such that twice as many patients will be enrolled in the EXOSURF groups as the control groups. Randomization will be coordinated at a central office located at Burroughs Wellcome Company.

## RESULTS

At this time, no patients have been enrolled in this protocol. Laboratory experience with the Visan nebulizer, which is to be used for the delivery of the aerosolized surfactant has revealed multiple problems. Until the nebulizer can be safely used in the animal model, patients will not be enrolled in this protocol.

## DISCUSSION

None.

## PRESENTATIONS/PUBLICATIONS

None.

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45. A Pilot 5-Day Inhalation Toxicity Study of EXOSURF Aerosol Formulation in the Cynomolgus Monkey (B.W. Document TTDR/87/0006).

46. A 14-Day Inhalation Toxicity Study of EXOSURF Aerosol Formulation in the Albino Rat (B.W. Document TTEP/87/0019).

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48. Effects of EXOSURF on Lung Conditioning and Static Pressure/Volume Characteristics of immature Rabbit Lungs. (CVRI #1, B.W. Document THZZ/85/0245).

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**RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY**

| 1. AGENCY ACCESSION | 2. DATE OF SUMMARY | REPORT CONTROL SYMBOL |
|---|---|---|
| DA346201 | 01 Oct 90 | DD-DR&E(AR) 636 |

| 3. DATE PREV SUM'RY | 4. KIND OF SUMMARY | 5. SUMMARY SCTY | 6. WORK SECURITY | 7. REGRADING | 8. DISP'N INSTR'N | 9. LEVEL OF SUM A. WORK UNIT |
|---|---|---|---|---|---|---|
| 11 Jun 90 | D. Chg | U | U | | CX | |

| 10. NO./CODES: | PROGRAM ELEMENT | PROJECT NUMBER | TASK AREA NUMBER | WORK UNIT NUMBER |
|---|---|---|---|---|
| a. PRIMARY | 62787A | 3M162787A874 | EA | 176 |
| b. CONTRIBUTING | | | | |
| c. CONTRIBUTING | | | | |

11. TITLE *(Precede with Security Classification Code)* (U) A Clinical Study of the Efficacy of Topical Silicone Gel in the Prevention of Hypertrophic Burn Scar Formation

12. SUBJECT AREAS 0605 Medicine and Medical Research
0612 Medical Facilities, Equipment, and Supplies

| 13. START DATE | 14. ESTIMATED COMPLETION DATE | 15. FUNDING ORGANIZATION | 16. PERFORMANCE METHOD |
|---|---|---|---|
| 9006 | 9309 | DA | C. In-House |

17. CONTRACT/GRANT MILITARY RELEVANCY CERTIFIED
a. DATE EFFECTIVE APPROVED BY
b. CONTRACT/GRANT NUMBER
c. TYPE d. AMOUNT
e. KIND OF AWARD f. CUM/TOTAL

18. RESOURCES ESTIMATE

| FISCAL YEARS | a. PROFESSIONAL WORKYEARS | b. FUNDS *(In thousands)* |
|---|---|---|
| 90 | 0.1 | 6 |
| 91 | 0.1 | 48 |

| 19. RESPONSIBLE DOD ORGANIZATION | 20. PERFORMING ORGANIZATION |
|---|---|
| a. NAME: US Army Institute of Surgical Research | a. NAME: US Army Institute of Surgical Research |
| b. ADDRESS *(include zip code)*: Fort Sam Houston, San Antonio, Texas 78234-5012 | b. ADDRESS: Fort Sam Houston, San Antonio, Texas 78234-5012 |
| c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A | c. NAME OF PRINCIPAL INVESTIGATOR: COZEAN, K |
| d. TELEPHONE NUMBER *(include area code)*: 512-221-2720 | d. TELEPHONE NUMBER *(include area code)*: 512-221-5957 |
| 21. GENERAL USE: FINA<br>MILITARY/CIVILIAN APPLICATION: M | e. NAME OF ASSOCIATE INVESTIGATOR *(if available)*: CIOFFI, W G<br>g. NAME OF ASSOCIATE INVESTIGATOR *(if available)*: RUE, L W |

22. KEYWORDS *(Precede EACH with Security Classification Code)* (U) Burns (Injuries); (U) Skin Grafts; (U) Scars; (U) Dressings

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS *(Precede text of each with Security Classification Code)*

23/24. (U) The objective of this work is to evaluate the efficacy of silicone gel in the prevention of hypertrophic burn scar formation. Improved management of hypertrophic scar formation will benefit military survivors of burn injury. Forty-two patients who have undergone meshed, split-thickness skin grafting of at least one upper extremity will be entered into the study. When the graft has healed and the wound is closed, Silastic gel sheeting will be applied to the grafted area. Data from treated and untreated sites of each patient will be subjected to a one-way, within subjects ANOVA. Data groups will be formed for treated and untreated sites and will undergo a one-way, between patients ANOVA.

25. (U) 8710 - 8809. Not applicable.

(U) 8810 - 8909. Not applicable.

(U) 8910 - 9009. This project was approved by the USAISR Research Council and US Army Institute of Surgical Research Human Use Committee during the third quarter of fiscal year 1990. Patient enrollment will begin during fiscal year 1991.

DD FORM 83 MAR 1498 EDITION OF MAR 88 IS OBSOLETE. * USGPO: 1988-491-003/50329

**SUPPLEMENTAL WORK UNIT INFORMATION FOR THE PROJECT ENTITLED "A CLINICAL STUDY OF THE EFFICACY OF TOPICAL SILICONE GEL IN THE PREVENTION OF HYPERTROPHIC BURN SCAR FORMATION"**

**Subrecord/Linking Accession Number:** Not applicable.

**Search Control Data:** W6007D/W6008D, 30 May 1990

**Product Identification:** Not applicable.

**Unclassified Special Categories:** Volunteers: Adults, RA II

**ANNUAL RESEARCH PROGRESS REPORT**

**PROJECT NUMBER:** 3MI62787A874-00, APPLIED RESEARCH AND EXPLORATORY DEVELOPMENT

**PROJECT TITLE:** A Clinical Study of the Efficacy of Topical Silicone Gel in the Prevention of Hypertrophic Burn Scar Formation

**US ARMY INSTITUTE OF SURGICAL RESEARCH**
**FORT SAM HOUSTON**
**SAN ANTONIO, TEXAS 78234-5102**

**1 October 1989 - 30 September 1990**

**INVESTIGATORS**

Stephen H. Luster, OTR, MS, Major, SP
William G. Cioffi MD, Jr., Major, MC
Loring W. Rue III, MD, Captain, MC
William F. McManus, MD, Colonel, MC

## ABSTRACT

**PROJECT NUMBER:** 3M162787A874-00, APPLIED RESEARCH AND EXPLORATORY DEVELOPMENT

**PROJECT TITLE:** A Clinical Study of the Efficacy of Topical Silicone Gel in the Prevention of Hypertrophic Burn Scar Formation

**INSTITUTION:** US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas 78234-5012 78234-5102

**PERIOD COVERED IN THIS REPORT::** 1 Oct 89 through 30 Sep 90

**INVESTIGATORS:** Stephen H. Luster, OTR, MS, Major, SP
William G. Cioffi, Jr., MD, Major, MC
Loring W. Rue III, MD, Captain, MC
William F. McManus, MD, Colonel, MC

Application of topical silicone gel material (Silastic Gel Sheeting) to healed burn wounds has been shown to smooth the texture of hypertrophic burn scar. Previous trials of silicone materials for scar reduction have been accomplished with existing hypertrophic burn scar. There have been no studies to assess the ability of silicone gel to prevent the development of hypertrophic burn scar. This study will assess the efficacy of silicone gel to prevent the development of hypertrophic burn scar in recently skin grafted patients. The results of this preliminary study will determine if Silastic gel is of clinical value in early post-graft scar management and if subsequent investigations into the mechanism of action of silicone gel are feasible within an acute care setting.

**A CLINICAL STUDY OF THE EFFICACY OF TOPICAL SILICONE GEL IN THE PREVENTION OF HYPERTROPHIC BURN SCAR FORMATION**

## INTRODUCTION

There has been interest in the use of silicone materials for the treatment of burns for many years. There have been reports of silicone oil and silicone dressings in the treatment of hand burns (1-3). In 1988, Dr. Ohmori of Tokyo reported successful treatment of keloid scars with Silastic (silicone) sheeting (4). Dow Corning Wright has developed and is now marketing a Silastic gel sheeting for clinical use with hypertrophic scars. The product, Silastic Gel Sheeting, is a 3.5 mm thick polyethyleneterephthalate mesh reinforced silicone gel sheet. Silicone gel sheets have been found to be bacteriologically inert and to have mechanical extensibility similar to skin (5). The mechanism of action of silicone gel on hypertrophic scar is unknown. After studying silicone gel sheets, Quinn concluded that its mechanism of action in altering scar tissue is not secondary to pressure, temperature, oxygen tension or skin hydration and occlusion and may therefore involve a chemical factor (5). Previous studies (5,6) have demonstrated that application of silicone gel sheets softens, flattens and increases the extensibility of existing hypertrophic burn scar. With the exception of a five patient clinical trial of silicone gel as a partial-thickness burn injury dressing (7) there have been no studies to assess its effect as treatment for the prevention of burn scar formation.

## MATERIALS AND METHODS

This study will utilize patients who have undergone meshed, split-thickness skin grafting of at least one upper extremity. When the graft has healed so that the interstices are closed, a 4cm square patch of Silastic silicone gel sheeting will be applied to a grafted area and secured with surgical netting (Surginete). The silicone gel sheeting will remain in contact with the graft for a total of 23 hours a day and be removed twice a day for cleaning, skin inspection and hygiene. Treatment will continue for three weeks. Treatment will be discontinued in the event of pruritus, pain, maceration, ulceration or other tissue degradation.

**Patient Inclusion Criteria:**

(1) Male and female patients 18 years of age and older who have been admitted to the US Army Institute of Surgical Research.

(2) Patients with burns > 1% TBSA but < 70% TBSA.

**Patient Exclusion Criteria:**

(1) Patients under 18 years of age.

(2) Patients with burns < 1% TBSA.

(3) Patients with toxic epidermal necrolysis (TEN).

(4) Patients who are pregnant or nursing.

Forty-two patients will be involved in the study based on an expected treatment effect difference of 25%, a 5% type I error and 10% type II error. The effect of treatment will be assessed by pre and post-treatment color and texture ratings. The ratings will be conducted by a panel of 5 disinterested observers. In addition, weekly measurements of scar pliability will be taken using a modified tonometer as described by Esposito et al. (8). The test sites and adjacent areas will also be photographed before and after treatment for later comparison. Weekly measurements will continue until the patient's discharge to assess the effect of treatment cessation.

The data from the treated and untreated sites of each subject will be subjected to a one way within-subjects ANOVA. Data groups will be formed for treated and untreated sites and will undergo a one way between-subjects ANOVA.

## RESULTS

All equipment and materials for the study have been assembled. A pre-study trial of one patient demonstrated that more training in the use of the Schiotz tonometer was required to assure reliable scar pliability measurements. This training is currently in progress.

## DISCUSSION

The principal investigator, Major Luster, is being transferred to another military medical facility effective 25 August 1990. CPT (P) Karen Cozean will be attached to ISR effective 9 October 1990 and has agreed to assume responsibilities of principal investigator.

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| RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY | 1. AGENCY ACCESSION | 2. DATE OF SUMMARY | REPORT CONTROL SYMBOL |
|---|---|---|---|
| | DA335466 | 01 Oct 90 | DD-DR&E(AR) 636 |

| 3. DATE PREV SUM'RY | 4. KIND OF SUMMARY | 5. SUMMARY SCTY | 6. WORK SECURITY | 7. REGRADING | 8. DISP'N INSTR'N | 9. LEVEL OF SUM A. WORK UNIT |
|---|---|---|---|---|---|---|
| 31 Jul 90 | D. Chg | U | U | | CX | |

| 10. NO./CODES: | PROGRAM ELEMENT | PROJECT NUMBER | TASK AREA NUMBER | WORK UNIT NUMBER |
|---|---|---|---|---|
| a. PRIMARY | 62787A | 3M162787A874 | AZ | 177 |
| b. CONTRIBUTING | | | | |
| c. CONTRIBUTING | | | | |

11. TITLE *(Precede with Security Classification Code)* (U) A Clinical Study of the Efficacy of Low-Dose Dopamine Therapy in Hospitalized Burn Patients

12. SUBJECT AREAS
0605 Medicine and Medical Research

| 13. START DATE | 14. ESTIMATED COMPLETION DATE | 15. FUNDING ORGANIZATION | 16. PERFORMANCE METHOD |
|---|---|---|---|
| 9007 | 9309 | DA | C. In-House |

17. CONTRACT/GRANT — MILITARY RELEVANCY CERTIFIED APPROVED BY [signature]

| a. DATE EFFECTIVE | | 18. RESOURCES ESTIMATE | | |
|---|---|---|---|---|
| b. CONTRACT/GRANT NUMBER | | FISCAL YEARS | a. PROFESSIONAL WORKYEARS | b. FUNDS *(In thousands)* |
| c. TYPE | d. AMOUNT | 90 | 0.1 | 46 |
| e. KIND OF AWARD | f. CUM/TOTAL | 91 | 0.1 | 48 |

| 19. RESPONSIBLE DOD ORGANIZATION | 20. PERFORMING ORGANIZATION |
|---|---|
| a. NAME: US Army Institute of Surgical Research | a. NAME: US Army Institute of Surgical Research |
| b. ADDRESS *(include zip code)*: Fort Sam Houston, San Antonio, Texas 78234-5012 | b. ADDRESS: Fort Sam Houston, San Antonio, Texas 78234-5012 |
| c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A | c. NAME OF PRINCIPAL INVESTIGATOR: CIOFFI, W G |
| d. TELEPHONE NUMBER *(include area code)*: 512-221-2720 | d. TELEPHONE NUMBER *(include area code)*: 512-221-4440 |
| 21. GENERAL USE: FINA | e. NAME OF ASSOCIATE INVESTIGATOR *(if available)*: HEIRONIMUS, J D |
| MILITARY/CIVILIAN APPLICATION: M | g. NAME OF ASSOCIATE INVESTIGATOR *(if available)*: VAUGHAN, G M |

22. KEYWORDS *(Precede EACH with Security Classification Code)* (U) Burns (Injuries); (U) Dopamine; (U) Therapy; (U) Kidney Function Tests

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS *(Precede text of each with Security Classification Code)*

23/24. (U) The objective of this work is to evaluate the efficacy of low-dose (2-5 mcg/kg/min) dopamine therapy in burn patients. Effective renal plasma flow, glomerular filtration rate, sodium and potassium excretion, and free water clearance will be measured utilizing radiolabelled tracers prior to and during a continuous intravenous dopamine infusion in 20 consecutive burn patients with burns > 30% of the total body surface area and 10 normal volunteers. Results will be evaluated using data tables prepared to compare both burn and control populations in terms of effective renal plasma flow, glomerular filtration rate, sodium and postassium osmolar excretion, serum and plasma variables, free water clearance, and cardiac output. ANOVA will include hour of infusion, burn size, age, and postburn day as main sources of variation for measured variables.

25. (U) 8710 - 8809. Not applicable.

(U) 8810 - 8909. Not applicable.

(U) 8910 - 9009. This project was approved by the USAISR Research Council, Brooke Army Medical Center Radiation Control Committee, US Army Institute of Surgical Research Human Use Committee, and The Surgeon General's Human Subjects Research Review Board during the fourth quarter of fiscal year 1990. Patient enrollment will begin during fiscal year 1991.

DD FORM 83 MAR 1498 EDITION OF MAR 68 IS OBSOLETE * USGPO 1986-491-003/50329

**SUPPLEMENTAL WORK UNIT INFORMATION FOR THE PROJECT ENTITLED "A CLINICAL STUDY OF THE EFFICACY OF LOW-DOSE DOPAMINE THERAPY IN HOSPITALIZED BURN PATIENTS"**

**Subrecord/Linking Accession Number:** Not applicable.

**Search Control Data:** W6K26L/W6K33L, 9 February 1990

**Product Identification:** For technical reports, refer to the US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Year 1990.

**Unclassified Special Categories:** Volunteers: Adults; RA II

**ANNUAL RESEARCH PROGRESS REPORT**

**PROJECT NUMBER:** 3M162787A874-00, APPLIED RESEARCH AND EXPLORATORY DEVELOPMENT

**PROJECT TITLE:** A Clinical Study of the Efficacy of Low-Dose Dopamine Therapy in Hospitalized Burn Patients

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

**1 October 1989 - 30 September 1990**

**INVESTIGATORS**

William G. Cioffi, Jr., MD, Major, MC
J.D. Heironimus
George M. Vaughan, MD, Colonel, MC

## ABSTRACT

**PROJECT NUMBER:** 3M162787A874-00, APPLIED RESEARCH AND EXPLORATORY DEVELOPMENT

**PROJECT TITLE:** A Clinical Study of the Efficacy of Low-Dose Dopamine therapy in Hospitalized Burn Patients

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

**PERIOD COVERED IN THIS REPORT:** 1 Oct 89 through 30 Sep 90

**INVESTIGATORS:** William G. Cioffi, Jr., MD, Major, MC
J.D. Heironimus
George M. Vaughan, MD, Colonel, MC

This project was approved by the USAISR Research Council, Brooke Army Medical Center Radiation Control Committee, US Army Institute of Surgical Research Human Use Committee, and the Surgeon General's Human Subjects Research Review Board during the fourth quarter of FY1990. Patient enrollment will begin during FY1991.

**A CLINICAL STUDY OF THE EFFICACY OF LOW-DOSE DOPAMINE THERAPY IN HOSPITALIZED BURN PATIENTS**

## INTRODUCTION

a. **Objective**. The objective of this study is to evaluate the efficacy of low-dose (2.0-5.0 mcg/kg/min) dopamine therapy in burn patients by documenting its effects on effective renal plasma flow (ERPF), glomerular filtration rate (GFR), sodium and potassium excretion, and free water clearance.

b. **Medical Application**. Dopamine (3,4-dihydroxy-phenylethylamine) is a precursor of norepinephrine and epinephrine. It is found in sympathetic nerves and ganglia; most prominently in the brain, heart, kidney, vasculature, and intestines. Depending upon the dosage used and thus the type of receptor stimulated, it has a wide variety of pharmacological actions. At low doses, 0.5-1.0 mcg/kg/min, dopamine-1 (DA1) receptors are primarily activated. The DA1 receptors are located in the renal, mesenteric, cerebral, and coronary vasculature (1). Their stimulation leads primarily to vasodilation. Additionally, stimulation of the DA1 receptors located in the renal juxtaglomerular apparatus and zona glomerulosa leads to inhibition of sodium transport in the kidney. At slightly higher doses, 2.0-5.0 mcg/kg/min, the beta-1 receptors are also activated. (1) This exerts a positive inotropic effect on the myocardium with a subsequent increase in cardiac output. At doses of approximately 10 mcg/kg/min, alpha-1 and alpha-2 receptors in the peripheral vasculature are activated, leading to vaso-constriction and increased systemic vascular resistance (SVR) (1). The combined renal and cardiovascular effects of "low-dose" dopamine therapy, 2.0-5.0 mcg/kg/min, result in increased renal blood flow, with an associated increased glomerular filtration rate (GFR), increased sodium excretion, and increased urine output (1-4).

Dopamine has a half-life of 1-2 min when administered parenterally. Monoamine oxidase rapidly metabolizes dopamine to sulfates and glucuronides by conjugation. This rapid elimination necessitates that dopamine be administered via a continuous infusion, using a metered pump for strict control of rate of flow. In addition, the dose-related pharmacological effects of dopamine occur gradually and the clinical response is variable from patient to patient. Therefore, careful monitoring of blood pressure, cardiac output, and urine flow is mandatory (5).

Side effects are rarely seen when low-dose dopamine infusion is administered. The renal clearance of other drugs which are eliminated primarily by glomerular filtration is increased and dosage adjustment may be required. The clearance of those drugs eliminated by hepatic degradation is likewise augmented secondary to mesenteric vasodilation and increased hepatic flow. Therefore,

drugs eliminated by this route must also be closely monitored. Extravasation of dopamine into the subcutaneous tissue can lead to an intense local vasoconstriction and subsequent necrosis. Therefore, dopamine should be administrated via a large, central vein. Phosphate levels must also be monitored due to increased urinary excretion. Hypoxemia must be avoided, since dopamine suppresses the ventilatory response to low oxygen tension at the carotid body. At higher doses, dopamine can cause tachycardia, arrhythmias, and ischemia. As a result, continuous cardiac monitoring is mandatory (1,5).

Dopamine's effect on renal perfusion and urine flow has been documented in a wide variety of clinical situations (3,6-9). Using radio labeled tracers, it has been shown to increase both ERPF and GFR with a resultant increase in urine output (1,2). These effects have been attributed to both the renal vasodilation and increased cardiac output observed with "low-dose" dopamine therapy. As a result, dopamine is considered to be efficacious when used in the management of cardiogenic, traumatic, and hypovolemic shock.

In these situations, it is hypothesized that renal function may be compromised due to low perfusion, possibly from increased sympathetic activity. Low-dose dopamine therapy is also occasionally utilized in burn patients when the fluid resuscitation requirements exceed the predicted rates; however, the efficacy of this therapy in burn patients has never been documented. It is uncertain if the effects of low-dose dopamine are the same in burn patients who are hypermetabolic and have elevated levels of aldosterone and ADH. The purpose of this study is to document the effect of low-dose dopamine therapy on the ERPF, GFR, solute excretion, and free-water clearance in burn patients who are approximately 4-7 days post injury and to compare the findings with previously documented effects seen in various other clinical situations (2,3,6-9).

If this study produces statistically significant results, a follow-up study will be designed to evaluate the effect of low-dose dopamine effect during the resuscitative phase immediately postburn.

## MATERIALS AND METHODS

a. **Design.** This protocol will study 20 consecutive burn patients with burns of > 30% of the total body surface area and 10 normal volunteers. ERPF and GFR of each patient will be measured utilizing radio labeled tracers, both prior to and during a continuous intravenous dopamine infusion between 1 and 30 days following injury.

The radiopharmaceuticals to be administered in this study include $^{99m}$Tc-diethylenetriamine penta-acetic acid (Tc-DTPA) and $^{131}$I-hippuran (I-HIP), used to measure GFR and ERPF, respectively.

These radiopharmaceuticals will be administered in a loading dose and then as a continuous infusion, providing an essentially minimal radiation exposure (less than a standard chest x-ray) and allowing for precise quantitation by gamma counting. The dose of Tc-DTPA and I-HIP will not exceed 4.0 mCi and 0.4 mCi, respectively. Clearance will be calculated by measurement of both plasma and urine levels of each radiopharmaceutical.

The patients will receive a priming bolus injection and sustaining infusion of each radiopharmaceutical which will be estimated based upon body size and renal function. The loading dose will be estimated to yield (after distribution) plasma levels of less than 40,000 counts per min per ml for the Tc-DTPA and < 1000 counts per min per ml for the I-HIP. The patient will then be begun on a continuous infusion of the two compounds to sustain these serum levels. After a 1-h equilibration period (during which distribution and adjustment in serum tracer levels are expected to occur), the infusion will then be continued for an 8 h study period (time 0-8 h). Blood will be collected from another site every 15 min for the 1st hour (time 0-1 h), then every 30 min during the remaining 7 h. The plasma will be separated and a measured volume will be counted in a well-type gamma counter for Tc-DTPA and I-HIP simultaneously within a few hours of collection. Timed urine samples, 1 h each, will be collected for 8 h. Aliquots of the urine samples will be counted for radioactivity in volumes and tubes equivalent to those used for plasma. Diluted proportions of the injectate will be counted to determine the dose actually given and to permit correction of spillover of counts for the $^{131}I$ channel into the $^{99m}Tc$ channel of the detector. A special computer program has been written to correct for physical decay. From blood and urine samples, we will determine GRF, ERPF, clearances of Na+, total osmolytes, $H_2O$, and creatinine, and changes in the serum thyrotropin (TSH) and plasma dopamine.

b. **Description of Procedures:**

(1) Treatment Period: Treatment should not exceed 10 days for burn patients and 32 hours for control subjects for the purpose of this study, including clinical observations after the infusion of renal function tracers and dopamine.

(2) Burn Patient Inclusion: Individuals meeting the following criteria will be eligible for entry into the study. Witnessed volunteer agreement affidavits will be obtained from each patient, or their legal guardian, prior to beginning the study.

(a) Patients admitted to the U.S. Army Institute of Surgical Research within 72 hours of the time of injury.

(b) Male or female patients older than 18 years of age. Female patients must have been surgically sterilized, be postmenopausal (over 45 years of age and the lack of menstrual

periods for at least one year), or have a negative pregnancy test prior to entry into the study.

(c) Patients with burns > 30% of the total body surface area.

(3) Burn Patient Exclusion: Patients meeting the following criteria will be excluded from the study.

(a) Patients younger than 18 years of age.

(b) Pregnant patients.

(c) Patients with burns < 30% of the total body surface area.

(d) Patients admitted to the U.S. Army Institute of Surgical Research > 3 days postburn.

(e) Patients with evidence of tachyarrhythmias, ventricular fibrillation, or evidence of cardiac ischemia on admission EKG.

(f) Patients with uncorrectable hypoxemia.

(g) Patients with uncorrectable hypovolemia as assessed by clinical and Swan-Ganz parameters.

(h) Patients with preexisting renal disease or a creatinine level > 2.0.

(i) Patients being treated with a monoamine oxidase inhibitor prior to injury.

(j) Patients with known dopamine or sulfite allergy or sensitivity.

(k) Patients with known pheochromocytomas.

(l) Patients with occlusive vascular disease, i.e., Raynaud's disease, diabetic endarteritis, and Buerger's disease.

(4) Control Subject Inclusion:

(a) Volunteers with no chronic medical problems and not currently on any medications.

(b) Male or female volunteers older than 18 years of age. Female volunteers must have been surgically sterilized, be post-menopausal (over 45 years of age and the lack of menstrual periods for at least one year), or have a negative pregnancy test prior to entry into the study.

(c) Volunteers will be obtained from the U. S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Maryland.

(5) Control Subject Exclusion:

(a) Subjects younger than 18 years of age.

(b) Pregnant subjects.

(c) Subjects with evidence of tachyarrhythmias, ventricular fibrillation, or evidence of cardiac ischemia on EKG.

(d) Subjects with preexisting renal disease or a creatinine level > 2.0.

(e) Subjects being treated with a monoamine oxidase inhibitor.

(f) Subjects with known dopamine or sulfite allergy or sensitivity.

(g) Subjects with known pheochromocytomas.

(h) Subjects with occlusive vascular disease, i.e., Raynaud's disease, diabetic endarteritis, and Buerger's disease.

(6) Procedures Prior to Study: The following information will be obtained for each patient/control subject prior to the initiation of the study.

(a) Medical history.

(b) Physical examination.

(c) ECG.

(d) Arterial blood gases (as part of routine care, only in burn patients).

(e) Laboratory profiles to include standard chemistries (phosphate included), hematology, and urine analysis.

(f) A central venous cordis catheter will be placed in burn patients only, with a pulmonary artery catheter advanced to measure cardiac output (CO), central venous pressure (CVP), and pulmonary capillary wedge pressure (PCWP). Control subjects will have a large bore peripheral IV catheter placed.

(g) A Foley catheter will be placed in each burn patient and hourly urine output prior to treatment will be recorded. Control subjects will stand to void and no catheter will be placed.

(h) The patient's and control subject's weights (in kilograms) will be obtained upon admission.

(i) A second IV catheter will be placed, at a site distant from the central venous cordis catheter, with a 3-way stopcock in place for blood withdrawal.

(j) The patient will be given an infusion (2 ml/kg/h $D_5W$ plus part of the previous ongoing fluid therapy) in order to produce a urine flow of 2 ml/min. The added infusion rate will include the infusion rate (as $D_5W$) that later (beginning at time=4 h) will be the dopamine infusion.

(k) A 2 ml/min urine flow will be established prior to time 0, at which time the bladder will be flushed with 100 ml of air. This air flush technique will be used to end all urine collections. Control subjects will stand to void and will not have a urinary catheter nor undergo bladder air flush.

(l) The urine produced during each hour of the 8-h study will be collected for electrolyte and osmolality determinations and a plasma sample, collected from the mid-point, will likewise be analyzed for electrolyte and osmolality determinations.

(m) One hour prior to time 0, the priming bolus of the Tc-DPTA and I-HIP will be injected into the infusion port of the central venus cordis catheter in burn patients and the catheter flushed with 5 ml of saline (t=0). Control subjects will have the same dosages injected into the large peripheral IV catheter. The constant infusion of the Tc-DPTA and I-HIP will then begin.

(n) Beginning at time 0, 2 ml of blood will be collected in heparinized tubes at 15-min intervals for 1 hr, then every 30 min for 7 h and placed on wet ice for gamma counting of plasma later in a well-type gamma counter. At the mid-point of each hour, a 3 ml blood sample will be taken for determination of serum electrolytes, creatinine, and osmolality. At time 0 and at each hour up to 8 h, a 6 ml blood sample will be taken for determination of serum TSH, a marker of dopamine effect, and plasma dopamine.

(7) Dosage and Administration: AT T=240 min, dopamine will be administered at a rate of 3.0 mcg/kg/min by continuous, intravenous infusion. It shall be infused through a central venous catheter, placed in one of the patient's central veins (femoral, subclavian, or internal jugular). The rate of infusion will be controlled by the use of a metered infusion pump. The weight used to calculate the dosage will be the patient's preburn weight. The control subjects will be weighed immediately prior to participating in the study. The infusion rate of dopamine (in $D_5W$) will replace a portion of the infusion rate of $D_5W$ that has been ongoing.

(8) Procedures during Study:

(a) Continuous blood pressure monitoring will be performed.

(b) Continuous cardiac monitoring will be performed using telemetry.

(c) Urine will be collected and the volume recorded each hour, using the bladder air flush technique previously described. Control subjects will stand to void.

(d) Cardiac output, CVP, and PCWP will be measured and recorded each hour in the burn patients.

(e) Continuous pulse oximetry will be performed.

(f) Dopamine infusion will be continued for 4 h (time 4-8 h), so that GFR, ERPF, all clearances, serum TSH, plasma dopamine, and hemodynamic measurements over the 4-h interval of dopamine infusion can be compared with those taken in the preceding 4 h without dopamine infusion. Pre-infusion periods of 4 h each are necessary because of hourly variation in urine residual volumes (even with a urinary catheter) and the need to allow time for the effect of dopamine to develop.

(g) The dopamine infusion will be discontinued at t=480 min.

(9) Procedures after Study:

(a) Continuous blood pressure monitoring will be continued for 24 hours post study.

(b) Continuous cardiac telemetry will be continued for 24 hours post study.

(c) Hourly urine outputs will be recorded for 24 hours post study.

(d) Cardiac output, CVP, and pulmonary capillary wedge pressure will be measured and recorded each hour for 4 hours post study.

(e) A laboratory profile, including standard chemistry and phosphate level will be obtained immediately post study and the following a.m.

(f) The clinical effectiveness of dopamine infusion will be evaluated using a standard statistical comparison of ERPF and GFR and other measurements pre-treatment and during treatment in both the burn patients and normal control groups.

c. **Determination of Number of Subjects Required.** This study is designed as a pilot study and there are no previous studies of its kind for reference. It is felt that 20 consecutive burn patients and 10 normal volunteers will provide an adequate number of subjects to assess the clinical effects of low-dose dopamine therapy in burn patients and provide some estimate of variation in measured variables with burn size and time postburn.

d. **Data Collection.** See Description of Procedures.

e. **Data Analysis Plan.** The results of this study will be evaluated using data tables prepared to compare the burn and control populations in terms of ERPF, GFR, sodium and potassium osmolar excretion, serum and plasma variables, free water clearance, and cardiac output. Analyses of variance will include hour of infusion, burn size, age, and postburn day as main sources of variation for measured variables.

## RESULTS

This protocol was approved by the Human Subjects Research Review Board located at the Office of the Surgeon General during this fiscal year. Enrollment of patients will begin during the second quarter of FY 1991.

## PRESENTATIONS/PUBLICATIONS

None.

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**RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY**

| 1. AGENCY ACCESSION | 2. DATE OF SUMMARY | REPORT CONTROL SYMBOL |
|---|---|---|
| DAOG6972 | 01 Oct 90 | DD-DR&E(AR) 636 |

| 3. DATE PREV SUM'RY | 4. KIND OF SUMMARY | 5. SUMMARY SCTY | 6. WORK SECURITY | 7. REGRADING | 8. DISP'N INSTR'N | 9. LEVEL OF SUM A. WORK UNIT |
|---|---|---|---|---|---|---|
| 01 Oct 89 | D. Chg | U | U | | CX | |

| 10. NO./CODES: | PROGRAM ELEMENT | PROJECT NUMBER | TASK AREA NUMBER | WORK UNIT NUMBER |
|---|---|---|---|---|
| a. PRIMARY | 61102A | 3M161102BS14 | FB | 301 |
| b. CONTRIBUTING | | | | |
| c. CONTRIBUTING | | | | |

11. TITLE *(Precede with Security Classification Code)* (U) Studies of Infection and Microbiologic Surveillance of Troops with Thermal Injuries

12. SUBJECT AREAS
0605 Medicine and Medical Research 0613 Microbiology 0615 Pharmacology

| 13. START DATE | 14. ESTIMATED COMPLETION DATE | 15. FUNDING ORGANIZATION | 16. PERFORMANCE METHOD |
|---|---|---|---|
| 7610 | 9909 | DA | C. In-House |

17. CONTRACT/GRANT MILITARY RELEVANCY CERTIFIED APPROVED BY [signature]

| a. DATE EFFECTIVE | d. AMOUNT |
|---|---|
| b. CONTRACT/GRANT NUMBER | |
| c. TYPE | |
| e. KIND OF AWARD | f. CUM/TOTAL |

18. RESOURCES ESTIMATE

| a. FISCAL YEARS | a. PROFESSIONAL WORKYEARS | b. FUNDS *(In thousands)* |
|---|---|---|
| 90 | 0.1 | 127 |
| 91 | 0.1 | 134 |

19. RESPONSIBLE DOD ORGANIZATION

a. NAME
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b. ADDRESS *(include zip code)*
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San Antonio, Texas 78234-5012

c. NAME OF RESPONSIBLE INDIVIDUAL
PRUITT, B A

d. TELEPHONE NUMBER *(include area code)*
512-221-2720

20. PERFORMING ORGANIZATION

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b. ADDRESS
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c. NAME OF PRINCIPAL INVESTIGATOR
MC MANUS, A T

d. TELEPHONE NUMBER *(include area code)*
512-221-3411

21. GENERAL USE
FINA

MILITARY/CIVILIAN APPLICATION M

f. NAME OF ASSOCIATE INVESTIGATOR *(if available)*

g. NAME OF ASSOCIATE INVESTIGATOR *(if available)*

22. KEYWORDS *(Precede EACH with Security Classification Code)* (U) Burns (Injuries); (U) Virulence; (U) Pseudomonas; (U) Klebsiella; (U) Staphylococcus; (U) Septicemia;

23. TECHNICAL OBJECTIVE 24 APPROACH 25 PROGRESS *(Precede text of each with Security Classification Code)*

22. (Continued) (U) Chemotherapy; (U) Chemotherapeutic Agents; (U) Antibiotics

23/24. (U) The objective of this work is to perform epidemiologic studies, study the response of significant species to topical chemotherapy modalities, and determine the relationship of antibiotic usage to sepsis control. 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) 8710 - 8809. During calander year 1987, microbiologic surveillance was carried out on 214 of the 221 admitted and discharged burn patients. More than 9,964 isolates were identified from 10,570 specimens. Gram-negative organisms represented less than 35% of isolates. Klebsiella pneumoniae was the most common Gram-negative isolate. The most common blood isolate was Staphylococcus aureus. Pseudomonas aeruginosa was recovered from the blood of one patient. No Pseudomonas aeruginosa wound infections were identified.

(U) 8810 - 8909. During calander year 1988, microbiologic surveillance was carried out on 220 of the 223 admitted and discharged burn patients. More than 9,794 isolates were identified from 11,788 specimens. Gram-negative organisms represented less than 33% of isolates. Klebsiella pneumoniae was the most common Gram-negative isolate. The most common blood

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isolate was Staphylococcus aureus. Pseudomonas aeruginosa was recovered from the blood of only three patients. No Pseudomonas aeruginosa wound infections were identified.

(U) 8910 - 9009. During calander year 1989, microbiologic surveillance was carried out on 207 of the 216 admitted and discharged burn patients. More than 7,598 isolates were identified from 12,861 specimens. Gram-negative organisms represented less than 33% of isolates. Klebsiella pneumoniae was the most common Gram-negative isolate. The most common blood isolate was Staphylococcus aureus. Pseudomonas aeruginosa was not recovered from any patients. No Pseudomonas aeruginosa wound infections were identified.

**SUPPLEMENTAL WORK UNIT INFORMATION FOR THE PROJECT ENTITLED "STUDIES OF INFECTION AND MICROBIOLOGIC SURVEILLANCE OF TROOPS WITH THERMAL INJURY"**

**Subrecord/Linking Accession Number:** Not applicable.

**Search Control Data:** W6L05K/W6L09M, 19 October 1989

**Product Identification:** For technical reports, refer to the US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Years 1977 through 1990.

**Unclassified Special Categories:** Volunteers: Adults; Children; Lab Animals: Rats; Mice; RA II

**ANNUAL RESEARCH PROGRESS REPORT**

**PROJECT NUMBER:** 3M161102BS14-00, 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 1989 - 31 December 1989**

**INVESTIGATORS**

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Basil A. Pruitt, Jr., MD, Colonel, MC

## ABSTRACT

**PROJECT NUMBER:** 3M161102BS14-00, 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 89 through 31 Dec 89

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During calendar year 1989, 207 burned patients were cultured and 7,598 isolates were identified. A relatively low colonization frequency(<33%) with Gram-negative organisms has continued for the 8th reporting period. This was also reflected in an increase in Gram-positive organisms in blood cultures. Staphylococcus aureus, Staphylococcus epidermidis, and Staphylococcus saprophyticus represented 53.9% of the bacteremia cases. The computerized microbial culture surveillance system now contains infection control and antibiotic usage data bases. This system is being evaluated for its use in predicting infecting organisms on the basis of previous sites of colonization and antibiotic usage.

## STUDIES OF INFECTION AND MICROBIOLOGIC SURVEILLANCE OF TROOPS WITH THERMAL INJURY

This report is produced from microbiology data collected for patients admitted during calendar year 1989. Data were collected from admission through disposition. This is the fifth report that is based on calendar year rather than fiscal year. This change 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 > 1,600 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 1989 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 Gram-negative organisms in sputum and urine cultures with $>10^5$ cfu/ml, *Staphylococcus aureus* isolates, *Pseudomonas aeruginosa* isolates, and other organisms as requested.

### MICROBIAL SURVEILLANCE

The microbial surveillance protocol established during fiscal year 1983 was continued during calendar year 1989 (1). The patient's wound, sputum, urine, and rectum were cultured on admission. Thereafter, sputum and urine were cultured 3X/week and stools and wound surfaces 2X/week. Patients transferred to the convalescent ward and hospitalized > 30 days were cultured 1X/week. Gentamicin-resistant Gram-negative organisms from sputum or stool specimens were screened by plating on MacConkey agar containing gentamicin sulfate (25 $\mu$g/ml).

### MICROBIOLOGIC FINDINGS IN BURN PATIENTS

A total of 207 patients admitted during 1989 were cultured. Species isolated and number of patients yielding each species are presented in Table 2. Because of the decreased host

**TABLE 1. *In vitro* Sensitivity Panels (1989)**

| Enteric Organisms | Nonenteric Gram-Negative Organisms | Gram-Positive Organisms |
|---|---|---|
| 1. Amikacin[a,b] | 1. Amikacin[a,b] | 1. Amikacin[a,b] |
| 2. Gentamicin[a,b] | 2. Gentamicin[a,b] | 2. Gentamicin[a,b] |
| 3. Ticarcillin[a] | 3. Tobramycin | 3. Tobramycin |
| 4. Mezlocillin[a,b] | 4. Ticarcillin[a] | 4. Mezlocillin[b] |
| 5. Piperacillin[a,b] | 5. Mezlocillin[a,b] | 5. Piperacillin[a,b] |
| 6. Cefotaxime[a] | 6. Piperacillin[a,b] | 6. Moxalactam[b] |
| 7. Cefoperazone | 7. Moxalactam[a,b] | 7. Cefotaxime |
| 8. Sulfadiazine | 8. Cefotaxime[a] | 8. Cefoperazone |
| 9. Netilmicin[a] | 9. Cefoperazone[a] | 9. Sulfadiazine |
| 10. Kanamycin | 10. Colistin | 10. Oxacillin[a] |
| 11. Choramphenicol | 11. Sulfadiazine[a] | 11. Cephalothin |
| 12. Tetracycline | 12. Netilmicin | 12. Vancomycin |
| 13. Cefoxitin[a] | 13. Kanamycin | 13. Chloramphenicol |
| 14. Cefamandole[a] | 14. Chloramphenicol | 14. Tetracycline |
| 15. Ampicillin[a] | 15. Tetracycline | 15. Ampicillin |
| 16. Trimethoprim | 16. Imipenem-Cilastatin[b] | 16. Imipenem-Cilastatin[b] |
| 17. Trimethoprim/Sulfamethoxazole | 17. Azlocillin[a] | 17. Clindamycin[a] |
| 18. Nalidixic Acid | 18. Norfloxacin | 18. Penicillin[a] |
| 19. Imipenem-Cilastatin[b] | 19. Aztreonam | 19. Erythromycin[a] |
| 20. Streptomycin | 20. Timentin | 20. Streptomycin |
| 21. Aztreonam | 21. Ceftazidime[a,b] | 21. Ceftazidime[a,b] |
| 22. Norfloxacin | 22. Ceftriaxone[a] | 22. Ceftriaxone |
| 23. Ceftazidime[a,b] | | |
| 24. Ceftriaxone[a] | | |

[a]Reported daily on daily clinical microbiology report (hard copy).

[b]Reported on computer screen from patient data base.

**TABLE 2. Distribution by Organism (1989)**

| Organism | Number of Isolates | Number of Patients Colonized | Organism | Number of Isolates | Number of Patients Colonized |
|---|---|---|---|---|---|
| Acinetobacter anitratus | 43 | 13 | Neisseria subflava | 1 | 1 |
| Acinetobacter lwoffii | 15 | 13 | Propionbacterium acnes | 1 | 1 |
| Aeromonas hydrophila | 8 | 2 | Proteus mirabilis | 254 | 34 |
| Alcaligenes xylosoxydans | 1 | 1 | Pseudomonas aeruginosa | 441 | 42 |
| Aspergillus flavus | 1 | 1 | Pseudomonas fluorescens | 1 | 1 |
| Bacillus | 64 | 49 | Pseudomonas maltophila | 33 | 11 |
| Bacteroides fragilis | 1 | 1 | Pseudomonas putida | 2 | 2 |
| Branhamella catarrhalis | 25 | 5 | Pseudomonas stutzeri | 1 | 1 |
| Candida albicans | 178 | 29 | Pseudomonas vesticularis | 1 | 1 |
| Candida parapsilosis | 2 | 2 | Proteus vulgaris | 10 | 3 |
| Candida rugosa | 20 | 8 | Providencia alcalifaciens | 4 | 1 |
| Candida stellata | 17 | 7 | Providencia rettgeri | 4 | 2 |
| Candida tropicalis | 15 | 4 | Providencia stuartii | 1 | 1 |
| Citrobacter diversus | 94 | 19 | Serratia marcescens | 82 | 17 |
| Citrobacter freundii | 17 | 5 | Staphylococcus aureus | 1568 | 144 |
| Clostridium clostridiiforme | 1 | 1 | Staphylococcus epidermidis | 559 | 143 |
| Clostridium difficile | 5 | 4 | Staphylococcus saprophyticus | 127 | 67 |
| Corynebacterium species | 5 | 4 | Alpha Streptococcus | 27 | 24 |
| Enterobacter aerogenes | 147 | 32 | Beta hemolytic Streptococcus | 3 | 3 |
| Enterobacter agglomerans | 36 | 15 | Beta Streptococcus, Not Group A, B, or D | 34 | 20 |
| Enterobacter cloacae | 206 | 47 | Group A Nonhemolytic Beta Streptococcus | 4 | 2 |
| Escherichia coli | 294 | 75 | Group B Streptococcus | 36 | 10 |
| Edwardsiella vulneris | 1 | 1 | Group C Streptococcus | 2 | 1 |
| Flavobacterium IIB | 1 | 1 | Group D Enterococcus | 352 | 75 |
| Haemophilus influenzae | 3 | 2 | Group D Streptococcus, Not Enterococcus | 235 | 104 |
| Haemophilus parainfluenzae | 2 | 2 | Group F Streptococcus | 36 | 10 |
| Klebsiella oxytoca | 25 | 14 | Nonhemolytic Streptococcus | 4 | 4 |
| Klebsiella ozaenae | 2 | 2 | Nonhemolytic Streptococcus, Not Group D | 442 | 143 |
| Klebsiella pneumoniae | 436 | 76 | Streptococcus intermedius | 1 | 1 |
| Micrococcus luteus | 1 | 1 | Streptococcus pneumoniae | 27 | 16 |
| Morganella morganii | 50 | 19 | Streptococcus viridans | 1213 | 177 |
| Morganella species | 2 | 2 | True Fungi Species (Other) | 147 | 52 |
| Neisseria lactamcia | 1 | 1 | | | |
| Neisseria mucosa | 202 | 77 | | | |
| Neisseria sicca | 44 | 26 | | | |
| Neisseria species | 3 | 2 | | | |

Total Number of Isolates=7598

Total Number of Patients=207

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 76% 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 yeasts are shown in Figure 2. Of particular note is the continued decline of Gram-negative isolates. Pseudomonas aeruginosa was not in the top 10 organisms, and was recovered from only 25 of the 207 patients. This frequency was not significantly different from calendar years 1984-88.

## FLORA RECOVERED FROM RESPIRATORY SYSTEM SPECIMENS

A total of 4,758 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 83.1% of the respiratory isolates.

## FLORA RECOVERED FROM WOUND SURFACE SPECIMENS

A total of 1,621 contact plate surface cultures were taken and 846 isolates were made. Relative frequencies of isolated species are presented in Figure 3. Subsurface flora, as measured by biopsy specimens, was measured in 495 biopsies taken from 120 patients. Organisms were recovered from 42 of the biopsied patients. The 10 most common organisms are presented in Table 5. Filamentous fungi remained the principal isolate. Pseudomonas aeruginosa was recovered from four biopsies taken from three 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 201 patients yielded 666 isolates. The 10 most common species are presented in Table 6. The top 10 organisms isolated from urine specimens with $> 10^5$ cfu/ml are presented in Table 7.

## FLORA RECOVERED FROM BLOOD CULTURES

Blood cultures were obtained from 119 patients for a total of 697 cultures. The principal organisms recovered are listed in Table 8. Positive cultures were obtained from 24 patients and 35 isolates were made from 33 positive cultures. Thirty cases of bacteremia were noted. A case of bacteremia was defined as isolation of an organism once or more than once within a 30-day period.

**TABLE 3. Ten Most Frequent Isolates (1989)**

| Organism | Number of Patients Colonized | % Patients | Number of Isolates | %Total Isolates |
|---|---|---|---|---|
| Staphylococcus aureus | 144 | 69.6 | 1568 | 20.6 |
| Streptococcus viridans | 177 | 85.5 | 1213 | 16.0 |
| Staphylococcus epidermidis | 143 | 69.1 | 559 | 7.4 |
| Nonhemolytic Streptococcus, Not Group D | 143 | 69.1 | 442 | 5.8 |
| Pseudomonas aeruginosa | 42 | 20.3 | 441 | 5.8 |
| Klebsiella pneumoniae | 76 | 36.7 | 436 | 5.7 |
| Group D Enterococcus | 75 | 36.2 | 352 | 4.6 |
| Escherichia coli | 75 | 36.2 | 294 | 3.9 |
| Proteus mirabilis | 34 | 16.4 | 254 | 3.3 |
| Group D Streptococcus, not Enterococcus | 104 | 50.2 | 235 | 3.1 |

Total Number of Patients Cultured= 207
Total Number of Isolates = 7598

1

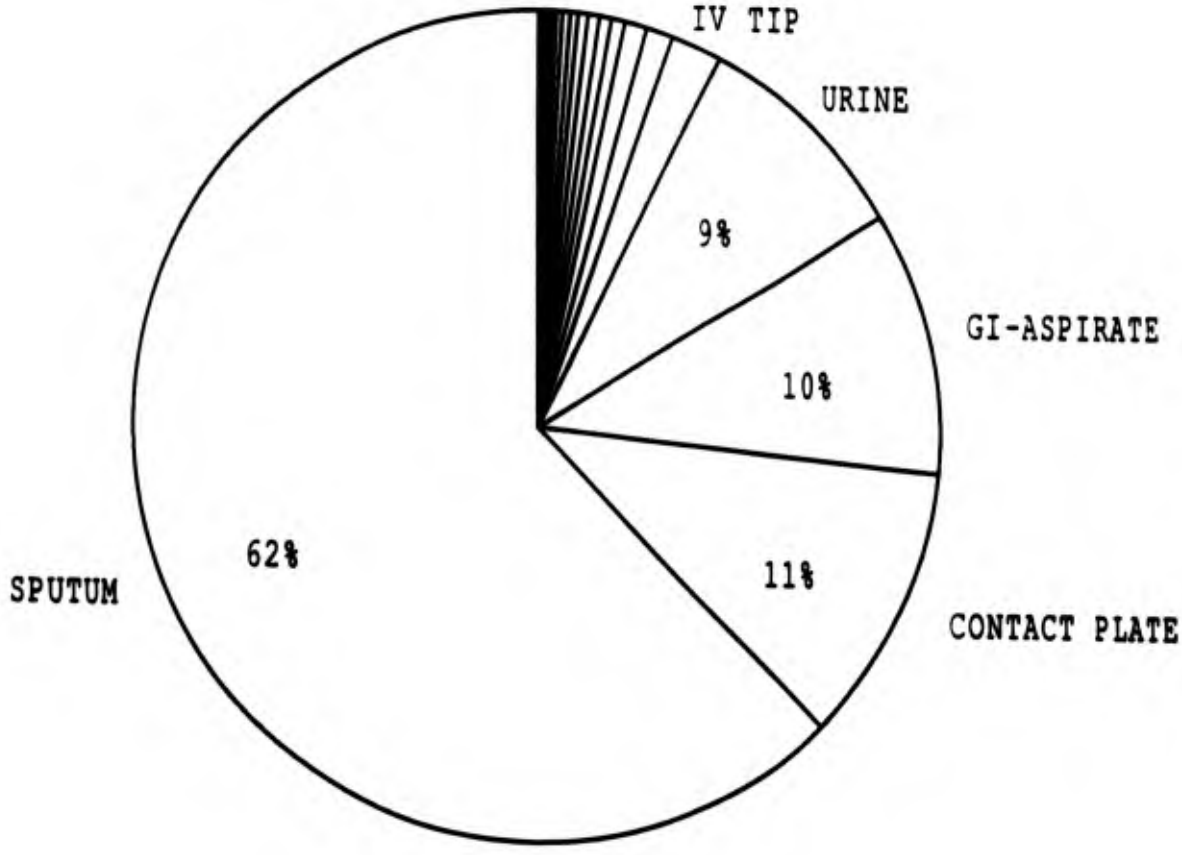



**FIGURE 1.** Display o the relative frequency of specimen sources yielding isolates in 1989.

2A

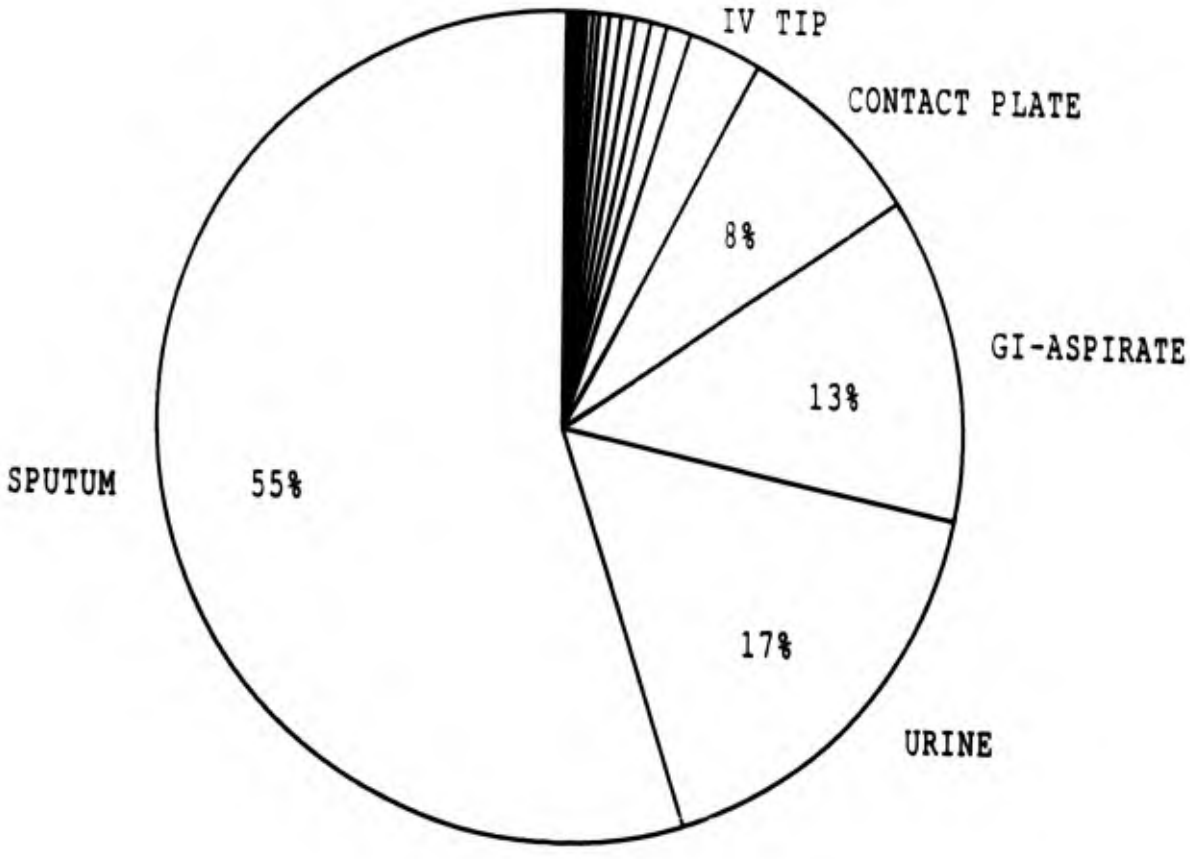



**FIGURE 2A.** Display of the relative frequency of specimen sources yielding Gram-negative organisms.

2B

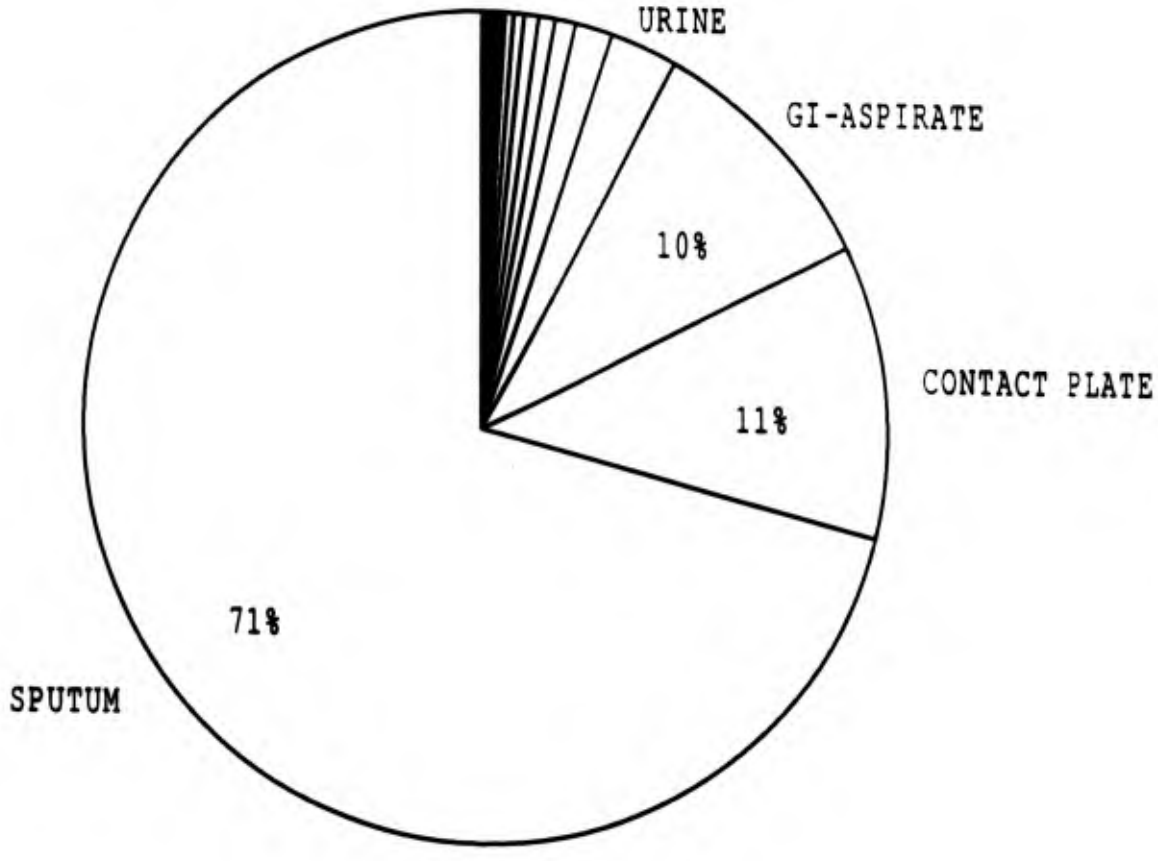



**FIGURE 2B.** Display of the relative frequency of specimen sources yielding Gram-positive organisms.

2C

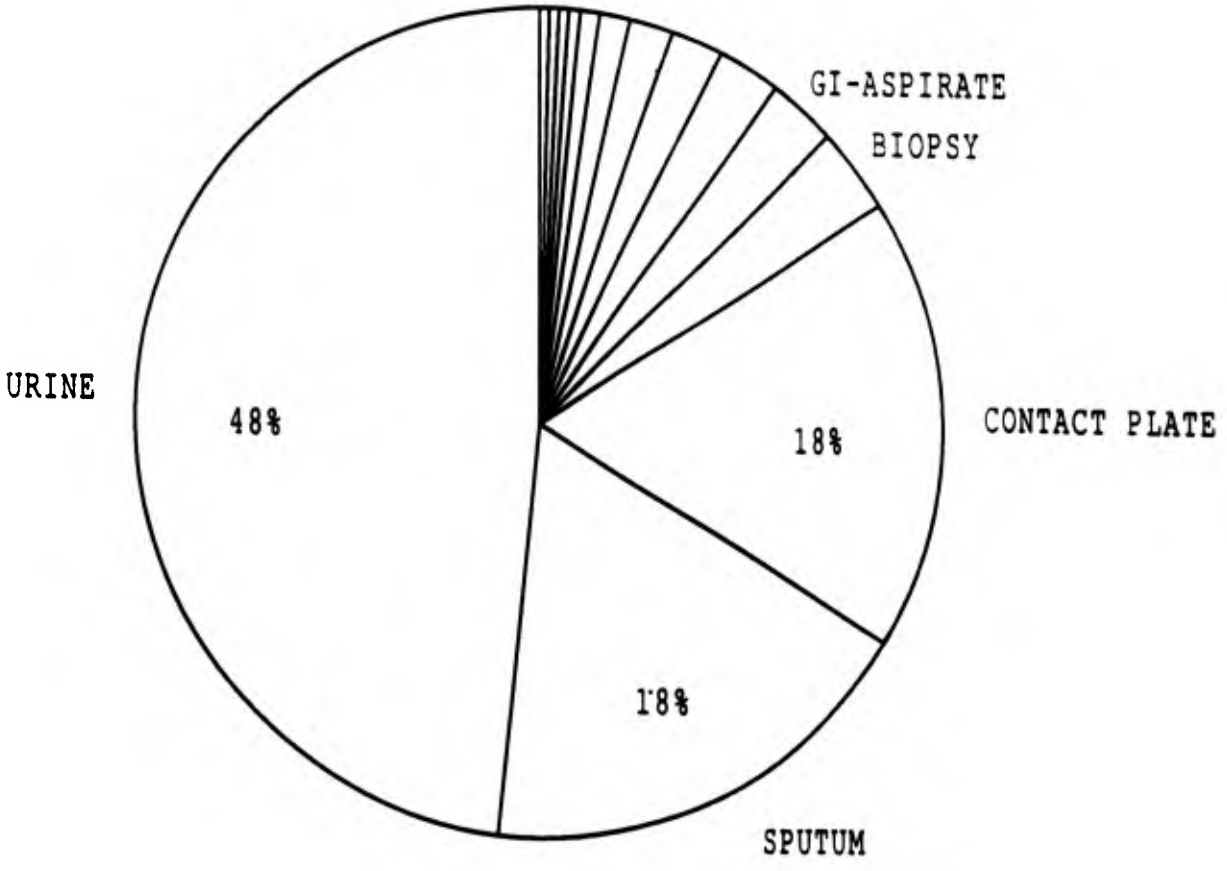



**FIGURE 2C.** Display of the relative frequency of specimen sources yielding yeast-like organisms.

**TABLE 4. Ten Most Frequent Isolates from Respiratory Sources (1989)**

| Organism | Number of Patients Colonized | % Patients | Number of Isolates | %Total Isolates |
|---|---|---|---|---|
| Streptococcus viridans | 173 | 94.5 | 1100 | 23.1 |
| Staphylococcus aureus | 117 | 63.9 | 1063 | 22.3 |
| Nonhemolytic Streptococcus, Not Group D | 137 | 74.9 | 386 | 8.1 |
| Pseudomonas aeruginosa | 21 | 11.5 | 296 | 6.2 |
| Staphylococcus epidermidis | 98 | 53.6 | 285 | 6.0 |
| Klebsiella pneumoniae | 48 | 26.2 | 222 | 4.7 |
| Neisseria mucosa | 69 | 37.7 | 180 | 3.8 |
| Group D Streptococcus, Not Enterococcus | 98 | 53.6 | 218 | 4.6 |
| Group D Enterococcus | 36 | 19.7 | 113 | 2.4 |
| Enterobacter cloacae | 27 | 14.8 | 107 | 2.2 |

Total Number of Patients Cultured = 183
Total Number of Isolates = 4758

3

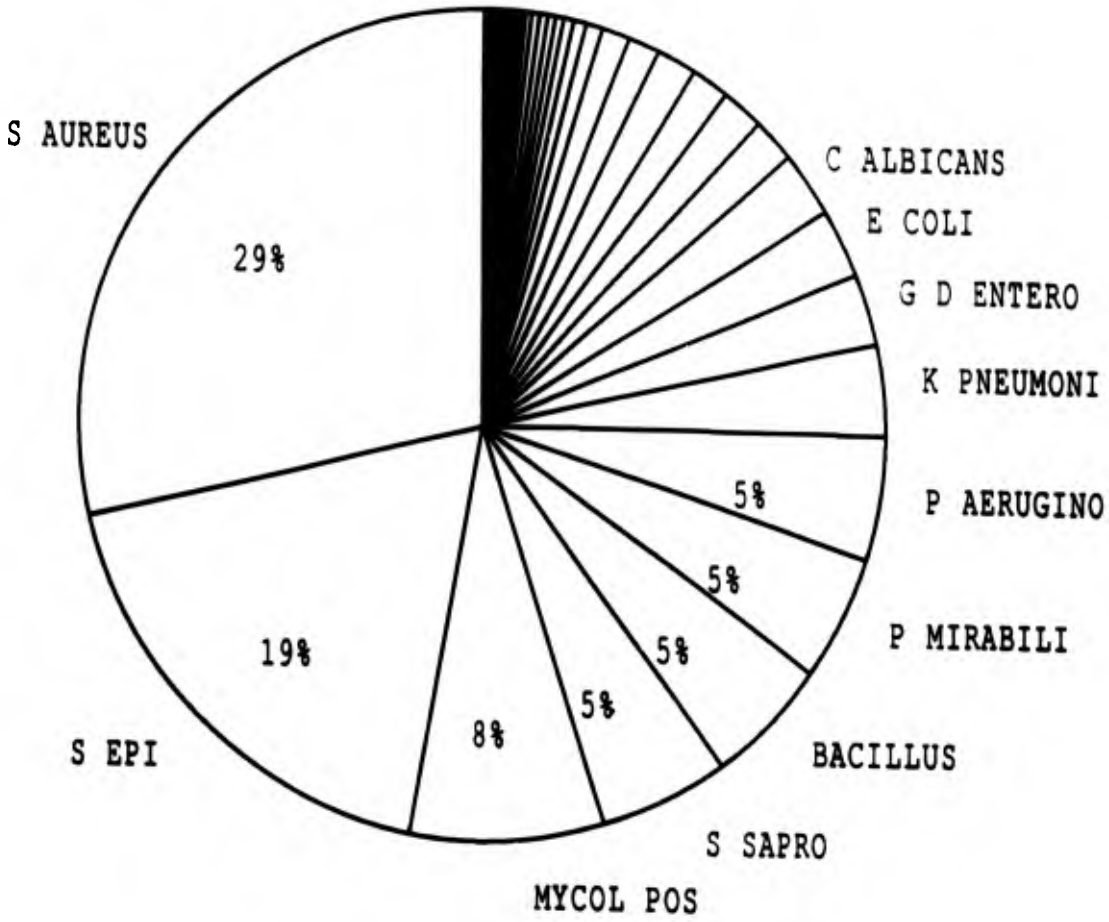



**FIGURE 3.** Display of the relative frequency of organism types isolated from surface wound cultures.

**TABLE 5. Principal Organisms Recovered in Biopsy Specimens (1989)**

| Organism | Number of Patients Colonized | % Patients | Number of Isolates | %Total Isolates |
|---|---|---|---|---|
| Filamentous fungi | 30 | 25.0 | 71 | 50.0 |
| Candida albicans | 7 | 5.8 | 16 | 11.3 |
| Staphylococcus aureus | 7 | 5.8 | 8 | 5.6 |
| Enterobacter aerogenes | 2 | 1.7 | 6 | 4.2 |
| Staphylococcus epidermidis | 5 | 4.2 | 5 | 3.5 |
| Klebsiella pneumoniae | 1 | 0.8 | 5 | 3.5 |
| Streptococcus viridans | 4 | 3.3 | 4 | 2.8 |
| Pseudomonas aeruginosa | 3 | 2.5 | 4 | 2.8 |
| Escherichia coli | 3 | 2.5 | 4 | 2.8 |
| Staphylococcus saprophyticus | 2 | 1.7 | 4 | 2.8 |

Total Number of Patients Biopsied=120
Total Number of Isolates =142
Biopsies Taken =495

**TABLE 6. Ten Most Frequent Organisms from Urinary Specimens (1989)**

| Organism | Number of Patients Colonized | %Patients | Number of Isolates | %Total Isolates |
|---|---|---|---|---|
| Escherichia coli | 46 | 22.9 | 108 | 16.2 |
| Candida albicans | 16 | 8.0 | 95 | 14.3 |
| Klebsiella pneumoniae | 38 | 18.9 | 84 | 12.6 |
| Proteus mirabilis | 27 | 13.4 | 81 | 12.2 |
| Group D Enterococcus | 29 | 14.4 | 51 | 7.7 |
| Pseudomonas aeruginosa | 21 | 10.4 | 36 | 5.4 |
| Streptococcus epidermidis | 15 | 7.5 | 29 | 4.4 |
| Morganella morganii | 13 | 6.5 | 25 | 3.8 |
| Staphylococcus aureus | 14 | 7.0 | 19 | 2.8 |
| Enterobacter aerogenes | 9 | 4.5 | 17 | 2.6 |

Total Number of Patients Cultured=201
Total Number of Isolates =666

**TABLE 7. Ten Most Frequent Organisms from Urinary Specimens with >$10^5$ cfu/ml (1989)**

| Organism | Number of Patients Colonized | %Patients | Number of Isolates | %Total Isolates |
|---|---|---|---|---|
| Escherichia coli | 25 | 36.2 | 44 | 15.4 |
| Candida albicans | 13 | 18.8 | 44 | 15.4 |
| Proteus mirabilis | 20 | 29.0 | 32 | 11.2 |
| Group D Enterococcus | 17 | 24.6 | 30 | 10.5 |
| Klebsiella pneumoniae | 18 | 26.1 | 27 | 9.4 |
| Pseudomonas aeruginosa | 16 | 23.2 | 20 | 7.0 |
| Staphylococcus epidermidis | 5 | 7.2 | 13 | 4.5 |
| Enterobacter cloacae | 6 | 8.7 | 11 | 3.8 |
| Morganella morganii | 7 | 10.1 | 10 | 3.5 |
| Enterobacter aerogenes | 8 | 11.6 | 9 | 3.1 |

Total Number of Patients with >$10^5$ cfu/ml=69
Total Number of Isolates =286

**TABLE 8. Principal Organisms Found in Blood Cultures (1989)**

| Organism | Number of Patients | %Patients Cultured | Number of Cases | %Cases | Number of Isolates | %Total Isolates |
|---|---|---|---|---|---|---|
| Staphylococcus aureus | 9 | 7.6 | 9 | 29.0 | 13 | 37.1 |
| Staphylococcus epidermidis | 5 | 4.2 | 5 | 16.1 | 5 | 14.3 |
| Proteus mirabilis | 2 | 1.7 | 2 | 6.5 | 2 | 5.7 |
| Streptococcus pneumoniae | 2 | 1.7 | 2 | 6.5 | 2 | 5.7 |
| Staphylococcus saprophyticus | 2 | 1.7 | 2 | 6.5 | 2 | 5.7 |
| Bacillus Sp | 1 | 0.8 | 1 | 3.2 | 1 | 2.9 |
| Bacteroides fragilis | 1 | 0.8 | 1 | 3.2 | 1 | 2.9 |
| Bacteroides Sp | 1 | 0.8 | 1 | 3.2 | 1 | 2.9 |
| Clostridium clostridiforme | 1 | 0.8 | 1 | 3.2 | 1 | 2.9 |
| Group D Streptococcus, not Enterococcus | 1 | 0.8 | 1 | 3.2 | 1 | 2.9 |
| Klebsiella pneumoniae | 1 | 0.8 | 1 | 3.2 | 1 | 2.9 |
| Nonhemolytic Streptococcus | 1 | 0.8 | 1 | 3.2 | 1 | 2.9 |
| Propionbactrium acnes | 1 | 0.8 | 1 | 3.2 | 1 | 2.9 |
| Pseudomonas maltophilia | 1 | 0.8 | 1 | 3.2 | 1 | 2.9 |
| Streptococcus intermedius | 1 | 0.8 | 1 | 3.2 | 1 | 2.9 |
| Streptococcus viridans | 1 | 0.8 | 1 | 3.2 | 1 | 2.9 |
| Total Number of Patients Cultured=119 | | Total Number of Patients Positives=24 | | | | |
| Total Number of Isolates =35 | | Total Cases (Patients/Species) =30 | | | | |
| Total Number of Cultures =697 | | | | | | |

Intravenous catheter tips were cultured from 89 patients. Isolations were made from 55 patients and 152 isolates were made. Data are presented in Table 9. These data show an unexpectedly high incidence of contamination or poor specimen collection technique.

## SUMMARY OF ANTIBIOTIC TESTING

A total of 4,062 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 3,204 isolates. Figure 6 displays the relative frequency of tested organisms. Figure 7 displays the frequency of resistant species. *Staphylococcus aureus* represented 49% of the gentamicin-resistant isolates. Only 49 Gram-negative isolates of 1577 strains tested were resistant to gentamicin (3.1%). This is the lowest percentage ever reported from the Institute and is a direct marker of the success of infection control isolation techniques in preventing the accumulation of a resistant Gram-negative flora.

**Staphylococcus aureus.** The sources of *Staphylococcus aureus* strains tested for *in vitro* antibiotic activity are presented in Figure 8. With the intention of using Gentamicin resistance as an indicator of a nosocomial versus a community strain origin, antiobiotic data are presented separately in Tables 10A and 10B and histograms are presented in Figures 9A and 9B. As can be seen, this segregation correlates well with the presence of other antibiotic resistances. Methicillin (Oxicillin) resistance remains endemic in the burn center despite highly successful control and prevention of cross contamination with antibiotic resistant strains of *P. aeruginosa* and other gram negative organisms.

**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 us 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 292 isolates were tested for *in vitro* sensitivities to antibiotics. The sources of isolation for tested strains are presented Figure 14. The results of *in vitro* antibiotic testing are presented in Table 12. Histogram displays of the distributions of zone sizes for selected antibiotics are presented in Figure 15.

**TABLE 9. Ten Most Frequent Organisms from Intravenous Catheters (1989)**

| Organisms | Number of Patients Colonized | %Patients | Number of Isolates | %Total Isolates |
|---|---|---|---|---|
| Staphylococcus epidermidis | 20 | 22.5 | 27 | 17.8 |
| Staphylococcus aureus | 12 | 13.5 | 20 | 13.2 |
| Proteus mirabilis | 9 | 10.1 | 17 | 11.2 |
| Klebsiella pneumoniae | 11 | 12.4 | 12 | 7.9 |
| Group D Enterococcus | 7 | 7.9 | 10 | 6.6 |
| Escherichia coli | 8 | 9.0 | 9 | 5.9 |
| Enterobacter cloacae | 7 | 7.9 | 9 | 5.9 |
| Enterobacter aerogenes | 3 | 3.4 | 9 | 5.9 |
| Pseudomonas aeruginosa | 6 | 6.7 | 8 | 5.3 |
| Staphylococcus saprophyticus | 4 | 4.5 | 5 | 3.2 |

Total Number of Patients Cultured=89
Total Number of Isolates =152

4

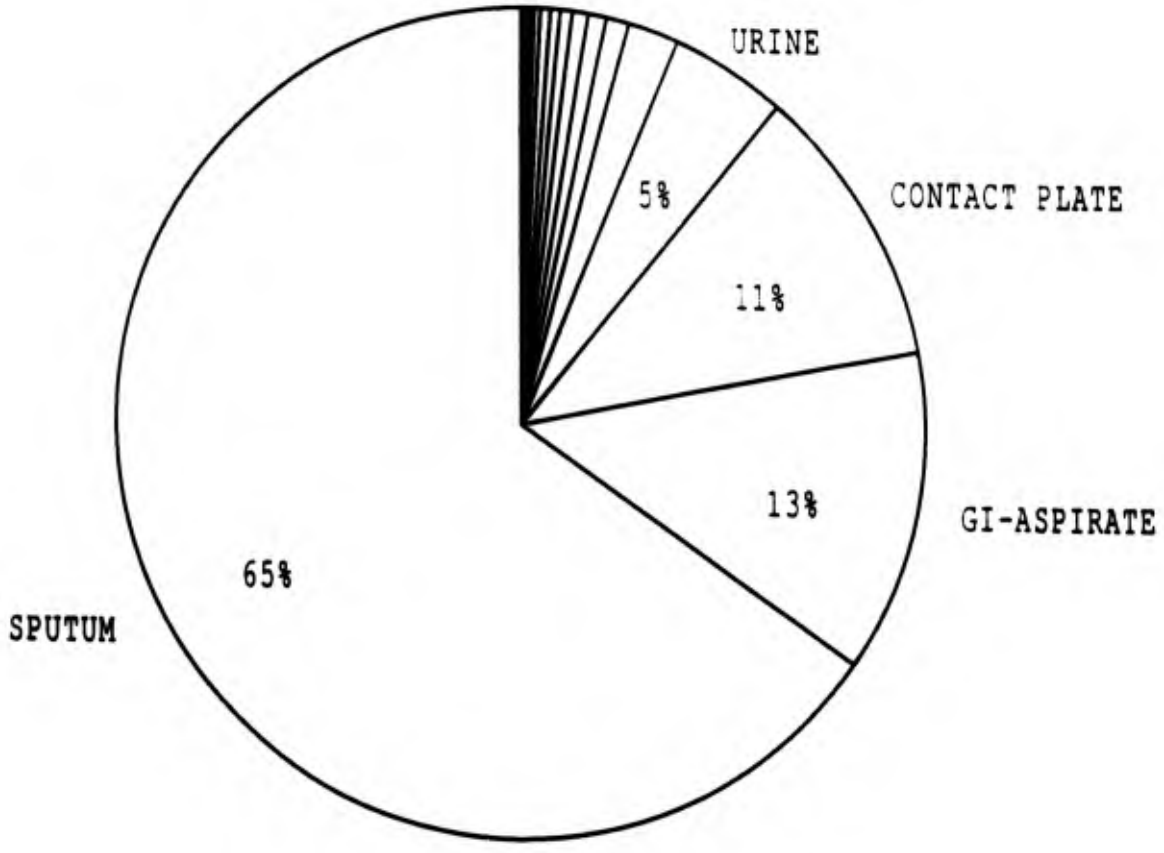



**FIGURE 4.** Display of the relative frequency of sources yielding organisms tested for <u>in vivo</u> sensitivity to antibiotics in 1989.

5

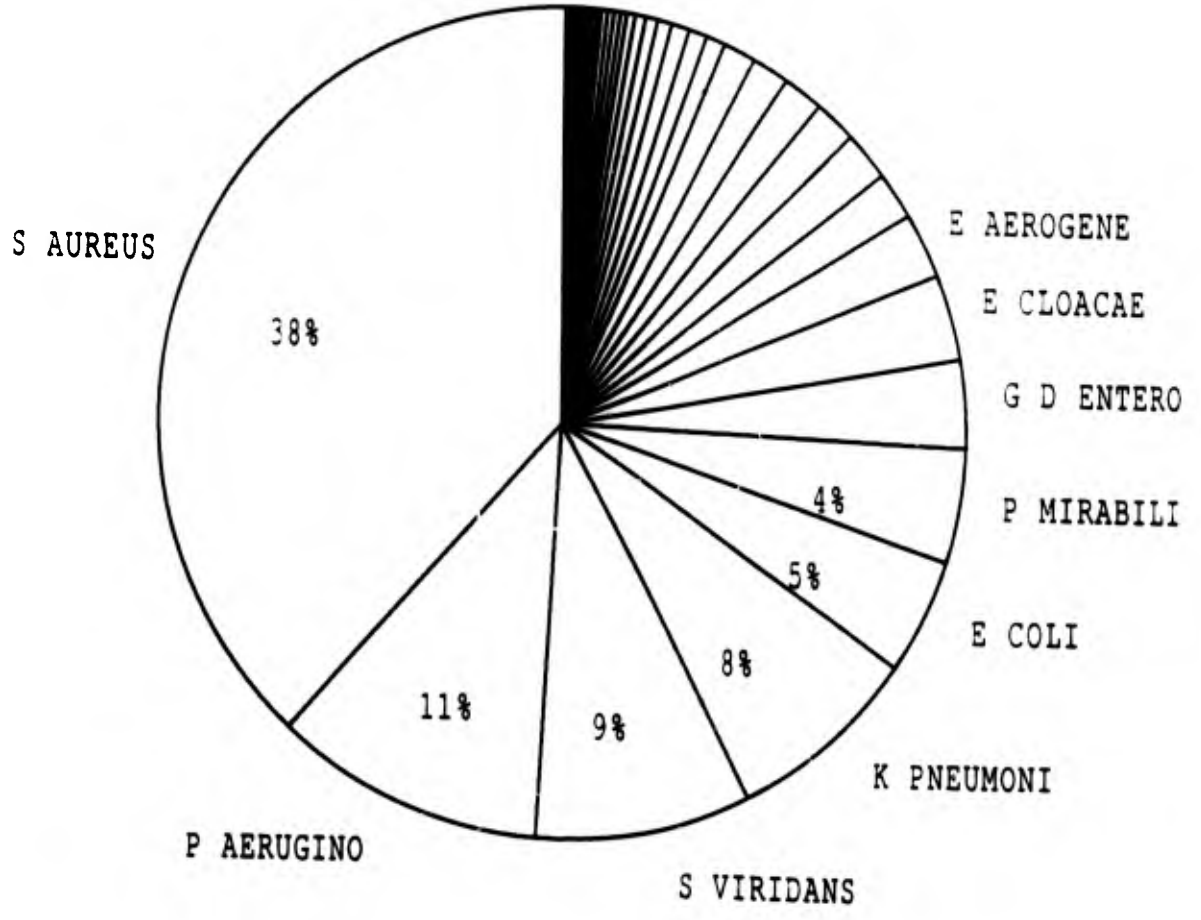



**FIGURE 5**. Display of the relative frequency of organisms tested for <u>in vitro</u> sensitivity to antibiotics in 1989.

6

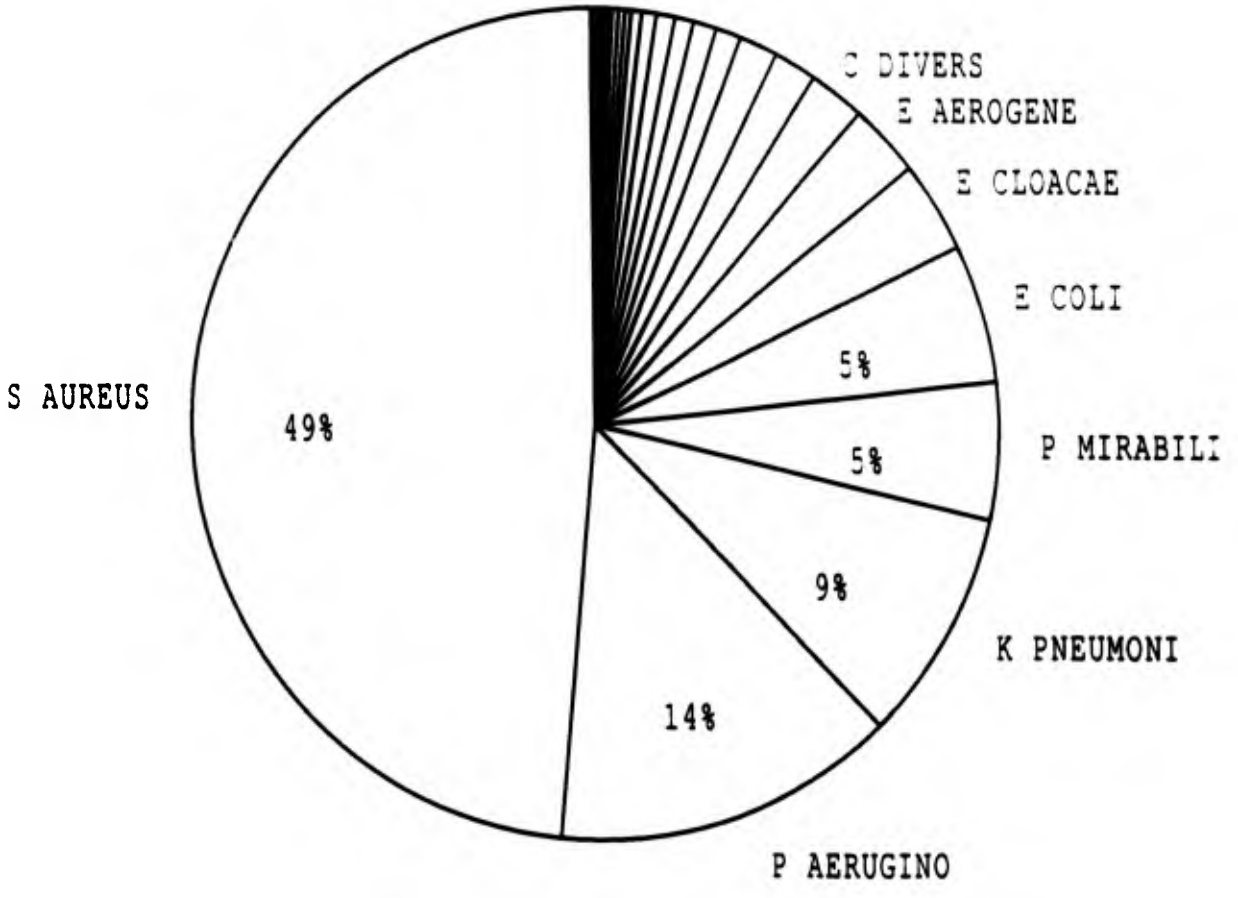



**FIGURE 6.** Display of the relative frequency of organisms tested for <u>in vitro</u> sensitivity to gentamicin in 1989.

7

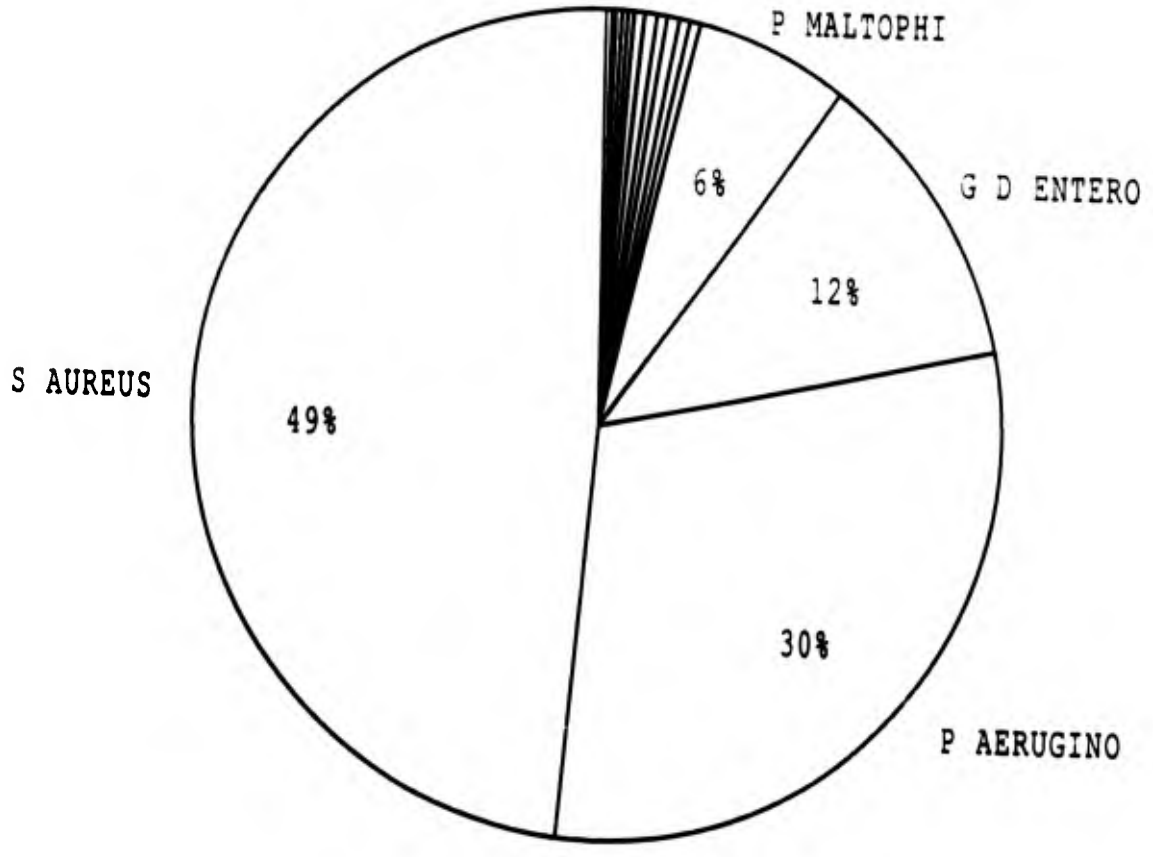



**FIGURE 7.** Display of the relative frequency of gentamicin-resistant organisms isolated in 1989.

8

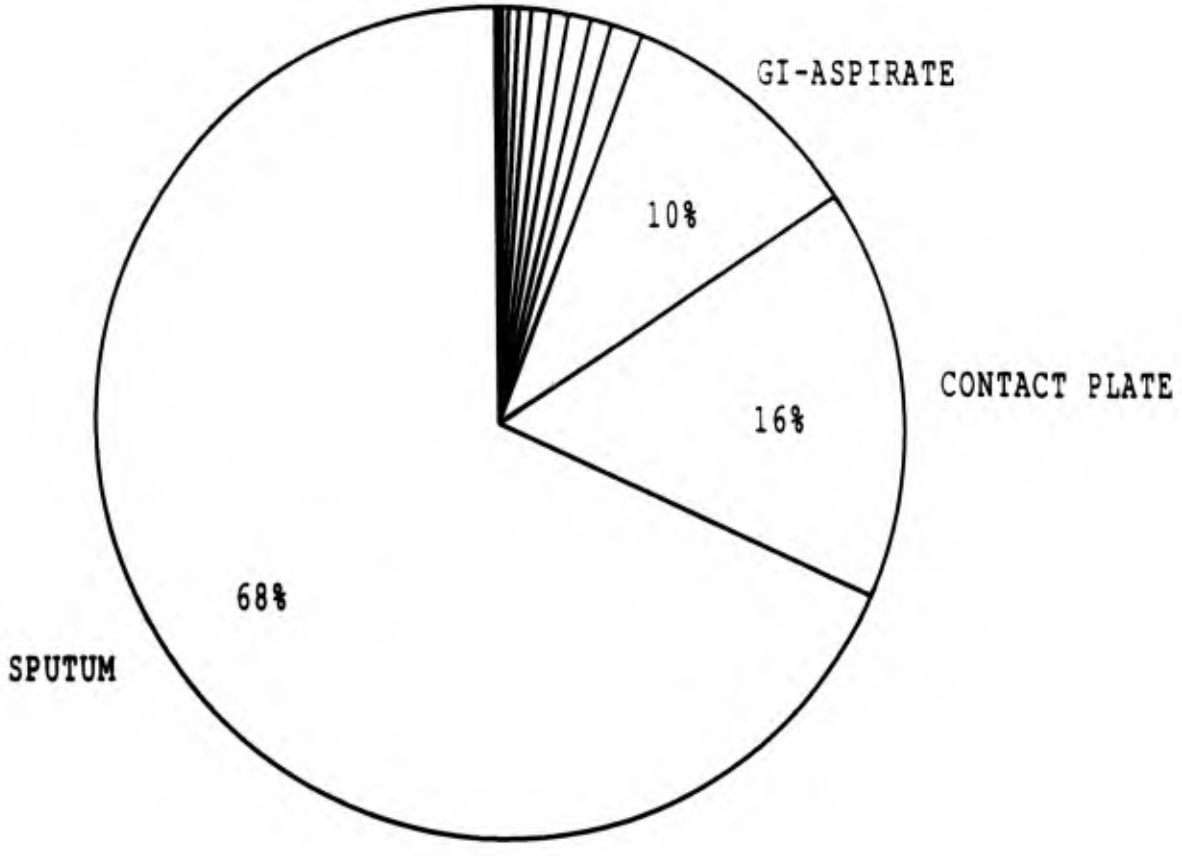


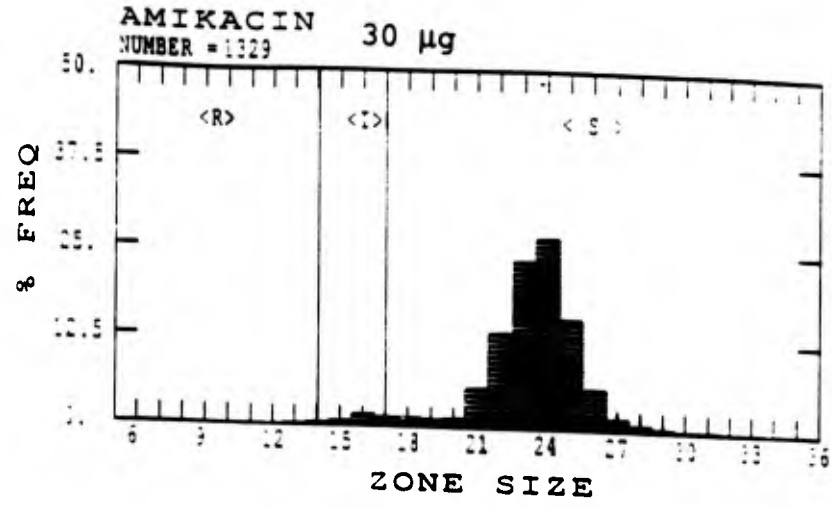

**FIGURE 8.** Display of the relative frequency of sources yielding Staphylococcus aureus tested for in vitro sensitivity to antibiotics in 1989.

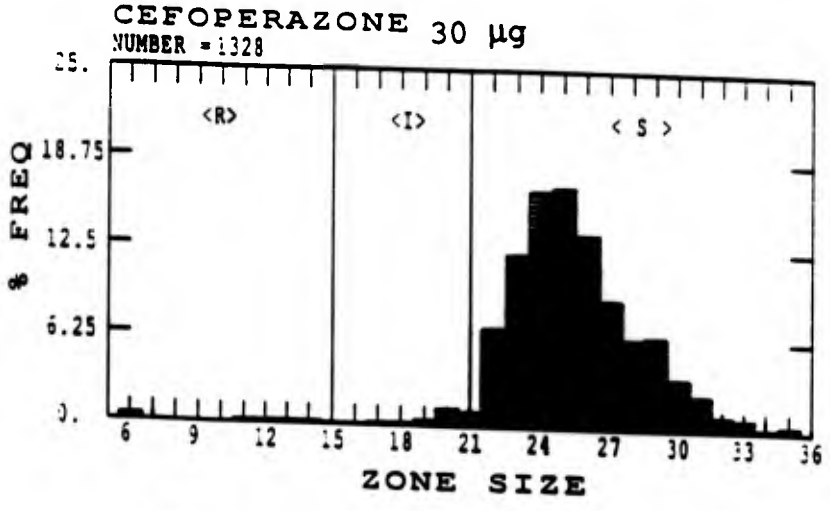
**TABLE 10A. Antibiotic Sensitivity Data for Staphylococcus aureus Sensitive to Gentamicin**

| Antibiotic | RESISTANT % | RESISTANT Number | INTERMEDIATE % | INTERMEDIATE Number | SENSITIVE % | SENSITIVE Number | Total Number |
|---|---|---|---|---|---|---|---|
| Amikacin | 0.61% | 8 | 3.13% | 41 | 96.27% | 1263 | 1312 |
| Ampicillin | 1.83% | 24 | 3.66% | 48 | 94.52% | 1241 | 1313 |
| Cefotaxime | 2.59% | 34 | 0.99% | 13 | 96.42% | 1266 | 1313 |
| Cefsulodin | 0.00% | 0 | 0.00% | 0 | 100.00% | 1 | 1 |
| Cefoperazone | 1.07% | 14 | 2.44% | 32 | 96.49% | 1265 | 1311 |
| Ceftazidime | 2.68% | 35 | 13.50% | 176 | 83.82% | 1093 | 1304 |
| Ceftriaxone | 0.00% | 0 | 0.00% | 0 | 100.00% | 1310 | 1310 |
| Cephalothin | 0.69% | 9 | 0.23% | 3 | 99.09% | 1301 | 1313 |
| Chloramphenicol | 0.31% | 4 | 0.00% | 0 | 99.69% | 1302 | 1306 |
| Clindamycin | 2.44% | 32 | 0.00% | 0 | 97.56% | 1282 | 1314 |
| Erythromycin | 11.66% | 153 | 0.59% | 9 | 87.65% | 1150 | 1312 |
| Gentamicin | 0.00% | 0 | 0.00% | 0 | 100.00% | 1314 | 1314 |
| Imipenim-Cilastatin sodium | 1.07% | 14 | 0.08% | 1 | 98.86% | 1299 | 1314 |
| Methicillin | 0.00% | 0 | 0.00% | 0 | 100.00% | 1 | 1 |
| Mezlocillin | 1.75% | 23 | 19.66% | 258 | 78.58% | 1031 | 1312 |
| Moxalactam | 3.58% | 47 | 22.39% | 294 | 74.03% | 972 | 1313 |
| Oxacillin | 5.18% | 68 | 2.52% | 33 | 92.30% | 1211 | 1312 |
| Penicillin | 57.99% | 762 | 31.43% | 413 | 10.58% | 139 | 1314 |
| Piperacillin | 1.75% | 23 | 21.19% | 278 | 77.06% | 1011 | 1312 |
| Streptomycin | 0.38% | 5 | 2.29% | 30 | 97.33% | 1277 | 1312 |
| Sulfadiazine | 1.88% | 24 | 3.52% | 45 | 94.61% | 1211 | 1280 |
| Tetracycline | 7.09% | 93 | 2.59% | 34 | 90.32% | 1185 | 1312 |
| Tobramycin | 3.82% | 50 | 1.15% | 15 | 95.04% | 1245 | 1310 |
| Vancomycin | 0.00% | 0 | 0.00% | 0 | 100.00% | 1314 | 1314 |

**TABLE 10B. Antibiotic Sensitivity Data for Staphylococcus aureus Resistant to Gentamicin (1989)**

| Antibiotic | RESISTANT % | RESISTANT Number | INTERMEDIATE % | INTERMEDIATE Number | SENSITIVE % | SENSITIVE Number | Total Number |
|---|---|---|---|---|---|---|---|
| Amikacin | 9.01% | 20 | 50.45% | 112 | 40.54% | 90 | 222 |
| Ampicillin | 60.81% | 135 | 1.35% | 3 | 37.84% | 84 | 222 |
| Cefoperazone | 1.81% | 4 | 58.37% | 129 | 39.82% | 88 | 221 |
| Cefotaxime | 22.97% | 51 | 54.05% | 120 | 22.97% | 51 | 222 |
| Cefsulodin | 0.00% | 0 | 0.00% | 0 | 100.00% | 1 | 1 |
| Ceftazidime | 68.78% | 152 | 14.48% | 32 | 16.74% | 37 | 221 |
| Ceftriaxone | 0.00% | 0 | 0.00% | 0 | 100.00% | 221 | 221 |
| Cephalothin | 2.25% | 5 | 1.80% | 4 | 95.95% | 213 | 222 |
| Chloramphenicol | 2.25% | 5 | 0.45% | 1 | 97.30% | 216 | 222 |
| Clindamycin | 4.50% | 10 | 0.90% | 2 | 94.59% | 210 | 222 |
| Erythromycin | 18.92% | 42 | 3.60% | 8 | 77.48% | 172 | 222 |
| Gentamicin | 96.85% | 215 | 3.15% | 7 | 0.00% | 0 | 222 |
| Imipenem-Cilastatin sodium | 0.00% | 0 | 0.45% | 1 | 99.55% | 220 | 221 |
| Mezlocillin | 62.16% | 138 | 3.60% | 8 | 34.23% | 76 | 222 |
| Moxalactum | 67.87% | 150 | 19.91% | 44 | 12.22% | 27 | 221 |
| Oxacillin | 65.77% | 146 | 13.06% | 29 | 21.17% | 47 | 222 |
| Penicilin | 85.91% | 189 | 14.09% | 31 | 0.00% | 0 | 220 |
| Piperacillin | 62.16% | 138 | 2.25% | 5 | 35.59% | 79 | 222 |
| Streptomycin | 83.33% | 185 | 2.70% | 6 | 13.96% | 31 | 222 |
| Sulfdiazine | 65.58% | 141 | 17.21% | 37 | 17.21% | 37 | 215 |
| Tetracyline | 6.31% | 14 | 2.70% | 6 | 90.99% | 202 | 222 |
| Tobramycin | 85.97% | 190 | 11.76% | 26 | 2.26% | 5 | 221 |
| Vancomycin | 0.00% | 0 | 0.00% | 0 | 100.00% | 222 | 222 |



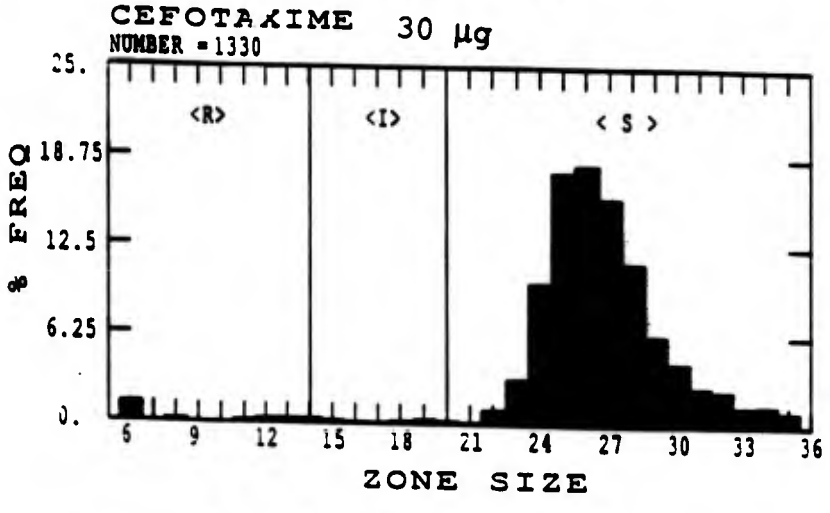




**FIGURE 9A.** Histogram display of the distribution of zones of inhibition of growth of gentamicin-sensitive Staphylococcus aureus.

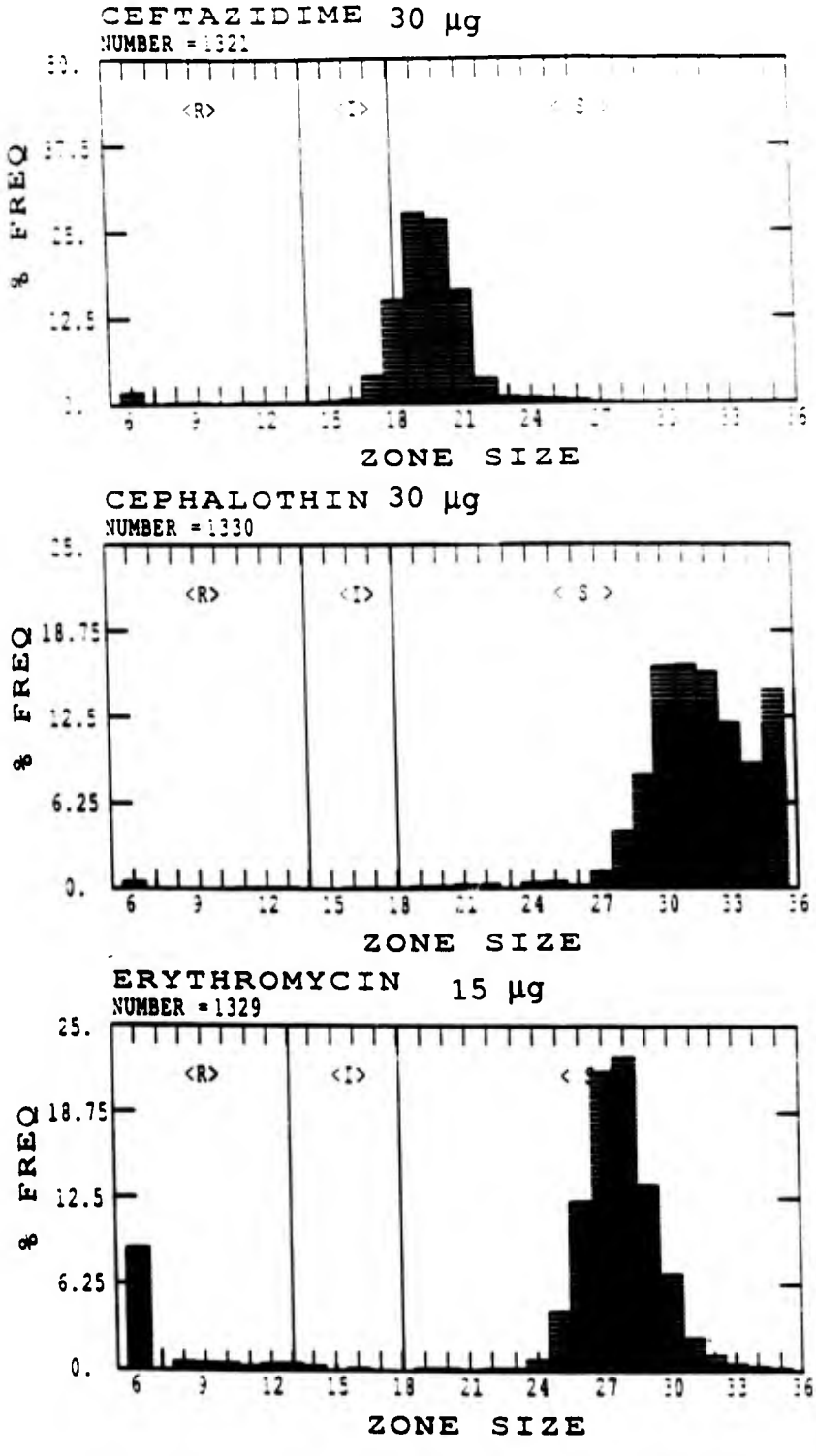



**FIGURE 9A.** Histogram display of the distribution of zones of inhibition of growth of gentamicin-sensitive Staphylococcus aureus. (Continued)

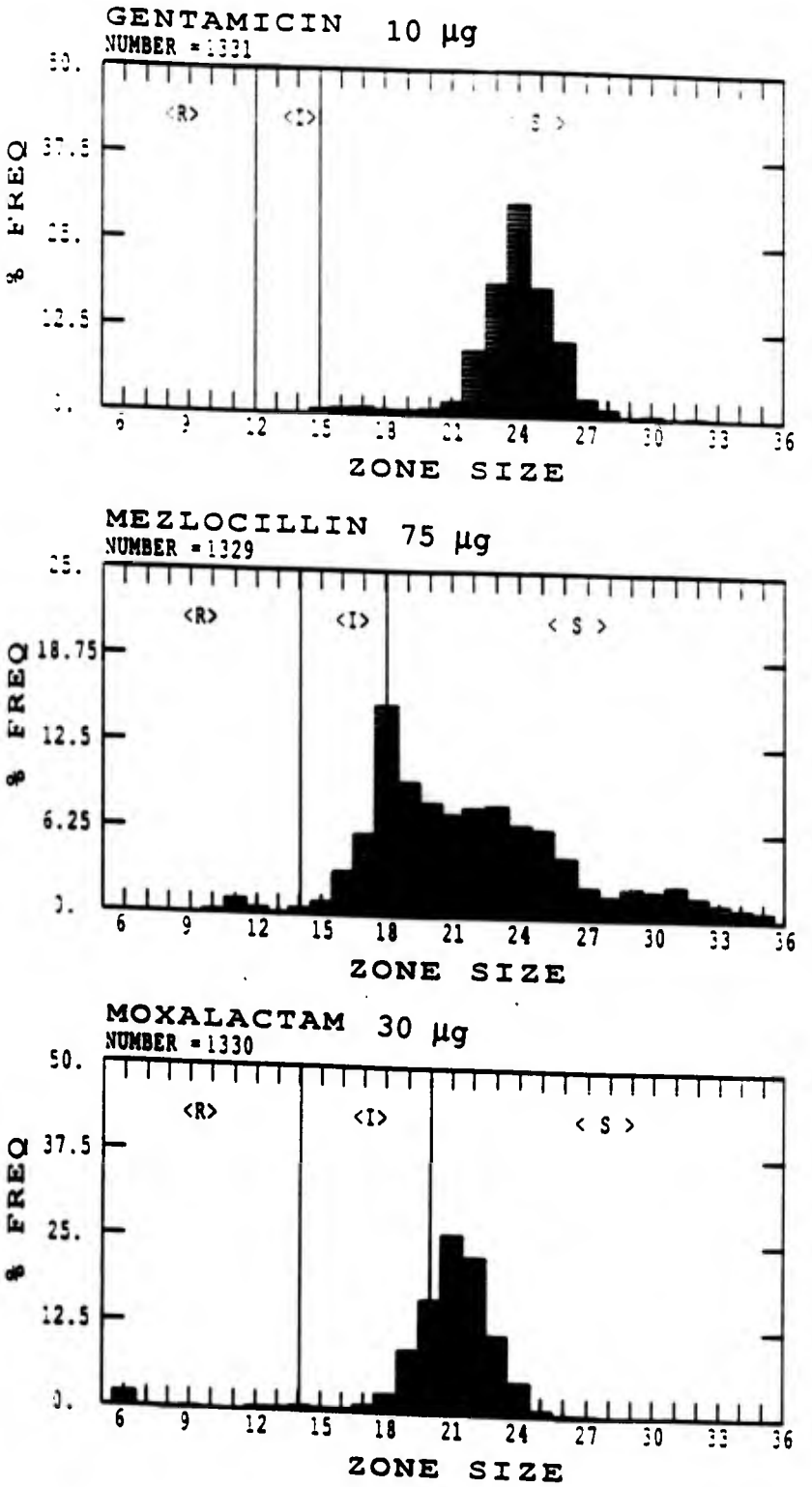



**FIGURE 9A.** Histogram display of the distribution of zones of inhibition of growth of gentamicin-sensitive Staphylococcus aureus. (continued)

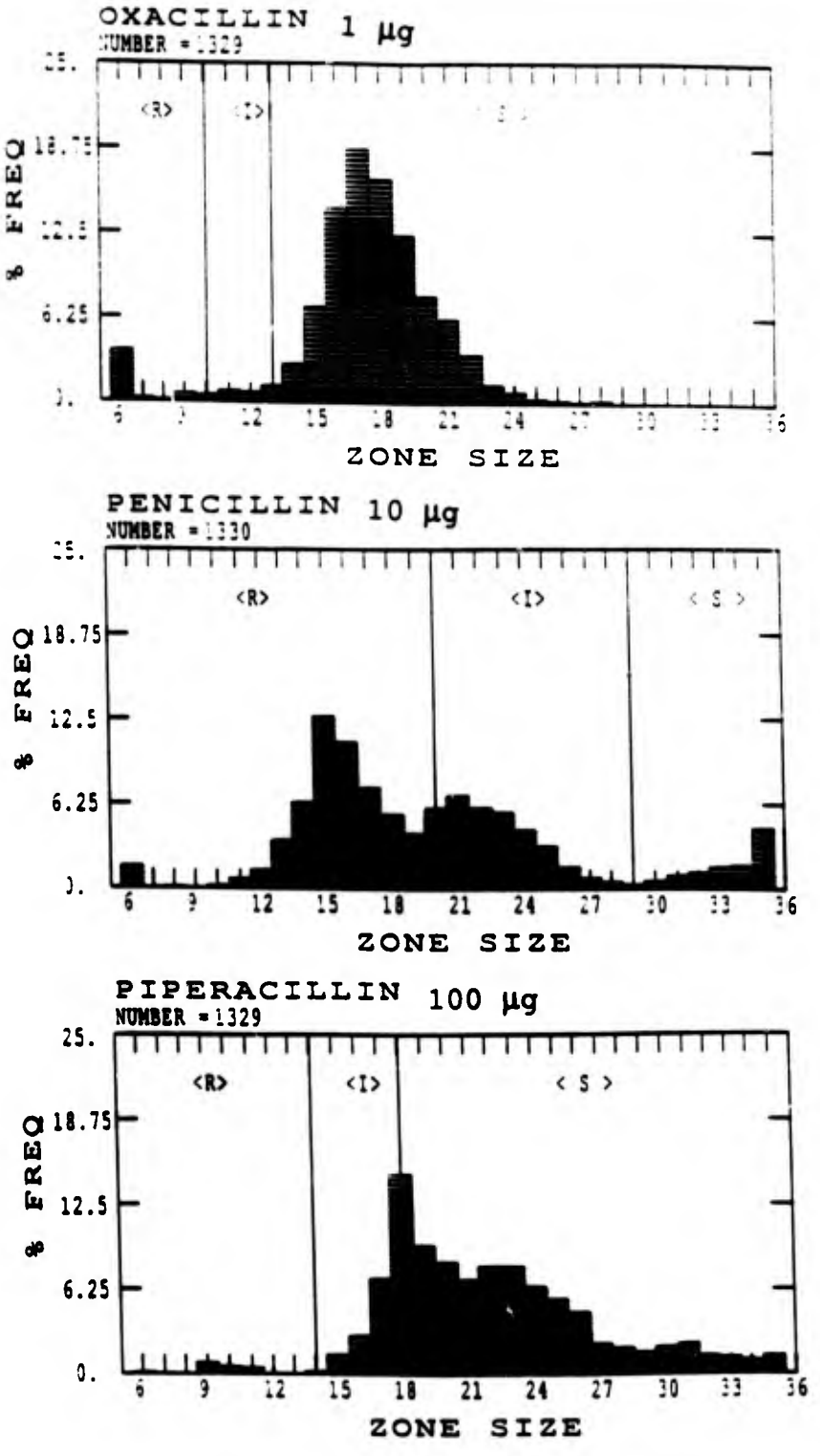



**FIGURE 9A.** Histogram display of the distribution of zones of inhibition of growth of gentamicin-sensitive Staphylococcus aureus. (continued)

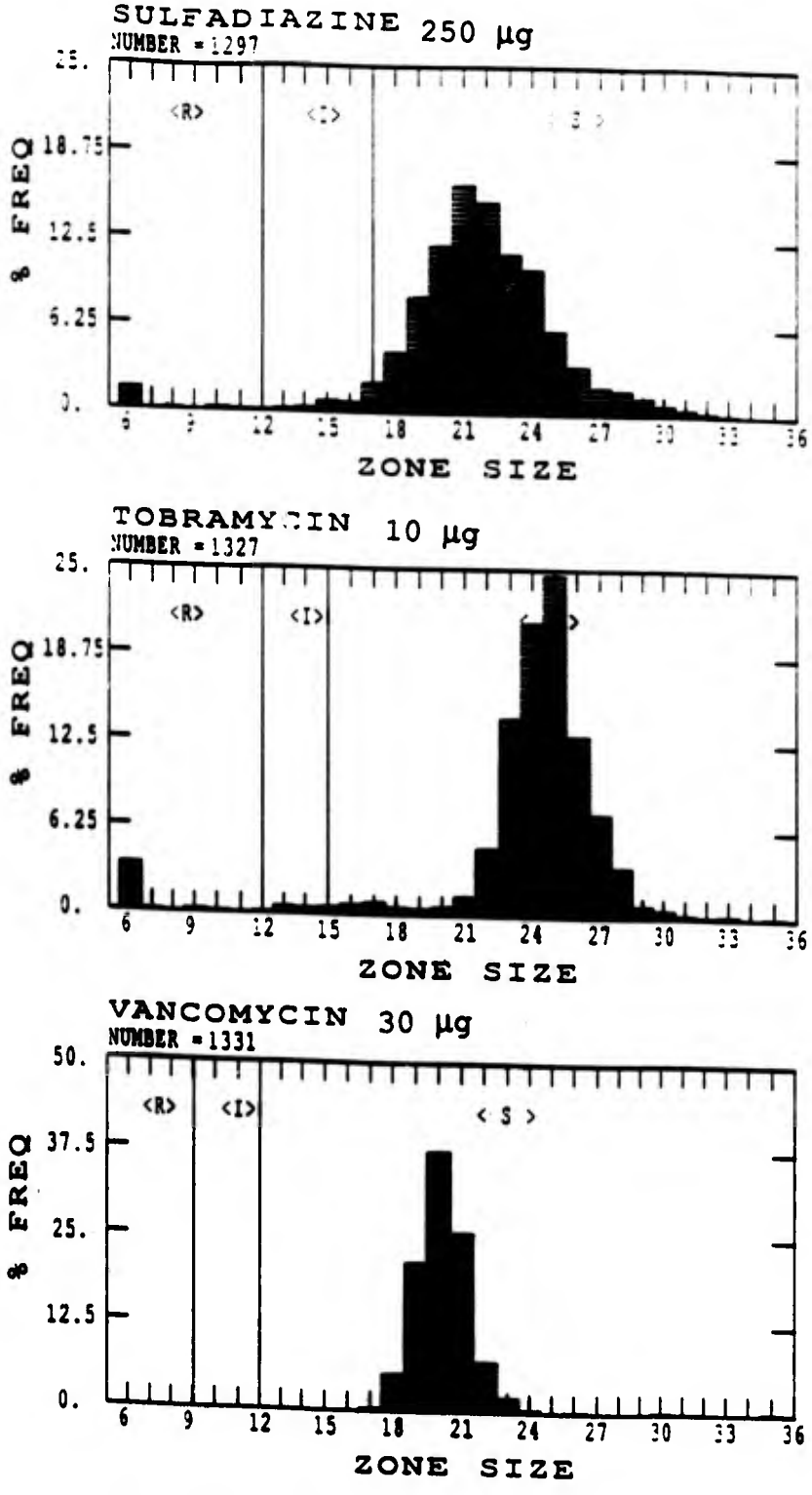



**FIGURE 9A.** Histogram display of the distribution of zones of inhibition of growth of gentamicin-sensitive Staphylococcus aureus. (continued)

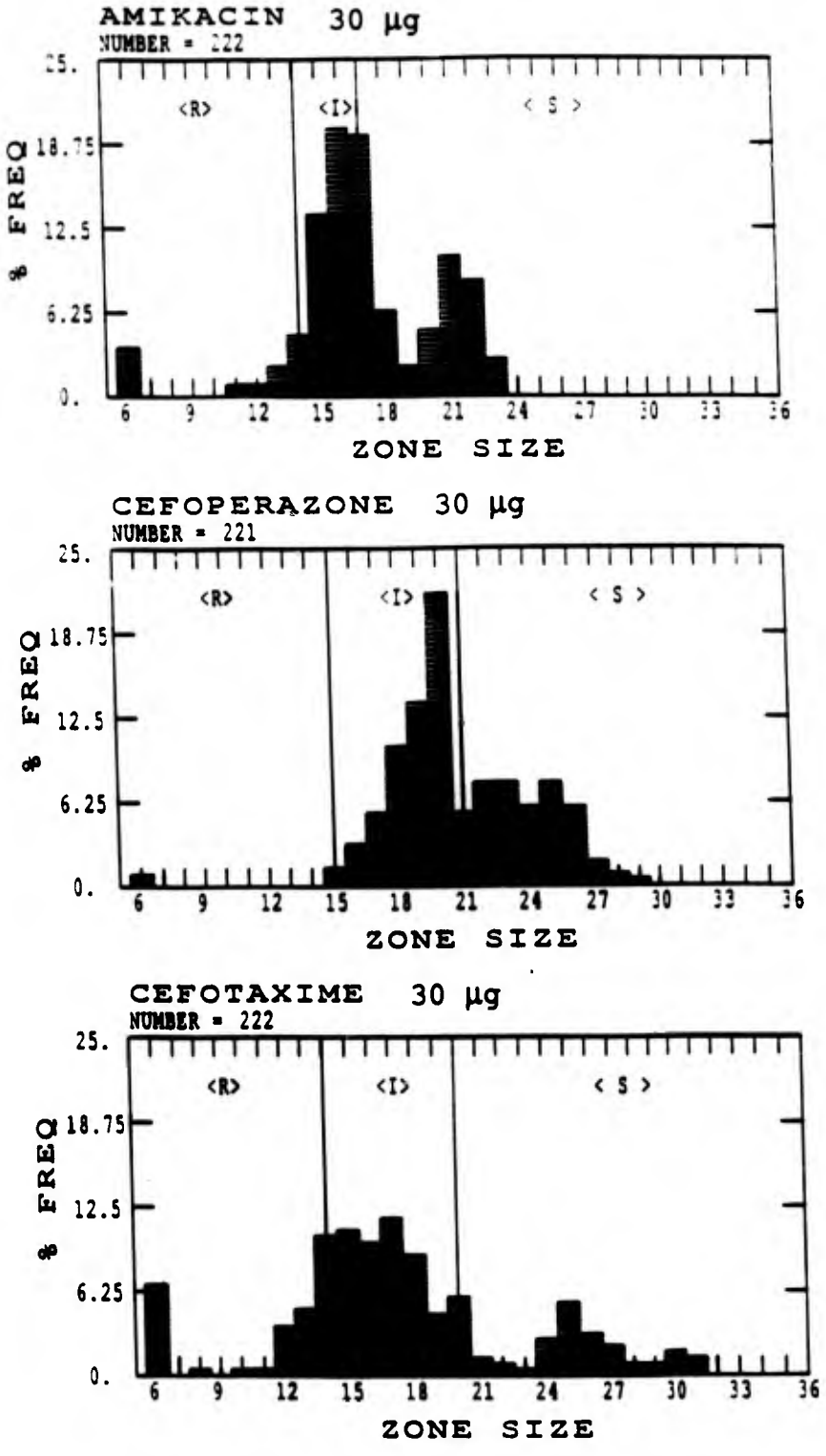



**FIGURE 9B.** Histogram display of the distribution of zones of inhibition of growth of gentamicin-resistant Staphylococcus aureus.

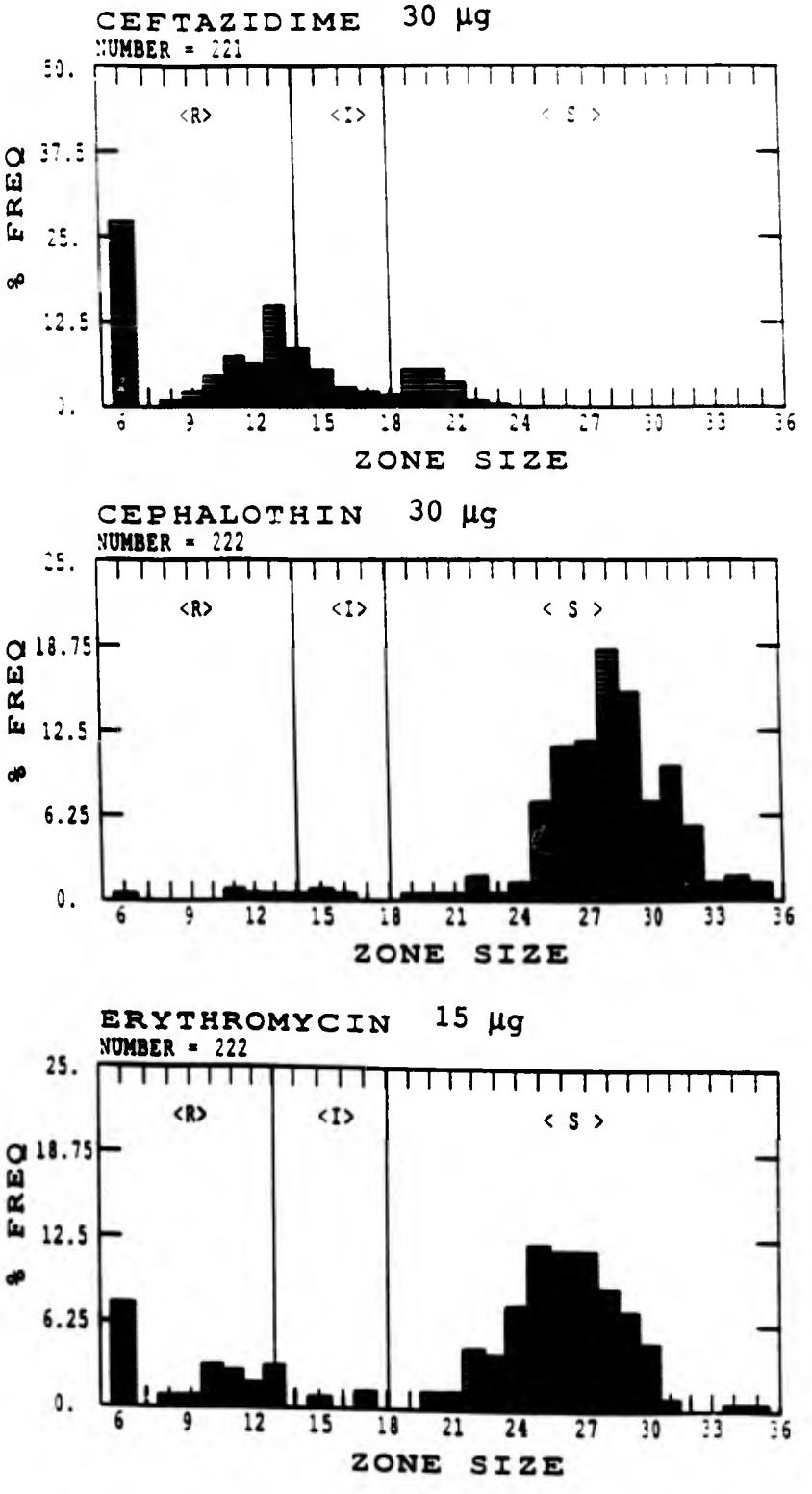



**FIGURE 9B.** Histogram display of the distribution of zones of inhibition of growth of gemtamicin-resistant Staphylococcus aureus. (Continued)

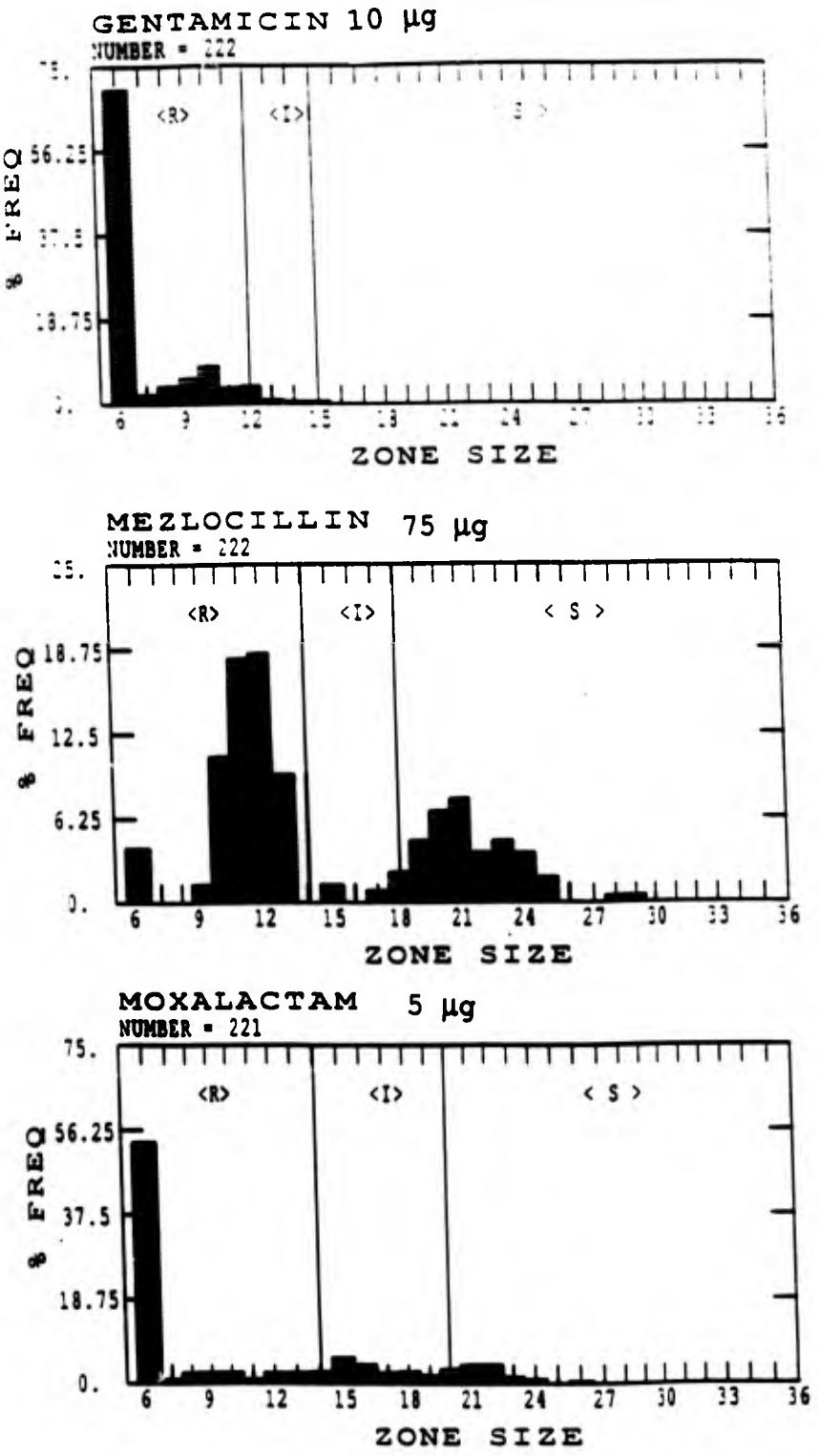



**FIGURE 9B.** Histogram display of the distribution of zones of inhibition of growth of gentamicin-resistant Staphylococcus aureus. (Continued)

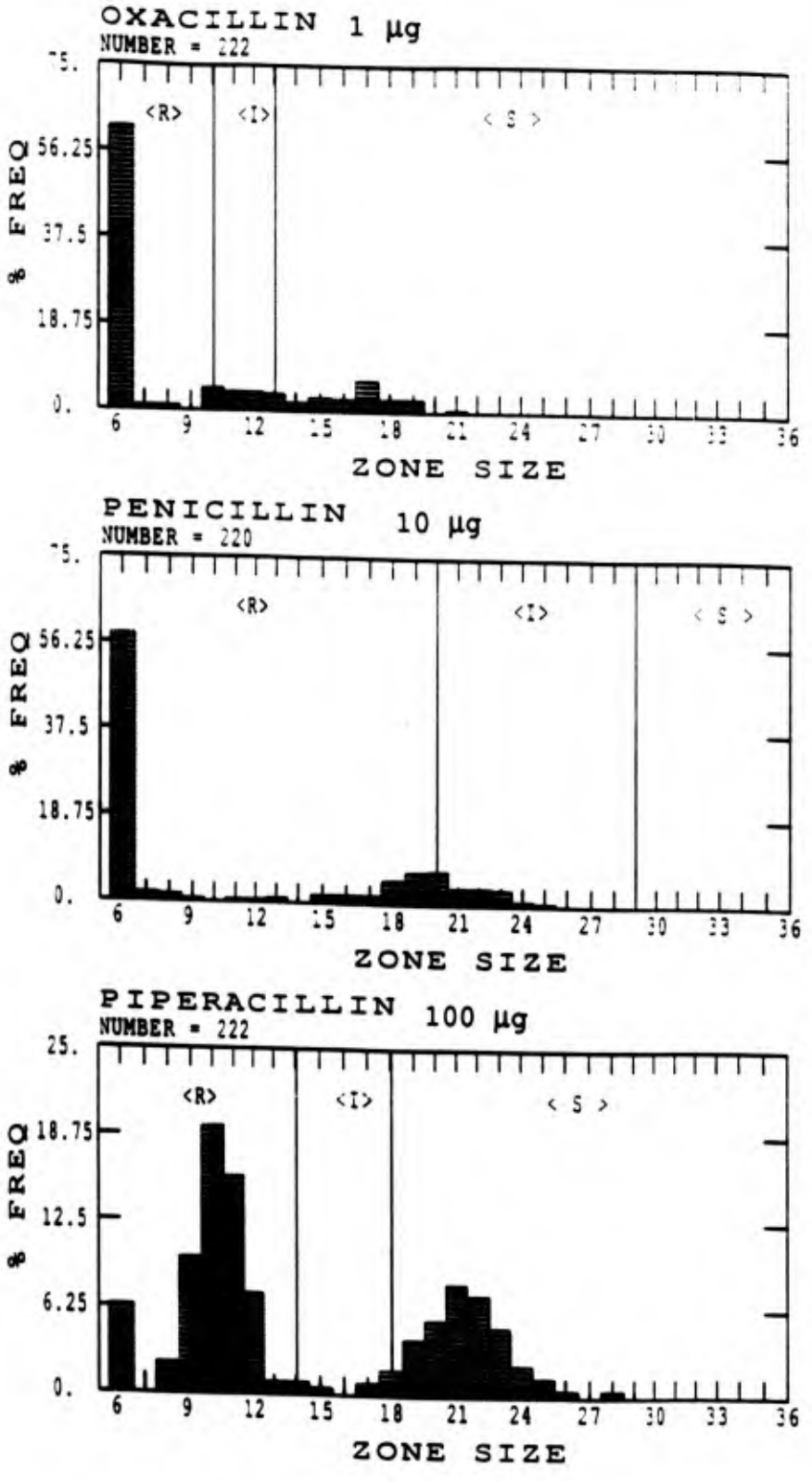



**FIGURE 9B.** Histogram display of the distribution of zones of inhibition of growth of gentamicin-resistant Staphylococcus aureus. (Continued)

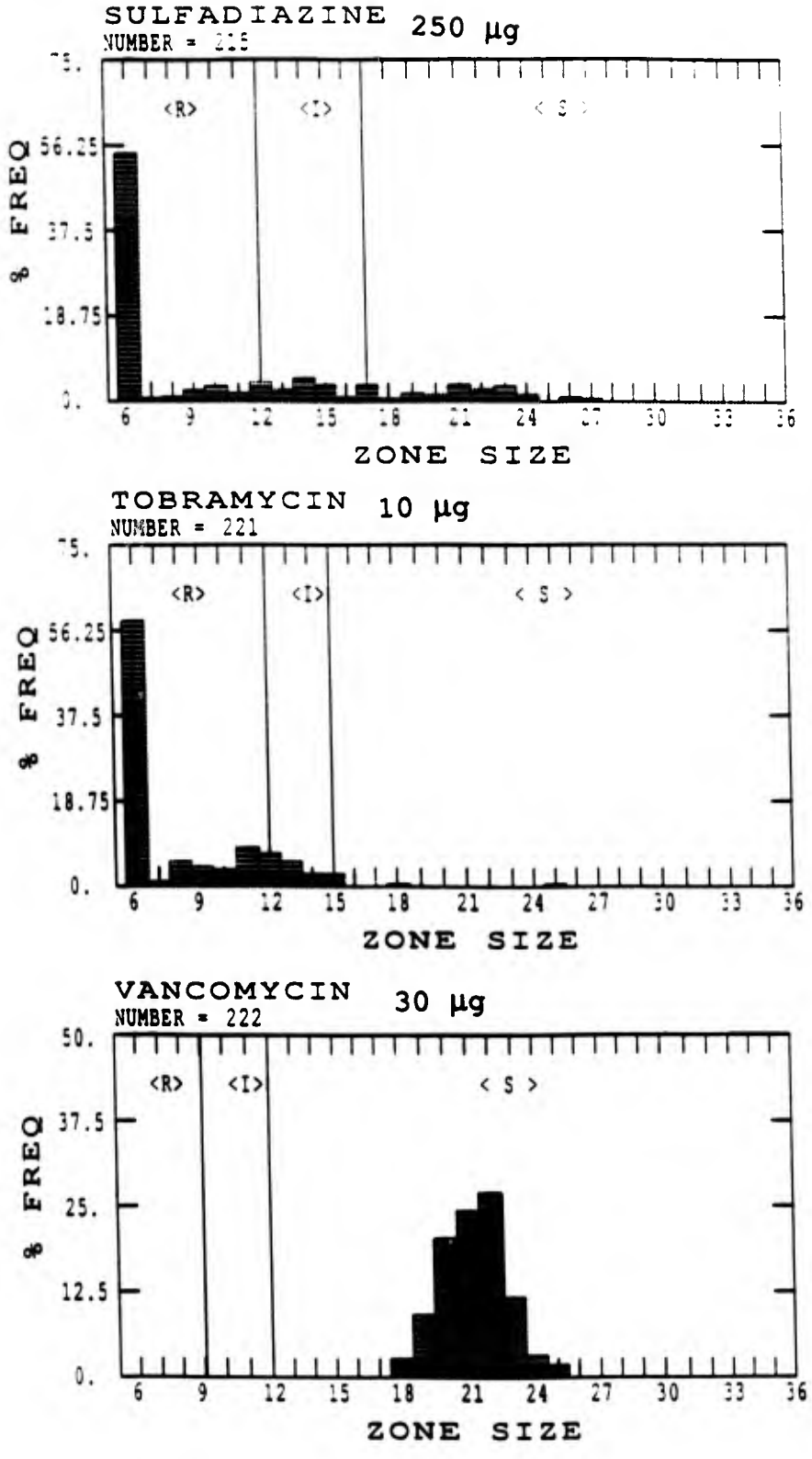



**FIGURE 9B.** Histogram display of the distribution of zones of inhibition of growth of gentamicin-resistant Staphylococcus aureus. (Continued)

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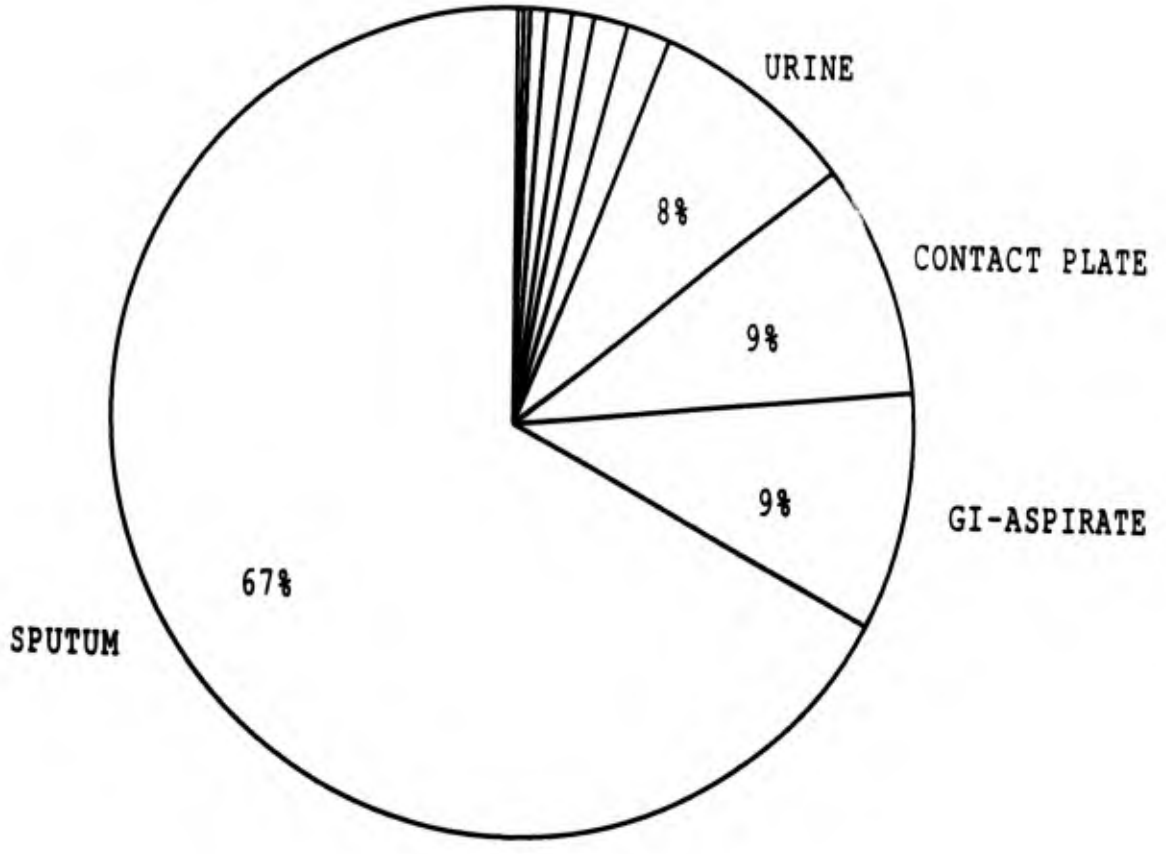



**FIGURE 10.** Display of the relative frequency of sources yielding Pseudomonas aeruginosa tested in vitro for sensitivity to selected antibiotics in 1989.

**TABLE 11. Antibiotic Sensitivity Data for *Pseudomonas aeruginosa* (1989)**

| Antibiotic | RESISTANT % | RESISTANT Number | INTERMEDIATE % | INTERMEDIATE Number | SENSITIVE % | SENSITIVE Number | Total Number |
|---|---|---|---|---|---|---|---|
| Amikacin | 5.01% | 22 | 17.08% | 75 | 77.90% | 342 | 439 |
| Azlocillin | 45.79% | 201 | 10.48% | 46 | 43.74% | 192 | 439 |
| Aztreonam | 46.70% | 205 | 27.79% | 122 | 25.51% | 112 | 439 |
| Cefoperazone | 42.82% | 188 | 17.08% | 75 | 40.09% | 176 | 439 |
| Cefotaxime | 77.01% | 335 | 22.07% | 96 | 0.92% | 4 | 435 |
| Ceftazidime | 33.49% | 147 | 12.98% | 57 | 53.53% | 235 | 439 |
| Ceftriaxone | 0.00% | 0 | 0.00% | 0 | 100.00% | 439 | 439 |
| Chloramphenicol | 98.40% | 430 | 1.14% | 5 | 0.46% | 2 | 439 |
| Colistin | 0.00% | 0 | 0.23% | 1 | 99.08% | 430 | 431 |
| Gentamicin | 3.64% | 16 | 32.35% | 142 | 64.01% | 281 | 439 |
| Imipenem-Cilastatin sodium | 13.90% | 61 | 1.59% | 7 | 84.51% | 371 | 439 |
| Kanamycin | 98.86% | 434 | 0.46% | 2 | 0.68% | 3 | 439 |
| Mezlocillin | 56.39% | 247 | 18.04% | 79 | 25.57% | 112 | 438 |
| Moxalactam | 61.28% | 269 | 31.21% | 137 | 7.52% | 33 | 439 |
| Netilmicin | 4.56% | 20 | 9.79% | 43 | 85.65% | 376 | 439 |
| Norfloxacin | 14.12% | 62 | 0.91% | 4 | 84.97% | 373 | 439 |
| Piperacillin | 38.95% | 171 | 13.21% | 58 | 47.84% | 210 | 439 |
| Sulfadiazine | 35.08% | 154 | 32.35% | 142 | 32.57% | 143 | 439 |
| Tetracycline | 91.57% | 402 | 7.97% | 35 | 0.46% | 2 | 439 |
| Ticarcillin | 52.39% | 230 | 6.38% | 28 | 41.23% | 181 | 439 |
| Tobramycin | 3.87% | 17 | 0.68% | 3 | 95.44% | 419 | 439 |
| Timentin | 51.03% | 224 | 2.96% | 13 | 46.01% | 202 | 439 |

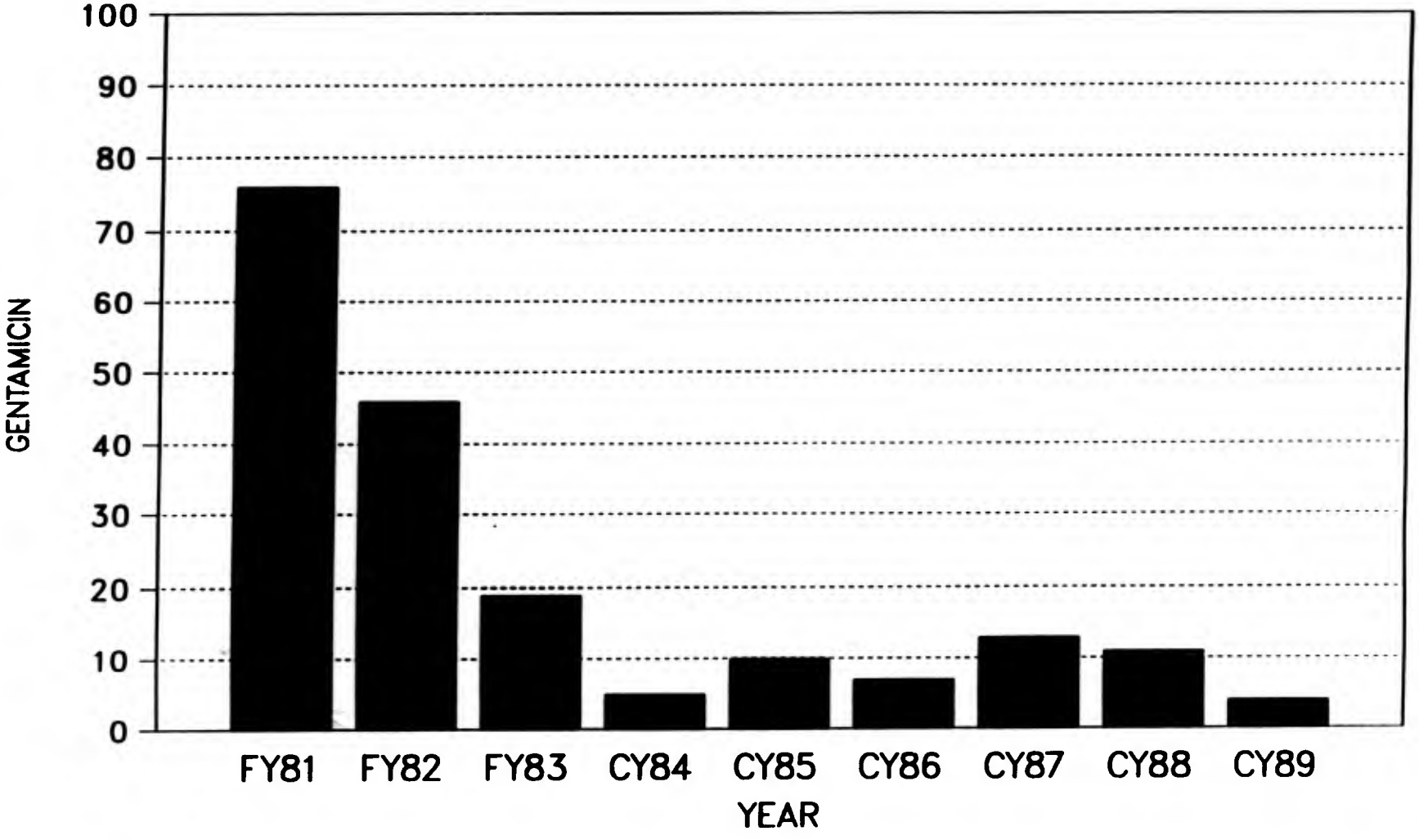



**FIGURE 11.** Relative frequency of (%) of Pseudomonas aeruginosa resistant to gentamicin for fiscal years 1981-3 and calendar years 1984-9.

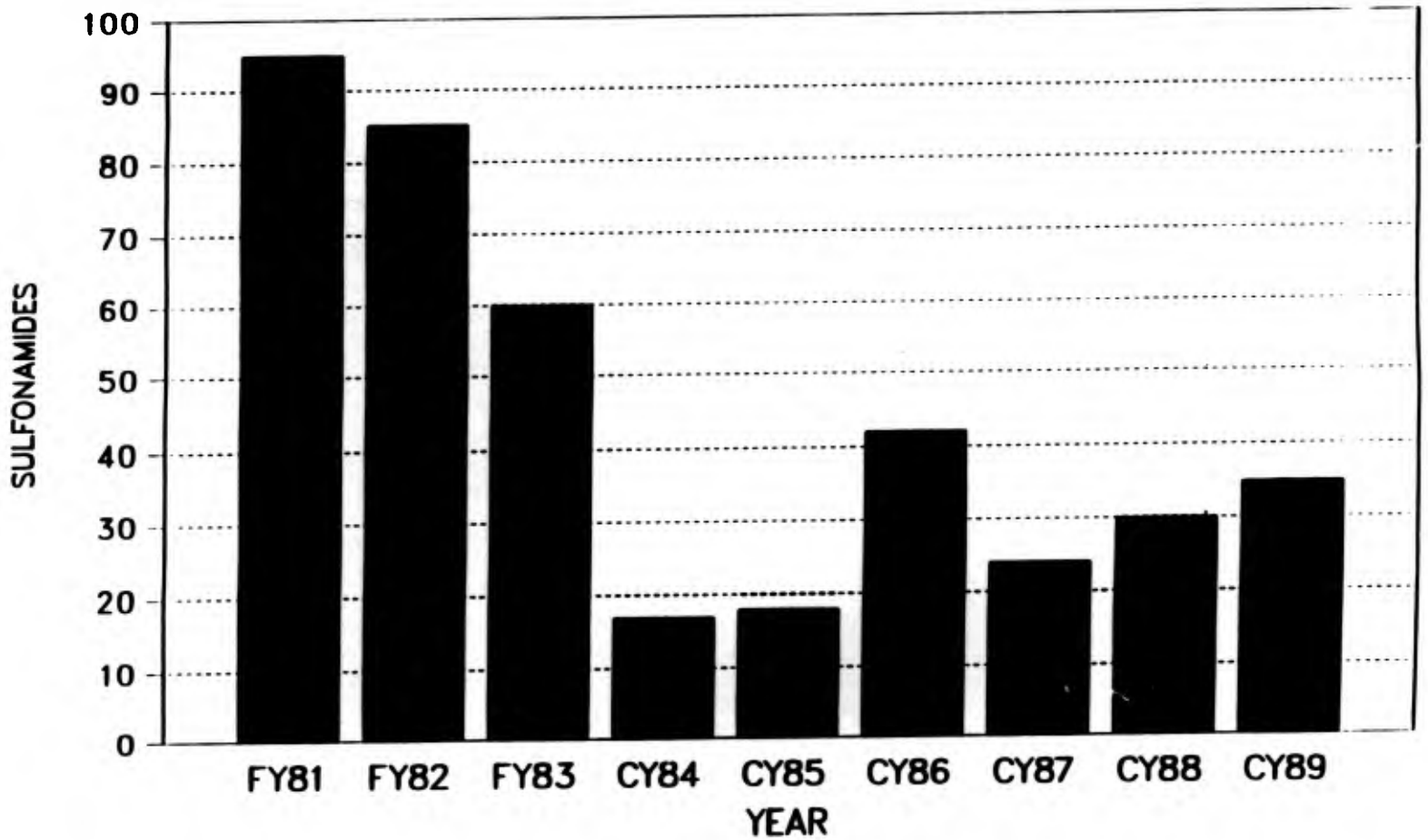





**FIGURE 12.** Relative frequency (%) of Pseudomonas aeruginosa resistant to sulfonamides for fiscal years 1981-3 and calendar years 1984-9.

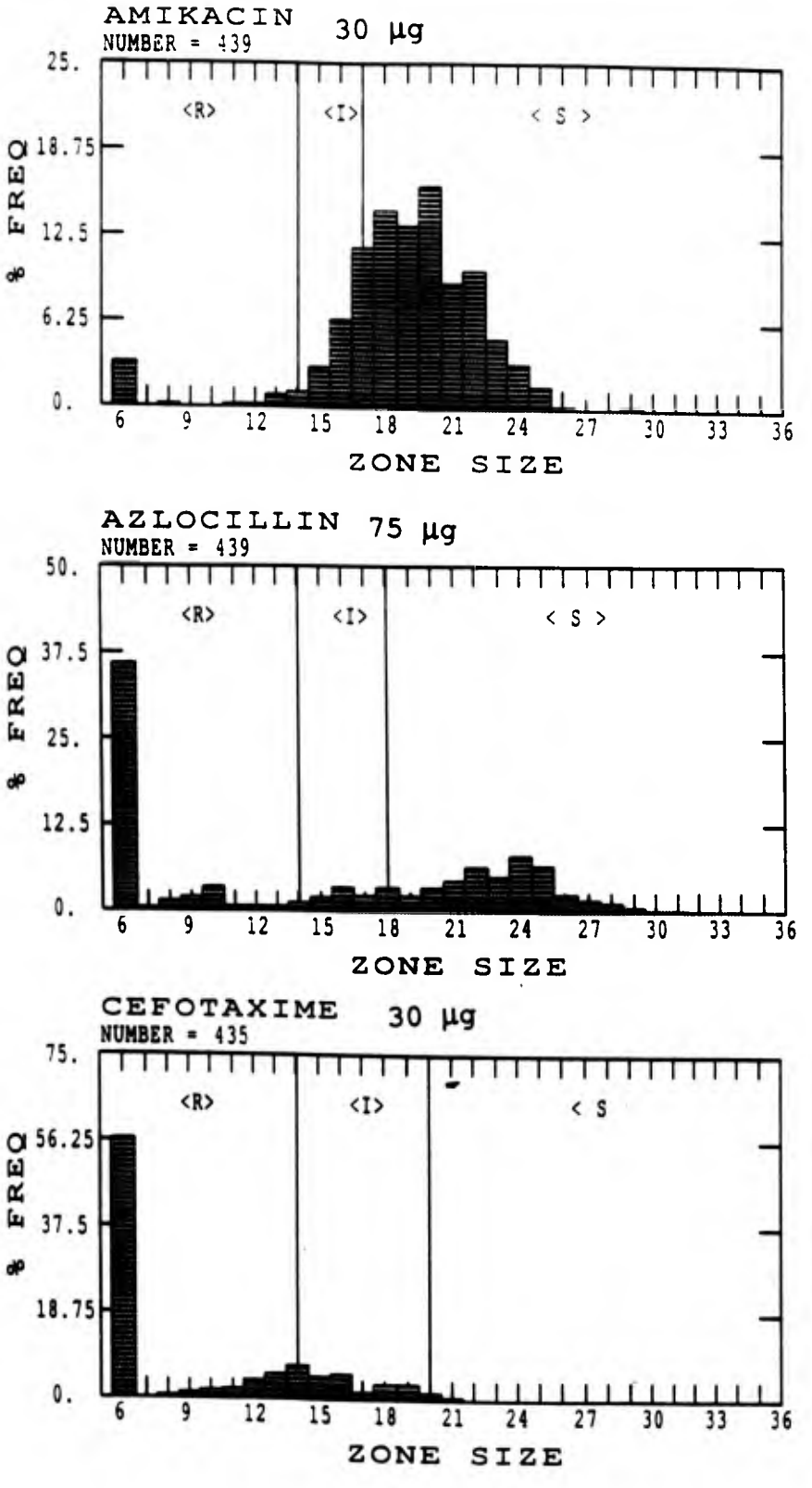



**FIGURE 13.** Histogram display of the distribution of zones of inhibition of growth of Pseudomonas aeruginosa.

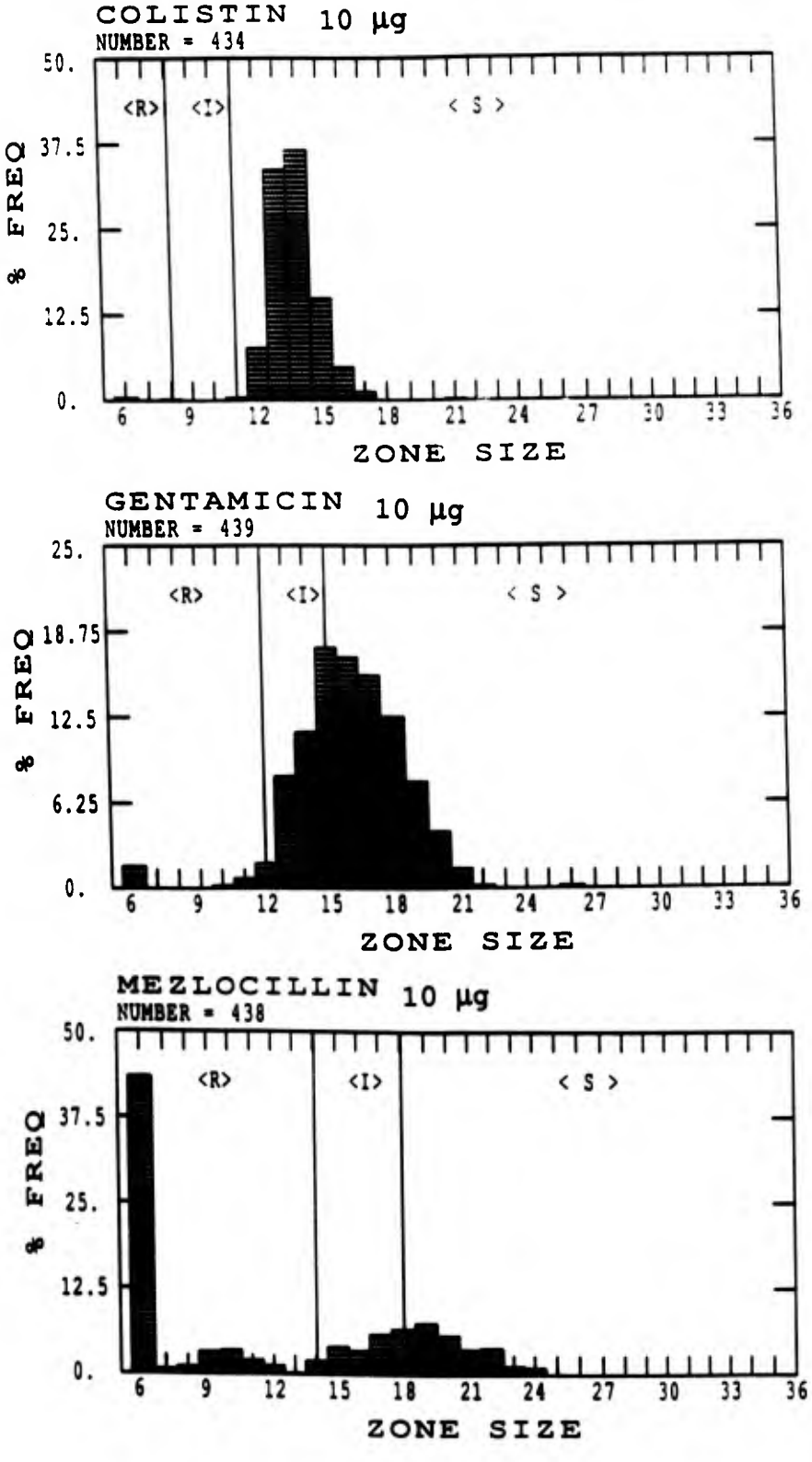



**FIGURE 13.** Histogram display of the distribution of zones of inhibition of growth of Pseudomonas aeruginosa.

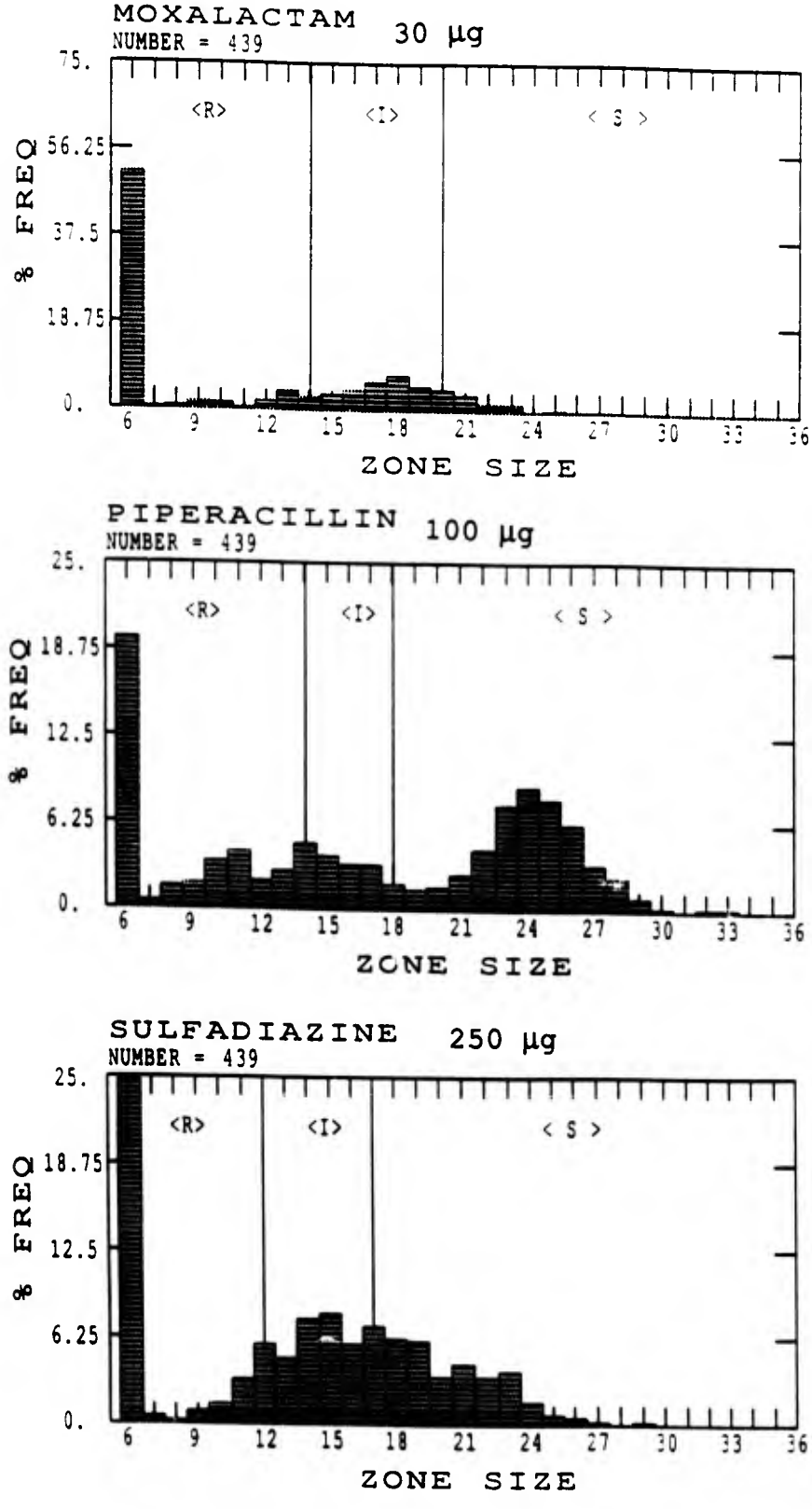



**FIGURE 13.** Histogram display of the distribution of zones of inhibition of growth of Pseudomonas aeruginosa.

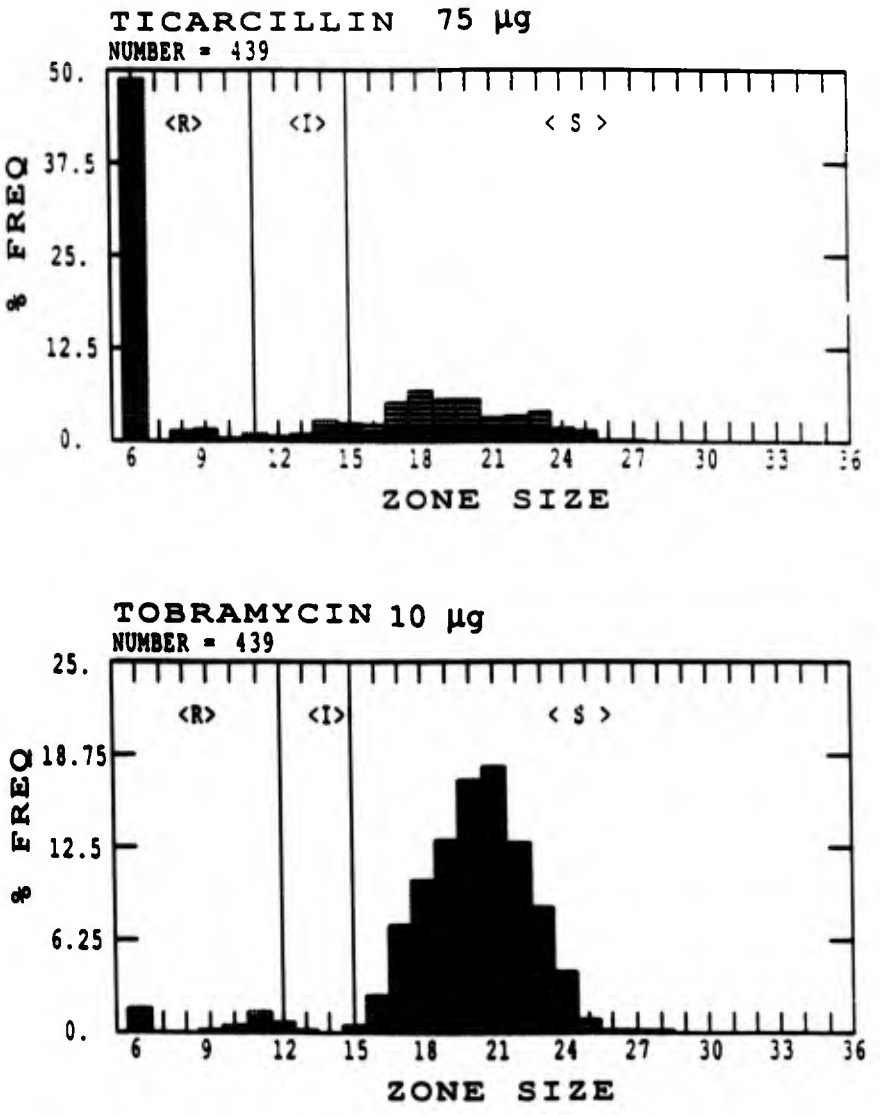



**FIGURE 13.** Histogram display of the distribution of zones of inhibition of growth of Pseudomonas aeruginosa.

KP

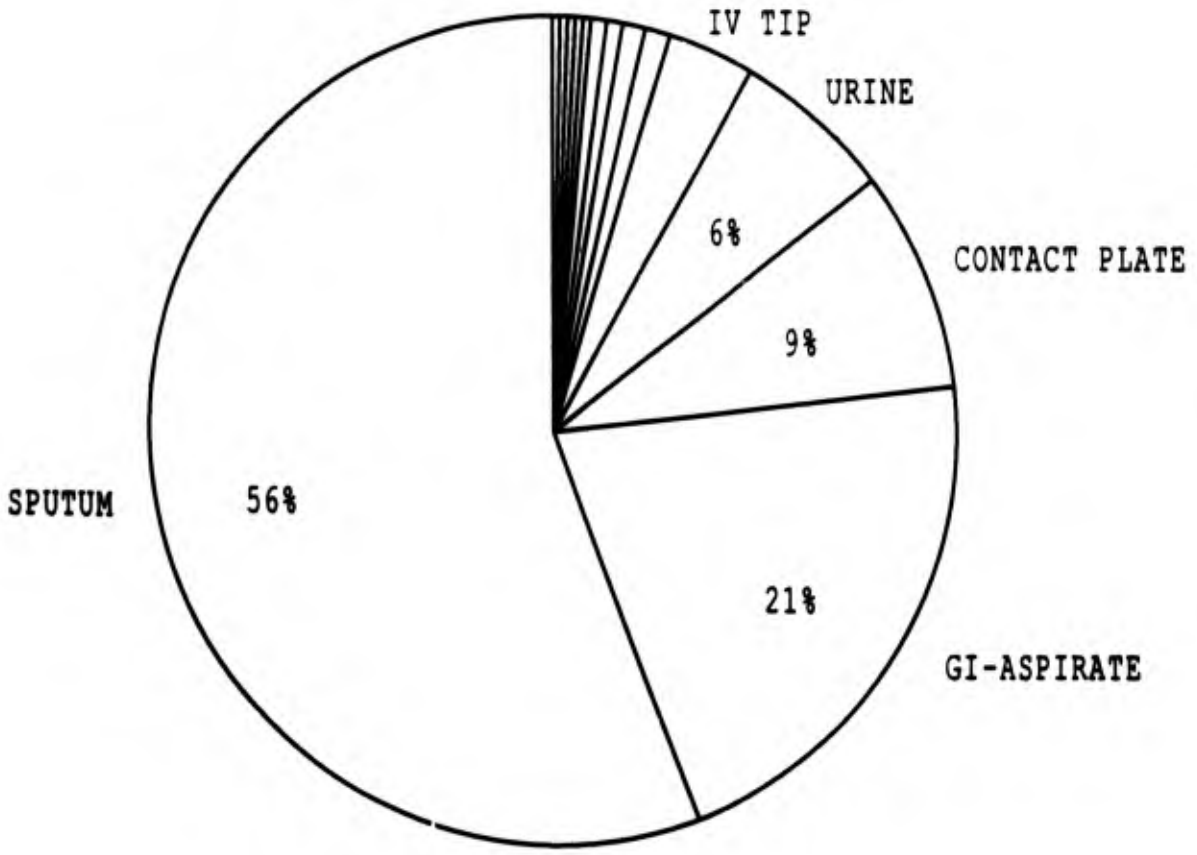



**FIGURE 14.** Display of the relative frequency of sources yielding Klebsiella penumoniae tested for in vitro sensitivity to antibiotics in 1989.

**TABLE 12. Antibiotic Sensitivity Data for *Klebsiella pneumoniae* (1989)**

| Antibiotic | RESISTANT % | RESISTANT Number | INTERMEDIATE % | INTERMEDIATE Number | SENSITIVE % | SENSITIVE Number | Total Number |
|---|---|---|---|---|---|---|---|
| Amikacin | 1.03% | 3 | 0.68% | 2 | 98.29% | 287 | 292 |
| Ampicillin | 77.74% | 227 | 14.73% | 43 | 7.53% | 22 | 292 |
| Aztreonam | 1.71% | 5 | 2.05% | 6 | 96.23% | 281 | 292 |
| Cefamandole | 2.05% | 6 | 6.51% | 19 | 91.44% | 267 | 292 |
| Cefoperazone | 2.05% | 6 | 8.56% | 25 | 89.38% | 261 | 292 |
| Cefotaxime | 0.68% | 2 | 0.00% | 0 | 99.32% | 290 | 292 |
| Cefoxitin | 2.07% | 6 | 1.03% | 3 | 96.90% | 281 | 290 |
| Ceftazidime | 4.81% | 14 | 4.47% | 13 | 90.72% | 264 | 291 |
| Ceftriaxone | 0.00% | 0 | 0.00% | 0 | 100.00% | 292 | 292 |
| Chloramphenicol | 5.14% | 15 | 0.00% | 0 | 94.86% | 277 | 292 |
| Gentamicin | 0.00% | 0 | 1.03% | 3 | 98.97% | 289 | 292 |
| Imipenem-Cilastatin sodium | 0.00% | 0 | 0.00% | 0 | 100.00% | 292 | 292 |
| Kanamycin | 1.37% | 4 | 1.71% | 5 | 96.92% | 283 | 292 |
| Mezlocillin | 12.67% | 37 | 9.59% | 28 | 77.74% | 227 | 292 |
| Nalidixic acid | 1.75% | 5 | 1.40% | 4 | 96.85% | 277 | 286 |
| Netilimicin | 0.34% | 1 | 0.00% | 0 | 99.66% | 291 | 292 |
| Norfloxacin | 0.00% | 0 | 1.37% | 4 | 98.63% | 288 | 292 |
| Piperacillin | 12.67% | 37 | 2.05% | 6 | 85.27% | 249 | 292 |
| Streptomycin | 10.96% | 32 | 47.26% | 138 | 41.78% | 122 | 292 |
| Sulfadiazine | 57.84% | 166 | 6.27% | 18 | 35.89% | 103 | 287 |
| Tetracycline | 23.02% | 67 | 1.37% | 4 | 75.60% | 220 | 291 |
| Ticarcillin | 82.53% | 241 | 13.36% | 39 | 4.11% | 12 | 292 |
| Trimethoprim | 15.41% | 45 | 1.03% | 3 | 83.56% | 244 | 292 |
| Trimeth & Sulfa | 14.48% | 42 | 2.76% | 8 | 82.76% | 240 | 290 |

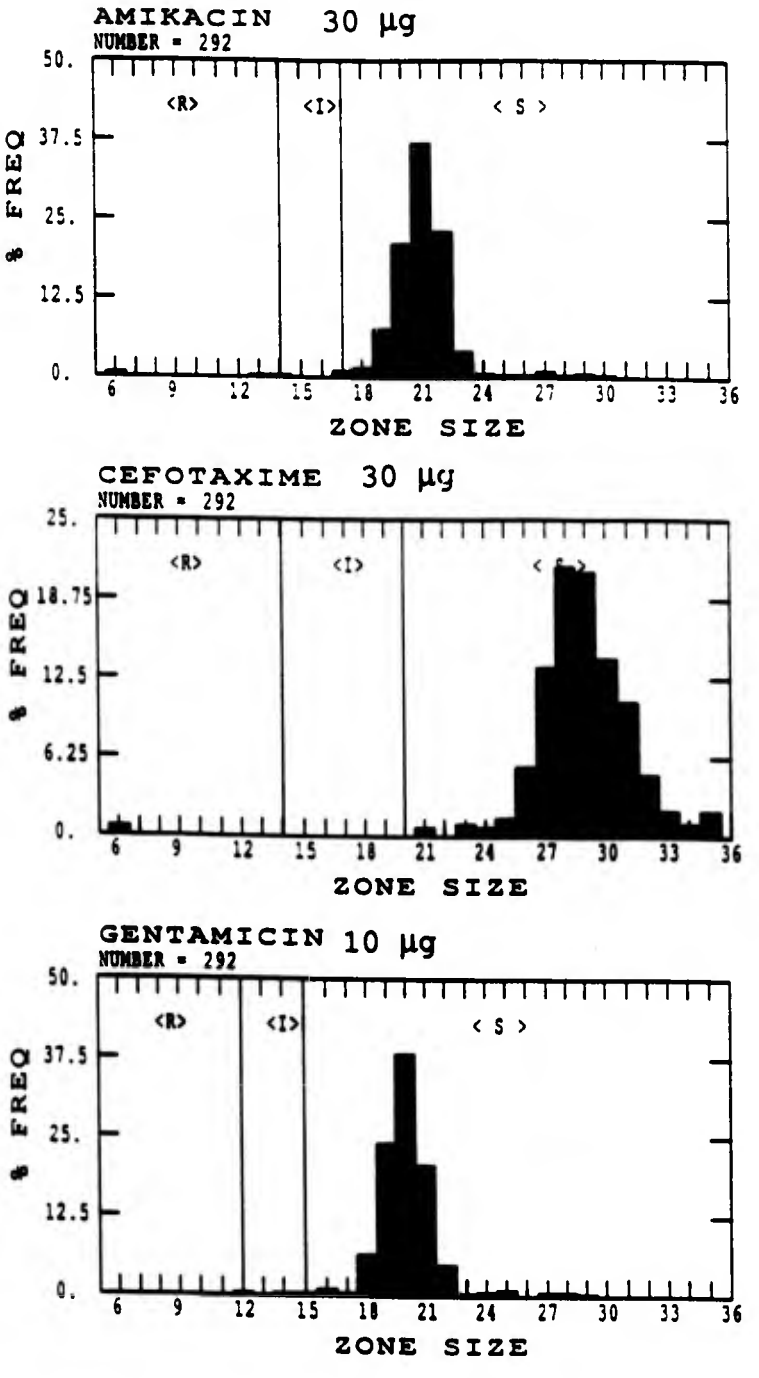



**FIGURE 15.** Histogram display of the distribution of zones of inhibition of growth of Klebsiella pneumoniae.

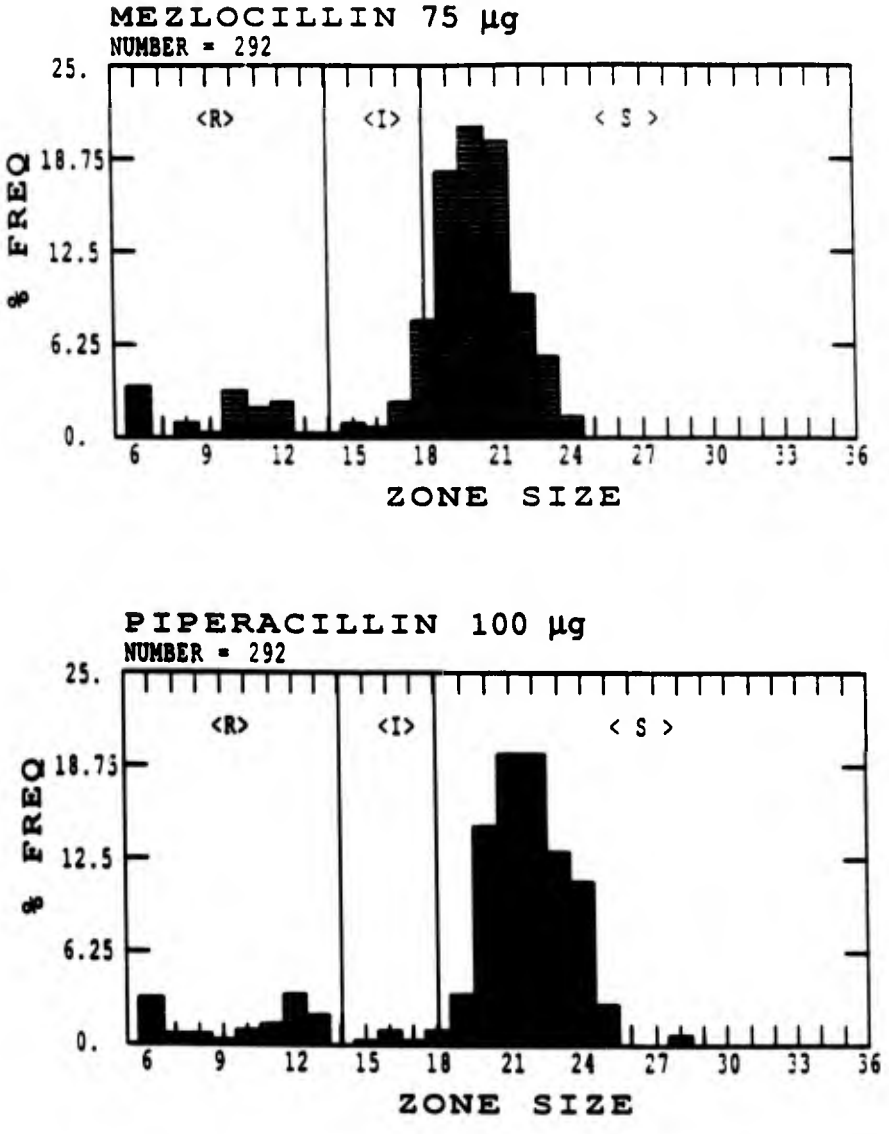
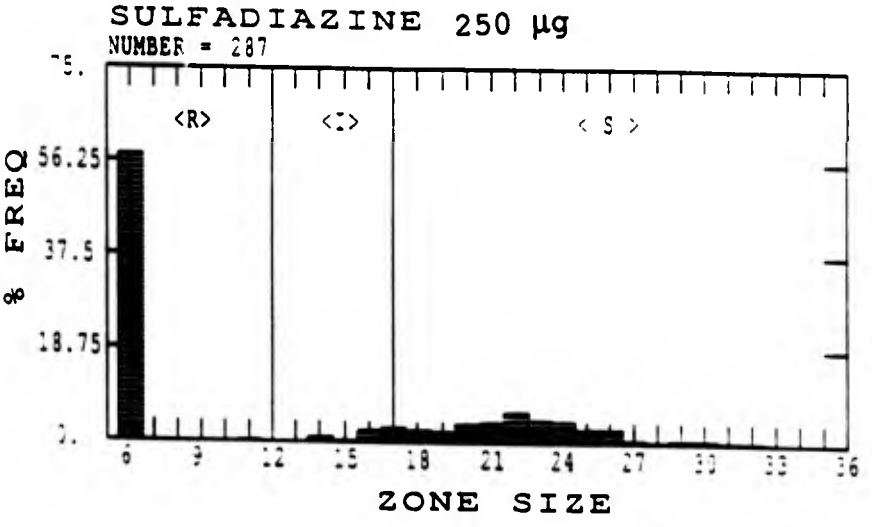



**FIGURE 15.** Histogram display of the distribution of zones of inhibition of growth of Klebsiella pneumoniae.



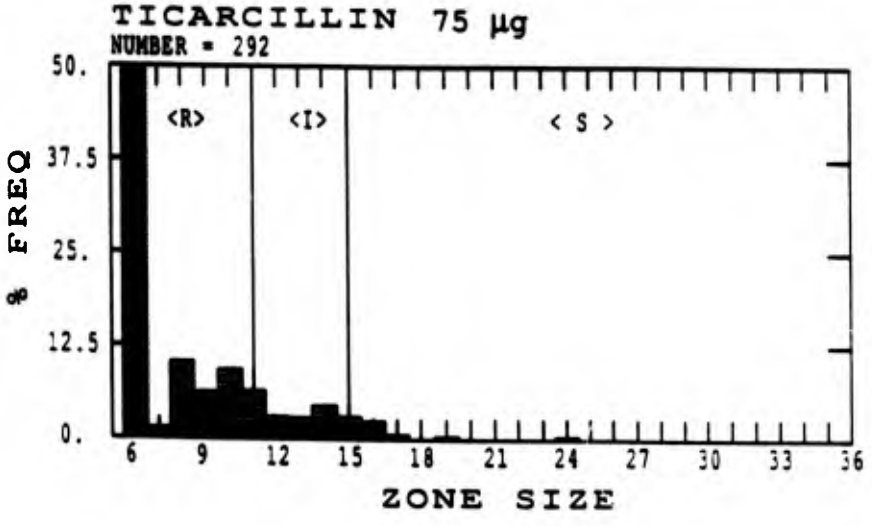




**FIGURE 15.** Histogram display of the distribution of zones of inhibition of growth of Klebsiella pneumoniae.

COLI

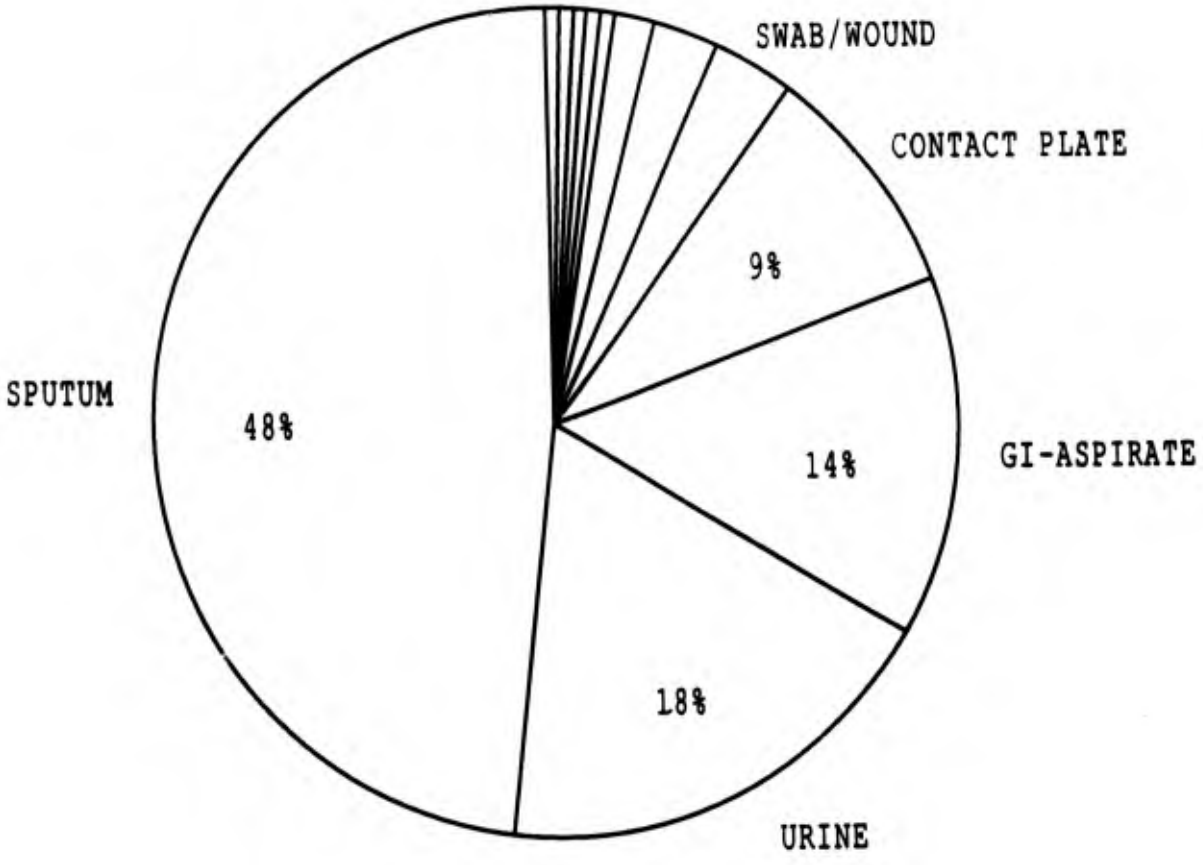



**FIGURE 16.** Display of the relative frequency of sources yielding Escherichia coli tested for in vitro sensitivity to antibiotics in 1989. Escherichia Coli from stool specimens were not tested.

**TABLE 13. Antibiotic Sensitivity Data for *Escherichia coli* (1989)**

| Antibiotic | RESISTANT % | RESISTANT Number | INTERMEDIATE % | INTERMEDIATE Number | SENSITIVE % | SENSITIVE Number | Total Number |
|---|---|---|---|---|---|---|---|
| Amikacin | 0.00% | 0 | 4.09% | 7 | 95.91% | 164 | 171 |
| Ampicillin | 38.95% | 67 | 2.91% | 5 | 58.14% | 100 | 172 |
| Aztreonam | 0.58% | 1 | 0.00% | 0 | 99.42% | 171 | 172 |
| Cefamandole | 0.58% | 1 | 13.37% | 23 | 86.05% | 148 | 172 |
| Cefoperazone | 1.17% | 2 | 8.19% | 14 | 90.64% | 155 | 171 |
| Cefotaxime | 0.00% | 0 | 0.00% | 0 | 100.00% | 172 | 172 |
| Cefoxitin | 0.58% | 1 | 2.33% | 4 | 97.09% | 167 | 172 |
| Ceftazidime | 0.58% | 1 | 0.00% | 0 | 99.42% | 171 | 172 |
| Ceftriaxone | 0.00% | 0 | 0.00% | 0 | 100.00% | 172 | 172 |
| Chloramphenicol | 12.79% | 22 | 3.49% | 6 | 83.72% | 144 | 172 |
| Gentamicin | 0.00% | 0 | 1.74% | 3 | 98.26% | 169 | 172 |
| Imipenim-Cilastatin sodium | 0.00% | 0 | 0.00% | 0 | 100.00% | 172 | 172 |
| Kanamycin | 10.47% | 18 | 9.30% | 16 | 80.23% | 138 | 172 |
| Mezlocillin | 20.35% | 35 | 18.60% | 32 | 61.05% | 105 | 172 |
| Nalidixic acid | 1.84% | 3 | 0.61% | 1 | 97.55% | 159 | 163 |
| Netilimicin | 0.00% | 0 | 0.00% | 0 | 100.00% | 172 | 172 |
| Norfloxacin | 0.00% | 0 | 0.00% | 0 | 100.00% | 172 | 172 |
| Piperacillin | 19.88% | 34 | 19.30% | 33 | 60.82% | 104 | 171 |
| Streptomycin | 38.95% | 67 | 17.44% | 30 | 43.60% | 75 | 172 |
| Sulfadiazine | 45.78% | 76 | 17.47% | 29 | 36.75% | 61 | 166 |
| Tetracycline | 34.30% | 59 | 1.16% | 2 | 64.53% | 111 | 172 |
| Ticarcillin | 38.37% | 66 | 0.58% | 1 | 61.05% | 105 | 172 |
| Trimethoprim | 9.88% | 17 | 1.74% | 3 | 88.37% | 152 | 172 |
| Trimeth & Sulfa | 9.94% | 17 | 1.17% | 2 | 88.89% | 152 | 171 |

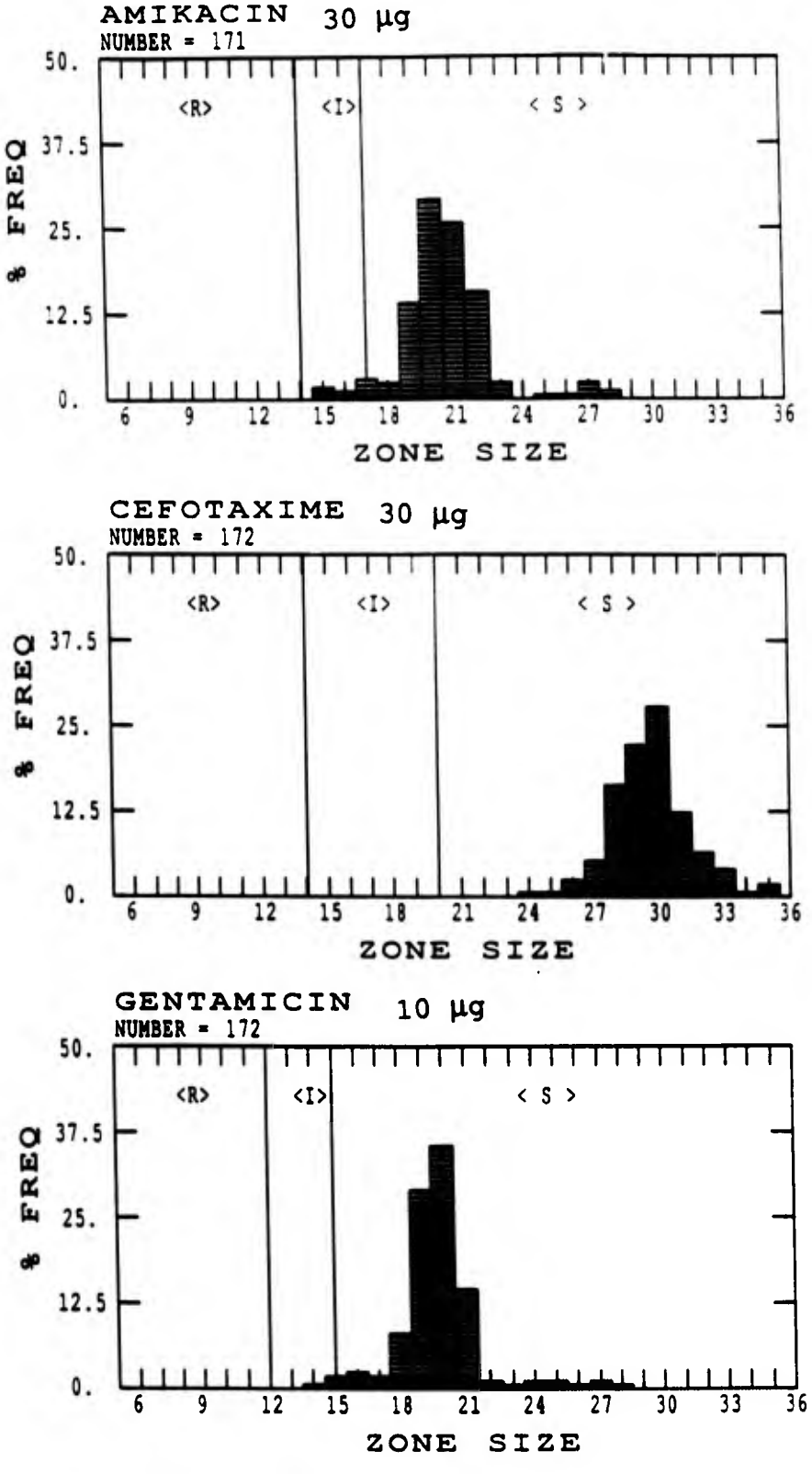
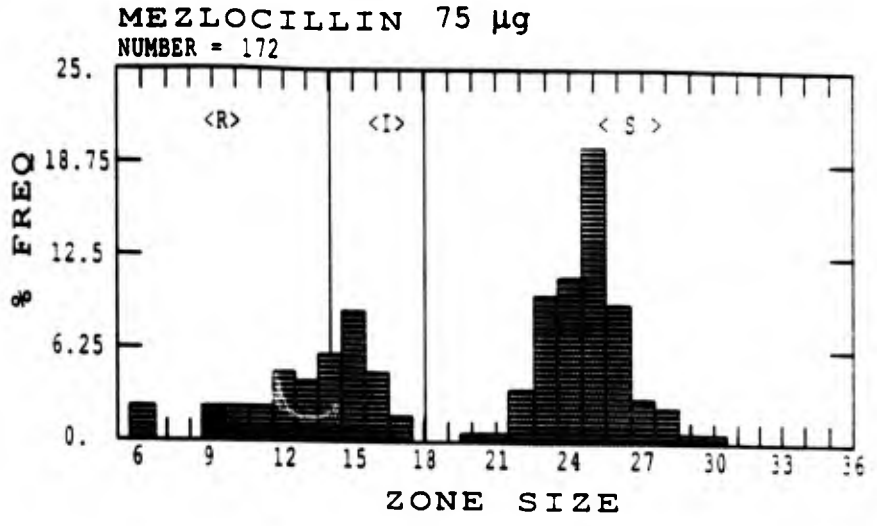



**FIGURE 17.** Histogram display of the distribution of zones of inhibition of growth of Escherichia coli.



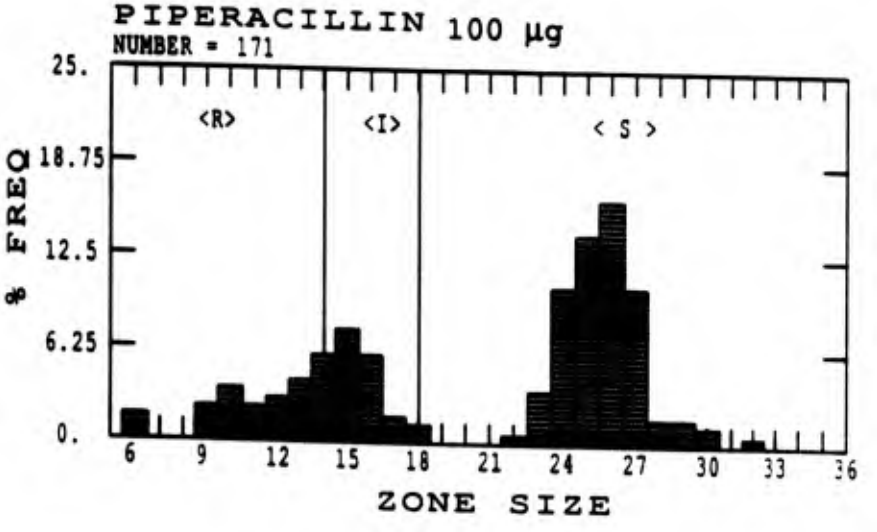




**FIGURE 17.** Histogram display of the distribution of zones of inhibition of growth of Escherichia coli.

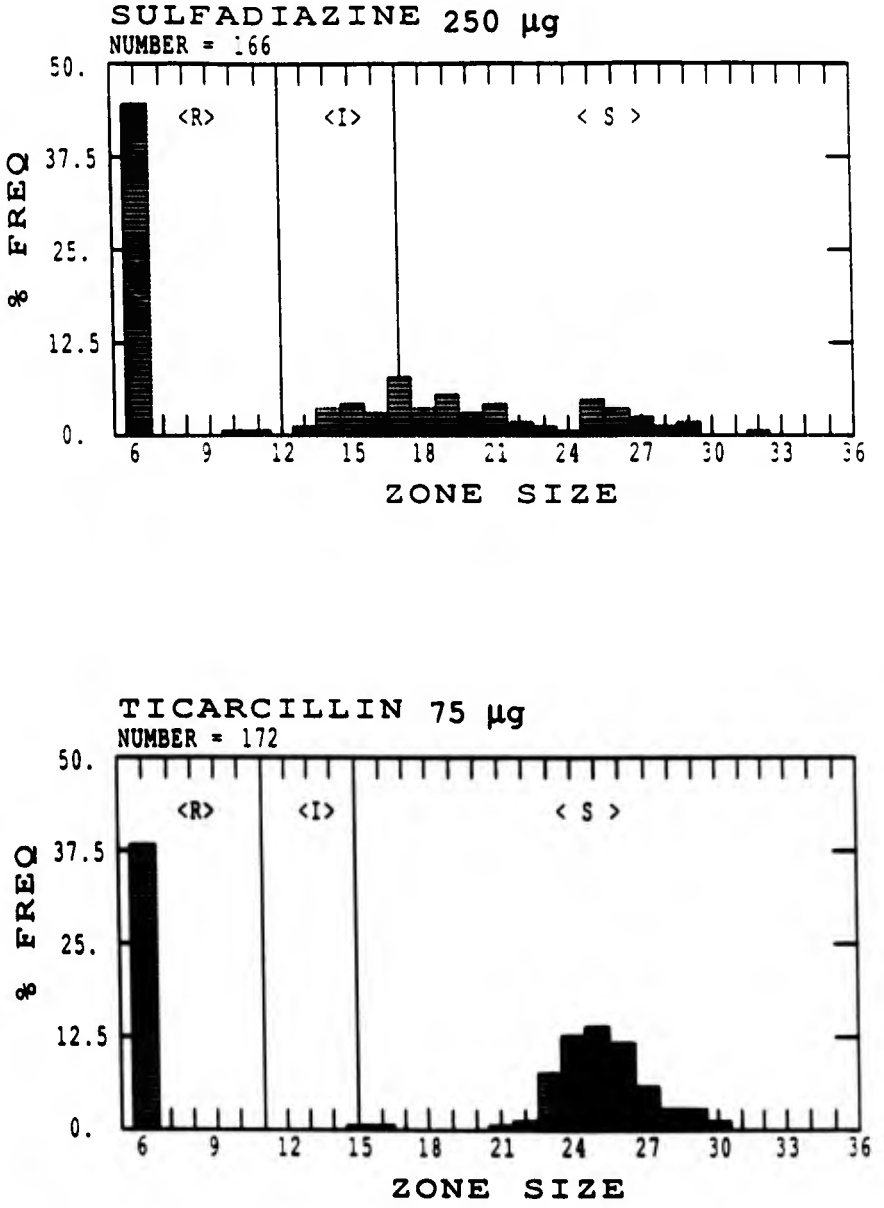



**FIGURE 17.** Histogram display of the distribution of zones of inhibition of growth of Escherichia coli.

PM

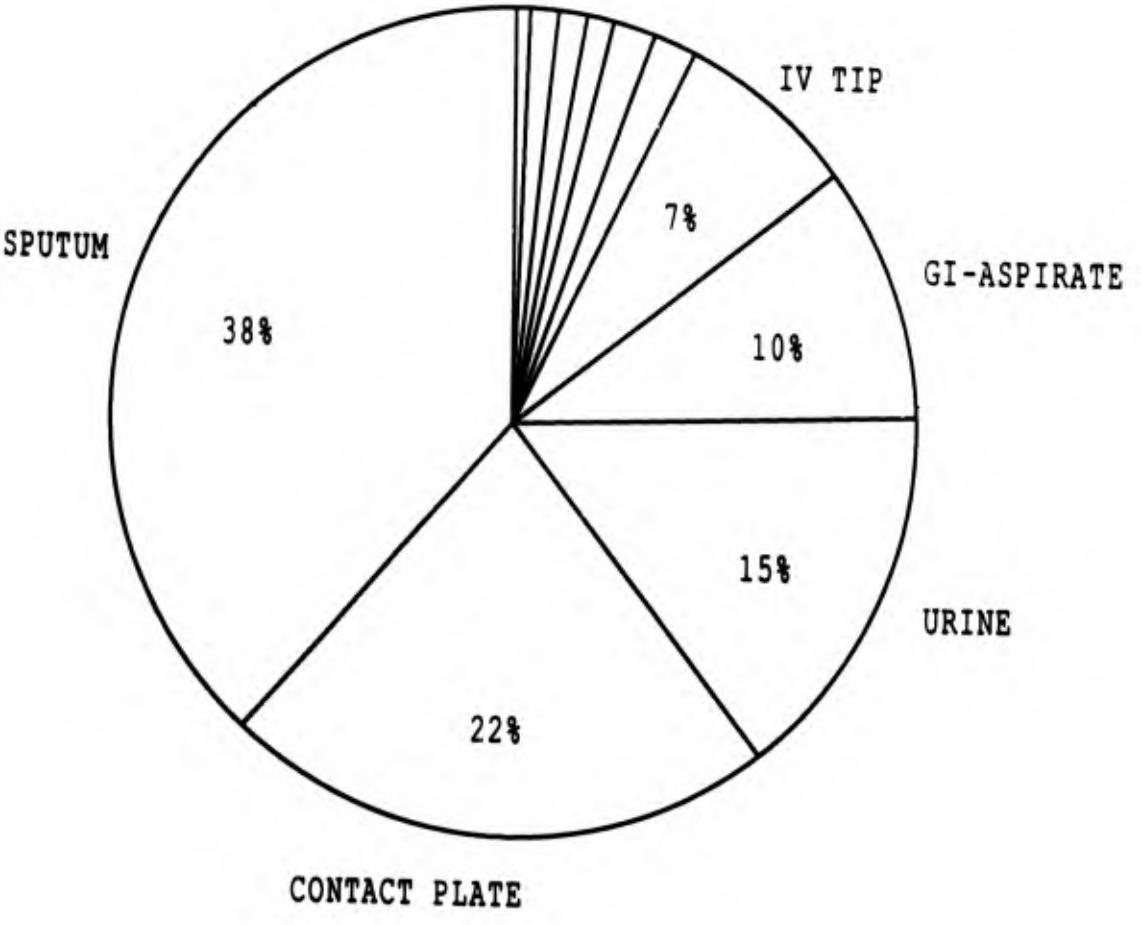



**FIGURE 18.** Display of the relative frequency of sources yielding Proteus mirabilis tested for in vitro sensitivity to antibiotics in 1989.

**TABLE 14. Antibiotic Sensitivity Data for *Proteus mirabilis* (1989)**

| Antibiotic | RESISTANT % | RESISTANT Number | INTERMEDIATE % | INTERMEDIATE Number | SENSITIVE % | SENSITIVE Number | Total Number |
|---|---|---|---|---|---|---|---|
| Amikacin | 0.00% | 0 | 0.00% | 0 | 100.00% | 172 | 172 |
| Ampicillin | 4.65% | 8 | 0.58% | 1 | 94.77% | 163 | 172 |
| Aztreonam | 0.00% | 0 | 1.74% | 3 | 98.26% | 169 | 172 |
| Cefamandole | 1.74% | 3 | 0.58% | 1 | 97.67% | 168 | 172 |
| Cefoperazone | 0.00% | 0 | 1.17% | 2 | 98.83% | 169 | 171 |
| Cefoxitin | 0.58% | 1 | 4.09% | 7 | 95.32% | 163 | 171 |
| Ceftazidime | 0.00% | 0 | 0.00% | 0 | 100.00% | 172 | 172 |
| Cefotaxime | 0.00% | 0 | 0.00% | 0 | 100.00% | 172 | 172 |
| Ceftriaxone | 0.00% | 0 | 0.00% | 0 | 100.00% | 172 | 172 |
| Chloramphenicol | 5.23% | 9 | 13.95% | 24 | 80.81% | 139 | 172 |
| Gentamicin | 0.00% | 0 | 0.00% | 0 | 100.00% | 172 | 172 |
| Imipenem-Cilastatin sodium | 0.00% | 0 | 0.00% | 0 | 100.00% | 172 | 172 |
| Kanamycin | 0.58% | 1 | 1.16% | 2 | 98.26% | 169 | 172 |
| Mezlocillin | 1.75% | 3 | 0.58% | 1 | 97.66% | 167 | 171 |
| Nalidixic acid | 1.20% | 2 | 0.60% | 1 | 98.19% | 163 | 166 |
| Netilimicin | 0.00% | 0 | 0.00% | 0 | 100.00% | 172 | 172 |
| Norfloxacin | 0.00% | 0 | 0.00% | 0 | 100.00% | 171 | 171 |
| Piperacillin | 1.17% | 2 | 0.00% | 0 | 98.83% | 169 | 171 |
| Streptomycin | 9.36% | 16 | 22.81% | 39 | 67.84% | 116 | 171 |
| Sulfadiazine | 10.98% | 18 | 6.10% | 10 | 82.93% | 136 | 164 |
| Tetracyline | 98.84% | 170 | 0.00% | 0 | 1.16% | 139 | 172 |
| Ticarcillin | 4.07% | 7 | 0.00% | 0 | 95.93% | 165 | 172 |
| Trimethoprim | 1.74% | 3 | 5.81% | 10 | 92.44% | 159 | 172 |
| Trimeth & Sulfa | 1.74% | 3 | 2.33% | 4 | 95.93% | 165 | 172 |

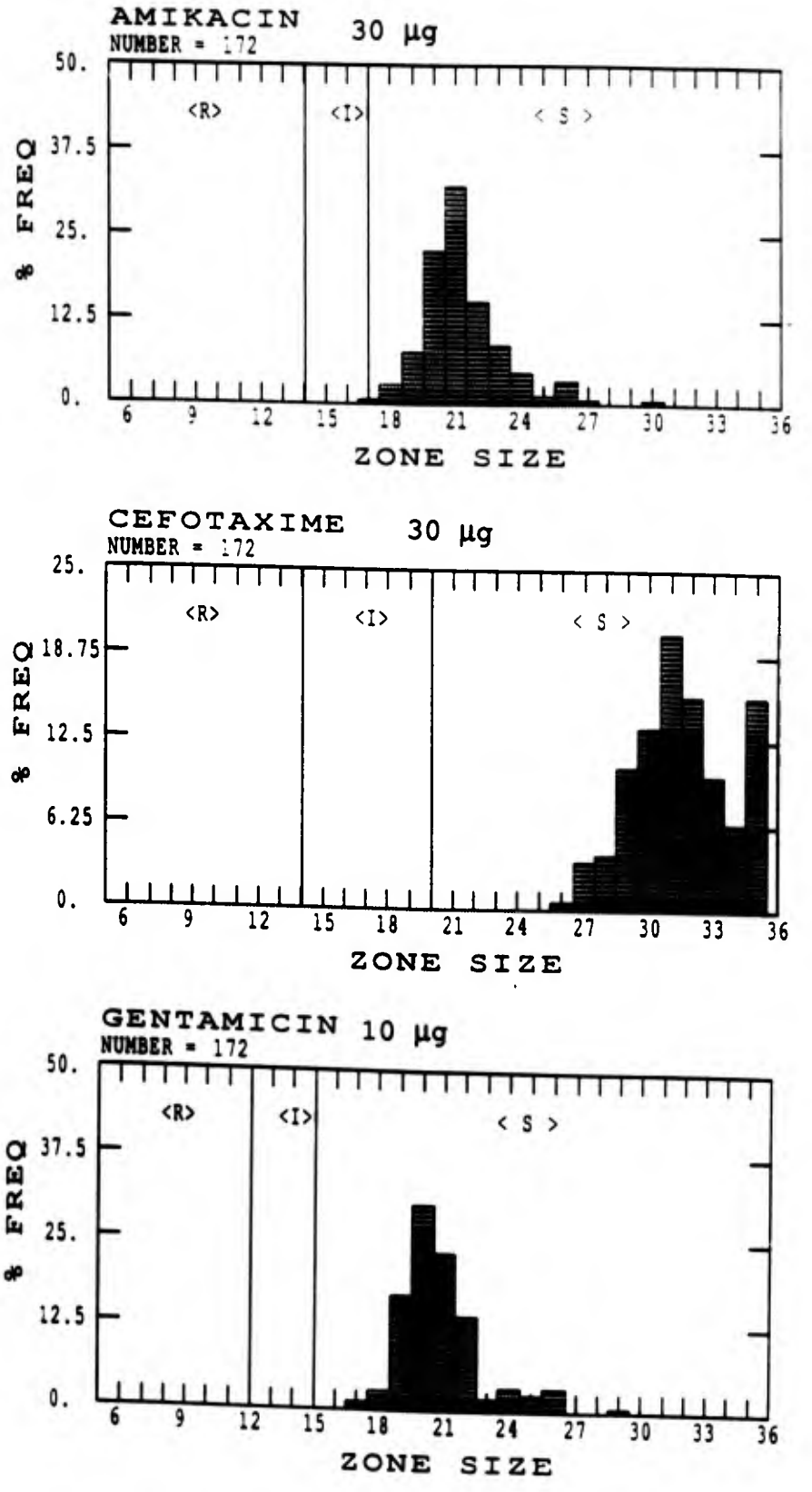
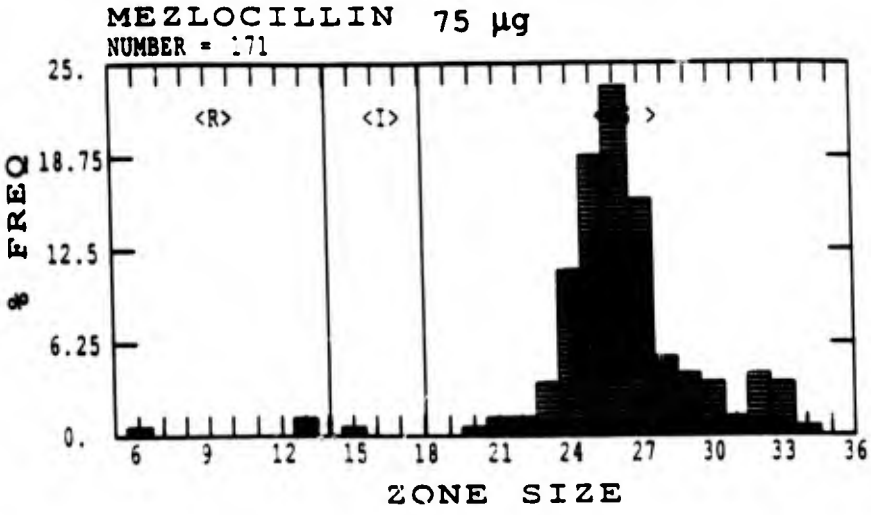



**FIGURE 19.** Histogram display of the distribution of zones of inhibition of growth of Proteus mirabilis.



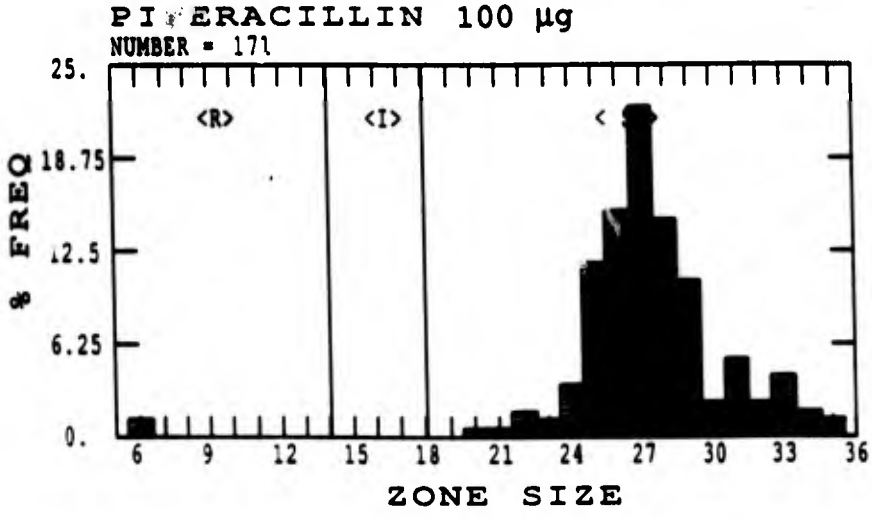
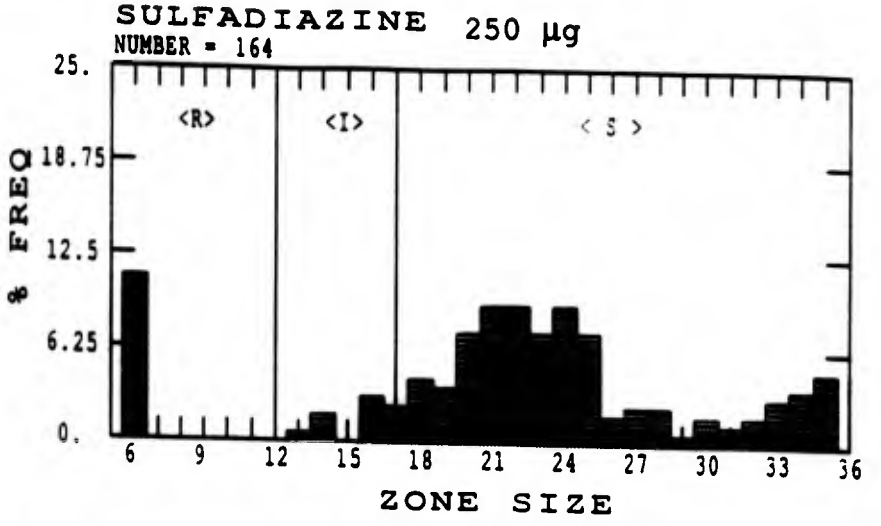




**FIGURE 19.** Histogram display of the distribution of zones of inhibition of growth of Proteus mirabilis.



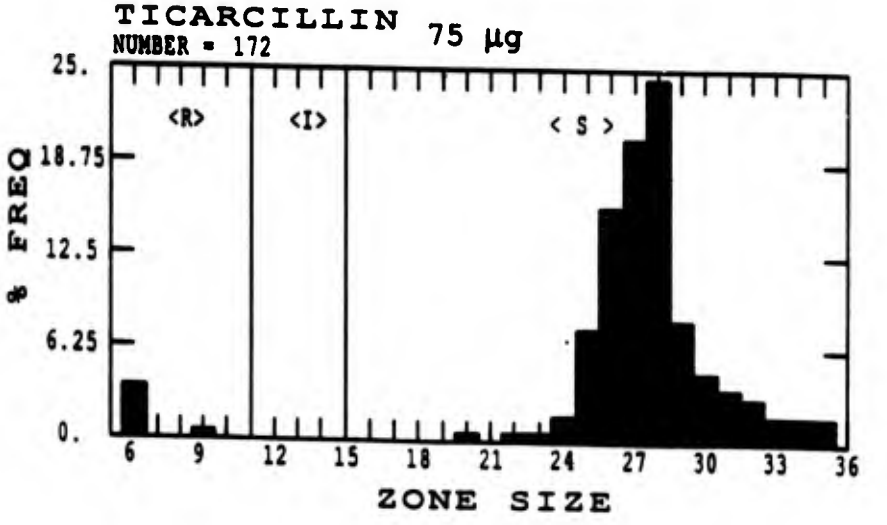




**FIGURE 19.** Histogram display of the distribution of zones of inhibition of growth of Proteus mirabilis.

GR D

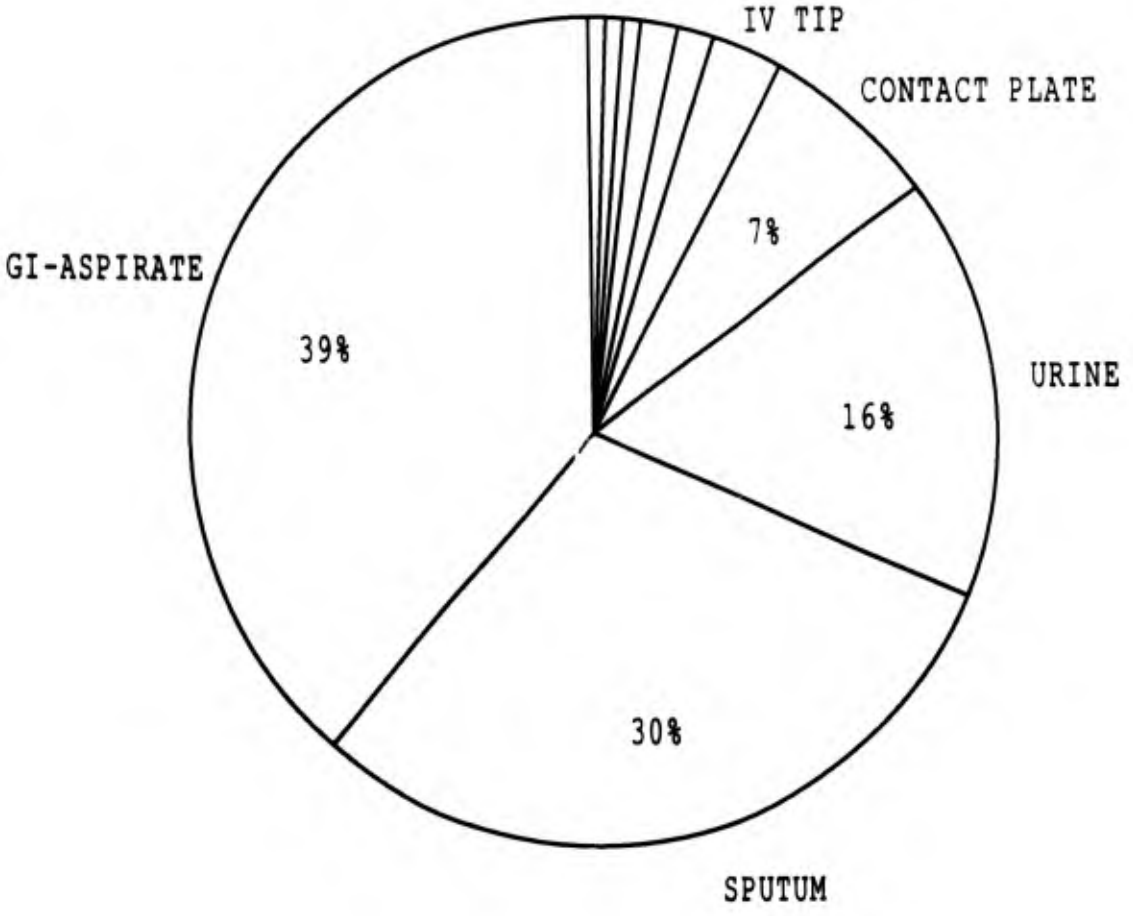



**FIGURE 20.** Display of the relative frequency of sources yielding Group D Enterococcus tested for in vitro sensitivity to antibiotics in 1989

**TABLE 15. Antibiotic Sensitivity Data for Group D Enterococcus (1989)**

| Antibiotic | RESISTANT % | RESISTANT Number | INTERMEDIATE % | INTERMEDIATE Number | SENSITIVE % | SENSITIVE Number | Total Number |
|---|---|---|---|---|---|---|---|
| Amikacin | 98.15% | 53 | 0.00% | 0 | 1.85% | 1 | 54 |
| Ampicillin | 0.00% | 0 | 1.85% | 1 | 98.15% | 53 | 54 |
| Cefoperazone | 7.41% | 4 | 83.33% | 45 | 9.26% | 5 | 54 |
| Cefotaxime | 57.41% | 31 | 33.33% | 18 | 9.26% | 5 | 54 |
| Ceftazidime | 98.15% | 53 | 0.00% | 0 | 1.85% | 1 | 54 |
| Ceftriaxone | 0.00% | 0 | 0.00% | 0 | 100.00% | 54 | 54 |
| Cephalothin | 59.26% | 32 | 37.04% | 20 | 3.70% | 2 | 54 |
| Chloramphenicol | 3.70% | 2 | 1.85% | 1 | 94.44% | 51 | 54 |
| Clindamycin | 96.30% | 52 | 0.00% | 1 | 3.70% | 2 | 54 |
| Erythromycin | 14.81% | 8 | 31.48% | 17 | 53.70% | 29 | 54 |
| Gentamicin | 96.30% | 52 | 3.70% | 2 | 0.00% | 0 | 54 |
| Imipenem-Cilastatin sodium | 1.85% | 1 | 1.85% | 1 | 96.30% | 52 | 54 |
| Mezlocillin | 0.00% | 0 | 1.85% | 1 | 98.15% | 53 | 54 |
| Moxalactam | 100.00% | 54 | 0.00% | 0 | 0.00% | 0 | 54 |
| Piperacillin | 0.00% | 0 | 1.85% | 1 | 98.15% | 53 | 54 |
| Streptomycin | 98.15% | 53 | 0.00% | 0 | 1.85% | 1 | 54 |
| Sulfadiazine | 98.08% | 51 | 0.00% | 0 | 1.92% | 1 | 52 |
| Tetracycline | 72.22% | 39 | 0.00% | 0 | 27.78% | 15 | 54 |
| Tobramycin | 98.11% | 52 | 1.89% | 1 | 0.00% | 0 | 53 |
| Vancomycin | 0.00% | 0 | 1.85% | 1 | 98.15% | 53 | 54 |

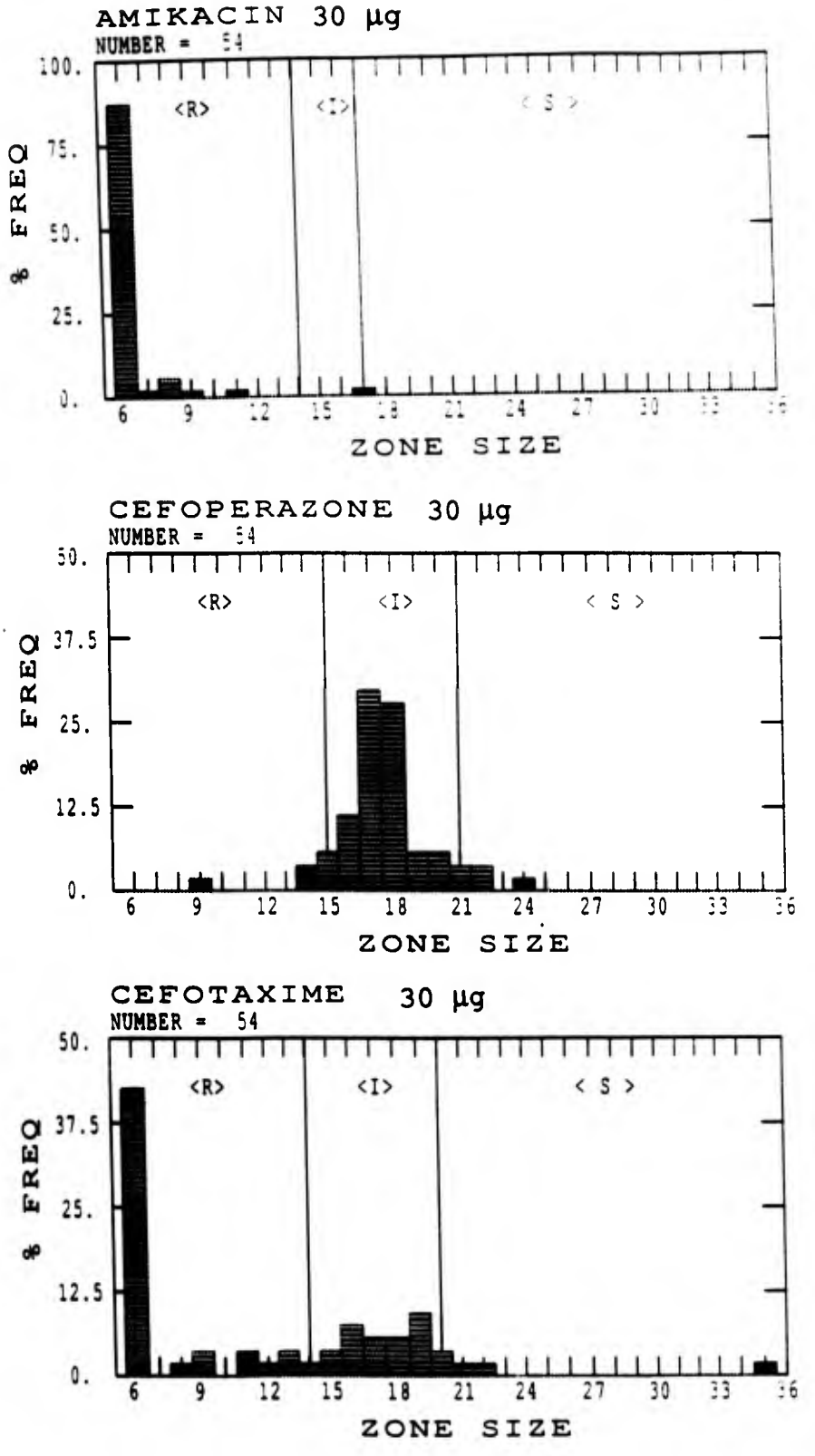



**FIGURE 21.** Histogram display of the distribution of zones of inhibition of growth of Group D. Enterococcus.

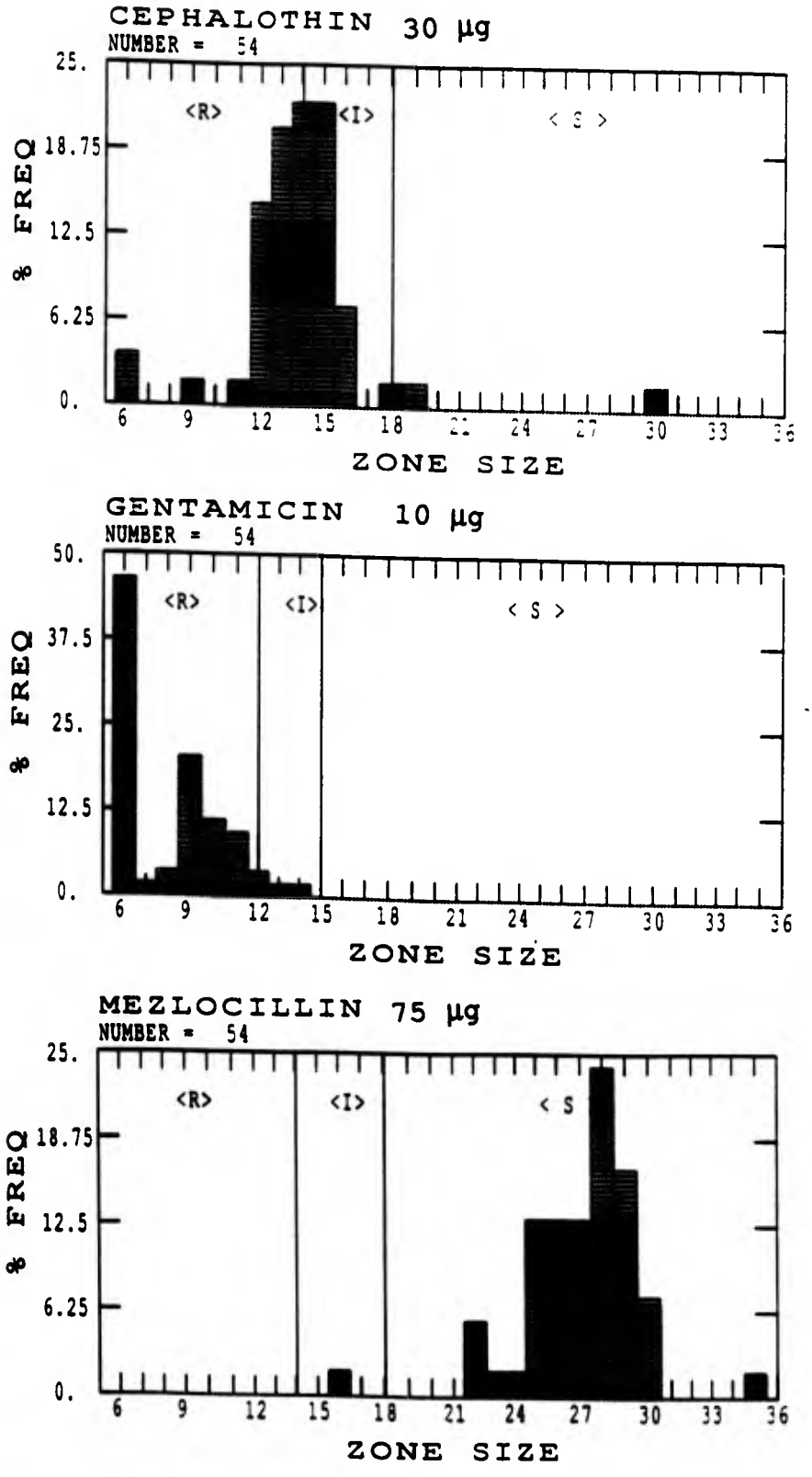



**FIGURE 21.** Histogram display of the distribution of zones of inhibition of growth of Group D. Enterococcus.

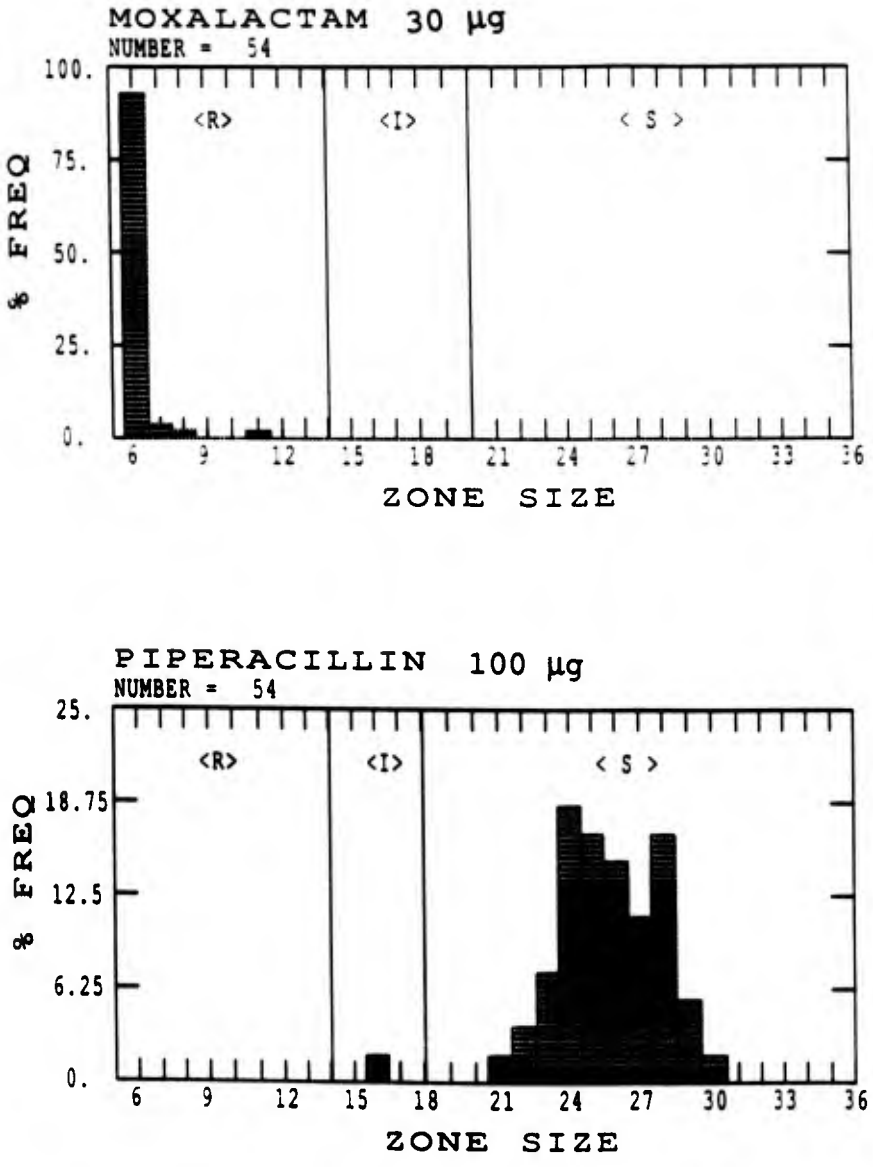
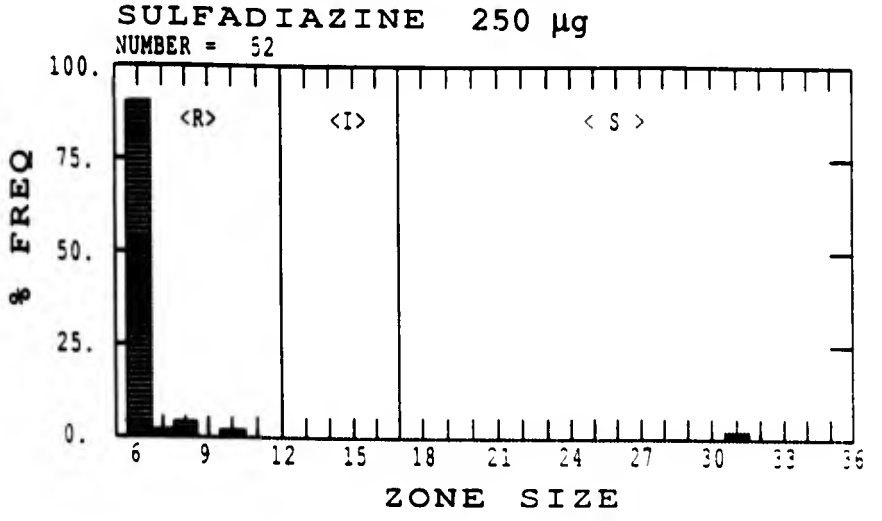



**FIGURE 21.** Histogram display of the distribution of zones of inhibition of growth of Group D. Enterococcus.



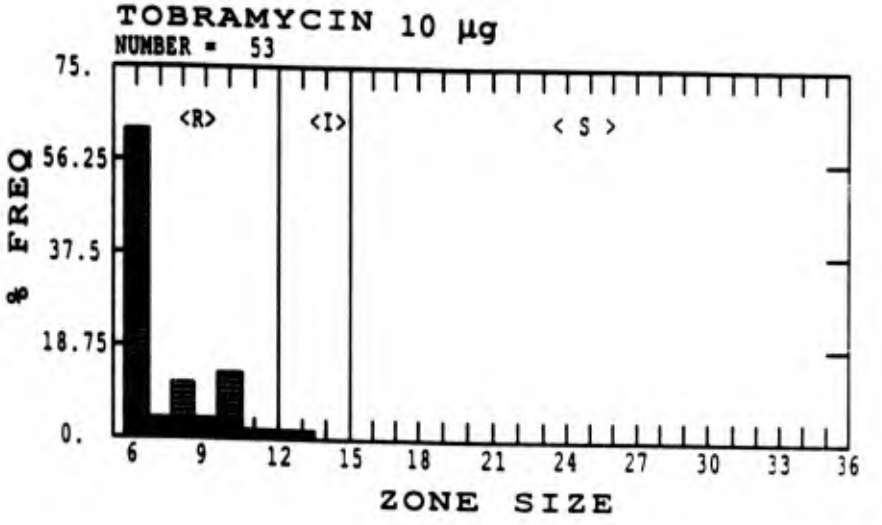




**FIGURE 21.** Histogram display of the distribution of zones of inhibition of growth of Group D. Enterococcus.

**Escherichia coli**. The sources of isolation for tested strains are presented in Figure 16. The results of in vitro antibiotic testing are presented in Table 13. Histogram displays of the distributions of zone sizes for selected antibiotics are presented in Figure 17.

**Proteus mirabilis**. The sources of isolation for tested strains are presented in Figure 18. The results of in vitro antibiotic testing are presented in Table 14. Histogram displays of the distributions of zone sizes for selected antibiotics are presented in Figure 19.

**Group D Enterococcus**. The sources of isolation for tested strains are presented in Figure 20. The results of in vitro antibiotic testing are presented in Table 15. Histogram displays of the distributions of zone sizes for selected antibiotics are presented in Figure 21.

## PRESENTATIONS/PUBLICATIONS

**Waymack JP, Guzman RF, Burleson DG, McManus AT, Mason AD Jr., and Pruitt BA Jr.**: Effect of prostaglandin E in multiple experimental models. VI. Effect on T-cell subsets. **Prostaglandins** 38 (3):345-353, Sept. 1989.

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**Kim SH, Mason AD, Jr., Okerberg CV, McManus AT, McManus WF, Pruitt BA, Jr.**: Viral infection in burn patients: A review of seven-year autopsies (1981-1987). **Proceedings of the Second Sino-American Conference on Burn Injury & Trauma**, Beijing, China, may 22-25, 1989. Abstract-page 82.

**Chu C-S, McManus AT, Mason AD, Jr., Pruitt BA, Jr.**: Multiple graft harvesting from donor wounds healed under the influence of weak direct current. **Proceedings of the Second Sino-American Conference on Burn Injury & Trauma**, Beijing, China, May 22-25, 1989. Abstract-page 101.

**Chu C-S, McManus AT, Mason Ad, Jr., Pruitt BA, Jr.**: Accelerating split-thickness graft healing on tangentially excised deep second degree burns wounds by weak direct current application. **Proceedings of the Second Sino-American Conference on Burn Injury & Trauma**, Beijing, China, May 22-25, 1989. Abstract-page 117.

**McManus AT, Mason AD, Jr.**: Organisms causing bacteremia (1987-1988): A survey of 5337 admissions to 30 North American burn units. **Proceedings of the Second Sino-American Conference on Burn Injury & Trauma**, Beijing, China, May 22-25, 1989. Abstract-page 149.

**McManus AT, Mason AD, Jr., Pruitt BA, Jr.**: Occurrence of Pseudomonas aeruginosa in seriously burned patients: A review of 950 patients (1983-1987). **Proceedings of the Second Sino-American Conference on Burn Injury & Trauma**, Beijing, China, May 22-25, 1989. Abstract-page 152.

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**McManus AT, Mason AD, Jr., McManus WF, Pruitt BA, Jr.**: Occurrence of Pseudomonas aeruginosa in seriously burned patients: A review of 950 patients. Hospital Epidemiology: New Challenges and Controversies, Baltimore, Maryland, March 10-12, 1989, sponsored by Infection Control Hospital Epidemiology and the Society of Hospital Epidemiology of America (SHEA). Abstract #1.

**McManus AT, Mason AD, Jr.**: Organisms causing bacteremia (1987-1988): A survey of 4659 admissions to 30 North American burn units. Hospital Epidemiology: New Challenges and Controversies, Baltimore, Maryland, March 10-12, 1989, sponsored by Infection Control and Hospital Epidemiology and the Society of Hospital Epidemiology of America (SHEA). Abstract#2.

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| RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY | | | | 1. AGENCY ACCESSION | 2. DATE OF SUMMARY | REPORT CONTROL SYMBOL |
|---|---|---|---|---|---|---|
| | | | | DA314656 | 07 Jul 90 | DD-DR&E(AR) 636 |
| 3. DATE PREV SUM'RY | 4. KIND OF SUMMARY | 5. SUMMARY SCTY | 6. WORK SECURITY | 7. REGRADING | 8. DISB'N INSTR'N | 9. LEVEL OF SUM A. WORK UNIT |
| 01 Oct 89 | K. Comp | U | U | | CX | |

| 10. NO./CODES: | PROGRAM ELEMENT | PROJECT NUMBER | TASK AREA NUMBER | WORK UNIT NUMBER |
|---|---|---|---|---|
| a. PRIMARY | 61102A | 3M161102BS14 | FA | 302 |
| b. CONTRIBUTING | | | | |
| c. CONTRIBUTING | | | | |

11. TITLE *(Precede with Security Classification Code)* (U) Investigation of the Physiologic and Immunologic Effects of Prostaglandin E in Septic and Traumatized Rats

12. SUBJECT AREAS
0605 Medicine and Medical Research 0615 Pharmacology

| 13. START DATE | 14. ESTIMATED COMPLETION DATE | 15. FUNDING ORGANIZATION | 16. PERFORMANCE METHOD |
|---|---|---|---|
| 8805 | 9007 | DA | C. In-House |

17. CONTRACT/GRANT — MILITARY RELEVANCY CERTIFIED — RESOURCES ESTIMATE

| a. DATE EFFECTIVE | APPROVED BY | FISCAL YEARS | a. PROFESSIONAL WORKYEARS | b. FUNDS *(In thousands)* |
|---|---|---|---|---|
| b. CONTRACT/GRANT NUMBER | | 89 | 0.5 | 30 |
| c. TYPE | d. AMOUNT | 90 | 0.5 | 35 |
| e. KIND OF AWARD | f. CUM/TOTAL | | | |

| 19. RESPONSIBLE DOD ORGANIZATION | 20. PERFORMING ORGANIZATION |
|---|---|
| a. NAME<br>US Army Institute of Surgical Research | a. NAME<br>US Army Institute of Surgical Research |
| b. ADDRESS *(include zip code)*<br>Fort Sam Houston<br>San Antonio, Texas 78234-5012 | b. ADDRESS<br>Fort Sam Houston<br>San Antonio, Texas 78234-5012 |
| c. NAME OF RESPONSIBLE INDIVIDUAL<br>PRUITT, B A | c. NAME OF PRINCIPAL INVESTIGATOR<br>WAYMACK, J P |
| d. TELEPHONE NUMBER *(include area code)*<br>512-221-2720 | d. TELEPHONE NUMBER *(include area code)*<br>512-221-2018 |
| 21. GENERAL USE<br>FINA<br>MILITARY/CIVILIAN APPLICATION: M | f. NAME OF ASSOCIATE INVESTIGATOR *(if available)*<br>MASON, A D<br>g. NAME OF ASSOCIATE INVESTIGATOR *(if available)*<br>MC MANUS, A T |

22. KEYWORDS *(Precede EACH with Security Classification Code)* (U) Burns (Injuries); (U) Prostaglandin; (U) Physiological Effects; (U) Immunosuppression; (U) Trauma; (U) Septicemia

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS *(Precede text of each with Security Classification Code)*

23/24. (U) The objective of this work was to determine the physiologic and immunologic effects of the elevations in prostaglandin E levels seen following trauma, sepsis, and tumor growth, to determine whether the net effect of the elevation in prostaglandin E levels of these disease states was beneficial or detrimental, and to determine whether pharmacologic manipulation of the prostaglandin E level was beneficial in terms of immunologic or physiologic function. Healthy adult Lewis rats were administered varying doses of the long-acting prostaglandin E derivative, 16,16-dimethyl-prostaglandin E, prior to receiving a number of physiologic challenges. Initial evaluations centered on the ability of prostaglandin E to alter resistance to mortality following challenge with endotoxin.

25. (U) 8710 - 8809. This project was approved by the USAISR Research Council and US Army Institute of Surgical Research Animal Care and Use Committee during the third quarter of fiscal year 1988. A lethal endotoxin model was developed and investigations began on the effect of prostaglandin E on resistance to endotoxin shock.

(U) 8810 - 8909. Prostaglandin E (PGE) was shown to enhance survival in mouse Escherichia coli peritonitis models and to improve survival in endotoxin shock models. These effects may have been secondary to decreased tumor necrosis factor production following infusion of PGE. PGE has not been shown to have a significant effect on T cell subsets or on immunologic function in tumor-bearing rats.

DD FORM 1498 83 MAR — EDITION OF MAR 68 IS OBSOLETE. — * USGPO: 1988-491-003/50329

**CONTINUATION OF DD FORM 1498 FOR THE PROJECT ENTITLED "INVESTIGATION OF THE PHYSIOLOGIC AND IMMUNOLOGIC EFFECTS OF PROSTAGLANDIN E IN SEPTIC AND TRAUMATIZED RATS"**

(U) 8910 - 9009. Investigations of the effect of other cyclooxygenase products were performed to further delineate the effect of PGE in septic and traumatized rats.

**SUPPLEMENTAL WORK UNIT INFORMATION FOR THE PROJECT ENTITLED "INVESTIGATION OF THE PHYSIOLOGIC AND IMMUNOLOGIC EFFECTS OF PROSTAGLANDIN E IN SEPTIC AND TRAUMATIZED RATS"**

**Subrecord/Linking Accession Number:** Not applicable.

**Search Control Data:** W6K44L/W6K45D, 20 October 1989

**Product Identification:** For technical reports, refer to the US Army Institute of Surgical Research Annual Research Progress Report for fiscal years 1988-90.

**Unclassified Special Categories:** Lab Animals: Rats; Mice; RA II

**ANNUAL RESEARCH PROGRESS REPORT**

**PROJECT NUMBER:** 3M161102BS14-00 RESEARCH

**PROJECT TITLE:** Investigation of the Physiologic and Immunologic Effects of Prostaglandin E in Septic and Traumatized Rats

**US ARMY INSTITUTE OF SURGICAL RESEARCH**
**FORT SAM HOUSTON**
**SAN ANTONIO, TEXAS, USA**

**1 October 1989 - 7 July 1990**

**INVESTIGATORS**

J. Paul Waymack, MD
Arthur D. Mason, Jr., MD
Albert T. McManus, PhD
Basil A. Pruitt, Jr., MD, Colonel, MC

## ABSTRACT

**PROJECT NUMBER:** 3M161102BS14-00 RESEARCH

**PROJECT TITLE:** Investigation of the Physiologic and Immunologic Effects of Prostaglandin E in Septic and Traumatized Rats

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

**PERIOD COVERED IN THIS REPORT:** 1 Oct 89 through 7 Jul 1990

**INVESTIGATORS:** J. Paul Waymack, MD
Arthur D. Mason, Jr., MD
Albert T. McManus, PhD
Basil A. Pruitt, Jr., MD, Colonel, MC

The immunosuppression seen following burn injury has frequently been attributed to elevated prostaglandin E levels. We evaluated the contribution of elevated prostaglandin E levels on susceptibility to infectious complications utilizing multiple mouse models. The administration of 100 $\mu$/kg of the long-acting derivative of prostaglandin E, 16,16-dimethyl-prostaglandin E was found to improve survival in C3/HEN mice challenged with $1x10^8$ Escherichia coli organisms intraperitoneally. The administration of indomethacin was found to decrease survival in the same model. With C3/HEJ (endotoxin-resistant) mice, indomethacin was found to increase mortality rates in animals challenged with $1x10^8$, $1x10^9$ or $1x10^{10}$ Escherichia coli organisms. These findings suggest that elevated prostaglandin E levels seen in burn patients may not be responsible for the postburn increased susceptibility to infectious complications.

# INVESTIGATION OF THE PHYSIOLOGIC AND IMMUNOLOGIC EFFECTS OF PROSTAGLANDIN E IN SEPTIC AND TRAUMATIZED PATIENTS

## INTRODUCTION

One of the primary etiologies for mortality following burn injury is the development of infectious complications (1). These infections have two main causes, a loss of the skin's natural barrier and the immunosuppression that results from burn injuries. This immunosuppression is due to a number of factors, including inadequate nutrition in the postburn period, the use of immunosuppressive agents such as anesthesia and blood transfusions, and the release of endogenous immunosuppressive metabolites.

Prostaglandin E (PGE) has been reported to be one of the immunosuppressive metabolites released following burn injury (2). The belief in an immunosuppressive nature of PGE has resulted from two areas of investigation. The first is the demonstration by Arturson (3) that PGE levels are increased following burn injury and that burn patients are immunosuppressed (4). The second is the demonstration that PGE impairs immune function in a number of *in vitro* leukocyte culture models (5).

There has been little investigation on the *in vivo* effects of PGE in animal models due to the extremely short half-life of parenterally administered PGE (6). The short half-life is the result of the near total clearance of PGE during each pass through the lungs. Derivatives of PGE that are resistant to enzymic degradation by the pulmonary parenchyma and thus have much longer half-lives have recently been developed. One of these derivatives is 16,16-dimethyl-prostaglandin E (dPGE). We herein report an evaluation of this agent on resistance to sepsis in multiple mouse models.

## MATERIALS AND METHODS

**Animals.** Five hundred adult male C3/HEJ mice and 210 adult male C3/HEN mice weighing approximately 25g were used in these studies. The mice were housed in stainless-steel hanging cages and allowed food and water ad libitum. The mice were observed for a minimum of 1 week prior to entry into the study to exclude the presence of pre-existing diseases.

**Drugs.** The dPGE was generously supplied by the Upjohn Company (Kalamazoo, MI, USA). The dPGE was diluted with sufficient normal saline to achieve a concentration that permitted the desired dose of drug to be administered in a final volume of 0.25 ml. The dPGE was injected intraperitoneally through a 25-gauge needle.

Indomethacin was generously supplied by Merck Sharp & Dohme (Columbus, OH, USA). The indomethacin was also diluted with sufficient normal saline to achieve a final concentration that

permitted the desired dose to be administered in a final volume of 0.25 ml. The indomethacin was injected intraperitoneally through a 25-gauge needle.

**Sepsis models.** Six sepsis models were chosen, all of which utilized intraperitoneal injections of varying quantities of Escherichia coli organisms. The E. coli were cultured in trypticase soy broth at 37°C for 16 h and then centrifuged at 3000 r.p.m. for 5 min. The supernatant was decanted and the E. coli pellet resuspended in a sufficient volume of saline to achieve the desired concentration of organisms. For the C3/HEN mice, two quantities of the E. coli were tested. For the first, $1x10^7$ c.f.u. of the E. coli organisms were given intraperitoneally in a volume of 0.5 ml saline. For the second, $1x10^8$ c.f.u. of the E. coli were given in 0.5 ml of saline.

The E. coli organisms were administered at four concentrations to the C3/HEJ mice. For the first, $1x10^7$ c.f.u. in 0.5 ml of saline were given. The second was $1x10^8$ c.f.u. in 0.5 ml of saline. For the third, $1x10^9$ c.f.u. in 0.5 ml of saline were given. The final concentration was $1x10^{10}$ c.f.u. in 0.5 ml of saline.

In each of the peritonitis models, the mice were randomized to one of four drug treatment groups. The first received twice daily injections of 0.25 ml of normal saline intraperitoneally. The second received twice daily intraperitoneal injections of 4 mg/kg of indomethacin dissolved in 0.25 ml of saline. The third group received twice daily injections of 50$\mu$g/kg of dPGE dissolved in 0.25 ml of saline intraperitoneally and the final group received twice daily injections of 100$\mu$g/kg of dPGE dissolved in 0.25 ml of saline intraperitoneally. Table 1 lists the number of animals in each drug treatment group for each of the models.

With each model, the mice were followed for seven days after peritoneal challenge to determine mean survival times and absolute survival rates. Those mice surviving to seven days had previously been noted to have no further mortalities. For the calculation of mean survival times, the mice which survived seven days were given a survival time of seven days.

All data are presented as mean ± SEM. Comparisons among groups were performed using chi-square and Kruskal-Wallis tests.

## RESULTS

The C3/HEN mice challenged with $1x10^7$ E. coli c.f.u. had a 100% survival rate in the saline control group, the 50$\mu$g/kg dPGE treatment group and the 100$\mu$g/kg dPGE treatment group (Table 2). Those mice receiving indomethacin had a decreased survival rate of 16% ($P<0.001$). The mean survival time for the indomethacin-treated

mice was also significantly decreased when compared with the other groups ($P<0.0001$) (Table 2).

For C3/HEN mice challenged with $1x10^8$ E. coli c.f.u., the survival rates of the saline-treated and 50μg/kg dPGE treated groups were 8% (Table 2). The indomethacin treatment groups had a 0% survival rate and the mice treated with 100μg/kg of dPGE had survival rate of 32%. These differences were statistically significant ($P<0.01$). The increased mean survival time of the mice treated with 100μg/kg of dPGE (Table 2) was also statistically significant when compared with the other groups ($P<0.005$).

The C3/HEJ mice injected with $1x10^7$ E. coli c.f.u. had a 94% survival rate in the saline treatment group (Table 3). Both the 50μg/kg and the 100μg/kg dPGE treatment groups had an 84% survival rate. The indomethacin-treated mice had a 76% survival rate. These differences were not statistically significant. The differences in the mean survival times among these four groups were also not statistically significant (Table 3).

**TABLE 1. Number of mice used for each sepsis model (represented by colonies of E. coli) in each treatment group**

| | C3/HEN Mice | | C3/HEJ Mice | | | |
|---|---|---|---|---|---|---|
| Treatment group | $1x10^7$ | $1x10^8$ | $1x10^7$ | $1x10^8$ | $1x10^9$ | $1x10^{10}$ |
| Saline | 35 | 25 | 50 | 25 | 50 | 25 |
| Indomethacin | 25 | 25 | 25 | 25 | 25 | 25 |
| 50 μg/kg dPGE | 25 | 25 | 25 | 25 | 50 | 25 |
| 100 μg/kg dPGE | 25 | 25 | 25 | 25 | 50 | 25 |

**TABLE 2. Mean survival times (mean ± SEM) and survival rates in C3/HEN mice challenged with $1x10^7$ or $1x10^8$ colonies of E. coli**

| | $1x10^7$ | | $1x10^8$ | |
|---|---|---|---|---|
| Treatment group | Mean survival time (days)**** | Survival rate(%)** | Mean survival time(days)*** | Survival rate%* |
| Saline | 7.00±0.00 | 100 | 1.52±0.33 | 8 |
| Indomethacin | 2.88±0.44 | 16 | 1.16±0.13 | 0 |
| 50 μg/kg dPGE | 7.00±0.00 | 100 | 1.52±0.33 | 8 |
| 100 μg/kg dPGE | 7.00±0.00 | 100 | 2.92±0.57 | 32 |

**** $P<0.0001$; ** $P<0.001$; *** $P<0.005$; * $P<0.01$.

**TABLE 3.** **Mean survival times (mean ± SEM) and survival rates of C3/HEJ mice challenged with $1X10^7$, $1X10^8$, $1X10^9$, and $1X10^{10}$ colonies of *E. coli***

| | $1X10^7$ | | $1X10^8$ | | $1X10^9$ | | $X10^{10}$ | |
|---|---|---|---|---|---|---|---|---|
| | Mean survival time (days) | Survival rate% | Mean Survival time (days) | Survival rate% | Mean Survival time (days)** | Survival rate%* | Mean Survival time (days)** | Survival rate%* |
| Saline | 6.72±0.16 | 94 | 7.00±0.00 | 100 | 6.20±0.38 | 92 | 6.84±0.16 | 96 |
| Indomethacin | 5.76±0.46 | 76 | 4.64±0.45 | 44 | 3.28±0.26 | 4 | 3.68±0.40 | 16 |
| 50µ/kg dPGE | 6.20±0.38 | 84 | 7.00±0.00 | 100 | 7.00±0.00 | 100 | 7.00±0.00 | 100 |
| 100µ/kg dPGE | 6.25±0.25 | 84 | 7.00±0.00 | 100 | 6.80±0.14 | 96 | 7.00±0.00 | 100 |

*P<0.001; **P<0.0001.

For C3/HEJ mice challenged with $1x10^8$ E. coli c.f.u., the indomethacin treatments significantly decreased both survival rates and mean survival times (Table 3). The 44% survival rate was statistically significant ($P<0.001$), as was the 4.64 ± 0.45 day mean survival time ($P<0.0001$) when compared with saline and dPGE treatment groups.

The detrimental effect of indomethacin treatment was also demonstrated in the C3/HEJ mice challenged with $1x10^9$ E. coli c.f.u. (Table 3). Both the 4% survival rate and the 3.28 ± 0.26 day mean survival time were statistically significant when compared with the other three groups ($P<0.001$ for survival rates and $P<0.0001$ for mean survival times).

Finally, at the $1x10^{10}$ E. coli c.f.u. challenge, indomethacin treatment adversely affected survival in the C3/HEJ mice. With this quantity of bacterial challenge, the indomethacin-treated mice had a 16% survival rate and a 3.68 ± 0.40 day mean survival time, which were both statistically significant when compared with remaining groups ($P<0.001$ for survival rates and $P<0.0001$ for mean survival times).

## DISCUSSION

Infection remains a major cause of morbidity and mortality following thermal injuries. The immunosuppression seen following burns is one of the main reasons for this elevated infection rate.

Elevated PGE levels have long been thought to be a contributing factor to the immunosuppression seen in burn patients because of the high levels demonstrated in burn patient serum and because of its toxic effects on leucocyte function in studies in vitro.

Attempts to quantitate the contribution of PGE to the postburn immumosuppression have been thwarted by the extremely short half-life of parenterally administered PGE. To avoid this limitation, we have utilized a long-acting derivative of PGE (dPGE) to quantitate the contribution of elevated PGE levels to susceptibility to infection-related mortality.

We have previously reported that the administration of dPGE to Lewis rats increased survival rates in an E. coli peritonitis model (7). This beneficial effect appears due, at least in part, to an increased resistance to endotoxin shock (8) when treatment with dPGE commenced prior to endotoxin challenge. When the administration of dPGE was delayed until after endotoxin challenge, the protective effect was no longer apparent. It was further shown that pretreatment with the PGE synthesis inhibitor indomethacin decreased survival rates in Lewis rats challenged with endotoxin (9). The protective effect of dPGE in endotoxin shock appears due, at least in part, to its ability to decrease the rate of release of

tumor necrosis factor (8). Finally, the administration of dPGE appears to exert a beneficial effect in septic rats by triggering an amino acid flux from skeletal muscle protein to acute phase proteins (10,11).

Our current studies have attempted to determine whether the beneficial effect of dPGE administration is a species-specific trait of rats. To answer this question, two strains of mice were utilized. The first group, C3/HEN mice, are endotoxin sensitive and the second group, C3/HEJ mice, are endotoxin resistant due to their inability to synthesize tumor necrosis factor in response to endotoxin exposure (12).

The results with C3/HEN (endotoxin-sensitive) mice demonstrated a protective effect of elevated PGE levels as evidenced by an increased survival in those animals receiving the dPGE and decreased survival among those receiving indomethacin. These findings strongly suggest that the previously demonstrated beneficial effect of dPGE administration in septic rats is not a species-specific response.

In C3/HEJ (endotoxin-resistant) mice, there was no significant mortality in the saline treatment groups, even at the $1x10^{10}$ c.f.u. of E. coli challenge. The ability of this strain of mouse to resist such a high concentration of E. coli organisms is probably due, at least in part, to its inability to synthesize the highly toxic macrophage metabolite tumor necrosis factor following the endotoxin exposure. As such, this prevented any possible demonstration of a beneficial effect of dPGE administration.

It was, however, noteworthy that indomethacin treatment of the C3/HEJ mice did result in a significant decrease in survival in three of the E. coli peritonitis models. Since C3/HEJ mice are not capable of synthesizing tumor necrosis factor, the detrimental effect of indomethacin cannot be attributed to the PGE/tumor necrosis factor interaction which has previously been demonstrated in rats (8). The increased mortality rate in the indomethacin-treated mice may be due to the effect of indomethacin on blood flow to various organs, on release of other toxic compounds of leucocytes in response to bacterial endotoxin exposure, or on the inhibition of the normal physiological response to sepsis. Such an inhibition has previously been demonstrated in burned septic rats treated with the cyclo-oxygenase inhibitor ibuprofen (13). The rats which received ibuprofen in that study failed to show the normal hypermetabolic response to sepsis and had a significantly higher mortality rate when compared to the rats treated with saline. Fink et al. reported that the use of cyclo-oxygenase inhibitors prevented the normal hyperdynamic response in septic dogs (14). When one considers the increased physiological workload septic patients must accomplish, the benefits of preventing a hyperdynamic/hypermetabolic state must be questioned. Further

studies will be required to determine the contribution of these factors to the increased mortality rate.

In conclusion, the elevation of PGE levels seen in burn patients may be a normal physiological response of the body to protect against infection-related mortality which is so common in burn patients. Attempts to decrease the rate of PGE synthesis in the burn patient through the use of cyclo-oxygenase inhibitors may only increase the mortality rate in those patients who develop infections. Further studies delineating the physiological importance of PGE in sepsis should be undertaken prior to initiation of trials using cyclo-oxygenase inhibitors in septic patients.

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| RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY | | | | 1. AGENCY ACCESSION | 2. DATE OF SUMMARY | REPORT CONTROL SYMBOL |
|---|---|---|---|---|---|---|
| | | | | DAOG6968 | 01 Oct 90 | DD-DR&E(AR) 636 |
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(U) Alteration of Host Resistance in Burned Soldiers

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|---|---|---|---|
| 7610 | 9909 | DA | C. In-House |

17. CONTRACT/GRANT MILITARY RELEVANCY CERTIFIED APPROVED BY [illegible]

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|---|---|
| a. NAME<br>US Army Institute of Surgical Research | a. NAME<br>US Army Institute of Surgical Research |
| b. ADDRESS *(Include zip code)*<br>Fort Sam Houston<br>San Antonio, Texas 78234-5012 | b. ADDRESS<br>Fort Sam Houston<br>San Antonio, Texas 78234-5012 |
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23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS *(Precede text of each with Security Classification Code)*

23/24. (U) The objective of this work is to define the microbial basis of opportunistic infection in susceptible burned patients, identify specific mechanisms of decreased host resistance that are targeted by opportunistic pathogens, and develop and evaluate countermeasures. The effect of in vitro alterations of specific microbial characteristics on infection will be investigated. Specific antimicrobial and immunostimulatory therapies will be examined.

25. (U) 8710 - 8809. The clinical trial of the parenteral antibiotic ceftazidime as monotherapy in infected burn patients continued. A newly described beta lactamase with activity against most of the third generation cephalosporins was identified in several isolates. The gene coding for the enzyme appeared chromosomal. The enzyme had an isoelectric point of 6.3. Investigations into the mechanisms of antimicrobial activity of mafenide acetate showed the compound was not an antagonist of dihydropteroate synthetase. This finding confirmed other biologic data, indicating that mafenide acetate is not a typical sulfonamide.

(U) 8810 - 8909. The mechanisms of resistance to the antimicrobial action of phosphanilic acid and sulfonamides have been examined using DNA probes specific for two plasmid-mediated dihydropteroate synthetase genes (R1drd19 and GS04). Examination of sulfonamide-resistant burn isolates showed that 97.9% were reactive (95/97) with the probes. None of the sulfonamide-sensitive control strains reacted with the probes. Phosphanilic acid cross-resistance was 24.7% (24/97). Transfer of sulfonamide resistance

plasmids showed that only plasmids with the GS04-specific probe co-transferred phosphanilic acid resistance.

(U) 8910 - 9009. Pulsed-field gel electrophoresis technology (PFGE) has been added to the molecular epidemiology techniques available at this Institute. This new technology allows DNA mapping of whole microbial chromosomes by identifying contiguous fragments produced by selective restriction enzyme digestion. This method allows endemic strains independent of the extra-chromosomal elements they may contain to be identified. PFGE was used to identify a single-source endemic of Serratia marcescens that could not be investigated using antibiotic resistance patterns or plasmid profiles.

**SUPPLEMENTAL WORK UNIT INFORMATION FOR THE PROJECT ENTITLED "ALTERATION OF HOST RESISTANCE IN BURNED SOLDIERS"**

**Subrecord/Linking Accession Number:** Not applicable.

**Search Control Data:** W6K56A/W6K56F, 20 October 1989

**Product Identification:** For technical reports, refer to the US Army Institute of Surgical Research Annual Research Progress Report for fiscal years 1976-90.

**Unclassified Special Categories:** Volunteers: Adults; Children; RA II

**ANNUAL RESEARCH PROGRESS REPORT**

**PROJECT NUMBER:** 3M161102BS14-00, 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 1989 - 30 September 1990**

**INVESTIGATORS**

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## ABSTRACT

**PROJECT NUMBER:** 3M161102BS14-00, 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 89 through 30 Sep 90

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**New Antibiotics in Clinical Use.** The parenteral antibiotic agents Ceftazidime, Ceftriaxone and Aztreonam were introduced for (nonprotocol) clinical use during this reporting period. As shown in Table 1, no increase in in vitro resistance resulted from the use of these agents. In fact, all three parenteral agents showed a significant improvement in sensitivity when compared to the previous reporting period ($p<0.05$). These improvements were most likely the results of decreases in cross-contamination of resistant strains that resulted from increased awareness of the presence of such strains.

**TABLE 1. Activity on Newly Released Antibiotics Fiscal Year 1990.**

| FISCAL YEAR | 1986 | 1987 | 1988 | 1989 | 1990 |
|---|---|---|---|---|---|
| | | | CEFTAZIDIME[a] | | |
| Resistant | 63 (6.4%) | 80 (6.7%) | 480 (15.4%) | 630 (21.0%) | 367 (11.1%) |
| Sensitive | 928 | 1,106 | 2,633 | 2,267 | 2,953 |
| | | | CEFTRIAZONE[a] | | |
| Resistant | 157 (16.8%) | 167 (14.4%) | 843 (37.0%) | 737 (24.6%) | 531 (16.0%) |
| Sensitive | 928 | 1,106 | 2,633 | 2,633 | 2,787 |
| | | | AZTREONAM[a] | | |
| Resistant | 1 (0.0%) | 81 (7.1%) | 540 (33.9%) | 616 (37.6%) | 381 (17.3%) |
| Sensitive | 928 | 1,106 | 1,053 | 1,022 | 1,891 |

() = Percent resistant. [a] Against all flora except oxacillin-resistant Staphylococcus aureus.

**Experimental Topical Agents.** Mafenide acetate was examined for in vitro activity against Pseudomonas aeruginosa isolated from 43 burn patients. Agar dilution minimal inhibitory concentraton (MIC) assays were completed on 202 strains. The mean MIC was 0.385 g/100 ml. The median MIC was 0.312 g/100ml. Data comparing the past three reporting periods are presented in Table 2.

A new active Mafenide compound has been synthesized at USAISR. The compound is designated ISR-55. Details of the structure and synthesis process will be released after completion of the U.S. Patent process. Summaries of therapeutic trials in infected burned rats are presented in Tables 3,4,5.

**TABLE 2. Minimal Inhibitory Concentration for *Pseudomonas aeruginosa* Strains to Mafenide Acetate**

| Mafenide Acetate Concentration (g/100 ml) | No. of Strains FY 1988 | No. of Strains FY 1989 | No. of strains FY 1990 |
|---|---|---|---|
| 0.019 | 10 | 24 | 7 |
| 0.039 | 16 | 11 | 19 |
| 0.078 | 36 | 26 | 17 |
| 0.156 | 39 | 50 | 55 |
| 0.312 | 42 | 59 | 66 |
| 0.625 | 13 | 23 | 2 |
| 1.250 | 2 | - | 6 |
| **Total Number of Strains** | **158** | **193** | **202** |

**TABLE 3. Relative *in vivo* Antimicrobial Activity of Compound ISR-55 Against *Ps. aeurginosa* strain 59-1244.**

| EXPERIMENTAL GROUP | MORTALITY | PERCENT SURVIVAL |
|---|---|---|
| BURN CONTROL | 1/63 | 98.4 |
| INFECTED CONTROL | 56/60 | 06.7 |
| 0.25% ISR-55 | 0/9 | 100.0 |
| 0.50% ISR-55 | 0/9 | 100.0 |
| 1.00% ISR-55 | 1/19 | 94.7 |
| 2.00% ISR-55 | 6/61 | 90.2 |
| SULFAMYLON | 9/61 | 86.7 |
| SILVADENE | 5/62 | 93.4 |

**TABLE 4. Relative *in vivo* activity of ISR-55 Against *Ps. aeruginosa* strain VA-134.**

| EXPERIMENTAL GROUP | MORTALITY | PERCENT SURVIVAL |
|---|---|---|
| BURN CONTROL | 1/24 | 95.8 |
| INFECTED CONTROL | 24/24 | 00.0 |
| 2.0% ISR-55 | 3/24 | 87.5 |
| 11.1% SULFAMYLON | 12/24 | 50.0 |
| 1.0% SILVADENE | 12/24 | 50.0 |

**TABLE 5. Relative *in vivo* Activity of ISR-Against *Proteus mirabilis*.**

| EXPERIMENTAL GROUP | MORTALITY | PERCENT SURVIVAL |
|---|---|---|
| BURN CONTROL | 0/34 | 100.00 |
| INFECTED CONTROL | 34/35 | 02.8 |
| 2.0% ISR-55 | 2/34 | 94.1 |
| 11.1% SULFAMYLON | 24/34 | 29.4 |
| 1.0% SILVADENE | 10/34 | 58.8 |

**Modification of the Standard Burned Infected Animal Model by Piperacillin alone or in Combination with Wound Excision.** The possible therapeutic effects of the antipseudomonal penicillin Piperacillin in delayed treatment of *Ps. aeruginosa* stain 59-1244 infected burned rats was investigated. Animals were infected within one hour postburn and antibiotic treatment (50 mg 2X/day S.C.) was initiated at 24,48,72 hours post inoculation and continued for seven days. The possible combined effect of excision of the infected wound at 72 hours with initiation of antibiotic treatment was also examined. Results are presented in Table 6. It appears that parenteral Piperacillin at a daily dose of 100mg/K is effective in treating Pseudomonas burn wound infection at up to 48 hours post inoculation. Excision of the infected wound with Piperacillin at 72 hours post inoculation appeared to extend the

therapeutic effect by at least one day. These experiments will be extended and include a control for the possible effects of excision alone.

**TABLE 6. Examination of Piperacillin (PIP) Activity in the Ps. aeurginosa 59-1244 Burn Rat Model.**

| EXPERIMENTAL GROUP | MORTALITY | PERCENT SURVIVAL |
|---|---|---|
| BURN CONTROL | 0/10 | 100.00 |
| INFECTED CONTROL | 10/10 | 00.00 |
| PIP 24 HOUR DELAY | 1/10 | 90.0 |
| PIP 48 HOUR DELAY | 0/10 | 100.0 |
| PIP 72 HOUR DELAY | 6/10 | 40.0 |
| PIP 72 HOUR DELAY + EXCISION OF WOUND | 1/10 | 90.0 |

**Serologic Types of Pseudomonas aeruginosa Isolated from Burn Patients During FY 1990.** Pseudomonas aeruginosa isolated from 53 burned patients were serotyped using the Difco International Typing Sera set and autoclaved bacterial suspensions. Strains were selected on the basis of having a distinct antibiotic sensitivity pattern for each patient. Data are presented in Figure 1 as the total number of patients per serotype and as total strains of each serotype in Figure 2.

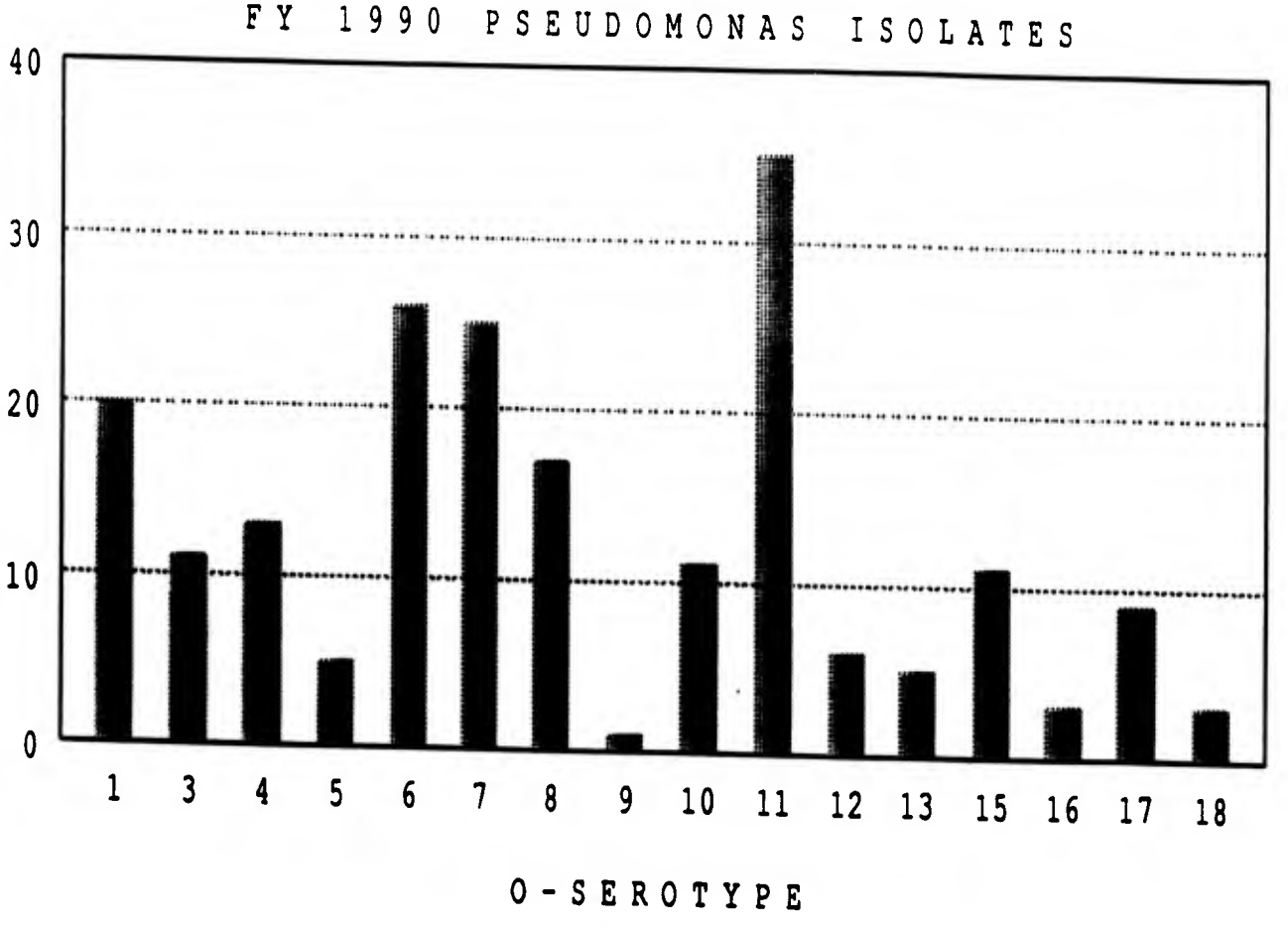


**FIGURE 1.** Histogram display of the frequency of FY 1990 P. aeruginosa O-serotypes (International). Type 18 indicates all strains not typeable by the standard 17 typing sera.

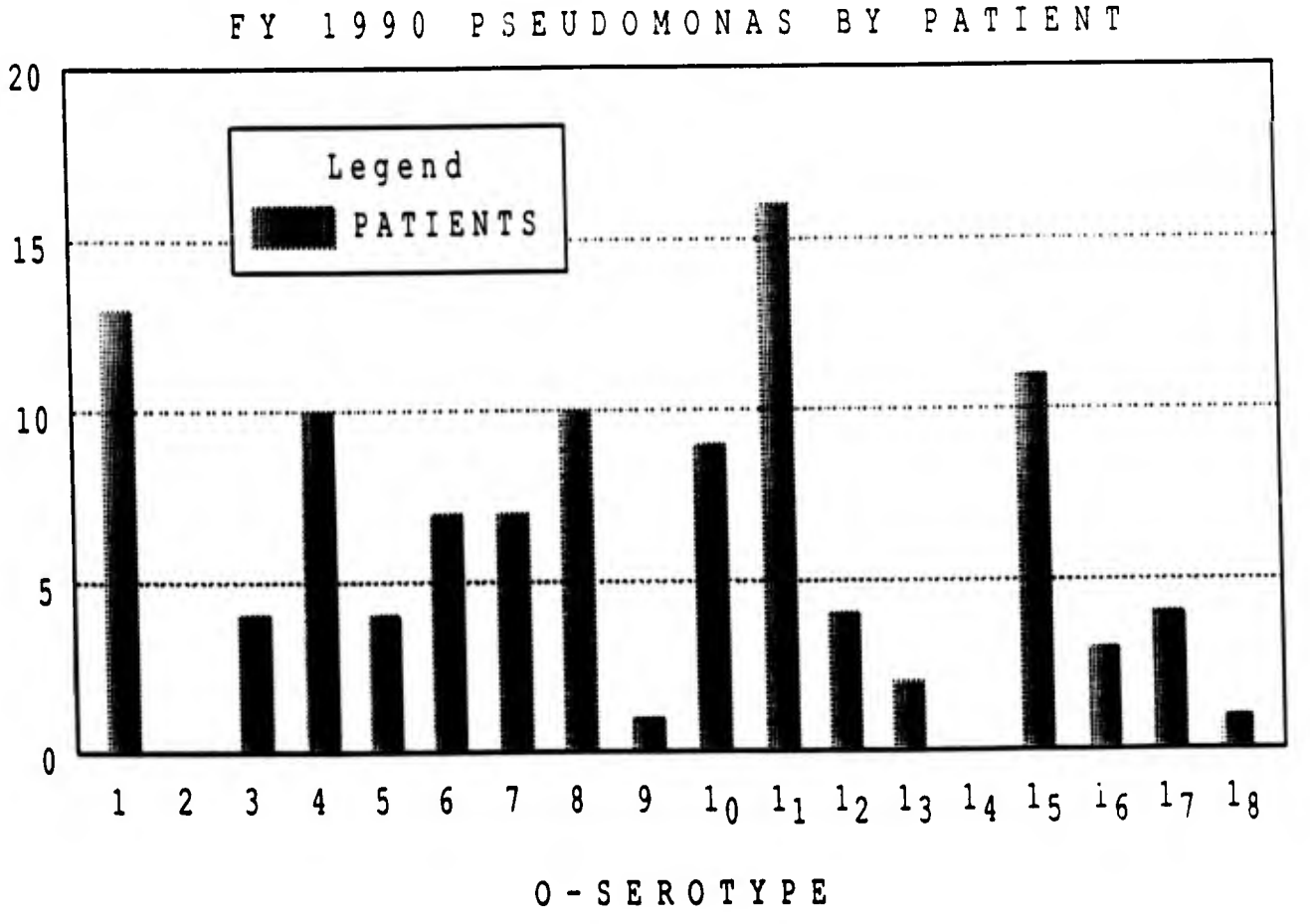


**FIGURE 2.** Histogram display of the frequency of FY 1990 P. aeruginosa O-serotypes (International) by the number of patients colonized with each serotype. Type 18 indicates all strains not typeable by the standard 17 typing sera.

**ANNUAL RESEARCH PROGRESS REPORT**

**PROJECT NUMBER:** 3M161102BS14-00, RESEARCH

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

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Several fluorescent substances are present in the serum of burned patients. We have previously reported that one of these substances is neopterin. In an attempt to determine whether neopterin might be useful as an indicator of infection, a preliminary study of selected sera from human burned patients was done. We measured the concentration of neopterin by reverse phase high pressure liquid chromatography (HPL). Extremely high serum neopterin levels were correlated with high levels of serum creatinine and presumed to indicate renal insufficiency. All samples with high creatinine (greater than 2 mg/dL) were eliminated from the study. We then retrospectively determined whether infection had been diagnosed at the time the sample had been taken and whether serum neopterin levels could be correlated with the infection. Serum neopterin was increased as early as ten days before infection diagnosis and remained elevated for approximately three weeks after. Elevated neopterin levels correlated best with samples from patients with bacteremia.

# CHARACTERIZATION OF BIOCHEMICAL INDICATORS OF INFECTION IN THE THERMALLY INJURED

## INTRODUCTION

Infection poses a serious threat to all severely burned patients and is a persistent obstacle to successful therapy. Prompt diagnosis of infection is crucial for timely treatment and for patient survival. Systemic changes induced by burn injury, such as leukocytosis and fever, hamper the early detection of systemic infection and make its diagnosis more difficult. Rapid biochemical detection of infection could provide diagnostic verification more promptly than is currently possible with standard microbiologic techniques.

We have measured serum neopterin levels in a selected group of burn patients and related those measurements to the presence or absence of infection in those patients.

## MATERIALS AND METHODS

Patient samples for study were remnants of serum samples that had been drawn for clinical chemistry analysis. The samples were analyzed for neopterin content and computer records were retrospectively screened to determine the date and type of infection episodes experienced by each patient.

Neopterin levels were determined by a modification of the method of Stea et al. (1). Serum (100 µl) was deproteinized by adding 200 µl of 0.12N acetic acid (final pH 4.5) and incubating at 100°C in an oil bath for 20 min. The mixture was centrifuged at 20,000g for 20 min. and 10 µl of the supernatant was injected directly on a Hewlett Packard (3200 Hillview Avenue, Palo Alto, CA 94304) model 1090 liquid chromatograph with a Biophase ODS reverse phase 4.6X250 mm column (Bioanalytical Systems, W. Lafayette, IN). The mobile phase consisted of .05M ammonium acetate (pH 7.0) for 7 min. followed by a methanol gradient reaching 30% in 4 min. Column temperature was maintained at 45°C and the flow rate was 1.0 ml/min. The HPL was equipped with a Kratos (Kratos Analytical, Ramsey, NJ) fluorescence detector, model 980 with a 25 µl flow cell. The excitation monochronometer was set at 350nm and the emission cutoff filter was at 389nm. The retention times for standard pterins (Sigma Chemical Co., St. Louis, MO) were determined using 10 µl of a standard solution of pterins (10 ng/ml). The amount of each fluorescent substance present was determined, using a Hewlett Packard model 3392A integrator.

## RESULTS

During the course of developing techniques to quantify serum neopterin by HPL, a group of burned patient sera had been selected because they had increased levels of fluorescence after acid

precipitation of the serum proteins. Neopterin concentration had been measured in each of these sera. As a preliminary evaluation of the usefulness of neopterin in infection diagnosis, these neopterin values were compared to the clinical microbiology information that was available for these patients.

Large increases in fluorescence were usually related to the deterioration of the patients condition (2) and the probable onset of renal failure. Neopterin is normally rapidly removed from the blood stream and excreted in the urine. Since renal failure is frequently a complication in failing patients, there was a concern that the increased fluorescence and neopterin concentration might be due to renal failure and not to infection. In order to examine the correlation between renal function and neopterin levels, we obtained serum creatinine values on each sample for which serum neopterin had been determined. When serum creatine levels were high (above 2mg/dL), serum neopterin levels were also high (Figure 1). These samples were presumed to have come from patients with renal insufficiency. The serum creatinine and the natural log of the serum neopterin concentration were highly correlated (correlation coefficient ($R^2$) = .415). When serum creatinine levels were low (<2mg/dL), there was little correlation between serum neopterin and serum creatine (Figure 2). The correlation coefficient was only 0.0217. Serum creatinine was used as a screening tool to select only those serum samples for analysis that were from patients with relatively good renal function. All samples with creatinine levels of 2.0 mg/dL or higher were excluded from the infection analysis.

There were 237 serum samples selected for this study. Of these, 30 samples had serum creatinine levels of 2 mg/dL or above and were removed from further analysis. The remaining 207 samples were from 19 patients. Of the 19 patients all but two experienced at least 1 infectious episode during the period when samples were collected. Most patients had more than one infection. The number and types of infections are shown in Table 1.

**TABLE 1. Number and Type of Infections.**

| Type | # Occurrences | # Patients | # Samples |
|---|---|---|---|
| Cellulitis | 6 | 6 | 14 |
| Pneumonia | 14 | 11 | 61 |
| Wound | 3 | 3 | 31 |
| Bactremia | 4 | 4 | 23 |
| Urinary Tract | 5 | 4 | 34 |
| Miscellaneous | 7 | 5 | 40 |
| No-infection | 0 | 2 | 4 |

Miscellaneous infections included: bronchitis, eye-infection, subcutaneous abscess, infection site unknown.

The mean neopterin concentrations from patients who had experienced various types of infections are shown in Figure 3. Samples from patients who experienced episodes of bacteremia had the highest levels of serum neopterin compared to samples from patients with other infections or no infection. The results were then analyzed with respect to time of infection diagnosis. As shown in Figure 4, mean serum neopterin concentrations from all patients with infections were highest in the periods from 10 days before infection to 19 days after infection compared to samples taken from other periods of time.

If we restricted the samples used in calculating the means of each type of infection to the period from ten days pre-infection to 19 days post infection as shown in Figure 4, bacteremia remains the only mean significantly different from the means for other infection types or no infection ($p < .001$). A temporal depiction of the course of serum neopterin concentrations for the four patients with bacteremia is shown in Figure 5. Serum neopterin was elevated as early as 9 days before the diagnosis of bacteremia and gradually declined after the sixth day post diagnosis.

## DISCUSSION

Neopterin is secreted by activated cultures of mononuclear leukocytes and is excreted in increased amounts by humans whose immune systems are responding to viral infection (3) including HIV (4,5), or tuberculosis (6). Neopterin has been used as a prognostic indicator for certain kinds of cancer (7) and as a measure of transplant rejection (8). Despite its widespread presence, no physiological role for neopterin has been found. Its precursor, dihydroneopterin triphosphate, is also a percursor of tetrahydrobiopterin which is a cofactor in hydroxylation reactions, particularly the hydroxylation of phenylalanine to form tyrosine, the precursor of serotonin and the catecholamines.

We have previously shown that serum neopterin levels in human burn patients are correlated with the levels of fluorescent factors previously discovered (2). These factors had been postulated to be potential indicators of infection in burned animals (9).

This preliminary evaluation indicates that neopterin may be a potential indicator of bacteremia. The values were consistently elevated over the few days before and after the diagnosis of bacteremia sepsis. However, this study included only four patients with bacteremia and two without any infection at all. More patient data needs to be collected to show definitively whether neopterin would be useful in determining the presence of bacteremia or other types of infection. A larger prospective study is underway to collect these data and determine if there is a correlation between serum neopterin levels and the presence of infection.

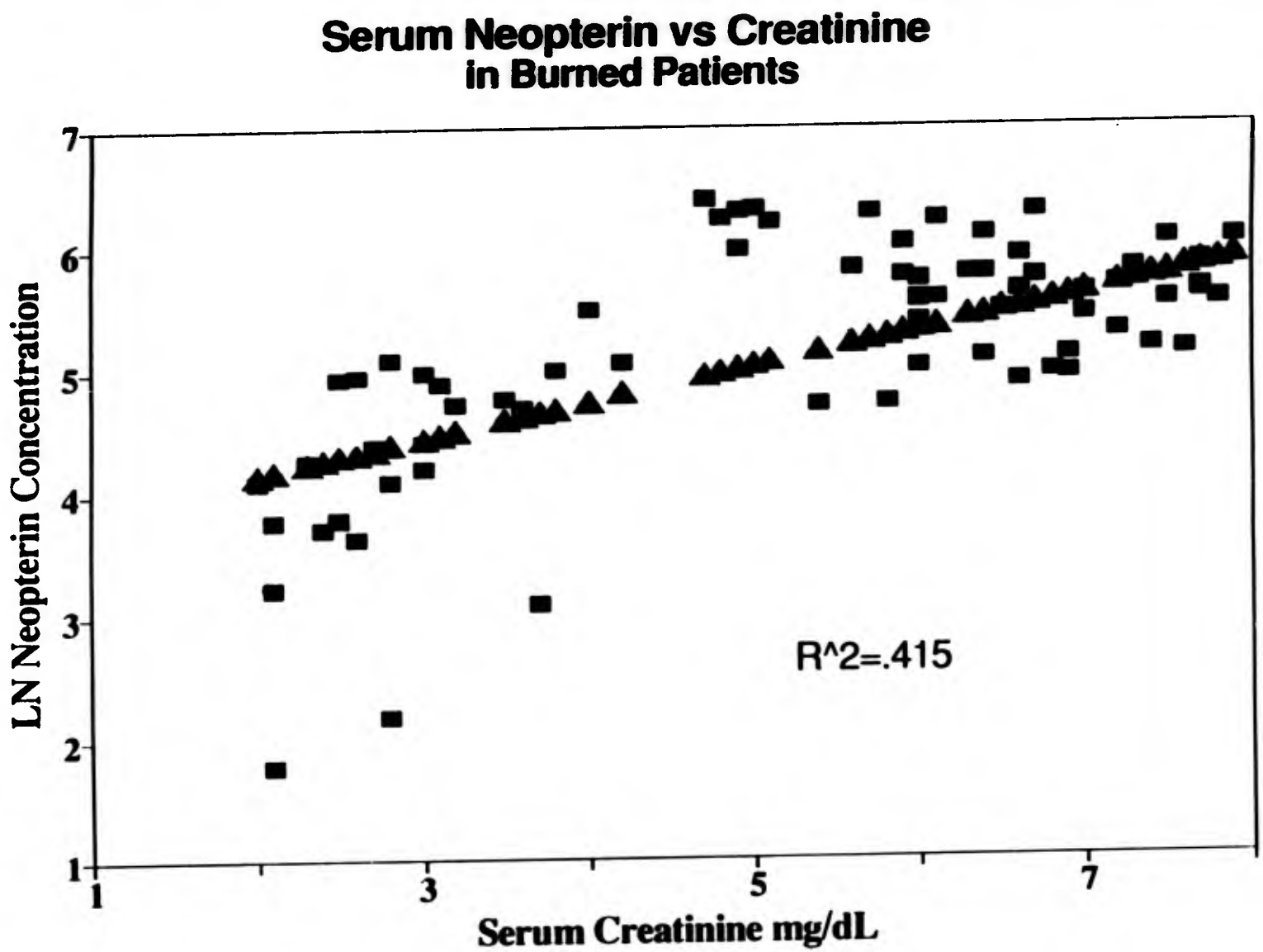


**FIGURE 1.** Correlation of high serum creatinine concentration with neopterin concentration. Creatinine and neopterin values were determined for each sample included in the study and the values correlated.

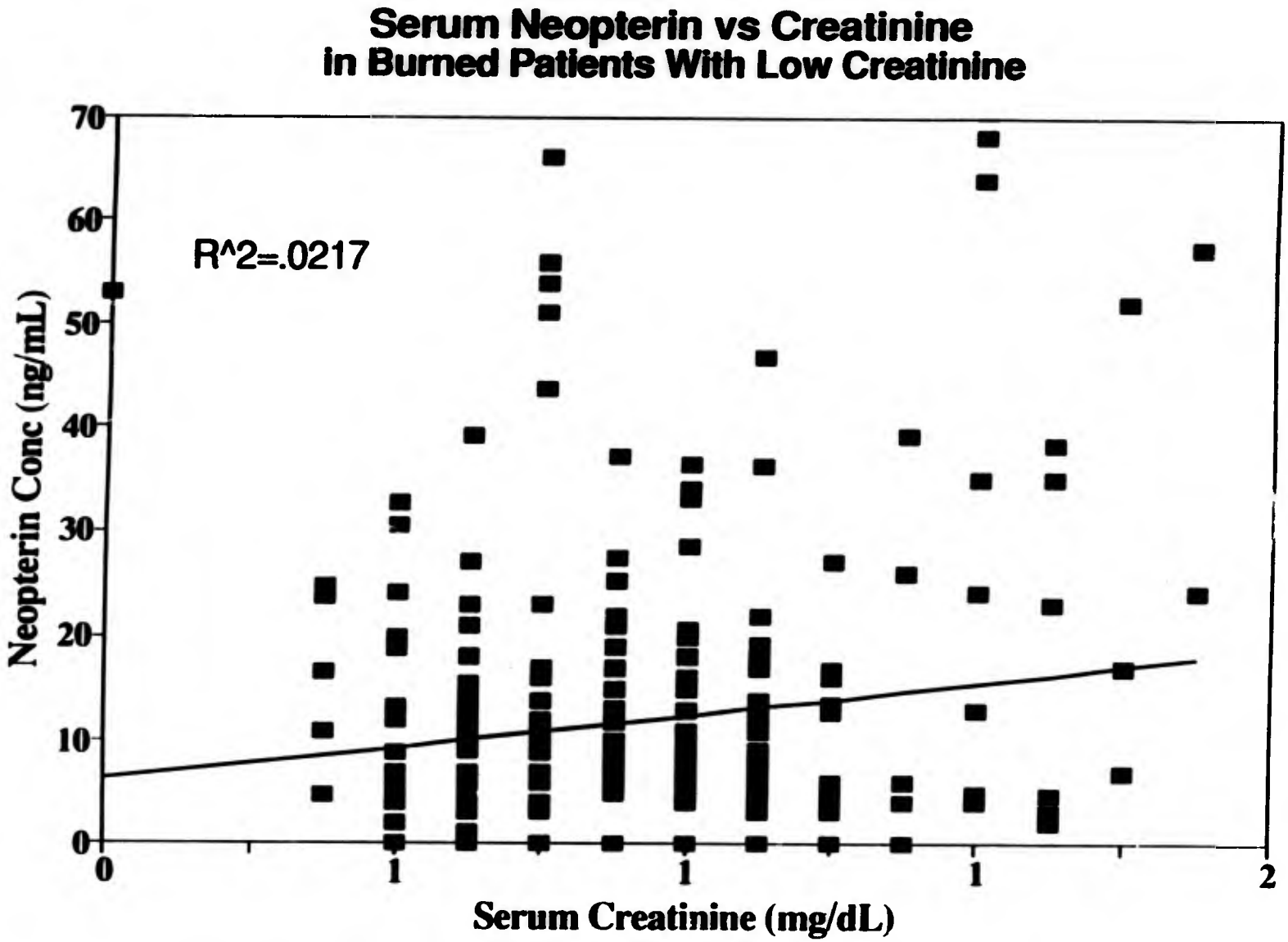


**FIGURE 2.** Lack of correlation between serum neopterin concentration and serum creatinine at low serum creatinine concentration.

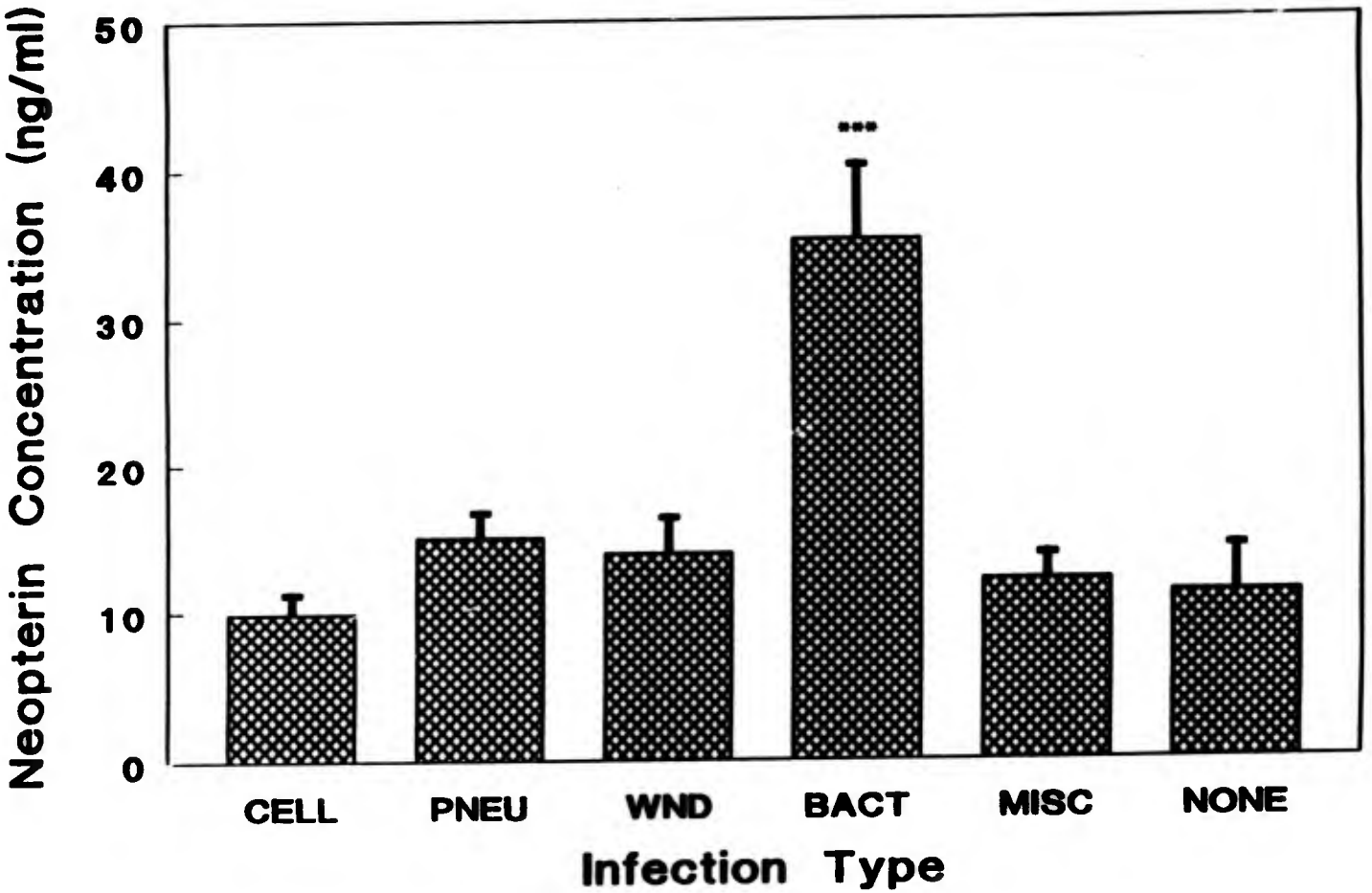


**FIGURE 3.** Mean serum neopterin concentration for sera from patients with various types of infection or no infection. The bars represent the mean values taken for all patients with a particular infection regardless of time of infection diagnosis. The error bar represents the standard error of the mean.

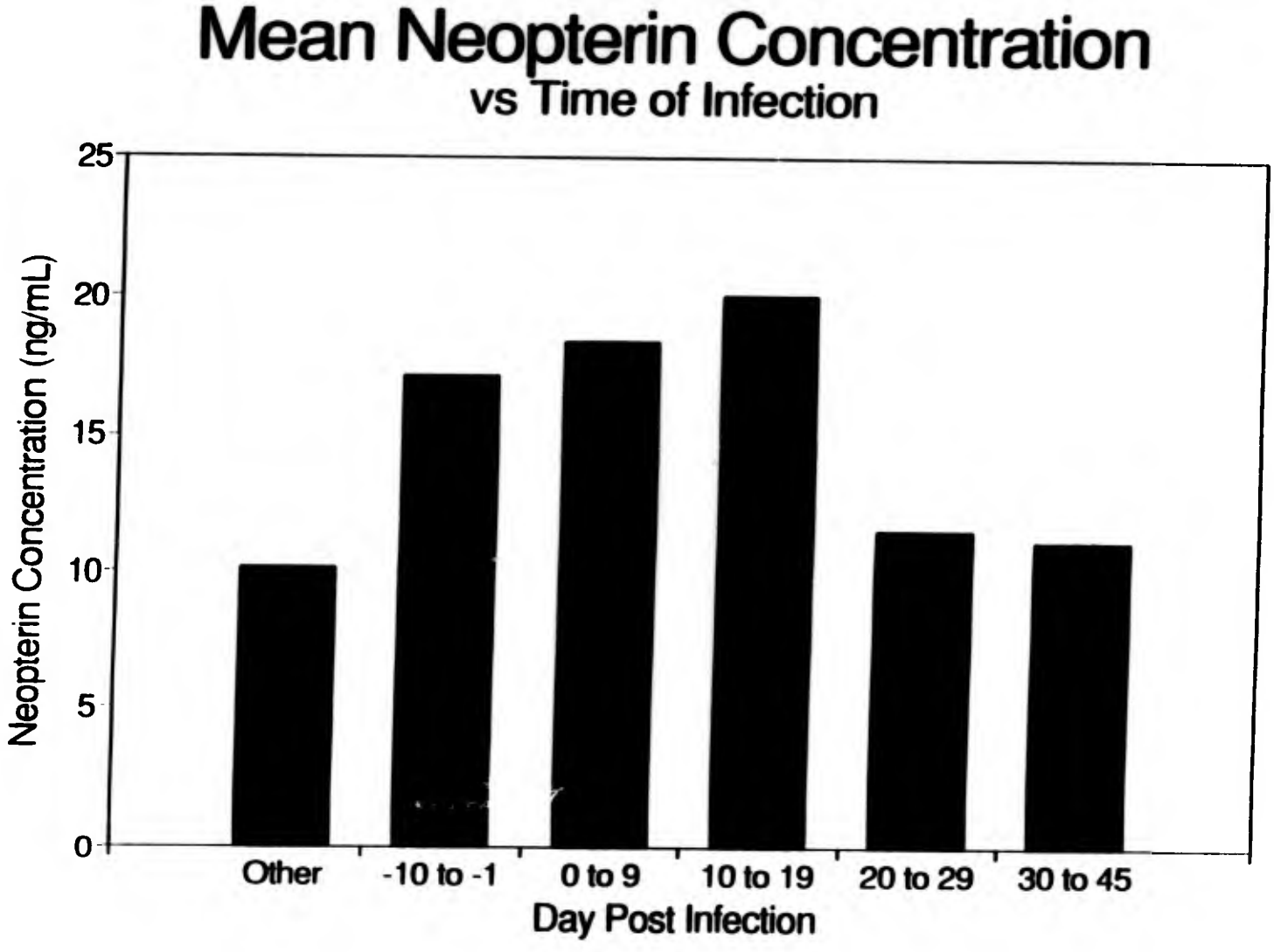




**FIGURE 4.** Mean serum neopterin values for various times pre and post infection. Values shown on the x axis are the days relative to the day of infection diagnosis and start of treatment. The bars represent the mean neopterin value for all patients with infection who fell within the time window indicated regardless of type of infection. The error bars represent the standard error of the mean.

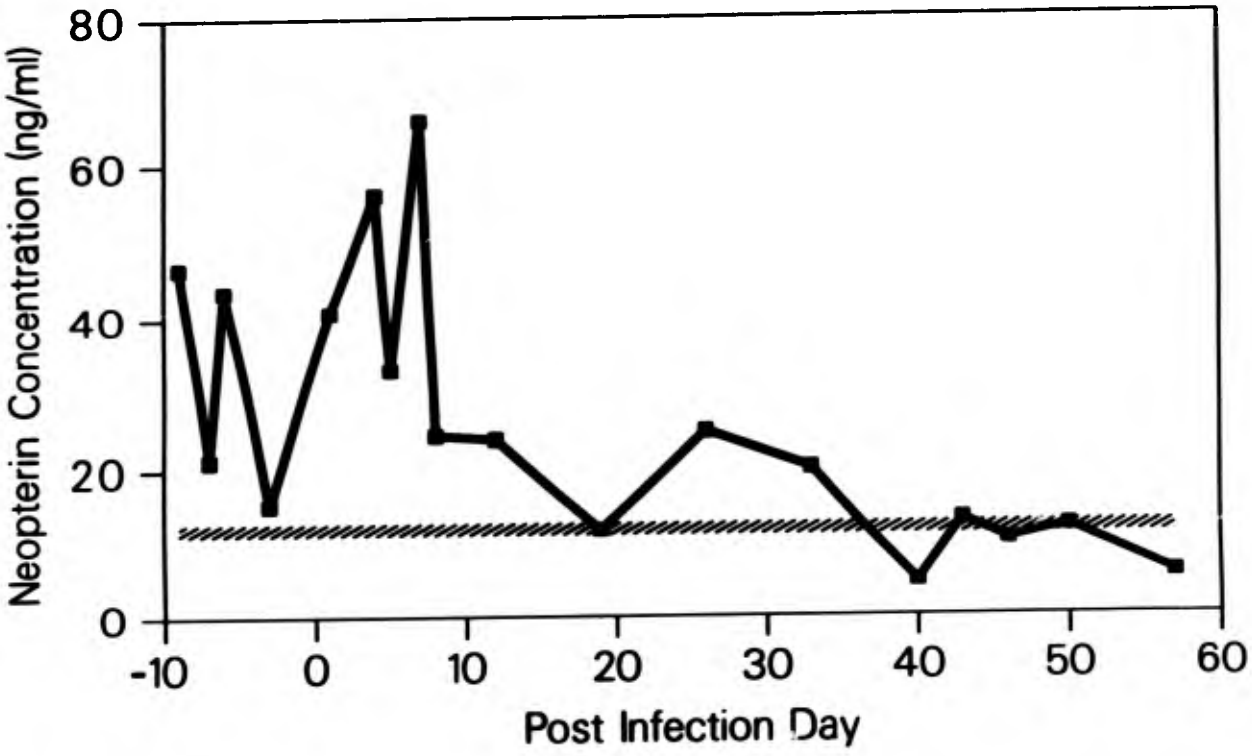




**FIGURE 5.** Temporal plot of serum neopterin concentration in septic burn patients. Serum neopterin levels are plotted for four patients who were septic during their course of recovery. Values are plotted with reference to the day that infection was diagnosed. Values occurring on the same post infection day were averaged. The horizontal bar represents non-infected patient values ± the standard error of the mean.

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| RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY | | | | 1. AGENCY ACCESSION: DAOG1842 | 2. DATE OF SUMMARY: 01 Oct 90 | REPORT CONTROL SYMBOL: DD-DR&E(AR) 636 |
|---|---|---|---|---|---|---|
| 3. DATE PREV SUM'RY: 01 Oct 89 | 4. KIND OF SUMMARY: D. Chg | 5. SUMMARY SCTY: U | 6. WORK SECURITY: U | 7. REGRADING: | 8. DISS'N INSTR'N: CX | 9. LEVEL OF SUM / A. WORK UNIT: |

| 10. NO./CODES | PROGRAM ELEMENT | PROJECT NUMBER | TASK AREA NUMBER | WORK UNIT NUMBER |
|---|---|---|---|---|
| a. PRIMARY | 61102A | 3M161102BS14 | CJ | 304 |
| b. CONTRIBUTING | | | | |
| c. CONTRIBUTING | | | | |

11. TITLE *(Precede with Security Classification Code)*
(U) Role of Thyroid Hormones in Burn Pathophysiology

12. SUBJECT AREAS
0601 Biochemistry 0605 Medicine and Medical Research

| 13. START DATE | 14. ESTIMATED COMPLETION DATE | 15. FUNDING ORGANIZATION | 16. PERFORMANCE METHOD |
|---|---|---|---|
| 7908 | 9909 | DA | C. In-House |

17. CONTRACT/GRANT — MILITARY RELEVANCY CERTIFIED APPROVED BY [signature]

a. DATE EFFECTIVE
b. CONTRACT/GRANT NUMBER
c. TYPE
d. AMOUNT
e. KIND OF AWARD
f. CUM/TOTAL

18. RESOURCES ESTIMATE

| FISCAL YEARS | a. PROFESSIONAL WORK YEARS | b. FUNDS *(In thousands)* |
|---|---|---|
| 90 | 0.1 | 154 |
| 91 | 0.1 | 161 |

19. RESPONSIBLE DOD ORGANIZATION

a. NAME: US Army Institute of Surgical Research
b. ADDRESS *(include zip code)*: Fort Sam Houston, San Antonio, Texas 78234-5012
c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A
d. TELEPHONE NUMBER *(include area code)*: 512-221-2720

20 PERFORMING ORGANIZATION

a. NAME: US Army Institute of Surgical Research
b. ADDRESS: Fort Sam Houston, San Antonio, Texas 78234-5012
c. NAME OF PRINCIPAL INVESTIGATOR: VAUGHAN, G M
d. TELEPHONE NUMBER *(include area code)*: 512-221-5416
f. NAME OF ASSOCIATE INVESTIGATOR *(if available)*
g. NAME OF ASSOCIATE INVESTIGATOR *(if available)*

21. GENERAL USE: FINA
MILITARY/CIVILIAN APPLICATION: M

22. KEYWORDS *(Precede EACH with Security Classification Code)* (U) Burns (Injuries); (U) Thyroid Hormones; (U) Thyroxine; (U) Thyrotropin

23. TECHNICAL OBJECTIVE 24 APPROACH 25. PROGRESS *(Precede text of each with Security Classification Code)*

23/24. (U) The objective of this work is to assess postburn alterations in thyroid function and develop treatment to improve survival in injured soldiers. Alterations in thyroid axis control will be characterized in a burned rat model.

25. (U) 8710 - 8809. Certain aspects of normal thyroid axis function must be better understood before optimal designs can be constructed to assess alterations in this axis after injury. Measurements of hormone dynamics during the dark phase of the lighting cycle have been helpful in elucidating the functions of other neuroendocrine axes. Therefore, we studied Sprague-Dawley (SD) and Fischer-344 (F) rats in different photoperiodic conditions. Around-the-clock radioimmunoassay measurements of serum thyroxine (T4), triiodothyronine (T3), their free indices (FT4I, FT3I), and thyrotrophin (TSH) were subjected to a statistical program we designed specially to assess the timing of nocturnal hormonal surges without bias due to other rhythmic parameters. Symmetrical lengthening of the dark phase engendered a nocturnal surge of T4 in SD rats. In F rats, nocturnal surges in total and free indices of both thyronines occurred even in a scotoperiod less than 12 h, and dark phase lengthening changed the surge timing to indicate entrainment control by lights-on rather than lights-off. Surprisingly, TSH measurements suggested mediation of thyronine changes by a nonpituitary mechanism in both strains. Alteration in assay techniques, leading to assessment of another source of these fluctuations (tissue stores), was begun.

DD FORM 1498 83 MAR — EDITION OF MAR 68 IS OBSOLETE. — * U.S.G.P.O. 1988 -491-003/50329

**CONTINUATION OF DD FORM 1498 FOR THE PROJECT ENTITLED "ROLE OF THYROID HORMONES IN BURN PATHOPHYSIOLOGY"**

(U) 8810 - 8909. Serum thyroxine (T4) and triiodothyronine (T3), in vitro T3 uptake (T3U), dialyzable fractions of T4 and T3, and thyrotropin (TSH) were measured after rats received a 17% total body surface area full-thickness standard scald burn or sham burn. The initial T4 and T3 fall was not TSH-dependent, as TSH was elevated at 1-2 days. Burn suppression of total T4 and T3 was greater than that explainable by variations in food intake. Iodothyronine binding was inhibited first (6 h, 1 day) to serum proteins and then later also to the T3U test matrix, compatible with a circulating inhibitor which changes character with time after burn. In another study, rats received a 25% burn (or sham) and infusions with either T4 or diluent until sacrificed at 6 days. Thyroidectomized controls received similar infusions. Serum TSH was inversely related to T4 and free T4, but in burned rats relatively more depressed than explained by normal feedback. Thus, after a transient early rise in TSH, burn injury appears to enhance negative feedback control of the TSH-thyroid axis, possibly contributing to suppression of the axis.

(U) 8910 - 9009. Changes in thyroxine (T4) and triiodothyronine (T3) disposal rates (kinetics) may constitute a portion of postburn thyroid axis abnormality. Usual methods to measure the amount of a radioactive tracer hormone present in serum have deficiencies related to specificity, recovery, and/or technical difficulty of the procedures. We are assessing a direct immunoprecipitation method involving reaction of tracer-spiked serum aliquots with four concentrations (including zero) of specific antibody and counting of the precipitation for logistic determination of the amount of tracer present. An antibody for T3 was found for which the amount of unlabelled T4 or T3 in the low or normal range and the amount of added T4 or reverse T3 radioactivity did not affect the determination of tracer T3. For T4 tracer determination, reaction of a T4 antibody with samples with low or normal unlabelled T4 and T3 gave the same result, and the proportion of total radioactivity precipitated was dependent on the radiochemical purity of the source of T4 label. If further testing confirms specificity and reliability of recovery, this will provide a method of measurement for kinetic studies of T4 and T3 in burn injury. In addition, a new direct method for determining free T4 by dialysis is being evaluated.

**SUPPLEMENTAL WORK UNIT INFORMATION FOR THE PROJECT ENTITLED "ROLE OF THYROID HORMONES IN BURN PATHOPHYSIOLOGY"**

**Subrecord/Linking Accession Number:** Not applicable.

**Search Control Data:** W6L23A/W6L24A, 20 October 1989

**Product Identification:** For technical reports, refer to the US Army Institute of Surgical Research Annual Research Progress Report for fiscal years 1979-90.

**Unclassified Special Categories:** Volunteers: Adults; Lab Animals: Rats; Hamsters; RA II

**ANNUAL RESEARCH PROGRESS REPORT**

**PROJECT NUMBER:** 3M161102BS14, RESEARCH

**PROJECT TITLE:** ROLE OF THYROID HORMONES IN BURN PATHOPHYSIOLOGY: Quantifying Isotopically Labelled Tracer Thyroxine ($T_4$) in Serum By Immunoprecipitation and Logistic Regression

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

**1 October 1989 - 30 September 1990**

**INVESTIGATORS**

George M. Vaughan, MD, Colonel, MC
Rita King
Leonard G. Seraile

**ABSTRACT**

**PROJECT NUMBER:** 3M161102BS14-00, RESEARCH

**PROJECT TITLE:** ROLE OF THYROID HORMONES IN BURN PATHOPHYSIOLOGY: Quantifying Isotopically Labelled Tracer Thyroxine ($T_4$) in Serum By Immunoprecipitation and Logistic Regression

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

**PERIOD COVERED IN THIS REPORT:** 1 Oct 89 through 30 Sep 90

**INVESTIGATORS:** George M. Vaughan, MD, Colonel, MC
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Leonard G. Seraile

These studies were done to lay the groundwork ultimately leading to assessment of thyroid hormone kinetics in burn injury. Conditions were found in which a monoclonal $T_4$ antibody together with a second antibody directed against the $T_4$ antibody can precipitate up to 82% of the radioactivity added to a serum sample, apparently depending on the radiochemical purity of the source of radioactively labelled $T_4$. These pilot results with this $T_4$ antibody system indicate that tracer binding at higher antibody concentrations (near those producing maximal binding) is similar among samples, although samples with more unlabelled $T_4$ may have relatively inhibited tracer $T_4$ binding at lower antibody concentrations. This was shown also by the similar projected maximal binding (PBmax) from logistic regression for charcoal thyronine-stripped serum (CTSS) and pooled rat serum (PRS), and for CTSS and normal human serum (NHS), when the same tracer source was used. This suggests that variation of unlabelled $T_4$ in the range expected for normal and burned individuals (who often have low $T_4$) may not affect the determination of the quantity of radiolabelled $T_4$ previously added to a sample as performed by this technique.

# QUANTIFYING ISOTOPICALLY LABELLED TRACER THYROXINE ($T_4$) IN SERUM BY IMMUNOPRECIPITATION AND LOGISTIC REGRESSION

## INTRODUCTION

Because of the marked alteration of thyroid function in burn injury (1) wherein the mechanism for the circulating $T_4$ deficit is not yet understood, it will be important to assess thyroid hormone kinetics in burn injury. If radioactively labelled $T_4$ is injected into an animal as a tracer for the purpose of determining clearance and secretary rates of $T_4$, there is a need to measure the amount of labelled $T_4$ in the presence of unlabelled $T_4$ and labeled $T_4$ metabolites. Methods involving differential extraction, column separation, or HPLC have been fraught with problems related to recovery, specificity, or inordinate complexity. A potentially simple method has been proposed in this laboratory, involving use of antibody precipitation and logistic regression. This method determines the amount of labelled $T_4$ in a serum sample by reacting several aliquots of the serum containing the labelled $T_4$ with several different dilutions of $T_4$ antibody in a radioimmuno-precipitation assay where the labelled $T_4$ bound to the $T_4$ antibody can be precipitated and separated from the labelled non-$T_4$ material. The counts (counts per min, cpm, radioactivity of tracer) in a given precipitate should then represent some portion of the total $T_4$ radioactivity in the sample. An initial approach, described herein, involves "spiking" a serum sample with a constant tracer amount of radioactive ($^{125}I$-labelled) $T_4$ from a label source. This source, however, may contain $^{125}I$ not in $T_4$ molecules. Greater amounts of $T_4$ antibody would be expected to precipitate greater amounts of tracer. The measurements can be expressed as percent of total cpm precipitated or bound with a given amount of antibody. By plotting the percent bound (PB) against an index of the quantity of antibody, determined by the antibody dilution, the PBmax where all the labelled $T_4$ would be antibody-bound can be extrapolated from the curve. This represents the amount of labelled $T_4$ in the serum sample.

This method for determining the quantity of labelled $T_4$ in a serum sample requires a $T_4$ antibody system that can bind most of the labelled $T_4$ in a serum aliquot even in the presence of varying amounts of unlabeled $T_4$. This report describes some of the steps taken to develop a system with sufficient binding of labelled $T_4$ with $T_4$ antibody in the presence of low or normal amounts of unlabelled $T_4$ for trial runs of a four-parameter logistic regression to extrapolate the quantity of labelled $T_4$ (expressed as PBmax) in a serum sample. The principles outlined in the published literature were used for guidance for designing antibody reactions (2-4) and logistic analysis (5). For the latter, we used the PAR routine of the BMDP software (6) on a VAX 3400 computer.

## METHODS

The reaction mixture components were patterned after those in conventional radioimmunoassays (RIA) for iodothyronines and more specifically after those used successfully in a previous investigation of a logistic approach for determining triiodothyronine ($T_3$) tracer levels in serum. Charcoal-thyronine-stripped serum (CTSS) of human origin containing only 0.4 µg/dl $T_4$, obtained from Chemicon, was spiked with $^{125}I$-$T_4$, 2700-58,000 cpm per 50 µl aliquot. Several tracer sources were used: a) DuPont Company, NEX111X, high specific activity, 4400 Ci/mmol, lot AV62200, approximately two months old; b) Nichols Institute kit for free $T_4$ by dialysis, one week postexpiration; c) Same as source a, except partially purified by ammonium sulfate precipitation as mentioned in "Results"; d) DuPont Company, NEX-111, low specific activity, 116 Ci/mmol, lot AT82400, approximately two weeks old.

Fifty microliters of tracer spiked CTSS was placed in a 12 X 75 mm glass test tube. Twenty-five microliters of a 1.25 mg/ml solution (in assay buffer, see below) of 8-aniline-1-naphthalene sulfonic acid, ANS, was added to the test tube, followed by 50 µl of a $T_4$ antibody diluted in assay buffer. Antibody sources are mentioned in "Results". ANS frees thyronines from serum binding proteins for better access between the $T_4$ and the antibody. The contents of the test tube were vortexed and incubated. After this first incubation, the test tubes were brought to room temperature in a water bath if the incubation temperature had been different. Then, 50 µl of a 2% normal rabbit or 1% normal mouse serum (carrier) was added, depending on the type of $T_4$ antibody (produced in rabbit or mouse, respectively). This carrier serum has nonimmune gamma globulin which greatly increases the mass of antibody-like protein as the target of the second antibody that precipitates the $T_4$ antibody along with the carrier globulin. The tubes were vortexed. Fifty microliters of a dilution of anti-rabbit (Sigma, RO881) or anti-mouse (Chemicon, AB26) gamma globulin (2nd antibody) was added to the tubes and vortexed. To promote 2nd antibody reaction and facilitate precipitation and separation of bound $T_4$ from unbound $T_4$, 400 µl of a 6% polyethylene glycol (PEG) solution was added to the tubes and vortexed. The tubes were incubated (second incubation) at room temperature for 5 or 15 minutes before adding a further 300 µl of PEG for adequate decanting volume. The test tubes were centrifuged at 2000G for 15 minutes at 4°C before decanting and counting of the precipitates in a well type scintillation gamma detector. The assay buffer was 0.075M barbital with 0.1% gelatin, pH 7.85, containing $NaN_3$ 0.05%. Assays were carried out in duplicate or triplicate and means are given in the tables. The logistic analysis is explained under "Results".

## RESULTS AND DISCUSSION

To obtain binding of tracer $T_4$ in CTSS, different polyclonal $T_4$ antibodies (developed in rabbit) from different companies (including Diagnostic Products No. 814 and Chemicon AB101) were tested. It was not possible to achieve greater than 50% binding with any of these polyclonal antibodies at a dilution compatible with adequate availability of the antibody for further use. The two antibodies mentioned above gave no more than 6% binding at any dilution. However, a mouse monoclonal antibody (Chemicon MAB086-156/7, lot 0629890CH2), appeared promising. In order to find the antibody dilution with the highest percent binding (near the PBmax) in the assay, serial dilutions of the antibody were made for incubation with tracer-spiked CTSS under the conditions specified in Table 1. Results in Table 1 suggested that PBmax appeared to be with $T_4$ antibody dilution in the range of 1:100 to 1:400. To improve the 52% binding, other variables of the assay still needed to be modified.

Since it was possible that not all of the $T_4$-bound first antibody was being precipitated, the next step was to use different dilutions of the second antibody, goat anti-mouse gamma globulin (GAMGG). The results from this test are shown in Table 2. The highest binding occurred with 1:15 dilution. This dilution was used in further testing.

Other variables in the assay that had the potential to improve the binding were the first incubation time after adding the $T_4$ antibody and the temperature of this incubation. Some results of testing these variables are shown in Table 3. These and other results indicated no differences in PB among conditions. One-hour incubation was chosen for convenience in further testing.

The next step was to observe the binding with use of a sample of normal human serum (NHS) containing 7.8 µg/dl $T_4$. NHS was spiked with $^{125}I$-$T_4$ and exposed to different dilutions of antibody and different incubation temperatures. The results from this trial are shown in Table 4. Room temperature incubation was chosen for further testing.

Another test was performed to determine binding as influenced be extending the 2nd antibody incubation time (after addition of 400 µl of PEG) from 5 to 15 minutes (both at room temperature). The results are given in Table 5. The extra 10 minutes of incubation may have enhanced the binding only slightly if at all. It was decided to use a 15-minute second incubation.

Since labelled $T_4$ must be measured in rat as well as human serum, pooled rat serum, PRS, was spiked with a labelled $T_4$ and the binding with the $T_4$ antibody was compared to that in spiked NHS and spiked CTSS. The results are shown in Table 6. The amount of unlabelled $T_4$ in the pooled serum was not directly determined, but

was estimated to be about 4 µg/dl from values obtained in individual rats. The data indicated a similar binding with the major difference being the binding with the lowest antibody level used.

Persistence of apparent maximal binding considerably less than 100% made it necessary to check for possible inhibitors in the sera. A binding assay was run, comparing spiked pooled serum with spiked assay buffer. The results shown in Table 7 are those expected in that PRS has unlabelled $T_4$ (and less binding at the lower antibody levels), while the assay buffer has no unlabelled $T_4$. However, at the 1:400 dilution, the PB in buffer similar to that in serum indicates that serum inhibitors do not explain the failure of PB to approach 100%.

Another explanation for this might be radiochemical impurity of the tracer, presumably from catastrophic decay and appearance of non-$T_4$ radioactivity. Stock label was evaporated in a vacuum centrifuge and taken up in CTSS. This is source a. A portion of this source of $^{125}I$-$T_4$ was cleansed by using 35 g/dl ammonium sulfate (2 ml to 0.5 ml labelled CTSS) to precipitate the $^{125}I$-$T_4$ bound to thyroxine binding proteins. The precipitate was extracted twice with 1-2 ml ethanol and centrifuged. The supernatants were pooled and evaporated. The residue was taken up in fresh CTSS. This is source c and was used to spike further test samples. See "Methods", first paragraph, for a description of the tracer sources. Next, a $T_4$ binding assay was run to compare CTSS spiked with cleaned and uncleaned tracer. The results of this assay are given in Table 8. The results show much better binding with the cleaned label.

Another source of $^{125}I$-$T_4$, low specific activity (source d), was tested, and as shown in Table 9, had a much higher percent binding. It is likely that this tracer was purer initially, or that the high specific activity tracer underwent more catastrophic decay before use, though this was not determined.

Some of the above results give an idea of the PBmax under various conditions. However, such values are too inaccurate to use quantitatively for several reasons. The quantities of $T_4$ antibody, being discontinuous by necessity, may not include that giving the actual PBmax. With antibody quantities above that for the actual PBmax, PB may fall off due to antibody-excess effects. The fall in binding with the highest antibody levels are possible due to insufficient second antibody for the amount of $T_4$ antibody plus carrier mouse gammaglobulin. The aliquots with $T_4$ antibody in amounts just above that for PBmax, which might begin to be thus susceptible to underestimates of PBmax, cannot be precisely identified. Consequently, data from antibody dilutions below that for PBmax, but including PB greater than half-maximal together with the PB at zero antibody were combined for a given sample to generate a best-fit curve through these observed data. Such a

curve, when projected toward an infinite antibody quantity index, asymptotically approaches PBmax. This projection was used as a quantitative index of PBmax. In this model, PB values were not corrected for nonspecific binding (NSB) as given by the PB at zero antibody, because NSB (assumed to be a constant fraction of the unbound radioactivity) varies from a maximum (without antibody) toward zero NSB as the amount of tracer bound approaches 100%. Even if non-$T_4$ counts are present in the sample and contribute to the PB at high antibody levels, this contribution would only be in the range of 2% of the excess counts. Values for binding in the data tables are corrected for the PB observed without antibody and thus are not precisely the same PB used in the logistic regression.

A four-parameter logistic regression model (typically fitting antibody and other reactions) was used in which the dependent variable (ordinate or Y axis) was PB and the independent variable (abscissa or x axis) was an index of the quantity of antibody present. The latter was used as the product of 1000 X the reciprocal of the denominator of the initial antibody dilution (in the preparation prior to adding it to the assay). A VAX 3400 computer utilizing the BMDP (UCLA) statistical software (PAR) was used to fit the data to the expression:

$$y = \frac{A - B}{1 + \left(\frac{x}{C}\right)^D} + B.$$

The four parameters obtained are labelled as A, B, C, and D. A represents the percent of counts precipitated in the absence of $T_4$ antibody (buffer substitute). B represents the counts bound (projected PBmax) expected for "infinite" antibody activity and precipitation of all the labelled $T_4$. C describes the antibody quantity index at which PB is half-way between PB with no antibody and the projected PBmax. D is an index of the slope at C.

It was found that if duplicate data for a sample were available including those for $T_4$ antibody dilutions down to 1:1600; if the antibody dilutions more concentrated than 1:400 were excluded from analysis, thereby avoiding the possibility of antibody excess in any sample; and if the data with zero antibody were included, then the range of data was wide enough to allow a logistic fit for serum samples with low or normal unlabelled $T_4$ concentration ($r^2 > 0.99$). One exception was the data in buffer (Table 7) in which PB variation was insufficient for a successful fit. Thus, data from zero antibody and antibody dilutions of 1:1600, 1:800, and 1:400 were used in logistic regressions for given serum samples to obtain a (projected) PBmax.

Pbmax obtained in this manner was 50.0% for pooled rat serum (PRS) in the data of Table 7. This compares with a value of 50.5% for charcoal-stripped serum (CTSS) (data from Table 8) for the same tracer (source a). Interestingly, for CTSS, when the tracer was treated with ammonium sulfate (tracer source c), the PBmax rose to 73.6% (based on data in Table 8). Further, when another tracer was used (source d, Table 9), CTSS and normal human serum (NHS) showed a PBmax of 81.3% and 81.9%, respectively (Table 10). The best-fit curves related to tables 9 and 10 are shown in Figure 1.

We observed widely different maximal tracer binding among sources of tracer (including greater binding after partial purification of one source), together with similar binding between samples of different unlabelled $T_4$ concentration with use of the same tracer source. This suggests that the regressionally projected PBmax represents the amount of radiolabelled $T_4$ in a sample, even in the presence of other radiolabelled material. However, it will be necessary to obtain further evidence that the PBmax in various samples can represent 100% of the total $T_4$ radioactivity present. This will be approached by obtaining tracer sources more closely approaching 100% radiochemical purity for the $T_4$ label. Finally, it will be necessary to assess specificity by determining whether the presence of known amounts of non-$T_4$ radioactivity (e.g., $T_3$ and reverse $T_3$) can alter the results.

Should these further assessments corroborate satisfactory performance, this system could be used to determine the level of tracer $T_4$ in serum after in vivo injection of $T_4$ tracer, enabling accomplishment of the ultimate goal of performaning kinetic studies of $T_4$ in normal and burn-injured humans and rats.

**TABLE 1. Monoclonal Antibody Binding Profile for Tracer $T_4$ In CTSS**

| $T_4$ Antibody Dilution | % Binding |
|---|---|
| 1:10 | 8.87 |
| 1:25 | 19.7 |
| 1:50 | 35.1 |
| 1:100 | 51.6 |
| 1:200 | 51.7 |
| 1:400 | 50.2 |
| 1:800 | 47.3 |
| 1:1600 | 43.2 |
| 1:3200 | 35.4 |

Labelled sample: charcoal-stripped serum
Label source: a
Monoclonal $T_4$ Ab incubation: 1h, 37 C
2nd antibody (GAMGG) incubation: 1:15 dil, 5 min
Percent "bound" without $T_4$ Ab: 1.74

**TABLE 2.** **$T_4$ Antibody Binding Profile for Initial Dilutions of Second Antibody**

| GOAT ANTI-MOUSE GAMMAGLOBULIN DILUTION | % BINDING |
|---|---|
| 1:5 | 51.7 |
| 1:10 | 52.1 |
| 1:15 | 52.1 |
| 1:20 | 50.5 |
| 1:40 | 27.1 |

Labelled sample: charcoal-stripped serum
Label source: a
Monoclonal $T_4$ Ab incubation: 1:200, 1 h. 37°C
2nd antibody (GAMGG) incubation: 5 min
Percent "bound" without $T_4$ Ab: 1.61 - 2.00

**TABLE 3.** **$T_4$ Antibody Binding - Incubation Time and Temperature**

| CONDITION | % BINDING |
|---|---|
| OVERNIGHT, 4°C | 49.7 |
| 4 h, 37°C | 51.3 |
| 4 h, room temperature | 50.0 |
| 1 h, 37°C | 49.9 |
| 1 h, room temperature | 48.2 |

Labelled sample: charcoal stripped serum
Label source: a
Monoclonal $T_4$ Ab incubation: 1:200
2nd antibody (GAMGG) incubation: 1:15 dil, 5 min
Percent "bound" without $T_4$ Ab: 1.44-3.16

**TABLE 4. $T_4$ Antibody Binding - Dilution of Antibody and Incubation Temperature**

| $T_4$ ANTIBODY DILUTION | INCUBATION TEMPERATURE | % BINDING |
|---|---|---|
| 1:25 | 37°C | 20 |
| 1:25 | Room temperature | 20.2 |
| 1:50 | 37°C | 34.6 |
| 1:50 | Room temperature | 36.3 |
| 1:100 | 37°C | 48.3 |
| 1:100 | Room temperature | 48.4 |
| 1:200 | 37°C | 47.7 |
| 1:200 | Room temperature | 47.5 |
| 1:400 | 37°C | 42.1 |
| 1:400 | Room temperature | 42.9 |

Labelled sample: normal human serum
Label source: a
Monoclonal $T_4$ Ab incubation: 4 h
2nd antibody (GAMGG) incubation: 1:15 dil, 5 min
Percent "bound" without T4 Ab: 1.64–1.73

**TABLE 5. $T_4$ Antibody Binding - Second Incubation Times**

| 1st INCUBATION | 2nd INCUBATION | % BINDING |
|---|---|---|
| 4 h | 5 min | 47.1 |
| 4 h | 15 min | 47.7 |
| 1 h | 5 min | 46.5 |
| 1 h | 15 min | 47.5 |

Labelled sample: normal human serum
Label source: a
Monoclonal $T_4$ Ab incubation: 1:100 dil, room temperature
2nd antibody (GAMGG) incubation: 1:15 dil
Percent "bound" without $T_4$ Ab: 1.56–1.76

**TABLE 6. $T_4$ Antibody Binding - Type of Spiked Serum and Dilution of Antibody**

| TYPE OF SERUM | ANTIBODY DILUTION | % BINDING |
|---|---|---|
| CTSS | 1:100 | 33.7 |
| CTSS | 1:200 | 34.4 |
| CTSS | 1:400 | 32.4 |
| CTSS | 1:800 | 31.6 |
| NHS | 1:100 | 30.0 |
| NHS | 1:200 | 32.2 |
| NHS | 1:400 | 30.3 |
| NHS | 1:800 | 22.8 |
| PRS | 1:100 | 21.2 |
| PRS | 1:200 | 32.1 |
| PRS | 1:400 | 34.4 |
| PRS | 1:800 | 30.4 |

Labelled samples: charcoal-stripped serum (CTSS), normal human serum (NHS), polled rat serum (PRS),
Label source: b
Monoclonal $T_4$ Ab incubation: 1 h, room temperature
2nd antibody (GAMGG) incubation: 1:15 dil, 15 min
Percent "bound" without $T_4$ Ab: 2.41-3.30

**TABLE 7. $T_4$ Antibody Binding - Buffer and Rat Serum**

| SAMPLE | ANTIBODY DILUTION | % BINDING |
|---|---|---|
| BUFFER | 1:200 | 48.0 |
| BUFFER | 1:400 | 47.5 |
| BUFFER | 1:800 | 46.0 |
| BUFFER | 1:1600 | 45.5 |
| PRS | 1:200 | 41.8 |
| PRS | 1:400 | 44.2 |
| PRS | 1:800 | 37.5 |
| PRS | 1:1600 | 24.9 |

Labelled samples: assay buffer, pooled rat serum (PRS)
Label source: a
Monoclonol $T_4$ Ab incubation: 1 h, room temperature
2nd antibody (GAMGG) incubation: 1:15 dil, 15 min
Percent "bound" without $T_4$ Ab: 2.12-2.30

**TABLE 8. $T_4$ Antibody Binding - Source of Tracer and Dilution of Antibody**

| TRACER | ANTIBODY DILUTION | % BINDING |
|---|---|---|
| SOURCE c | 1:200 | 69.0 |
| SOURCE c | 1:400 | 67.6 |
| SOURCE c | 1:800 | 62.6 |
| SOURCE c | 1:1600 | 55.1 |
| SOURCE a | 1:200 | 44.5 |
| SOURCE a | 1:400 | 43.2 |
| SOURCE a | 1:800 | 40.6 |
| SOURCE a | 1:1600 | 35.1 |

Labelled sample: charcoal-stripped serum
Labelled sources: c (ammonium-sulfate-cleaned), a (untreated)
Monoclonal $T_4$ Ab incubation: 1 h, room temperature
2nd antibody (GAMGG) incubation:1:15 dil, 15 min
Percent "bound" without $T_4$ Ab: 2.60 (labelled $T_4$ SOURCE c)
1.78 (labelled $T_4$ SOURCE a)

**TABLE 9. $T_4$ Antibody Binding - Type of Serum and Dilution of Antibody**

| SERUM | ANTIBODY DILUTION | % BINDING |
|---|---|---|
| CTSS | 1:100 | 76.6 |
| CTSS | 1:200 | 79.4 |
| CTSS | 1:400 | 76.3 |
| CTSS | 1:800 | 72.6 |
| CTSS | 1:1600 | 64.8 |
| NHS | 1:100 | 78.6 |
| NHS | 1:200 | 82.0 |
| NHS | 1:400 | 70.2 |
| NHS | 1:800 | 55.5 |
| NHS | 1:1600 | 33.1 |

Labelled samples: charcoal-stripped serum (CTSS), normal human serum (NHS)
Monoclonal $T_4$ Ab incubation: 1 h, room temperature
2nd antibody (GAMGG) incubation: 1:15 dil, 15 min
Percent "bound" without $T_4$ Ab: 2.01-2.21

**TABLE 10. Logistic Regression Results from Table 9 Data**

| SERUM | ANTIBODY DILUTIONS | PARAMETERS A | B | C | D |
|---|---|---|---|---|---|
| CTSS | 0, 1:1600–1:400 | 2.01 | 81.3 | .191 | 1.27 |
| NHS | 0, 1:1600–1:400 | 2.21 | 81.9 | .766 | 1.69 |

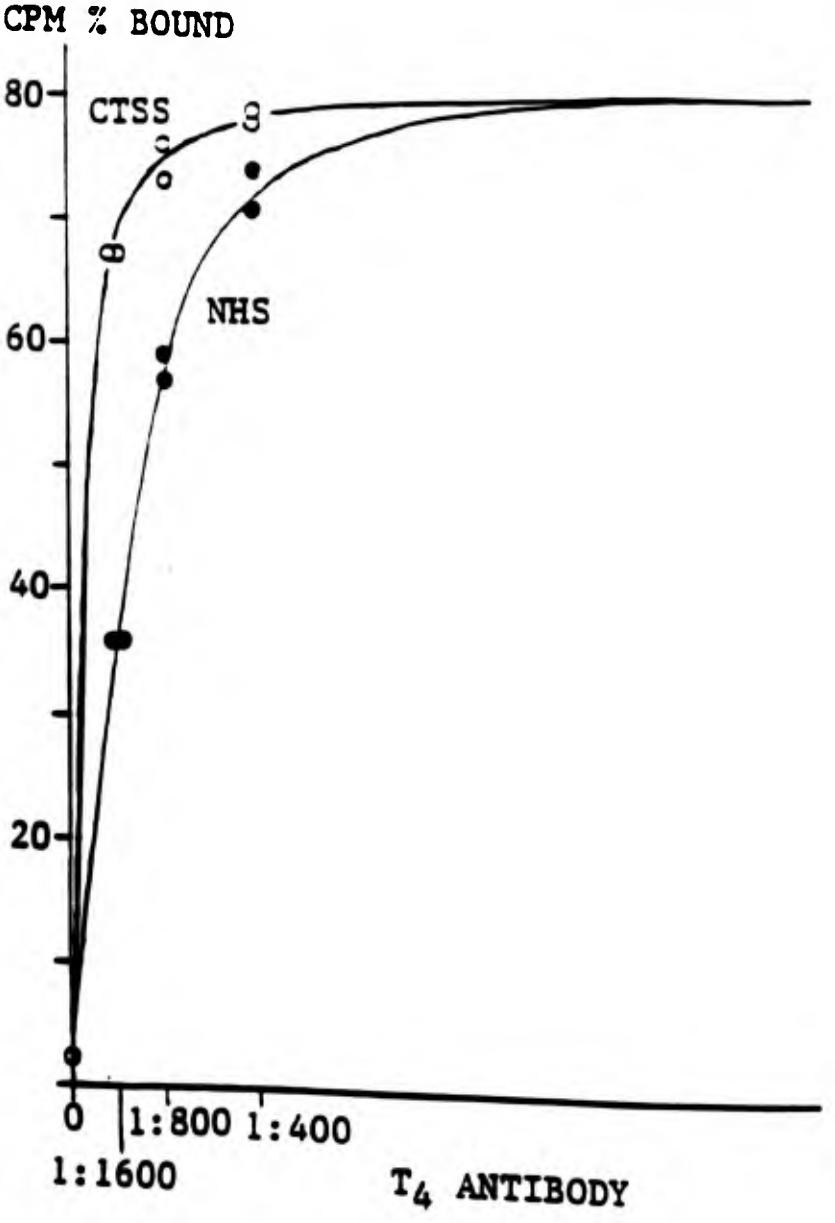


**FIGURE 1.** Percent bound from data contributing to Table 9 for the antibody levels shown on the abscissa, together with the best-fit curves from the parameters in Table 10.

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**ANNUAL RESEARCH PROGRESS REPORT**

**PROJECT NUMBER:** 3M161102BS14-00, RESEARCH

**PROJECT TITLE:** ROLE OF THYROID HORMONES IN BURN PATHOPHYSIOLOGY: Assessment of Tetraiodothyroine by Direct Dialysis in Burn Injury

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

**1 October 1989 - 30 September 1990**

**INVESTIGATORS**

George M. Vaughan, MD, Colonel, MC
Leonard G. Seraile
Rita King

## ABSTRACT

**PROJECT NUMBER:** 3M161102BS14-00, RESEARCH

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Recent attention has been given to serum dialysis performed without the classical serum dilution and addition of radioactive tracer $T_4$ but, instead, with direct radioimmunoassay of the endogenous $T_4$ in the dialysate. It has been claimed that the reduced serum free $T_4$ ($FT_4$) concentration sometimes seen in non-thyroidal illness (NTI) with the classical tracer method is not found with direct dialysis, implying absence of suppression of the pituitary-thyroid axis in NTI. However, there is a lack of direct comparisons in NTI between the methods. With use of a direct comparison in the NTI model of burn injury, we were not able to substantiate the previous claims. We found that $FT_4$ was depressed similarly by either method in burn patients (mean burn size 66%). Direct dialysis disclosed that the free fraction of serum $T_4$ (and $FT_4$ concentration) may be generally higher than previously thought from tracer dialysis results in rats, but the results were similar between methods in humans. Rats with burns (burn size 28%) showed some (but little) depression of $FT_4$ by either method and those with burn size of 20% showed no depression of $FT_4$ by either method. In both species, both dialysis methods disclosed an appreciable postburn serum $T_4$ binding defect. In the rats, this was seen at both burn sizes. Though the newer direct dialysis method appears considerably less difficult to perform, both methods provide similar assessments of the effects of burn injury on serum $T_4$ binding and $FT_4$ concentration.

# ASSESSMENT OF TETRAIODOTHYRONINE BY DIRECT DIALYSIS IN BURN INJURY

## INTRODUCTION

The ability of burn injury, other trauma, and critical illness to cause a serum binding defect for tetra- and triiodothyronine ($T_4$ and $T_3$) on circulating transport proteins (1,2) has complicated the determination of whether there is a deficit of circulating free $T_4$ and $T_3$ in illness and injury (nonthyroidal illness, NTI). Serum total $T_4$ and $T_3$ concentrations are depressed in severe NTI often in the face of normal thyrotropin (TSH) levels, and the level of free $T_4$ is considered to provide the best index of the status of the thyroid axis in such a situation. In that the pituitary can make its own $T_3$ by conversion from circulating $T_4$ and this process is related to the control of TSH secretion, the role of bioavailable circulating $T_4$ is considered key in evaluating the status of the thyroid axis (1,2). Consequently, most observations have assessed free $T_4$ ($FT_4$) as determined by conventional tracer dialysis of a diluted serum sample. They indicate that in some NTI cases, the binding defect offsets the depression of (total) $T_4$ and allows a normal $FT_4$ even in the face of a low $T_4$ (1,2).

However, the binding defect does not always offset the depression of $T_4$ enough to normalize the $FT_4$ in all cases of NTI, particularly in severe burn injury (1,2). This has suggested that in severe NTI and burns with apparently low or normal TSH, low $T_4$, and low $FT_4$ by tracer dialysis, the thyroid axis is depressed. However, the conventional dialysis method utilizes radioactive tracer $T_4$ added to the serum to track the ability of the sample's $T_4$ to occupy the unbound pool and traverse the dialysis membrane. The results are particularly sensitive to error from small amounts of contaminating radioactive iodide not incorporated into $T_4$ molecules. Furthermore, this method involves preliminary dilution of the sample (usually 1:10) prior to dialysis, so that the collection of free tracer $T_4$ in the dialysate is facilitated for adequate measurement. It has recently been suggested that such dilution may prevent the full effect of binding inhibitors present in NTI serum, thus underestimating the binding defect as reflected by the dialyzable fraction, $DFT_4$. That is, according to such a formulation, the full elevation of the $DFT_4$ in NTI samples may not be appreciated, with resultant underestimation of the calculated concentration of $FT_4$ (3-5).

Those authors, therefore, developed a "direct" dialysis technique wherein an undiluted serum sample without added tracer $T_4$ is dialyzed against a larger volume of dialysate, a buffer that mimics serum except that it contains no $T_4$ binding proteins but does contain HEPES buffer, gelatin, rabbit IgG, three antibiotics, and sodium azide (4). The $FT_4$ concentration is calculated from direct radioimmunoassay (RIA) of the dialysate $T_4$ with a very sensitive antibody. They found that in a group of NTI patients

with low $T_4$ the $FT_4$ by direct dialysis ($FT_4DD$) was normal. Interestingly, there is no direct comparison between tracer and direct techniques in individuals with NTI. However, because of their extensive studies indicating that primary dilution has much more effect on $FT_4$ in NTI than in normal serum they have developed the $FT_4DD$ technique into a kit marketed by Nichols Institute of San Juan Capistrano, California, and promoted it as a means to distinguish the hypothyroxinemia of NTI (normal $FT_4DD$) from that of genuine hypothyroidism (low $FT_4DD$). This formulation thus embodies the conclusion that NTI is not associated with suppression of the thyroid axis. The present study was undertaken to test this as an hypothesis by the use of $FT_4DD$ in the burn model of NTI.

## METHODS

In experiment 1, six male burn patients [mean age 25, mean total burn size (TBS) 66% of body surface] were each sampled by venipuncture once on postburn days 2-7 (mean PBD 5). Six healthy men, mean age 37, provided control samples. In experiment 2, male Sprague-Dawley rats were given a standard full-thickness scald burn, with 15 ml physiologic saline resuscitation given intraperitoneally between the application of back followed by abdominal burns, under pentobarbital anesthesia. Hair was shaved from the skin prior to the scalding procedure. An equal number of rats received the sham procedure (anesthesia, shaving, and resuscitation) at the same time. In groups 1 through 4, the rats weighed 325 g at the time of burning or sham, the TBS was 20% of body surface area (groups 2 and 4), and the animals were sampled on PBD 4 or 10 in groups of 9. In groups 5 through 8, the rats weighed 220 g, the TBS was 28% (groups 6 and 8), and the animals were sampled on PBD 7 or 15 in groups of 9. Prior to any procedures, the rats were adapted to a light/dark cycle of 14/10 h. Prior to sampling by guillotine decapitation and collection of trunk blood, the animals were housed singly in hanging wire cages at an ambient 25°C and given water and laboratory chow ad libitum. All rat procedures and sampling occurred at 4 to 6 h into the light phase.

All samples were promptly separated by centrifugation after clotting and the serum frozen in aliquots at -60°C until the time of thawing and assay. Serum $T_4$ and $T_3$ were determined by RIA with commercially available kits (Biotecx, Houston, Texas) and in vitro $T_3$ uptake ($T_3U$) by radioassay with kits (Coat-a-Count®) from Diagnostic Products, Los Angeles, California. The free $T_4$ index ($FT_4I$) was calculated as the product of the $T_4$ X $T_3U$. Conventional tracer equilibrium dialysis at 37°C (serum dilution 1:10) for determination of the $DFT_4Tr$ and calculation of the $FT_4Tr$ (as $T_4$ X $DFT_4Tr$) was performed in duplicate at the Nichols Institute, San Juan Capistrano, California. $FT_4DD$ was determined in our laboratory with the direct equilibrium dialysis kits obtained from the Nichols Institute, which included dialysis cells reusable up to 20 times each, plastic holder trays to secure the cells during

incubation in a flowing water bath at 37°C overnight (18 h), dialysate buffer, and special RIA materials to determine $T_4$ on the dialysate. The sample (0.2 ml) was undiluted and the dialysate volume was 2.4 ml. Each dialysis allowed duplicate RIA determination of $T_4$ on the dialysate with a least detectable concentration of 0.2 ng/dl and a normal expected range of 0.8 to 2.7 ng/dl (both determined by the supplier) for measurement of $FT_4DD$ concentration in the dialysate, taken to represent $FT_4$ in the sample. For the dialysis procedure, close attention was paid to the written instructions and orally emphasized cautions provided by the supplier, including prevention of membrane drying (storage in 4°C deionized $H_2O$) and proper washing technique between runs; and for a given run, prevention of evaporation with Parafilm®, maintenance of 37°C until separation of sample and dialysate, and rapid sampling of the separated dialysates into the antibody-coated RIA tubes at room temperature prior to addition of labelled $T_4$ for the 2-h room-temperature RIA incubation. Standard sera showed no consistent deviation from run to run in a given cell. Each run was performed with two trays of cells in the water-flow incubator in which the water level reached the dialysate level in all cells, but did not contaminate the contents. The experimental samples were dialyzed in 2 to 4 replicates per sample. Assessment of these and of standard sera together with results from preliminary runs disclosed no significant variation among cells within a tray, but did disclose a slight variation between the two tray positions, with the dialysis cells in the second tray position always giving slightly lower results than those given by cells in the first tray position. Experimental samples were distributed with replicates of most samples in both tray positions and in a given run with similar representation by tray position for any two comparable burn and control groups. For each replicate, the dialysate $T_4$ was determined in duplicate. Further, all second tray results were corrected by regression to the value expected for the first tray position. This correction, related to the magnitude of the $FT_4DD$, amounted to a mean of 4 to 20% in different runs. For a given sample, the mean of all replicate dialyses was used as the result.

For four standard sera provided in the kits and run in a total of 7 or more dialyzed replicates each (along with the experimental samples), the means ranged from 1.57 to 2.97 and the overall mean between-dialysis within-sample coefficient of variation was 11.9%. The mean between-dialysis within-sample coefficient of variation was 14% and 22% for human control and burn samples respectively, and 7% for both rat sham and burn samples. Six dialyses with fresh dialysate buffer as the retentiate sample gave $FT_4DD$ values on the dialysate from 0.31 to 0.49 ng/dl, though fresh dialysate buffer put directly into the RIA gave an undetectable response.

The $FT_4DD$ result for serum samples was used to back-calculate the dialyzable fraction ($DFT_4DD$) as the quotient of $FT_4DD/T_4$.

Burn and control means were compared by t test with the assumption of separate variances and the use of the BMDP (P7D) (6) program on a VAX 3400 computer.

## RESULTS

Serum $T_4$, $T_3$, and $FT_4I$ were markedly depressed in burned humans (Table 1), though $T_3$ was only minimally depressed in burned rats (Table 3). Though there was a clear elevation of $T_3U$ in burned humans (Table 1), there was little consistent elevation of $T_3U$ in burned rats (Table 3).

Both the $DFT_4Tr$ and $DFT_4DD$ were elevated in the burned humans (Table 2) and in all burned rat groups (Table 4). $FT_4Tr$ was depressed in burned humans, but in rats, only in both burned groups with TBS 28%. $FT_4DD$ was depressed in burned humans and in only one (PBD 15) burned rat group with TBS 28%. Burned rats with TBS of 20% had no detectable depression of $FT_4Tr$ or $FT_4DD$.

**TABLE 1. HUMAN NON-DIALYSIS RESULTS**

| | $T_4$ (μg/dl) | $T_3$ (ng/dl) | $T_3U$ (%) | $FT_4I$ |
|---|---|---|---|---|
| CONTROL | 8.85 ± .38 *** | 143 ± 3 *** | 27.94 ± .82 ** | 2.47 ± .11 *** |
| BURN | 3.20 ± .54 | 54 ± 5 | 33.13 ± 1.30 | 1.04 ± .16 |

$T_4$, thyroxine; $T_3$, triiodothyronine; $T_3U$, in vitro $T_3$ uptake; $FT_4I$, free $T_4$ index ($T_4$ X $T_3U$); results are in serum, mean ± SE; **p < .01; ***p < .001.

**TABLE 2. HUMAN DIALYSIS RESULTS**

| | $DFT_4Tr$ (%) | $FT_4Tr$ (ng/dl) | $FT_4DD$ (ng/dl) | $DFT_4DD$ (%) |
|---|---|---|---|---|
| CONTROL | .024 ± .001 * | 2.14 ± .10 * | 1.80 ± .18 * | .021 ± .002 * |
| BURN | .045 ± .005 | 1.36 ± .22 | 1.24 ± .15 | .043 ±. 008 |

$DFT_4Tr$, tracer $T_4$ dialyzable fraction; $FT_4Tr$, free $T_4$ by tracer dialysis ($T_4$ X $DFT_4Tr$); $FT_4DD$, free $T_4$ by direct dialysis; $DFT_4DD$, dialyzable fraction by direct dialysis ($FT_4DD/T_4$); results are in serum, mean ± SE; *p < .05.

**TABLE 3. RAT NON-DIALYSIS RESULTS**

| GRP | TBS | PBD | CONDITION | $T_4$ (µg/dl) | $T_3$ (ng/dl) | $T_3U$ (%) | $FT_4I$ |
|---|---|---|---|---|---|---|---|
| 1 | - | 4 | Sham | 3.75 ± .28 *** | 60.4 ± 3.4 * | 44.22 ± .25 * | 1.66 ± .12 *** |
| 2 | 20% | 4 | Burn | 2.24 ± .14 | 52.0 ± 1.5 | 45.30 ± .36 | 1.01 ± .06 |
| 3 | - | 10 | Sham | 4.28 ± .16 *** | 70.4 ± 4.0 | 44.70 ± .30 | 1.91 ± .06 *** |
| 4 | 20% | 10 | Burn | 2.49 ± .11 | 61.2 ± 4.0 | 44.88 ± .35 | 1.12 ± .05 |
| 5 | - | 7 | Sham | 5.81 ± .24 *** | 87.1 ± 2.9 *** | 44.37 ± .27 ** | 2.58 ± .11 *** |
| 6 | 28% | 7 | Burn | 2.35 ± .13 | 60.4 ± 2.9 | 46.63 ± .58 | 1.10 ± .06 |
| 7 | - | 15 | Sham | 4.69 ± .19 *** | 74.6 ± 2.1 * | 45.25 ± .68 | 2.12 ± .08 *** |
| 8 | 28% | 15 | Burn | 2.55 ± .16 | 67.6 ± 2.3 | 43.66 ± .39 | 1.11 ± .07 |

TBS, total burn size as % body surface; PBD, postburn day; for explanation of hormonal variables, see Table 1; results are in serum, mean ± SE; *p < .05; **p < .01; ***p < .001.

## TABLE 4. RAT DIALYSIS RESULTS

| GRP | TBS | PBD | CON-DI-TION | $DFT_4Tr$ (%) | $FT_4Tr$ (ng/dl) | $FT_4DD$ (ng/dl) | $DFT_4DD$ (%) |
|---|---|---|---|---|---|---|---|
| 1 | - | 4 | Sham | .035 ± .002 ** | 1.33 ± .06 | 2.84 ± .11 | .079 ± .006 *** |
| 2 | 20% | 4 | Burn | .051 ± .004 | 1.21 ± .10 | 2.77 ± .22 | .124 ± .007 |
| 3 | - | 10 | Sham | .035 ± .001 *** | 1.49 ± .07 | 3.10 ± .09 | .073 ± .002 *** |
| 4 | 20% | 10 | Burn | .059 ± .003 | 1.46 ± .07 | 3.35 ± .11 | .136 ± .006 |
| 5 | - | 7 | Sham | .030 ± .001 *** | 1.74 ± .08 ** | 3.87 ± .11 | .067 ± .002 *** |
| 6 | 28% | 7 | Burn | .060 ± .003 | 1.39 ± .04 | 4.05 ± .16 | .175 ± .009 |
| 7 | - | 15 | Sham | .031 ± .001 *** | 1.48 ± .09 * | 3.95 ± .07 * | .085 ± .002 *** |
| 8 | 28% | 15 | Burn | .047 ± .002 | 1.19 ± .05 | 3.67 ± .10 | .147 ± .006 |

TBS, total burn size as % body surface; PBD, postburn day; for explanation of hormonal variables, see Table 2; results are in serum, mean ± SE; *p < .05; **p < .01; ***p < .001.

## DISCUSSION

The depression of serum $T_3$ (relative to the depression of $T_4$) was less in rats than in humans. This relative sparing of $T_3$ in several rat models of NTI, including burns, has been discussed previously (1,2). The inhibition of peripheral $T_4$ to $T_3$ conversion by NTI is apparently less well reflected by serum $T_3$ in models of NTI in rats, whose serum $T_3$ concentration is relatively more dependent upon some thyroidal secretion of $T_3$ than are $T_3$ levels in humans. Also, the serum protein iodothyronine binding defect, reflected by the elevated $T_3U$ in burned humans is less well reflected by the $T_3U$ in burned rats. This has been noted before (7), and may result from greater ability in rats of a burn-induced serum protein-binding inhibitor to inhibit $T_3$ binding also to the competing matrix (in the present study, immobilized $T_3$ antibody) in the $T_3U$ test. It is not likely entirely a result of a different protein binding system for $T_3$ in rats (versus that in humans) because of previous observation of an elevated tracer $T_3$ dialyzable fraction in the presence of non-elevated $T_3U$ (charcoal matrix) in burned rats (7). Because of the presence of inhibited binding of iodothyronines to living cells in the presence of human NTI serum and the better prediction of cellular $T_4$ binding by the $T_3U$-

dependent $FT_4I$ than by the tracer dialysis dependent $FT_4$ in such human NTI sera (8), it has been proposed that the better index of the $T_4$ available to tissues in NTI is the $FT_4I$ (2). Whether this may be the case in rats is not yet addressed. The $FT_4I$ was depressed in burn injury in both species.

The serum binding defect for $T_4$ in burn injury was well shown by the $DFT_4Tr$ as well as the $DFT_4DD$, in that both these variables representing the free fraction of serum $T_4$ were elevated after burn in both species. Though the free fraction result was nearly identical between both procedures for the human samples, the $DFT_4DD$ result was approximately two-fold that of the $DFT_4Tr$ in the various rat groups. This suggests that primary dilution of samples may enhance binding more in rats than in humans. This would be compatible with binding inhibitors normally present in rat serum that become relatively inactive when diluted, though other explanations are possible. However, in rat sera, both the tracer and direct (undiluted) procedure showed reduction in binding (elevation of the free fraction) after burn injury without a large difference in this effect as manifested by the two procedures. Thus, for the conditions of the humans and rats of this study, the effect of any burn-induced serum binding inhibitors was not influenced much by factors inherently different between tracer dialysis with preliminary serum dilution and direct dialysis without it. However, it is possible that even in the direct method, the larger (12-fold) dialysate versus sample (retentate) volume affords a functional dilution (if the burn-induced binding inhibitors are themselves dialyzable), thus possibly obscuring any difference of burn effect between direct and tracer results.

For the humans, the burn-induced binding defect was not sufficient to prevent observation of a depressed $FT_4$ by either tracer or direct dialysis. This suggests that direct dialysis does not necessarily allow the interpretation that thyroid axis suppression is absent in burn patients. Previously reported normal $FT_4$ by either method in low $T_4$ NTI may reflect injury or illness severe enough to affect serum $T_4$ binding but not severe enough to depress thyroidal secretion below that necessary to keep $FT_4$ normal for whatever the $T_4$ degradation rate happens to be. Major burns may produce the equivalent of more severe NTI, capable of suppression of both $T_4$ binding and thyroid axis function. Previous observation of normal or low TSH in the presence of low $FT_4Tr$ supports the hypothesis of blunted thyroid axis function in burn patients (1), as does the excessively negative serum TSH response to $T_4$ replacement in burned rats (1,2,9).

In the present results with rats, the depressive effect of burn on $FT_4$ was evident in the rats with TBS of 28% (PBD 7 and 15 for $FT_4Tr$ and PBD 15 for $FT_4DD$) but not in those with TBS of 20%. This probably represents an effect of injury severity. Beyond 24 h after burn, $FT_4Tr$, not depressed in rats with a 17% burn (10),

was depressed in those with 25% burn (9) and 60% burn (7). It will be of interest to observe the $FT_4DD$ in rats with larger burns.

$FT_4$ by direct dialysis confirms low $FT_4$ concentration in burn patients and the serum $T_4$ binding defect in burned humans and rats.

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| RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY | | | | 1. AGENCY ACCESSION DA318018 | 2. DATE OF SUMMARY 7 Jul 90 | REPORT CONTROL SYMBOL DD-DR&E(AR) 636 |
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| 3. DATE PREV SUM'RY 1 Oct 89 | 4. KIND OF SUMMARY H. Comp | 5. SUMMARY SCTY U | 6. WORK SECURITY U | 7. REGRADING | 8. DISP'N INSTR'N CX | 9. LEVEL OF SUM A. WORK UNIT |

| 10. NO./CODES | PROGRAM ELEMENT | PROJECT NUMBER | TASK AREA NUMBER | WORK UNIT NUMBER |
|---|---|---|---|---|
| a. PRIMARY | 61102A | 3M161102BS14 | F | 305 |
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18. RESOURCES ESTIMATE

| FISCAL YEARS | a. PROFESSIONAL WORKYEARS | b FUNDS *(In thousands)* |
|---|---|---|
| 89 | 0.1 | 30 |
| 90 | 0.1 | 35 |

| 19. RESPONSIBLE DOD ORGANIZATION | 20. PERFORMING ORGANIZATION |
|---|---|
| a. NAME US Army Institute of Surgical Research | a. NAME US Army Institute of Surgical Research |
| b. ADDRESS *(include zip code)* Fort Sam Houston San Antonio, Texas 78234-5012 | b. ADDRESS Fort Sam Houston San Antonio, Texas 78234-5012 |
| c. NAME OF RESPONSIBLE INDIVIDUAL PRUITT, B A | c. NAME OF PRINCIPAL INVESTIGATOR WAYMACK, J P |
| d. TELEPHONE NUMBER *(include area code)* 512-221-2720 | d. TELEPHONE NUMBER *(include area code)* 512-221-2018 |
| 21. GENERAL USE FINA<br>MILITARY/CIVILIAN APPLICATION: M | f. NAME OF ASSOCIATE INVESTIGATOR *(If available)* BURLESON, D G<br>g. NAME OF ASSOCIATE INVESTIGATOR *(If available)* OKERBERG, C V |

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23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS *(Precede text of each with Security Classification Code)*

23. (U) A DTIC literature search was conducted under DTIC request number W6Q35J dated 19 October 1989 for the technical report database and request number W6Q51L dated 19 October 1989 for the work unit database. The proposed effort will not result in the duplication of effort. The objective of this work is to identify the immunological effects of blood transfusions in traumatized patients utilizing a rat transfusion model and determine whether pharmacological manipulation can prevent the adverse immunologic sequelae of the blood transfusions.

24. (U) Healthy and traumatized Lewis rats will be administered blood transfusions. The animals will then be evaluated for blood transfusion effects on their resistance to bacterial infection.

25. (U) 8901 - 9007. Allogeneic blood transfusions were documented to impair NK cell function at both 1 and 2 weeks following blood transfusion. This impairment was further evidenced by an increased rate of metastatic tumor growth. Transfusions were not found to significantly alter the percentage of helper T cells, suppressor T cells, IL-2 bearing lymphocytes or transferrin-bearing lymphocytes. Transfusions were found to enhance survival in endotoxin shock. For technical reports, refer to the US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Year 1990.

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ANNUAL RESEARCH PROGRESS REPORT

**PROJECT NUMBER:** 3M161102BS14-00, RESEARCH

**PROJECT TITLE:** Investigation of the Immunologic Sequelae of Blood Transfusions in Rats

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

**1 October 1989 - 7 July 1990**

**INVESTIGATORS**

J. Paul Waymack, MD
David G. Burleson, PhD, Lieutenant Colonel, MS
Carlin V. Okerberg, DVM, Lieutenant Colonel, VC

**ABSTRACT**

**PROJECT NUMBER:** 3M161102BS14-00, RESEARCH

**PROJECT TITLE:** Investigation of the Immunologic Sequelae of Blood Transfusion in Rats

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

**PERIOD COVERED IN THIS REPORT:** 1 Oct 89 through 7 Jul 90

**INVESTIGATORS:** J. Paul Waymack, MD
David G. Burleson, PhD, Lieutenant Colonel, MS
Carlin V. Okerberg, DVM, Lieutenant Colonel, VC

Blood transfusions have previously been demonstrated in Wistar-Furth rat models to impair resistance to pulmonary metastases from a colonic tumor, but not the growth of the primary tumor. Utilizing the same tumor cell line, we evaluated the effect of transfusion on resistance to peritoneal spread of the tumor and on macrophage response to the transfusion. Transfusions were found to have no significant effect on survival in animals with peritoneal carcinomatosis, nor on the host response to the tumor. Transfusion failed to alter macrophage function or metabolism as measured by macrophage cytotoxicity against YAC-1 cells, by macrophage production of adenosine deaminase, by glucose metabolism, or by macrophage ATP levels. In summary, transfusions failed to alter the peritoneal resistance to tumor spread or the activity of the predominant resident peritoneal leukocyte.

# INVESTIGATION OF THE IMMUNOLOGIC SEQUELAE OF BLOOD TRANSFUSIONS IN RATS

## INTRODUCTION

It has previously been demonstrated in a number of retrospective studies that there is an adverse correlation between perioperative blood transfusions and long-term survival in colon cancer patients (1-4). However, this correlation has not been noted universally (5). Part of the difficulty with such retrospective studies is the possibility that those patients with more advanced or aggressive tumors (lead time or length bias) received a greater proportion of the transfusions. To eliminate the potential for such bias, animal studies have been utilized. These have also yielded contradictory results, depending on the species and strain of animal utilized, the type of tumor, and the method of transfusion (6).

We have previously demonstrated, utilizing a Wistar-Furth colon cancer model, that allogeneic transfusions do not increase the rate of primary tumor growth, at least with this tumor line. When the same tumor was utilized in a pulmonary metastasis model, we found an enhanced rate of growth of pulmonary metastases and a decreased long-term survival rate in allogeneically transfused rats as compared to a control group which received lactated Ringer's. The purpose of our current study was to determine if this alteration in host response to pulmonary metastases is representative of a universal response to metastases in transfused rats or if it is unique to pulmonary metastases.

## MATERIALS AND METHODS

**Animals.** Adult male Wistar-Furth rats weighing approximately 250 g were used in these studies. Adult male A'Sogaloff Cancer Institute (ACI) rats were used as blood donors. All animals were housed in individual stainless steel, hanging cages and allowed food and water ad libitum throughout the study. The animals were observed for one week prior to entry into the study to exclude the possibility of any preexisting diseases.

**Transfusion Protocol.** Blood was obtained from the donor rats by vena cava puncture and mixed at a 4:1 volume ratio with CPDA-1 anticoagulant. Animals in the control group received 3 ml of lactated Ringer's solution intravenously and those in the transfusion group were given 1 ml of ACI rat blood intravenously. The increased volume of lactated Ringer's was chosen since it was believed that this would closely approximate the intravascular volume changes achieved in the rats receiving 1 ml of whole ACI rat blood.

**Tumor Protocol.** Two tumor models were used to evaluate the transfusion effect on tumor growth. In the first model, 45 rats

(control=28, transfusion=27) received $1x10^6$ tumor cells suspended in 1 ml of complete RPMI injected intraperitoneally through a 23-ga needle. The tumor cell suspension was prepared as previously described (6). Briefly, viable tumor was obtained by excision of a rapidly growing syngeneic 1,2-dimethylhydrazine-induced colon carcinoma. The tumor was mechanically disaggregated by first slicing the tumor into approximately 1-$mm^3$ pieces and vigorously shaking the suspension in complete RPMI-1640 media with penicillin, streptomycin, and 10% fetal calf serum. The cells were washed 3X in the same media. An aliquot of this suspension was stained with trypan blue and the number of viable tumor cells determined. The cells were centrifuged and resuspended in sufficient RPMI to achieve a final concentration of $1x10^6$ viable cells/ml. A 1-ml aliquot of this suspension was injected intraperitoneally through a 23-ga needle into the Wistar-Furth rats immediately following the administration of either lactated Ringer's or blood. Animals were followed to death and mean survival times were determined. All animals were necropsied to confirm that death was due to tumor growth. Eighty-five days after implantation of the tumor cell suspension, all rats remaining alive appeared healthy and had no evidence of viable tumor growing intraperitoneally by physical examination. These rats were sacrificed by a lethal sodium pentobarbital injection and were necropsied. None were found to have any macroscopic evidence of tumor present. For the purpose of calculating mean survival time, all of these animals were given a survival time of 85 days, and for calculating survival rates, these rats were considered to be permanent survivors.

Leukocyte infiltration of the tumors present in the animals described above was assayed as previously described (7). Briefly, wedge biopsies of the periphery of the tumors were performed at the time of necropsy. The biopsies were fixed in formalin, sectioned, and stained with hematoxylin-eosin. Cellular infiltrates were quantified by enumeration of cells at the tumor periphery in 15hpf with an image analysis system (Optomax$^R$, Hollis, NH).

For the second tumor model, a similar tumor cell suspension was prepared. Twenty-two Wistar-Furth rats (control=10, transfusion=12) were anesthetized with intraperitoneal sodium pentobarbital (35mg/kg). A midline celiotomy was performed and 1 ml of the tumor cell suspension was injected into the retroperitoneum. The incisions were closed in multiple layers and the animals were allowed to awaken in their cages. These animals were followed to mortality and mean survival times determined.

To evaluate the effect of transfusion on macrophage cytotoxic response to tumor, elicited peritoneal macrophages were studied. Twenty Wistar-Furth rats were administered either 1 ml of ACI rat blood (N=10) or 3 ml of lactated Ringer's (n=10). Three days following administration of the transfusion or lactated Ringer's, 4 ml of brain-heart infusate was administered intraperitoneally through a 23-ga needle. Four days later, the animals were

sacrificed by decapitation. A midline celiotomy was performed and the peritoneal cavity lavaged with 20 ml of Hank's balanced salt solution (HBSS) without calcium or magnesium and with 0.25 mm EDTA. The resulting suspensions were hypotonically lysed of any contaminating red cells and washed 3X in standard HBSS. They were resuspended in RPMI-1640 with 10% fetal calf serum. The resulting macrophage suspensions were assayed for macrophage purity and for macrophage cytotoxic activity against YAC-1 cells.

Macrophage purity was tested for by both adherence to flat-bottomed cell well plates (96 wells) (Corning Glass Works, Corning, NY) and by nonspecific esterase staining. Macrophage adherence was tested for by placing $5x10^5$ cells in 0.1 ml of media per well. The plates were cultured for 1 hour at 37°C in 5% $CO_2$. Nonadherent cells were then removed and the remaining cells were counted. Ninety percent of the cells from both the transfused and control rats were found to be adherent. Nonspecific esterase staining was assayed using a standard esterase staining kit (Kit #90-A1, Sigma Technical Bulletin #90). Ninety-eight percent of the cells from both the transfused and control rats were found to be positive for esterase staining.

Macrophage cytotoxicity against YAC-1 cells was assayed as follows. Briefly, $1X10^6$ YAC-1 cells/ml RAMI-1640 with 10% fetal calf serum and containing 5$\mu$Ci/ml of $^{125}$I-labelled 5-iodo-2'-deoxyuridine (Dupont Corp, NEN Research Products, Boston, MA) plus $10^{-6}$M 5-fluorouridine (Sigma Chemical Corporation) were cultured at 37°C in 7.5% $CO_2$ for 4 hours. The cultures were then washed three times to remove all nonincorporated radioactivity. $5x10^4$ of the labelled YAC-1 cells in 0.1 ml RPMI-1640 with 2.5% fetal calf serum were added to each well in the cell well plates which contained $5x10^5$ macrophages in 0.1 ml RPMI with 2.5% fetal calf serum. Additional wells containing only target cells were utilized for measuring spontaneous release. Total counts were measured by lysis of target cells utilizing detergent. All wells were cultured at 37°C for 24 hours. Supernatants from each well were harvested and assayed for radioactivity present. Specific release for each well was calculated as follows:

$$\frac{\text{cpm released in test well} - \text{cpm released in spontaneous control sample}}{\text{cpm in total lysis well} - \text{cpm in spontaneous release sample}}$$

Data are presented as percent of mean of the value for macrophages obtained from the control group.

The effect of transfusion on macrophage metabolism was assayed by measuring the amount of adenosine deaminase produced by cultured macrophages, the intracellular levels of adenosine triphosphate (ATP) and finally glucose metabolism by the macrophages. Briefly, 20 Wistar-Furth rats were transfused (n=10) or given lactated

Ringer's (n=10) and had peritoneal macrophages elicited as described above 3 days after transfusion. Four days after BHI injection, the macrophages were harvested and purified as described above. $1x10^7$ of the cells were suspended per milliliter of complete RPMI media and 1-ml aliquots of the suspension were cultured in standard flat-bottomed polystyrene culture plates with concanavalin A stimulation for 6 hours at 37°C. The plates were then frozen at -70°C and thawed at room temperature three times. Aliquots of the lysed suspensions were analyzed for glucose, adenosine deaminase, and adenosine triphosphate levels. Glucose content of the RPMI media prior to macrophage addition was also assayed.

Glucose was determined on Beckman Synchron CX3 System (Beckman Instruments, Inc., Brea, CA). The Synchron CX3 glucose chemistry determines glucose by means of the oxygen-rate method employing a Beckman oxygen electrode.

Adenosine deaminase activity was assayed using a colorimetric method described by Giusti (8). Briefly, 0.05 ml aliquots of the thawed macrophage lysate were added to 1 ml of buffered adenosine solution (21 mM adenosine, 50 mM phosphate buffer, pH 6.5). After incubation for 60 min at 37°C, 3.0 ml of phenol/nitroprusside solution ($10^6$ mM phenol, 0.17 mM sodium nitroprusside) and 3.0 ml of alkaline hypochlorite solution (11mM NaOCl, 125mM NaOH) were added. After incubation for 30 min. at 37°C, absorbance at 628nm (E) was measured. Volume activity was determined as follows:

(E sample - E sample blank) ÷ (E standard - E reagent blank)

Adenosine triphosphate levels were measured by a coupled enzymatic reation (9). Briefly, adenosine triphosphate was measured using the coupled enzymatic reation: ATP + 3-phosphoglycerate → ADP + 1,3-diphosphoglycerate [1] 1,3-diphosphoglycerate + NADH → glyceraldehyde 3-phosphate + NAD + phosphate [2]. Reaction[1] was catalyzed by phosphoglycerate kinase and reaction [2] was catalyzed by glyceraldehyde phosphate dehydrogenase. The change in absorbance at 340nm that results when NADH is oxidized to NAD was measured on a Gilford 240 spectrophotometer (Gilford Instruments, Gerdin, OH). Enzymes and reagents were obtained from Sigma Chemical (St. Louis, MO).

**Statistical Analysis.** All data are expressed as mean ± SEM. Comparisons among groups were made using Fischer's exact, generalized Savage (Mantel-Cox), and ANOVA.

## RESULTS

The survival curves for the animals challenged with intraperitoneal tumor cell suspensions are shown in Figure 1. There were 19 deaths and nine survivors in the control group. In the transfused group, there were 12 deaths and 15 survivors. This

difference was not statistically significant (P=0.106). The mean survival time for the control group is 60.71 ± 3.80 days and for the transfusion group, 70.81 ± 3.58 days. This difference was also not statistically significant (P=0.0629). Necropsy of each of the deaths revealed a generalized peritoneal carcinomatosis.

Analysis of leukocyte infiltration of the tumors revealed 123.0 ± 38.4 cells/hpf in the tumors obtained from the control group and 143.9 ± 41.9 cells/hpf in the tumors obtained from the transfused group. This difference was not statistically significant (P=0.196).

The survival curves for the animals challenged with retroperitoneal tumor cell suspensions are shown in Figure 2. The mean survival time for the control group was 34.50 + 2.15 days and for the transfusion group, 33.67 ± 2.63 days. This difference was not statistically significant (P=0.883).

The macrophages obtained from the control group had 100.0 ± 11.8% of the predicted lysis of the YAC-1 cells. The macrophages obtained from the rat had 121.1 ± 16.3% of the predicted lysis of the YAC-1 cells. This difference was not statistically significant (P=0.305).

The glucose level in the lysed suspensions of macrophages obtained from the control rats was 188.4 ± 0.5 mg/dl. The glucose level in the lysed suspensions of macrophages obtained from the transfused rats was 189.7 ± 1.1 mg/dl. This difference was not statistically significant (P=0.387). The glucose level from five samples of RPMI media prior to macrophage addition was 192.0 ± 0.7 mg/dl, indicating a similar slow rate of glucose metabolism in both groups.

The adenosine deaminase level in the lysed suspensions of macrophages obtained from the control rats was 3.33 ± 1.78 U/ml and with supernatants from the lysed suspensions of macrophages obtained from transfused rats, 1.06 ± 0.19 U/ml. This difference was not significant (P=0.147). The greater mean and the large standard error of the mean for the control group was due to a single sample which had a value far in excess of all other samples in both groups.

The adenosine triphosphate level of the lysed suspensions of macrophages obtained from the control rats was 9.57 ± 1.26 $\mu g/1x10^7$ cells and for the lysed suspensions of macrophages obtained from transfused rats, 10.66 ± 1.56 $\mu g/1 \times 10^7$ cells. This difference was not significant (P=0.638).

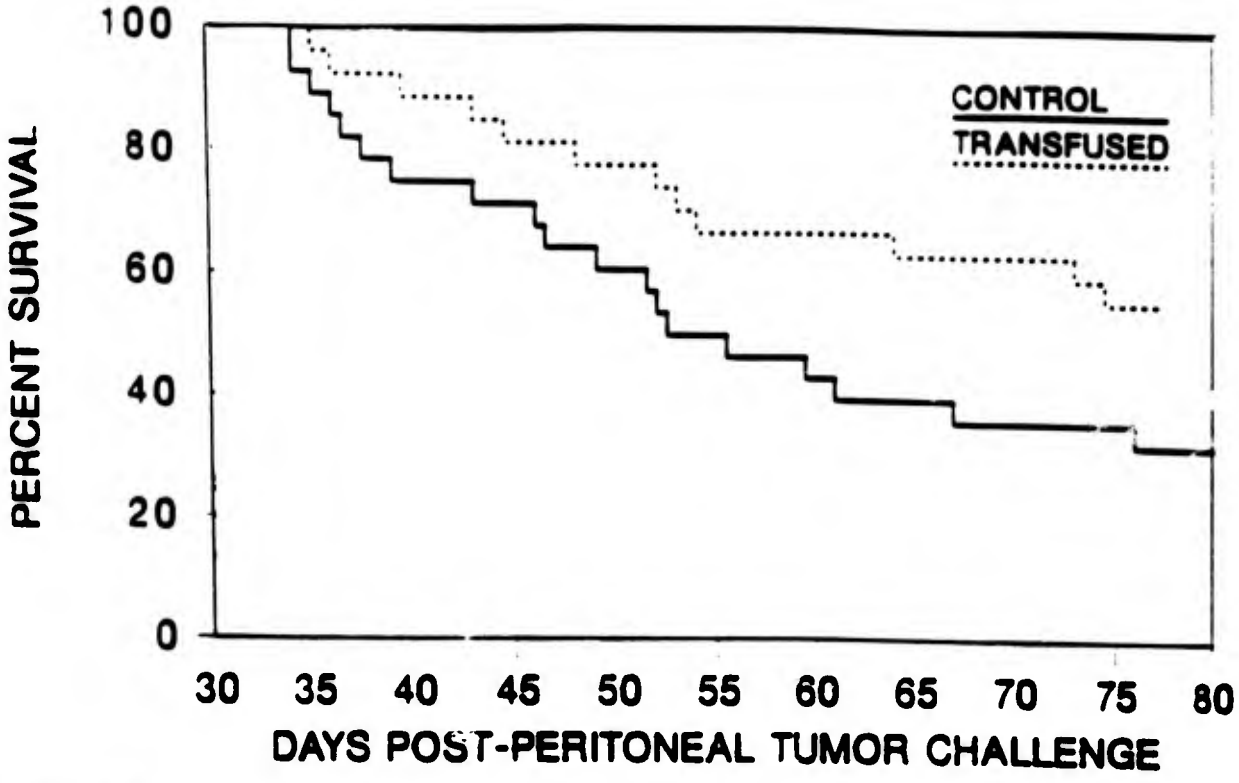


**FIGURE 1:** Survival curves for control and transfused groups challenged with $1x10^6$ tumor cells intraperitoneally.

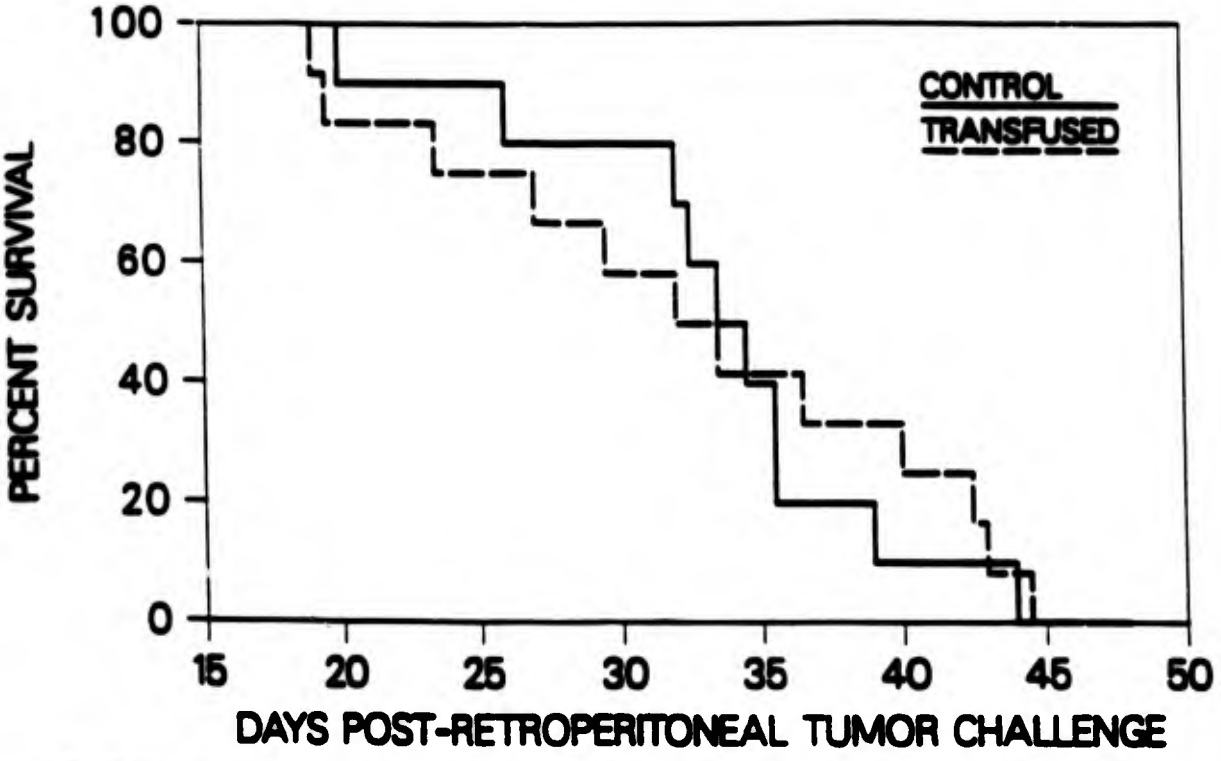


**FIGURE 2:** Survival curves for control and transfused groups challenged with $1x10^6$ tumor cells retroperitoneally.

## DISCUSSION

Appropriate surgical intervention is normally able to control the primary site of common solid malignant neoplasms. It is, rather, the distant metastases which eventually lead to a fatal outcome. Such metastases can take place by three methods, migration through coelomic cavities, spread through lymphatic vessels, and spread through blood vessels.

Tumor spread by these methods does not always lead to a successful metastasis. For such tumor spread to eventually become a metastasis, the tumor cells, or group of tumor cells, must implant in a distant site and escape control by the host's immune system. The patient's immune system can generate a complex immune response to both primary tumor and metastatic sites. Among the more important components of this response are helper/inducer T lymphocytes, cytotoxic T lymphocytes, natural killer cells, and cytotoxic macrophages.

We have previously reported, utilizing the same tumor cell line in Wistar-Furth rats, that blood transfusions can alter certain components of the host's response to this tumor when the tumor is presented in a particular manner (6). In that study allogeneic blood transfusions were demonstrated to have no significant effect on the host's response to the primary tumor site with this cell line. This finding was confirmed in our current study in that, when all the tumor cells were injected into a single site in the host's retroperitoneum, transfusions exerted no effect on survival. Our current study also confirmed our previous finding that transfusions do not appear to alter the host's response to tumor growths once they become established since, as in our earlier study, (6) transfusions did not alter the leukocyte infiltration of established tumor growths, in this case, the peritoneal carcinomatosis growths.

Our previous study with this tumor cell line did demonstrate that transfusions increased the rate of growth of pulmonary metastases and decreased long-term survival in rats bearing pulmonary metastases when the tumor cells were given intravenously and thus allowed to implant throughout the lungs as multiple single-cell emboli. Since natural killer cells are particularly important in a successful host response to blood borne tumor cells, we also evaluated the effect of allogeneic transfusions on natural killer cell function. It was found that such transfusions significantly impaired natural killer cell function. It was found that such transfusions significantly impaired natural killer cell cytotoxicity against YAC-1 cells at both one and two weeks following the transfusions. This finding would appear to indicate that a selective impairment of a single component of the immune system may alter the clinical response of the host, at least to a particular method of tumor spread.

Our current study was therefore designed to determine if the alteration in host response to pulmonary metastases following transfusion was representative of a universal response to metastases or merely of a particular immune component and a selective organ response to metastases. Although the allogeneic blood source, the tumor host, and the tumor cell line were identical to our previous study, the host's response to the metastatic tumor cell challenge (peritoneal) and the response of the particular immune cell studied (macrophage) were different. There was noted to be no effect of the transfusion on macrophage cytotoxicity against YAC-1 cells. The transfusions also failed to alter the metabolic activity of the macrophages, as measured by glucose metabolism, adenosine deaminase levels, or adenosine triphosphate levels. These findings would appear to indicate that there was no significant effect of the transfusions on the ability of the macrophages to be stimulated to a metabolically excited and immunologically enhanced state by either concanavalin A mitogen or tumor cell targets. This could explain the lack of a transfusion effect on the peritoneal metastatic carcinoma model used in our study, since such macrophages are the initial immune response cell in the host's peritoneum.

The lack of a transfusion effect on the macrophage's metabolic parameters measured in our current model is somewhat surprising in view of the previous demonstration of a transfusion effect on macrophage arachidonic acid metabolism (10). That study found that transfusions increased the rate of metabolism of arachidonic acid by the macrophage's cyclooxygenase enzyme system. This discrepancy may reflect that the alterations in macrophage activity following transfusion relate primary to alterations in macrophage regulation of other components of the immune system (11), rather than alterations in the macrophage's response to invading pathogens by the tumor or microorganisms (12).

Our current study thus provides further confirmation of the selectivity of the transfusion effect. Just as the transfusion effect has been shown to alter resistance to gram-negative infections (13,14) but not to gram-positive infections (15,16), so it also impairs resistance to primary tumor growth of certain tumor cell lines (17,18) but not to the primary growth of other tumor lines (19). Of even greater interest is the finding that its ability to alter resistance to gram-negative infections and tumor cell metastases is dependent upon whether the challenge is intravenous or intraperitoneal (6,20).

Finally, our current study indicates that the transfusion-induced alterations in immune function may provide a model for investigating the effect of various components of host immune function on the response to tumor challenge. In the future, such an understanding might assist in the formulation of immunostimulatory protocols for the treatment of oncology patients.

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| RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY | | | | 1. AGENCY ACCESSION | 2. DATE OF SUMMARY | REPORT CONTROL SYMBOL |
|---|---|---|---|---|---|---|
| | | | | DA311499 | 01 Oct 90 | DD-DR&E(AR) 636 |
| 3. DATE PREV SUM'RY | 4. KIND OF SUMMARY | 5. SUMMARY SCTY | 6. WORK SECURITY | 7. REGRADING | 8. DISB'N INSTR'N | 9. LEVEL OF SUM A. WORK UNIT |
| 01 Oct 89 | D. Chg | U | U | | CX | |

| 10. NO./CODES: | PROGRAM ELEMENT | PROJECT NUMBER | TASK AREA NUMBER | WORK UNIT NUMBER |
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| a. PRIMARY | 61102A | 3M161102BS14 | CK | 306 |
| b. CONTRIBUTING | | | | |
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11. TITLE *(Precede with Security Classification Code)* (U) Preliminary Studies on Zinc Homeostatic Control and Immunocompetence in a Burned Animal Model

12. SUBJECT AREAS
0601 Biochemistry 0613 Microbiology

| 13. START DATE | 14. ESTIMATED COMPLETION DATE | 15. FUNDING ORGANIZATION | 16. PERFORMANCE METHOD |
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| 8610 | 9109 | DA | C. In-House |

17. CONTRACT/GRANT MILITARY RELEVANCY CERTIFIED APPROVED BY [illegible]

| a. DATE EFFECTIVE | b. CONTRACT/GRANT NUMBER | c. TYPE | d. AMOUNT | e. KIND OF AWARD | f. CUM/TOTAL |
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| | | | | | |

18. RESOURCES ESTIMATE

| FISCAL YEARS | a. PROFESSIONAL WORKYEARS | b. FUNDS *(In thousands)* |
|---|---|---|
| 90 | 0.1 | 101 |
| 91 | 0.1 | 107 |

| 19. RESPONSIBLE DOD ORGANIZATION | 20. PERFORMING ORGANIZATION |
|---|---|
| a. NAME: US Army Institute of Surgical Research | a. NAME: US Army Institute of Surgical Research |
| b. ADDRESS *(include zip code)*: Fort Sam Houston, San Antonio, Texas 78234-5012 | b. ADDRESS: Fort Sam Houston, San Antonio, Texas 78234-5012 |
| c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A | c. NAME OF PRINCIPAL INVESTIGATOR: SHIPPEE, R L |
| d. TELEPHONE NUMBER *(include area code)*: 512-221-2720 | d. TELEPHONE NUMBER *(include area code)*: 512-221-7138 |
| 21. GENERAL USE: FINA<br>MILITARY/CIVILIAN APPLICATION: M | e. NAME OF ASSOCIATE INVESTIGATOR *(if available)*<br>g. NAME OF ASSOCIATE INVESTIGATOR *(if available)* |

22. KEYWORDS *(Precede EACH with Security Classification Code)* (U) Burns (Injuries); (U) Nutrition; (U) Septicemia; (U) Bioassay; (U) Immunosuppression

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS *(Precede text of each with Security Classification Code)*

23/24. (U) The objective of this work is to determine changes in zinc metabolism caused by burn and infection in a murine model as manifested by altered mechanisms at the whole body, organ, and molecular levels, relate the role of these changes to septic complications, and determine optimal levels of zinc supplementation in burned humans. The interaction between zinc nutriture, burn injury, and immunocompetence will be studied. Studies planned for the next year will use additional functional tests of immunocompetence, such as mixed lymphocyte assays, to determine the perturbations in the immune response related to zinc nutriture and burn injury. Work will continue on purifying cell types to be used in functional tests of immunocompetence to further delineate effects at the cellular level.

25. (U) 8710 - 8809. Burn injury and burn injury plus zinc deficiency caused a decrease in total peripheral blood T cells. However, burned/zinc-deficient animals showed an increase in T suppressor cell populations. The results from the plaque assay showed a supression in antibody response in burned/zinc-deficient animals. These studies demonstrated a modulating effect of zinc nutriture on various aspects of the immunological system after burn injury.

(U) 8810 - 8909. The effect of burn injury and zinc nutriture on the anamnestic response was investigated. The kinetics of the anamnestic response to sheep RBCs showed that burn injury did not affect the peak day response; both burned and nonburned rats gave a peak response on the third

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day following immunization. The response for burn-injured rats was significantly lower than for nonburned control rats. Preliminary data indicated that this response was further suppressed by placing burn-injured rats on a zinc-restricted regimen. Initial data using isolated lymphocytes and monocytes in crossover-type studies indicated that burn injury suppresses monocyte function and that this phenomena was aggravated by zinc restriction.

(U) 8910 - 9009. The increased humoral response shown in burned rats maintained on adequate zinc intake as determined by the Jerne plaque assay was further investigated because the results were contradictory to previous reports in the literature using a similar animal model. A number of studies were performed to investigate the effect of burn injury on total serum antibody levels as determined by radial immunodiffusion and on specific serum antibody levels determined by hemagglutination with sheep RBCs. Our results confirmed the data from the plaque assay that burn injury causes an increased primary humoral response in the burned rat model.

**SUPPLEMENTAL WORK UNIT INFORMATION FOR THE PROJECT ENTITLED "PRELIMINARY STUDIES ON ZINC HOMEOSTATIC CONTROL AND IMMUNOCOMPETENCE IN A BURNED ANIMAL MODEL"**

**Subrecord/Linking Accession Number:** Not applicable.

**Search Control Data:** W6L28F/W6L29E, 20 October 1989

**Product Identification:** For technical reports, refer to the US Army Institute of Surgical Research Annual Research Progress Report for fiscal years 1987-90.

**Unclassified Special Categories:** Lab Animals: Rats; RA II

**ANNUAL RESEARCH PROGRESS REPORT**

**PROJECT NUMBER:** 3M161102BS14-00, RESEARCH

**PROJECT TITLE:** Preliminary Studies on Zinc Homeostatic Control and Immunocompetence in a Burned Animal Model

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

**1 October 1989 - 30 September 1990**

**INVESTIGATORS**

Ronald L. Shippee, PhD, Major, MS
Thomas Koppenheffer, PhD*

*Chairman, Biology Department, Trinity University

## ABSTRACT

**PROJECT NUMBER:** 3M161102BS14-00, RESEARCH

**PROJECT TITLE:** Preliminary Studies on Zinc Homeostatic Control and Immunocompetence in a Burned Animal Model

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

**PERIOD COVERED IN THIS REPORT:** 1 Oct 89 through 30 Sep 90

**INVESTIGATORS:** Ronald L. Shippee, PhD, Major, MS
Thomas Koppenheffer, PhD*

*Chairman, Biology Department, Trinity University

We have used the Hemolytic Plaque Assay to determine the interaction of zinc nutriture and burn injury on immunocompetence in a 30% total body surface scald burn rat model. Major discrepancies between our results and those previously reported in the literature have prompted us to further investigate the humoral response in the burn rat model. Data from a radial immunodiffusion assay are in agreement with published data on humans not shown to be septic. An early decrease in all three classes of immunoglobulin followed with an increase in IgG to the high normal range and an increase in IgM and IgA to low normal range. Hemagglutination titers from burned rats given normal and low dose levels of sheep red blood cells (SRBC) gave an increase humoral response compared to control rats. This finding supports our earlier work using the Plaque assay. The results of work to date with this protocol demonstrate that although the rat appears to be a valuable tool to study the physiological effects of thermal injury, many aspects of the host defense system in the rat during recovery from burn injury need to be defined before observed phenomena are applied to a clinical situation.

## INTRODUCTION

We have used the Hemolytic Plaque Assay to determine the interaction of zinc nutriture and burn injury on immunocompetence afteera 30% Total Body Surface (TBS) scald burn in rats. Animals maintained on adequate zinc nutriture had a significantly ($p<0.05$) higher primary Plaque Forming Cell (PFC) response to Sheep Red Blood Cells than sham burned animals on day 10 postburn. Although zinc restriction for 10 days postburn caused a lower PFC response, the response was still higher than the sham control zinc adequate or restricted groups. Results from experiments to assess the secondary response indicate suppression of PFC in both zinc restricted and zinc adequate burned groups when compared to sham burned animals.

After review of the data collected to this point, two issues appeared to need further investigation. First, the increase in the primary response was not consistent with reported studies using a similar rat model (1). The second issue centers around certain aspects of the secondary response. When compared to a primary response the secondary humoral response classically is characterized by an earlier response to antigen, and greater magnitude of the response, with the primary class of antibody being IgG as opposed to IgM in the primary response. Although our kinetics experiments with the rats consistently gave an earlier response, the response was not much greater than the primary response and although there was an increase in IgG antibody, it represented only about 22% of the total PFC response.

We decided first to determine the kinetics of plasma immunoglobulin concentration after burn injury and then to investigate the kinetics of serum antibody titers to SRBC, using a hemagglutination assay.

## MATERIALS AND METHODS

Adult Sprague-Dawley rats were anesthetized and administered a 30% TBS burn by scalding with 90°C water for 10 sec. Animals were housed individually and fed Purina Chow and deionized water ad libitum. Blood was drawn prior to burn injury and then 1, 4, 8, 12 and 16 days postburn. IgM, IgG and IgA subclasses of immunoglobulin were determined by radial immunodiffusion (Serotec, 22 Bankside, Killington, Oxford, England).

In a second set of experiments, rats were burned and fed as described above. One day after burn injury, a primary immunization was given with 0.5ml of either 2% or 0.02% SRBC suspensions in saline, administered intraperitoneally (IP). Animals were bled from a tail vein on days 4, 6, 8, 11, and 15 postburn. Ninety-six well culture plates were used to prepare serial 1:2 saline dilutions of 0.025 ml of plasma. Twenty-five microliters of a 1%

suspension of SRBC were added to each well. Titers were determined after four hours of incubation at 25°C.

The secondary response was determined by administering a primary dose of 0.2 ml of 20% SRBC intravenously four weeks prior to burn injury. These animals were then treated as described above except that blood was drawn prior to the burn and then on days three and nine postburn.

The plasma immunoglobulin concentration data were analyzed using a two factor repeated measures analysis of variance (SAS/STAT User's Guide, Release 6.03 edition, SAS Institute Inc., Cary, NC 27512-8000, page 602-609). The data were described as percentage values of the preburn concentration within each animal. The resulting percentage data were converted using an arcsin transformation as suggested by Snedecor (2). The hemagglutination data were defined descriptively in terms of central tendency using mode values.

## RESULTS AND DISCUSSION

Data for plasma concentrations of IgM, IgA and IgG for individual animals are shown in Tables 1, 2, and 3, respectively and summarized in Table 4. The effects of day and burn treatment were significant for IgM ($p<0.01$, $p=0.02$) and IgG ($p=0.03$, $p=0.04$), while only the effect of day was significant for IgA ($p<0.01$).

We are unable to explain why there was an increase in all classes of immunoglobulin with time, regardless of treatment. Whether this was due to handling or some environmental variable, is impossible to determine from the present experimental design. Of interest is an early study of the effect of thermal injury on serum immunoglobulin by Munster et al. (3). Although the control subjects were only followed to the seventh day of the study, a gradual increasing trend is shown for all three classes of immunoglobulin.

The kinetics of the three classes of immunoglobulin in burn patients in this early study is in general agreement with our rat data. An early decrease in all three classes occurred, presumably due to leakage from the burn wound, with an increase in IgG to the upper portion of the normal range and IgA and IgM increasing to the mid and lower portion of the normal range, respectively. When sepsis was taken into account (3) the patterns of IgG and IgA postburn were not influenced by the presence of sepsis, but the IgM pattern was altered sharply by fungal invasion of the burn wound. The four patients with phycomycosis had very high serum IgM levels at the time of diagnosis.

A more recent study has confirmed these early findings with the additional conclusion that normal or supranormal quantities of serum immunoglobulin exist concurrent with the presence of

suppression of T cell related immune function (4). Shorr et al. (4) have studied in vitro immunoglobulin production by lymphocytes isolated form the blood of burn patients and cultured in the presence of polkweed mitogen (PWM). The lymphocytes from burn patients produced significant quantities of IgG and IgM without PWM and failed to increase production with PWM stimulation.

Table 5 shows the $Log_2$ hemagglutination titers in each individual animal for the primary response. The data are summarized in Table 6. Both the rapid appearance of hemagglutinating antibody in burned rats given a high dose of SRBC, and the slight but consistently higher antibody titers produced by these animals as compared to sham-burned rats during later stages of the response, are consistent with our previous findings of heightened primary PFC responses following thermal injury. More striking, however, is the antibody response generated by burned rats given a subimmunogenic dose of SRBC.

The heightened primary response that we consistently observe is in contrast to the results observed by Alexander and Moncrief (1) using a similar model. In those studies, rats administered a 20% burn and injected IP with a primary immunization of 2% Human Red Blood Cells (HRBC) 24 hours after injury showed no difference in serum antibody titers. Animals administered a 30% burn and immunized with 0.02% suspension of HRBC given 1,2 and 3 hours postburn showed a dramatic decrease in serum antibody titers. The authors give no explanation for the different immunizing concentrations of HRBC given to the different burn size groups.

The only major difference between our study and the Alexander and Moncrief study is the species of red blood cells used as immunogen. SRBC have been historically used in the Jerne Plaque forming assay. In fact, we have attempted to use HRBC in our plaque forming assay and have never shown a plaque forming response in the rat with 2 to 20% suspensions.

The hemagglutination data for the secondary response in individual animals are shown in Table 7 and summarized in Table 8. We were surprised to find that four weeks after primary immunization, the plasma titers were quite elevated. It appears that the rat has a sustained response to primary immunization with a 20% solution of SRBC. This may explain why we have been unable to produce an appropriate anamnestic response to SRBC in the rat.

The burn rat model has been used at this Institute and by researchers in other laboratories for investigations into many physiological and metabolic sequelae of burn injury. There is no doubt that the rat model should be a valuable model to study the interaction of nutrition, burn injury and immunocompetence. However, based on results to this point, it is obvious that the many aspects of the host defense system of the rat during recovery

from burn injury must be defined before phenomena observed in this species can be applied clinically.

**TABLE 1**

PLASMA IgM
DAY

| | 0 | | 1 | | 4 | | 8 | | 12 | | 16 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | mg/L | % | mg/L | % | mg/L | % | mg/L | % | mg/L | % | mg/L | % |
| **BURN** | | | | | | | | | | | | |
| 1 | 961 | 100 | 565 | 59 | 1102 | 115 | 919 | 95 | 800 | 83 | 800 | 83 |
| 2 | 1132 | 100 | 606 | 54 | 1220 | 108 | 867 | 77 | 961 | 85 | 774 | 68 |
| 3 | 1073 | 100 | 584 | 54 | 1251 | 117 | 1190 | 111 | 1059 | 99 | 947 | 88 |
| 4 | 1132 | 100 | 575 | 51 | 919 | 81 | 947 | 84 | 1073 | 95 | 1075 | 95 |
| 5 | 828 | 100 | 401 | 48 | 919 | 111 | 794 | 96 | 853 | 103 | 880 | 106 |
| 6 | 1073 | 100 | 593 | 55 | 1117 | 104 | 1059 | 99 | 1281 | 119 | 1159 | 108 |
| **CONTROL** | | | | | | | | | | | | |
| 1 | 1015 | 100 | 961 | 95 | 1132 | 112 | 1146 | 113 | 1220 | 120 | 974 | 96 |
| 2 | 1251 | 100 | 1102 | 88 | 1345 | 108 | 1220 | 98 | 1102 | 88 | 1190 | 95 |
| 3 | 853 | 100 | 1015 | 119 | . | . | 1251 | 147 | 1073 | 128 | 1376 | 161 |
| 4 | 987 | 100 | 1102 | 112 | 1190 | 121 | 1281 | 130 | 961 | 97 | 1030 | 104 |
| 5 | 961 | 100 | 905 | 94 | 1132 | 118 | 1296 | 135 | 1251 | 130 | 987 | 103 |
| | **MEAN** | | **BURN** | 54 | | 107 | | 94 | | 97 | | 91 |
| | | | CONTROL | 102 | | 115 | | 125 | | 113 | | 112 |

**TABLE 2**

PLASMA IgG
DAY

| | 0 | | 1 | | 4 | | 8 | | 12 | | 16 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | mg/L | % | mg/L | % | mg/L | % | mg/L | % | mg/L | % | mg/L | % |
| **BURN** | | | | | | | | | | | | |
| 1 | 2020 | 100 | 790 | 34 | 2260 | 112 | 2720 | 135 | 2480 | 123 | 3720 | 184 |
| 2 | 2260 | 100 | 1190 | 53 | 2960 | 131 | 2260 | 100 | 1810 | 80 | 2260 | 100 |
| 3 | 2260 | 100 | 1810 | 80 | 3720 | 165 | 3720 | 165 | 4240 | 188 | 5368 | 237 |
| 4 | 1590 | 100 | 790 | 50 | 2020 | 127 | 1810 | 114 | 1810 | 114 | 2720 | 171 |
| 5 | 4240 | 100 | 1810 | 43 | 3980 | 94 | 2720 | 64 | 3480 | 94 | 5360 | 126 |
| 6 | 2720 | 100 | 1390 | 51 | 2960 | 109 | 2020 | 74 | 3980 | 146 | 5080 | 187 |
| **CONTROL** | | | | | | | | | | | | |
| 1 | 2260 | 100 | 2260 | 100 | 2480 | 110 | 2960 | 131 | 2960 | 131 | 2480 | 110 |
| 2 | 3200 | 100 | 3720 | 116 | 4240 | 133 | 4520 | 141 | . | . | 4800 | 150 |
| 3 | 2480 | 100 | 3460 | 140 | . | . | 2960 | 119 | 3460 | 140 | 5080 | 205 |
| 4 | 2720 | 100 | 2960 | 109 | 2960 | 109 | 2720 | 100 | . | . | 2720 | 100 |
| 5 | 3590 | 100 | 3200 | 89 | . | . | 3460 | 96 | 5080 | 142 | 4820 | 126 |
| | **MEAN** | | **BURN** | 53 | | 123 | | 109 | | 124 | | 168 |
| | | | CONTROL | 111 | | 117 | | 117 | | 138 | | 138 |

**TABLE 3**
PLASMA IgA
DAY

| | 0 | | 1 | | 4 | | 8 | | 12 | | 16 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | mg/L | % | mg/L | % | mg/L | % | mg/L | % | mg/L | % | mg/L | % |
| **BURN** | | | | | | | | | | | | |
| 1 | 83 | 100 | 48 | 58 | 75 | 91 | 75 | 91 | 106 | 129 | 91 | 110 |
| 2 | 96 | 100 | 70 | 74 | 86 | 90 | 80 | 83 | 99 | 104 | 89 | 93 |
| 3 | 80 | 100 | 48 | 60 | 75 | 94 | 86 | 108 | 89 | 112 | 80 | 100 |
| 4 | 73 | 100 | 48 | 65 | 59 | 80 | 70 | 96 | 99 | 135 | 106 | 145 |
| 5 | 78 | 100 | 51 | 64 | 92 | 118 | 78 | 100 | 99 | 126 | 99 | 126 |
| 6 | 70 | 100 | 53 | 76 | 73 | 104 | 67 | 96 | 96 | 136 | 78 | 110 |
| **CONTROL** | | | | | | | | | | | | |
| 1 | 86 | 100 | 83 | 96 | 96 | 111 | 96 | 111 | 116 | 136 | 92 | 108 |
| 2 | 75 | 100 | 80 | 106 | 106 | 141 | 106 | 141 | 104 | 139 | 106 | 141 |
| 3 | 81 | 100 | 96 | 118 | . | . | 96 | 118 | 106 | 131 | 96 | 118 |
| 4 | 89 | 100 | 83 | 93 | 86 | 97 | 99 | 111 | 96 | 108 | 106 | 120 |
| 5 | 102 | 100 | 89 | 87 | 92 | 90 | 102 | 100 | 116 | 114 | 104 | 102 |
| **MEAN** | **BURN** | | 66 | | 96 | | 96 | | 124 | | 114 | |
| | **CONTROL** | | 100 | | 110 | | 116 | | 126 | | 118 | |

**TABLE 4**

DAY
(% of DAY 0)

| | | 1 | 4 | 8 | 12 | 16 |
|---|---|---|---|---|---|---|
| IgM | Burn | 54 | 107 | 94 | 97 | 91 |
| | Control | 102 | 115 | 125 | 113 | 112 |
| IgG | Burn | 53 | 123 | 109 | 124 | 168 |
| | Control | 111 | 117 | 117 | 138 | 138 |
| IgA | Burn | 66 | 96 | 96 | 124 | 114 |
| | Control | 100 | 110 | 116 | 126 | 118 |

**TABLE 5**

LOG$^2$ HEMAGGLUTINATION TITERS
FOR THE PRIMARY SRBC RESPONSE

Burn, 2% Dose

| Animal | Day 4 | 6 | 8 | 11 | 15 |
|---|---|---|---|---|---|
| 1 | 16 | 64 | 64 | 32 | 32 |
| 2 | 16 | 64 | 64 | 64 | 64 |
| 3 | 16 | 64 | 64 | 32 | 32 |
| 4 | 16 | 64 | 64 | 64 | 32 |
| 5 | 0 | 32 | 64 | 32 | 32 |
| 6 | 16 | 64 | 64 | 32 | 32 |

Sham-Burn 2% Dose

| Animal | Day 4 | 6 | 8 | 11 | 15 |
|---|---|---|---|---|---|
| 1 | 0 | 32 | 32 | 32 | 32 |
| 2 | 0 | 32 | 32 | 32 | 32 |
| 3 | 0 | 16 | 16 | 16 | 32 |
| 4 | 4 | 32 | 32 | 32 | 64 |
| 5 | 0 | 32 | 16 | 32 | 32 |

Burn, 0.2% Dose

| Animal | Day 4 | 6 | 8 | 11 | 15 |
|---|---|---|---|---|---|
| 1 | 0 | 32 | 32 | 32 | 32 |
| 2 | 0 | 16 | 16 | 16 | 0 |
| 3 | 0 | 32 | 16 | 32 | 16 |
| 4 | 0 | 32 | 32 | 32 | 32 |

Sham-Burn 0.2% Dose

| Animal | Day 4 | 6 | 8 | 11 | 15 |
|---|---|---|---|---|---|
| 1 | 0 | 0 | 0 | 0 | 0 |
| 2 | 0 | 0 | 0 | 0 | 0 |
| 3 | 0 | 16 | 0 | 0 | 0 |
| 4 | 16 | 16 | 0 | 0 | 0 |
| 5 | 0 | 0 | 0 | 0 | 0 |

**TABLE 6**

SUMMARY TABLE OF LOG$^2$ HEMAGGLUTINATION TITERS
FOR THE PRIMARY SRBC RESPONSE

| | Dose | Burn 2.0% | Control 2.0% | Burn 0.2% | Control 0.2% |
|---|---|---|---|---|---|
| Day | 4 | 16 (1) | 0 | 0 | 0 |
| | 6 | 64 | 32 | 32 | 0 |
| | 8 | 64 | 32 | 32 | 0 |
| | 11 | 32 | 32 | 32 | 0 |
| | 15 | 32 | 32 | 32 | 0 |

(1) mode values

**TABLE 7**

LOG$^2$ HEMAGGLUTINATION TITERS FOR THE SECONDARY SRBC RESPONSE

Burn, 2% Dose

| Animal | Day 0 | Day 3 | Day 9 |
|---|---|---|---|
| 1 | 64 | 32 | 64 |
| 2 | 64 | 64 | 64 |
| 3 | 32 | 32 | 64 |
| 4 | . | 16 | 16 |
| 5 | 64 | 32 | 64 |
| 6 | 16 | 16 | 64 |

Sham-Burn 2% Dose

| Animal | Day 0 | Day 3 | Day 9 |
|---|---|---|---|
| 1 | 128 | 64 | 64 |
| 2 | 32 | 32 | 64 |
| 3 | 32 | 16 | 32 |
| 4 | 32 | 32 | 64 |
| 5 | . | 64 | 128 |
| 6 | 32 | 32 | 64 |

Burn, 0.2% Dose

| Animal | Day 0 | Day 3 | Day 9 |
|---|---|---|---|
| 1 | . | 16 | 32 |
| 2 | 64 | 64 | 64 |
| 3 | 64 | 32 | 16 |
| 4 | 32 | 16 | 64 |
| 5 | 64 | 16 | 32 |
| 6 | 16 | . | 32 |

Sham-Burn 0.2% Dose

| Animal | Day 0 | Day 3 | Day 9 |
|---|---|---|---|
| 1 | 32 | 16 | 32 |
| 2 | 64 | 32 | 64 |
| 3 | 32 | 32 | 32 |
| 4 | 64 | 32 | 32 |
| 5 | 128 | 64 | 128 |
| 6 | 32 | 32 | 64 |

**TABLE 8**

SUMMARY TABLE OF LOG$^2$ HEMAGGLUTINATION TITERS FOR THE SECONDARY SRBC RESPONSE

| | Dose | Burn 2.0% | Control 2.0% | Burn 0.2% | Control 0.2% |
|---|---|---|---|---|---|
| Day | 0 | 64 (1) | 32 | 64 | 32 |
| | 3 | 32 | 32 | 16 | 32 |
| | 9 | 64 | 64 | 32 | 32 |

(1) mode values

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3. Munster AM, Hoagland HC, Pruitt BA Jr: The effect of thermal injury on serum immunoglobulins. **Ann Surg** 172:965-969, 1970.

4. Shorr RM, Ershler WR, Gamelli RL: Immunoglobulin production in burned patients. **J Trauma** 24:319-322, 1984.


## PUBLICATIONS


1. Shippee RL, Watiwat S: Effect of zinc nutriture on postburn amamnestic response. **Federation of the American Society of Experimental Biology**. Abstract #3875, April 1990.

| RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY | | | | 1. AGENCY ACCESSION | 2. DATE OF SUMMARY | REPORT CONTROL SYMBOL |
|---|---|---|---|---|---|---|
| | | | | DA318368 | 01 Oct 90 | DD-DR&E(AR) 636 |
| 3. DATE PREV SUM'RY | 4. KIND OF SUMMARY | 5. SUMMARY SCTY | 6. WORK SECURITY | 7. REGRADING | 8. DISP'N INSTR'N | 9. LEVEL OF SUM A. WORK UNIT |
| 01 Oct 89 | D. Chg | U | U | | CX | |

| 10. NO./CODES | PROGRAM ELEMENT | PROJECT NUMBER | TASK AREA NUMBER | WORK UNIT NUMBER |
|---|---|---|---|---|
| a. PRIMARY | 61102A | 3M161102BS14 | FA | 307 |
| b. CONTRIBUTING | | | | |
| c. CONTRIBUTING | | | | |

11. TITLE *(Precede with Security Classification Code)* (U) Interleukin 1 (IL1) Activity in the Serum of Burned Rats and Thermally Injured Patients

12. SUBJECT AREAS
0601 Biochemistry 0605 Medicine and Medical Research

| 13. START DATE | 14. ESTIMATED COMPLETION DATE | 15. FUNDING ORGANIZATION | 16. PERFORMANCE METHOD |
|---|---|---|---|
| 8905 | 9109 | DA | C. In-House |

17. CONTRACT/GRANT — MILITARY RELEVANCY CERTIFIED — APPROVED BY [illegible]

| a. DATE EFFECTIVE | | b. RESOURCES ESTIMATE | | |
|---|---|---|---|---|
| b. CONTRACT/GRANT NUMBER | | c. FISCAL YEARS | a. PROFESSIONAL WORKYEARS | b. FUNDS *(in thousands)* |
| c. TYPE | d. AMOUNT | 90 | 0.1 | 112 |
| e. KIND OF AWARD | f. CUM/TOTAL | 91 | 0.1 | 118 |

| 19. RESPONSIBLE DOD ORGANIZATION | 20. PERFORMING ORGANIZATION |
|---|---|
| a. NAME: US Army Institute of Surgical Research | a. NAME: US Army Institute of Surgical Research |
| b. ADDRESS *(Include zip code)*: Fort Sam Houston, San Antonio, Texas 78234-5012 | b. ADDRESS: Fort Sam Houston, San Antonio, Texas 78234-5012 |
| c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A | c. NAME OF PRINCIPAL INVESTIGATOR: BURLESON, D G |
| d. TELEPHONE NUMBER *(Include area code)*: 512-221-2720 | d. TELEPHONE NUMBER *(Include area code)*: 512-221-7138 |
| 21. GENERAL USE: FINA<br>MILITARY/CIVILIAN APPLICATION: M | f. NAME OF ASSOCIATE INVESTIGATOR *(If available)*: DROST, A C<br>g. NAME OF ASSOCIATE INVESTIGATOR *(If available)*: CIOFFI, W G |

22. KEYWORDS *(Precede EACH with Security Classification Code)* (U) Burns (Injuries); (U) Blood; (U) Immunosuppression

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS *(Precede text of each with Security Classification Code)*

23/24. (U) The objective of this work is to delineate the role of IL 1, IL 6, and TNF in burn patients, with emphasis on establishing a correlation between serum IL 1 levels, IL 6, and TNF and the degree of burn injury or infection. The first part of this study includes duplication of the methodology used to detect serum IL 1, IL 6, and TNF activity in a burned rat model. The second part of the study will involve detection of serum IL 1, IL 6, and TNF activity in burned patients. If serum IL 1, IL 6, and TNF activity is significantly increased in burn patients above that of healthy controls, serum IL 1, IL 6, and TNF activity will be correlated with time postburn, burn size, infection, or other burn-associated manifestations.

25. (U) 8710 - 8809. Not applicable.

(U) 8810 - 8909. This project was approved by the USAISR Research Council, US Army Institute of Surgical Research Human Use Committee, and US Army Institute of Surgical Research Animal Care and Use Committee during the second and third quarters of fiscal year 1989. Nine patients were enrolled in the study during this reporting period.

(U) 8910 - 9009. Nine patients and 17 control subjects were enrolled in the study during this reporting period and a total of 409 serum samples were analyzed. Overall, IL 1 and IL 6, but not TNF, were increased in burn patients above that found in control subjects. In surviving patients, IL 1 levels were positively correlated with burn size, IL 6 level, and episodes

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of infection and negatively correlated with time postburn. IL 6 levels were increased in patients who died as compared to survivors.

**SUPPLEMENTAL WORK UNIT INFORMATION FOR THE PROJECT ENTITLED "INTERLEUKIN 1 (IL 1) ACTIVITY IN THE SERUM OF BURNED RATS AND THERMALLY INJURED PATIENTS"**

**Subrecord/Linking Accession Number:** Not applicable.

**Search Control Data:** W6L37A/W6L37E, 20 October 1989

**Product Identification:** For technical reports, refer to the US Army Institute of Surgical Research Annual Research Progress Report for fiscal years 1989-90.

**Unclassified Special Categories:** Volunteers: Adults; RA II

**ANNUAL RESEARCH PROGRESS REPORT**

**PROJECT NUMBER:** 3M161102BS14-00, RESEARCH

**PROJECT TITLE:** Interleukin 1 (IL1) Activity in the Serum of Burned Rats and Thermally Injured Patients

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

**1 October 1989 - 30 September 1990**

**INVESTIGATORS**

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

## ABSTRACT

**PROJECT NUMBER:** 3M161102BS14-00, RESEARCH

**PROJECT TITLE:** Interleukin 1 (IL1) Activity in the Serum of Burned Rats and Thermally Injured Patients

**INSTITUTION:** US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas 7823-5012

**PERIOD COVERED IN THIS REPORT:** 1 Oct 89 through 30 Sep 90

**INVESTIGATORS:** David G. Burleson, Ph.D., Lieutenant Colonel, MS
Adriane C. Drost, MS
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We measured plasma interleukin 1ß (IL1ß), tumor necrosis factorα (TNFα), and interleukin 6 (IL6) in burned patients for up to six weeks postburn. The cytokines were measured by Enzyme Linked Immunosorbent Assay (ELISA) and the correlations between cytokine concentrations and burn size, postburn time, mortality, and infection were determined. Mean IL1ß and IL6 concentrations were significantly increased in the plasma of burn patients as compared to healthy laboratory employees.

There was a positive correlation between IL1ß and postburn time, with significantly elevated IL1ß levels during postburn week 1 and 2, whereas IL6 was significantly elevated only during the first week postburn. A positive correlation between IL1ß and burn size was observed. Mean IL1ß concentrations in patients with total body surface burns between 20 and 39% were significantly lower than those of patients with a total body surface burn greater than 40%. On the other hand, mean TNFα levels were significantly higher in patients with burn size ranging from 20 to 39% total body surface burn as compared to those patients with larger burns. Nonsurviving patients had significantly increased mean IL6 levels compared to surviving patients and controls. All three cytokines were significantly increased in patients who had infections during their recorvery, compared to patients who remained infection free.

These results suggest a direct relationship between plasma IL1ß and the extent of thermal injury, whereas IL6 appeared elevated in almost all burn patients regardless of burn size and further increased during infections. TNFα appeared more closely related to infection than to burn injury itself.

# INTERLEUKIN 1 (IL1) ACTIVITY IN THE SERUM OF BURNED RATS AND THEREMALLY INJURED PATIENTS

## INTRODUCTION

The survival of patients with thermal injuries remains jeopardized by infection, despite improvements in treatment during the last decade. Many aspects of the immune system are altered after burn injury, and these alterations may influence the susceptibility to infection in these patients. Several cytokines have been shown to modulate a number of immunological reactions throughout the body, including the response to infection. Changes in the production of cytokines may influence resistance to infection in burned patients.

Interleukin 1β (IL1) is an endogenous pyrogen which induces a variety of acute-phase reactions (1). IL2 dependent T cell proliferation is induced by IL1, leading to specific antigen dependent immune responses (2). Furthermore, IL1 stimulates the production of other cytokines such as interleukin 6 (IL6) (3-5).

Originally IL6 was identified as a B cell differentiation factor (6) which induced antibody production. Now its biological effects are known to include T cell activation (7), hepatic acute phase protein synthesis (8), stimulation of hematopoiesis (9), and inhibition of tumor cell growth (10).

TNF or cachectin is classically known for its ability to cause necrosis of tumors (11). It also has effects on normal cells, such as stimulation of fibroblast growth (12) and modulation of granulopoiesis. TNF affects bone resorption, the production and function of granulocytes, hemostasis, and lipid metabolism (13). TNF can induce IL6 (14,15) and IL1 (16). Most importantly, TNF is thought to be a primary mediator of host inflammatory response (17).

All three cytokines have been extensively studied in infectious disease. IL1β, which is one of two existing forms of IL1, is increased in the plasma of patients with septic shock (18) and in the colonic mucosa of patients with active inflammatory bowel disease (19). IL1α, IL1β, and TNFα production is increased in cultured peripheral blood monocytes from patients with scleroderma (20). IL6 levels in several body fluids are elevated in patients with bacterial infections (21). Patients with mechanical trauma synthesize increased amounts of IL6 over a 14 day period after injury (22). Plasma IL6 levels are increased in patients with sepsis and septic shock (23). Patients with rheumatoid arthritis and other inflammatory arthritides have high levels of IL6 in synovial fluids (24). The production of all three cytokines appear to be altered following major trauma and infection. This suggests that they may be altered following thermal injury as well.

The aim of the current study was to monitor plasma cytokines following thermal injury and establish relationships between these cytokines and the clinical course of the patients. The potential usefulness of monitoring cytokine levels as a diagnostic tool and as a basis for planning therapeutic regimes was explored.

## METHODS

**Specimens**. The patients entered into the study had burns ranging from 17.5 to 89% of total body surface area and an average age of 35.8 years. They were normotensive and hemodynamically stable after uneventful rescucitation. Healthy laboratory personnel with an average age of 37.9 years served as the control group. Blood was drawn three times a week between 5:00-6:00 a.m. into EDTA blood collection tubes. The specimens were centrifuged at 3,000 rpm for 20 min. and 1 ml aliquots of plasma were stored at -70°C until assay. Platelets were removed by centrifugation at 10,000 rpm for 1 min. immediately before assaying.

Plasma IL1ß concentrations were measured in 253 samples from 21 patients. 409 samples from 28 patients were tested for TNF content and IL6 was measured in 399 samples from 28 patients. Cytokine levels were compared to 17 samples from 16 healthy laboratory employees.

**ELISA**. Plasma levels of the cytokines were measured by ELISA. IL1ß and TNFα ELISAs were purchased from Cistron Biotechnology, NJ and the IL6 ELISA was purchased from Genzyme Corporation, MA. All three kits were sandwich ELISAs in which the measured molecule was first bound by a primary monoclonal antibody and subsequently by a rabbit polyclonal antibody. The reactions were amplified by a goat anti-rabbit IgG conjugated to horseradish peroxidase enzyme, and visualized by production of colored substrates in a peroxide substrate system. The color intensity was proportional to the amount of bound conjugate and, therefore, to the amount of cytokine present.

**Test Standards**. The amount of each cytokine bound to its respective antibodies was measured with a MR600 microplate reader (Dynatech Laboratories Inc., VA) at 490 nm or 450 nm. The cytokine concentrations were calculated by comparing the absorbance of each sample to the absorbance of a standard curve consisting of pooled control plasma enriched with known amounts of recombinant cytokine. Sample measurements were accepted as different from 0 when their absorbance was two standard deviations above that of nonspecific binding (NSB). Pooled control plasma without cytokine added served as NSB in all ELISAS.

**Data Analysis**. Statistical significance was determined by T-test and One-Way Analysis of Variance in conjunction with Tukey (BMDP Statistical Software, Los Angeles, CA), rejecting the null hypothesis at $p < .05$.

## RESULTS

The ELISA kits were tested for interference from nonspecific plasma factors. Increasing amounts of hrIL1ß, hrIL6 or hrTNFα were added to bovine serum albumin (BSA) or pooled control plasma and run in the appropriate ELISA. Standard curves were calculated and recoveries compared. Figure 1 shows that IL1ß standard curves coincided when either BSA or control plasma served as buffer. Furthermore, the average recovery of hrIL1ß in control plasma was 95.8%. Increasing amounts of hrTNFα were suspended in BSA or control plasma and run in the TNF-ELISA (Fig 1). The standard curve in BSA had a steeper slope than in control plasma. Furthermore, adding 200 pg/ml hrTNFα to patient plasma resulted in a calculated recovery of 238 pg/ml. These data suggest that a factor present in control plasma interfered with TNFα detection, decreasing the absorbance of samples at high concentrations more than at low concentrations, thereby increasing the calculated recovery in plasma samples. As a result, the concentrations in this report are relative rather than absolute TNFα concentrations.

Standard curves in the IL6-ELISA also had different slopes depending on the matrix used (Fig 2). Since hrIL6 diluted in BSA had a higher absorbance than hrIL6 diluted in control plasma, adding 1.25 ng/ml hrIL6 to patient plasma resulted in a calculated recovery of 1.17 ng/ml. Thus, it appeared that an endogenous plasma factor also interfered slightly with IL6 measurements.

**Plasma Cytokines Following Thermal Injury**. Free circulating IL1ß, IL6 and TNFα were measured three times a week for up to six weeks in the plasma of 27 patients with severe thermal injury. Plasma cytokine concentrations in burn patients were compared to those of healthy laboratory personnel. Table 1 shows that mean levels of IL1ß and IL6 were significantly increased in burned patients as compared to healthy laboratory personnel. The mean TNFα concentration was also higher, but not significantly different from controls.

**Cytokines And Time Postburn**. To explore the course of IL1ß and IL6 following thermal injury, results from patient plasma samples were grouped by postburn week for comparison. As displayed in Figure 3, mean plasma IL1ß concentrations were significantly increased during postburn week 1 and 2 ($p < 0.01$ and $p < 0.05$ respectively) and decreased until almost normal levels were reached in postburn week six.

Mean plasma IL6 levels (Fig 4) were significantly increased during the first postburn week ($p < 0.01$), but were nearly normal by the second postburn week.

Mean plasma TNFα concentration was also high during the first postburn week and decreased thereafter, but the mean concentration was not significantly different from control (data not shown).

**Plasma Cytokines And Burn Size.** The relationship between cytokine levels and extent of total body surface burn was also examined. Plasma IL1ß concentrations positively correlated with percent total body surface burn (r=.3597). When grouped by burn size (Fig 5), the mean plasma IL1ß concentration from patients with burn sizes between 40-59% total body surface was higher than the mean IL1ß concentration of healthy laboratory employees ($p < 0.01$). The same was true for samples from patients with a total body surface burn larger than 60%. The average plasma IL1ß concentration in patients with burn injuries ranging from 40-59% total body surface was significantly higher than that of patients with burn sizes of 20-39% total body surface ($p < 0.01$), but the average IL1ß concentration in patients with burn sizes ranging from 40-59% were not different from those with burn sizes greater than 60%.

Plasma IL6 did not correlate with the extent of burn injury. Interestingly, plasma TNFα levels were higher in patients with small burn sizes (20-39% total body surface burn) than in patients with burn sizes ranging from 40-60% total body surface burn ($p < 0.01$) (data not shown).

**Plasma Cytokines and Mortality.** The plasma levels of TNFα and IL6 from 24 survivors and four nonsurvivors were compared. The number of IL1ß samples from nonsurviving patients was too small to be included in the data analysis.

Figure 6 shows that the mean plasma IL6 concentration of nonsurviving patients was significantly above that of surviving patients or controls ($p < 0.01$). Plasma TNFα levels from surviving patients were also increased, but the difference was not statistically significant (data not shown).

Figure 7 displays the temporal plasma IL6 profile of three burn patients who subsequently died. Patient five, who died on postburn day 13, had very high IL6 levels between postburn days one and five which declined thereafter. The patient was diagnosed as having pneumonia (S. aureus) one day after the peak plasma IL6 concentration on postburn day six (designated in the figure by an arrow) and wound invasion (aspergillus) on postburn day seven. Treatment started on the days of diagnosis. Patient 13 expired on postburn day 18. His plasma IL6 profile peaked on postburn day three, one day before he was diagnosed and treated for sepsis (S. aureus) (arrow). Patient 25 was diagnosed as having sepsis on postburn day three and died on postburn day five. The plasma IL6 levels in patients five and 13 were at relatively low levels a few days before the patients died.

**Plasma Cytokines And Infection.** In view of the apparent relationship between infection and IL6 levels in nonsurviving patients, we further evaluated the relationship between plasma cytokines and infection. Of 27 patients studied, 16 had at least

one infection during the study period. There were seven instances of pneumonia, seven of bronchitis, two of vaginitis, one of wound invasion, one of enterocolitis, and three of sepsis. Cytokine levels in all samples from infected patients were compared to all samples from patients who stayed infection free. Table 2 shows that patients who suffered from infections had increased mean IL1ß, IL6 and TNFα plasma levels as compared to infection free patients.

In order to examine the relationship between plasma cytokines and infection in more detail, infection windows of 10 days were assigned where day 5 was the day of infection diagnosis and start of treatment. Figure 8 shows IL6 plasma levels in control subjects and uninfected patients as compared to infected patients within and outside the assigned infection window. IL6 levels within the infection window (infected) were significantly higher than plasma IL6 levels of patients who stayed infection free (no infection) ($p < 0.05$). There was a tendency for plasma IL6 levels to increase further during the course of infection (infected) as compared to before or after infectious periods (uninfected).

Results of IL1ß comparisons are displayed in Figure 9. Samples from infected patients outside the infection window (uninfected) were significantly higher than those from uninfected patients (no infection) and controls (ctr) ($p < 0.01$). There was no statistical difference between plasma IL1ß levels inside or outside the infection window, although IL1ß concentrations were lower within the infection window.

TNFα levels were similar to IL1ß (Fig 10). Plasma samples from patients who did not have an infection (no infection) had significantly lower TNFα levels than samples from infected patients outside the assigned infection window (uninfected) ($p < 0.01$). Again, there was no statistical difference between samples outside and inside the infection window. Thus, TNFα and IL1ß were higher in patients who subsequently became infected as compared to patients who remained infection free, but were highest before diagnosis and start of treatment. IL6, on the other hand, was highest within the infection window.

## DISCUSSION

The results of this study indicate that mean plasma IL1ß and IL6 measured by ELISA were significantly increased in patients with thermal injury as compared to healthy controls. Mean TNFα did not appear significantly different from controls although 10 out of 27 burn patients examined had plasma TNFα levels up to 30 fold higher than healthy laboratory employees. On the other hand, 24 out of 27 burn patients had elevated plasma IL6 levels at some point in their postburn course above that of unburned controls. The TNFα distribution, often seen in patient screening studies, indicates that changes in TNFα plasma levels occurred in some cases following

thermal injury, but were not consistently elevated in all burn patients.

**TABLE 1. PLASMA CYTOKINE LEVELS IN BURN PATIENTS**

| LABEL | IL1β [pg/ml] | IL6 [pg/ml] | TNFα [pg/ml] |
|---|---|---|---|
| CTR | 0.346±0.205 n=17 | 0.298±0.15 n=17 | 0.303±0.303 n=17 |
| BURN | 1.798±0.144 n=254 | 6.461±1.26 n=419 | 1.012±0.192 n=407 |
| SIGNIFICANCE | p <0.01 | p <0.01 | n.s. |

Table 1: Plasma cytokine levels were measured by ELISA in healthy laboratory personnel (CTR) and burn patients over 6 weeks (BURN). n reflects the number of plasma samples included in the group.

**TABLE 2. CYTOKINE LEVELS IN INFECTED AND UNINFECTED PATIENTS**

| LABEL | CTR | NO INFECTION | INFECTION |
|---|---|---|---|
| IL6 in ng/ml | 0.030±0.015 n=17 | 0.220±0.027 n=169 | 0.034±0.209 n=250 a |
| IL1β in pg/ml | 0.346±0.205 n=17 | 1.339±0.201 n=101 | 2.184±0.2 n=147 b |
| TNFα in pg/ml | 0.303±0.303 n=17 | 0.139±0.081 n=156 | 1.540±0.3 n=253 c |

Table 2: Plasma cytokine levels were measured by ELISA in healthy laboratory personnel (CTR), uninfected patients (NO INFECTION) and patients who had at least one infection during the course of the study (INFECTION). N is the number of samples included in the group. a = $p < 0.05$ between no infection and infection; b = $p < 0.05$ between no infection and infection, $p < 0.01$ between infection and ctr; c = $p < 0.01$ between no infection and infection.

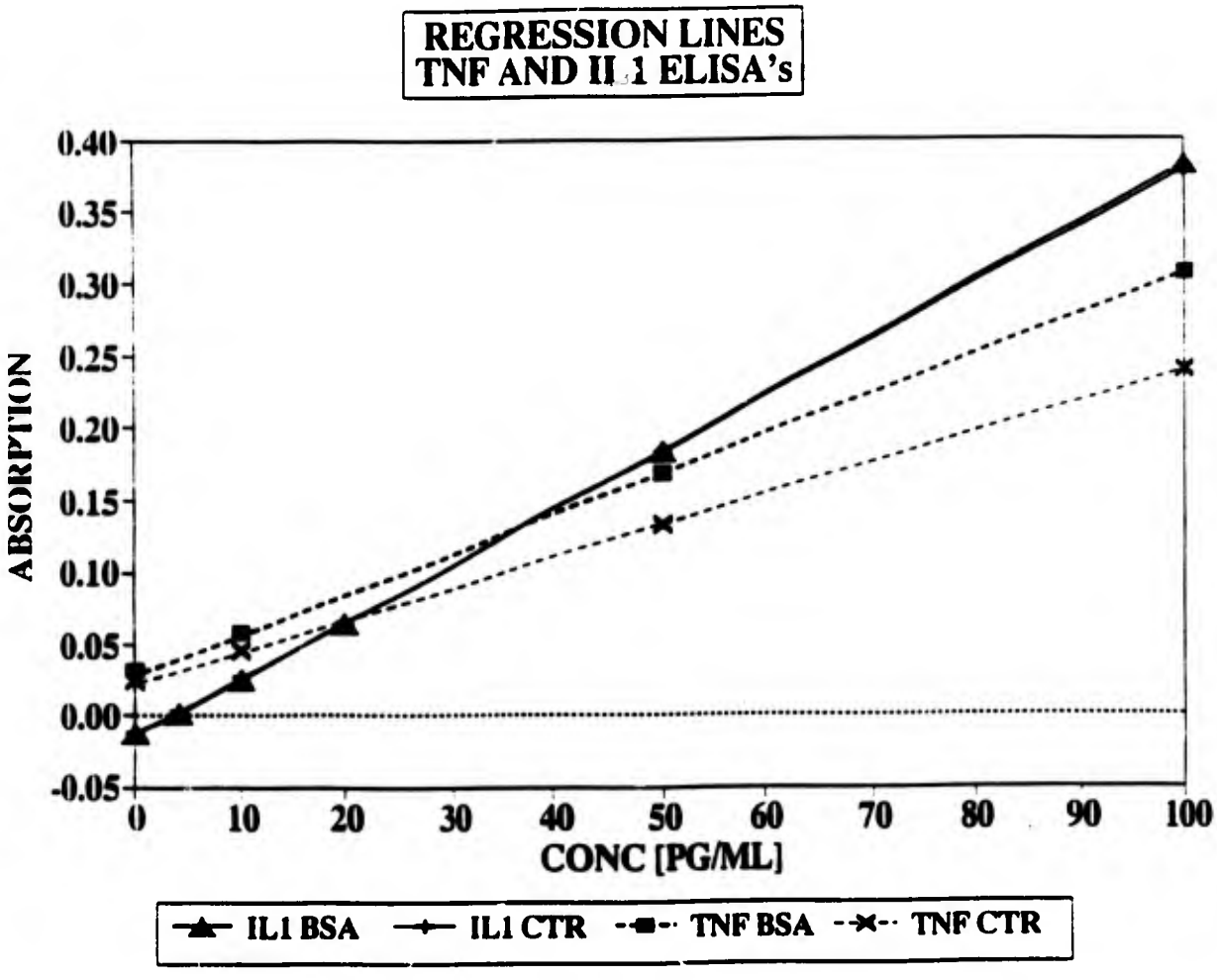


**FIGURE 1:** Regression lines of TNFα and IL1β ELISA's comparing standards suspended in BSA or in control plasma. The regression lines were calculated by reverse regression of the absorbance of different concentrations of cytokine in the appropriate buffer.

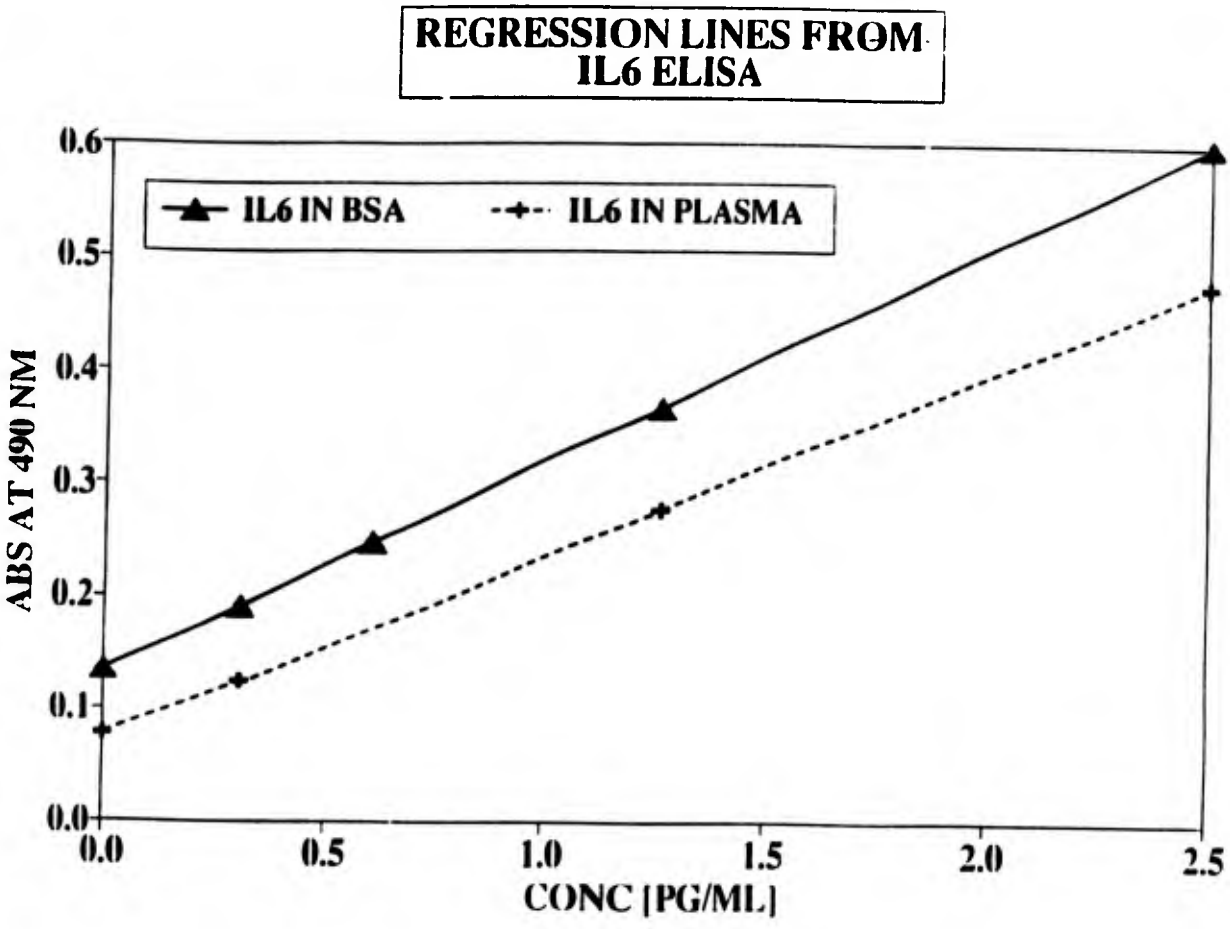


**FIGURE 2:** Regression line of IL6 ELISA comparing standards suspended in BSA or control plasma. The regression lines were calculated by reverse regression of the absorbance of different concentrations of cytokine in the appropriate buffer.

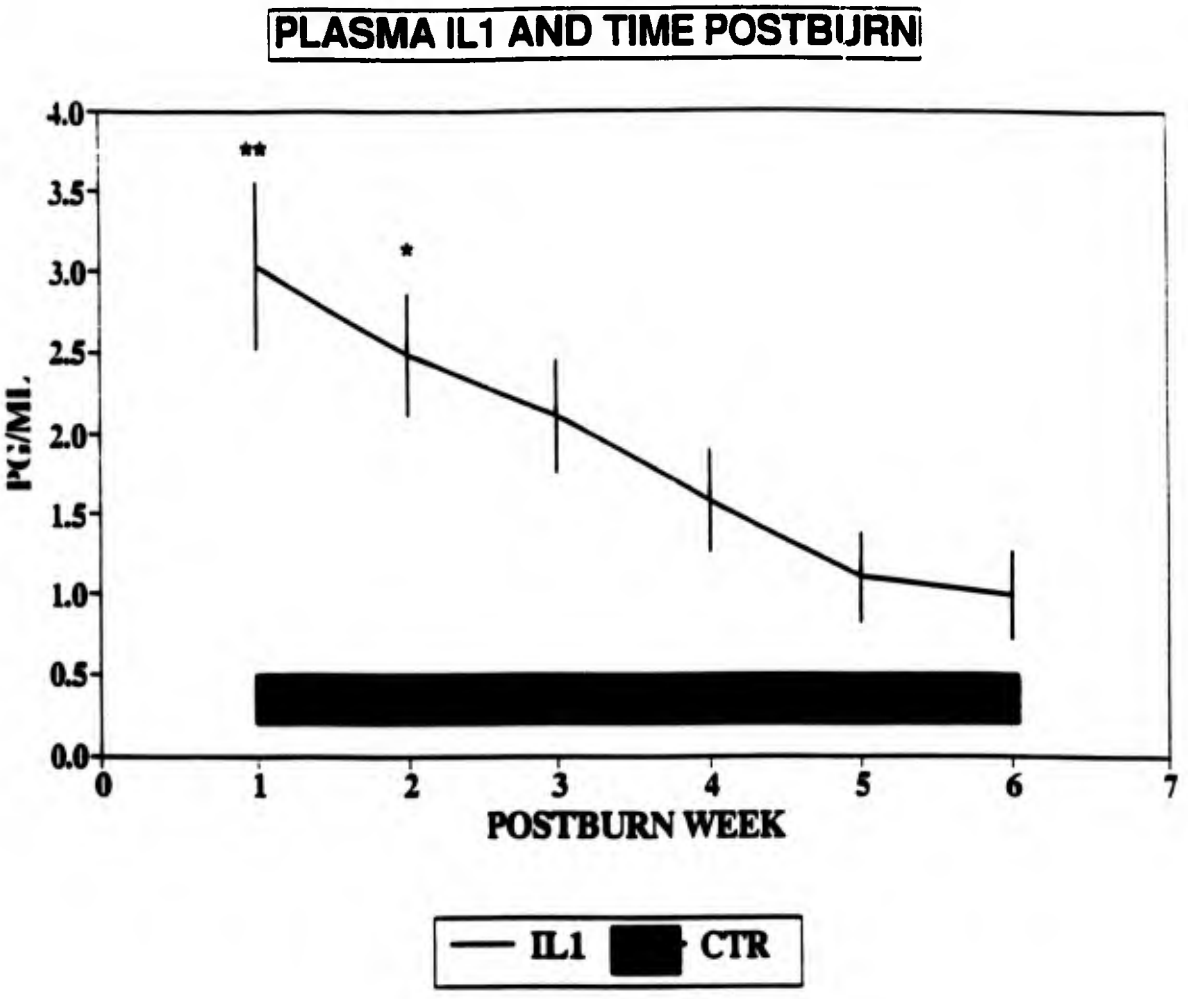


**FIGURE 3:** Plasma IL1β in surviving burn patients over time. IL1β values from different patients were grouped by postburn week and compared to controls. ** = $p < 0.01$; * = $p < 0.05$; +/- SEM.

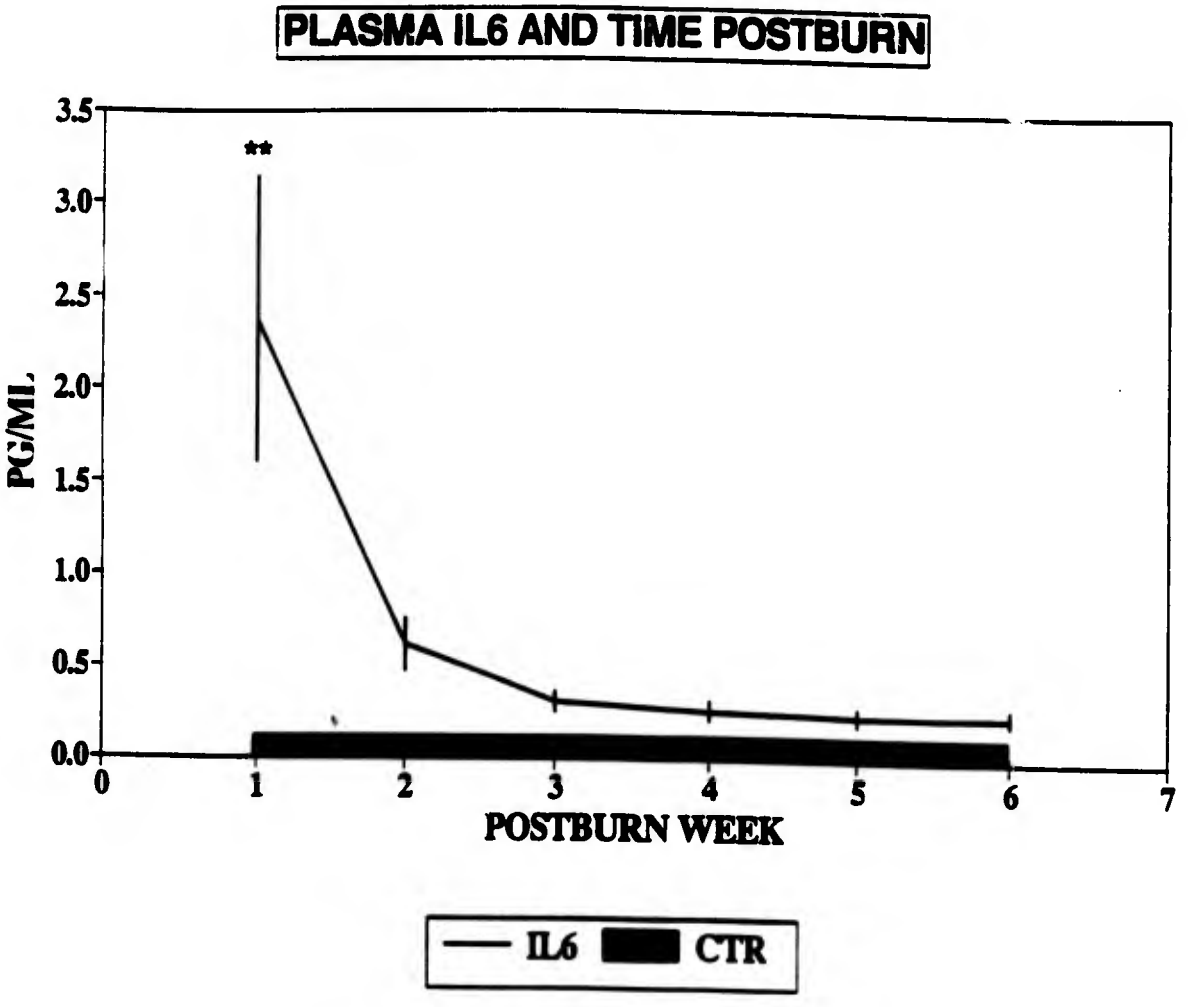


**FIGURE 4**: Plasma IL6 in burn patients over time. IL6 values from different patients were grouped by postburn week and compared to controls. ** = $p < 0.01$s; +/- SEM

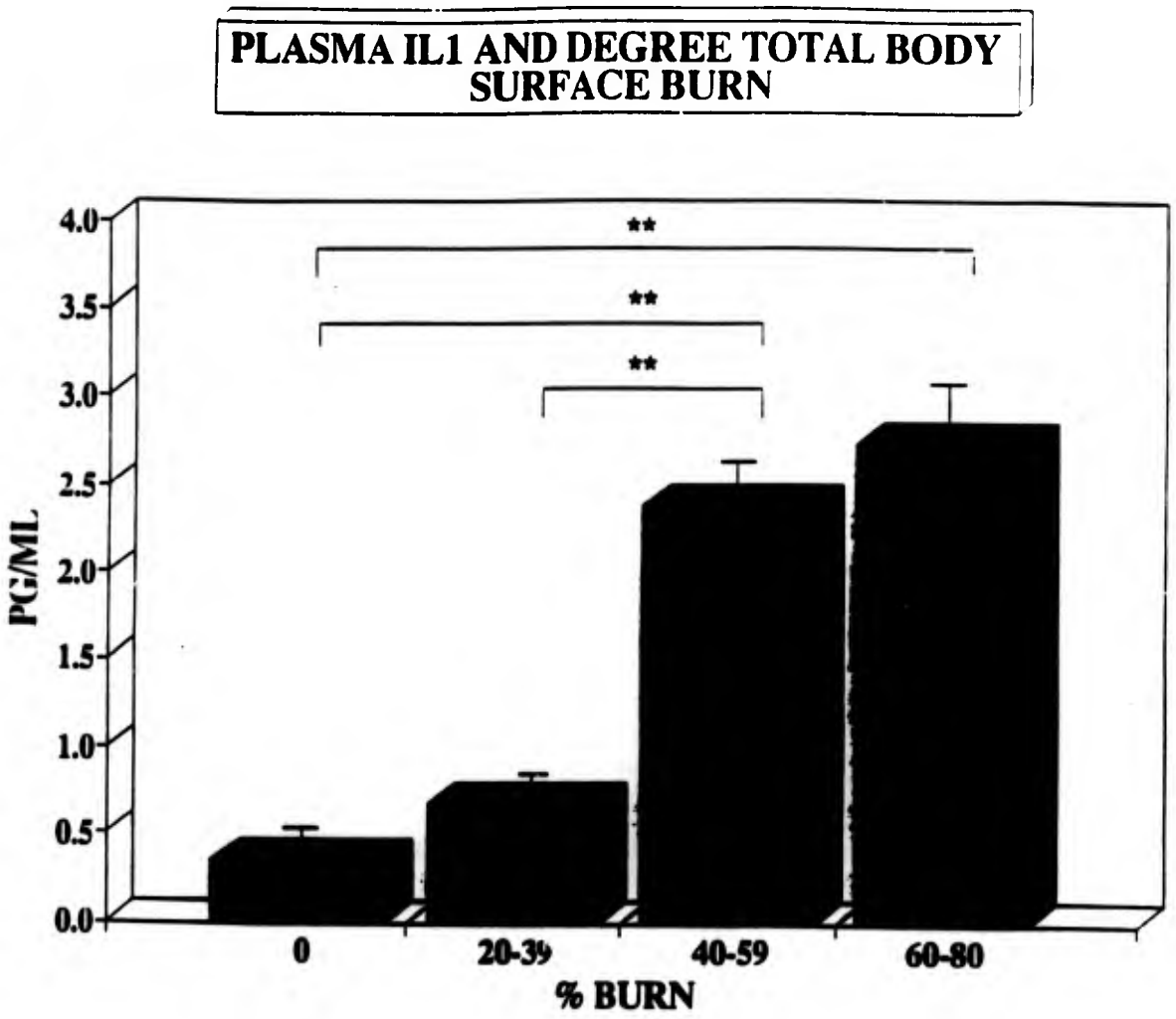


**FIGURE 5:** Plasma IL1β levels and the degree of total body surface burn in surviving burn patients. The bars represent averages of IL1β levels from different burn patients grouped by burn size + SEM. ** = $p < 0.01$.

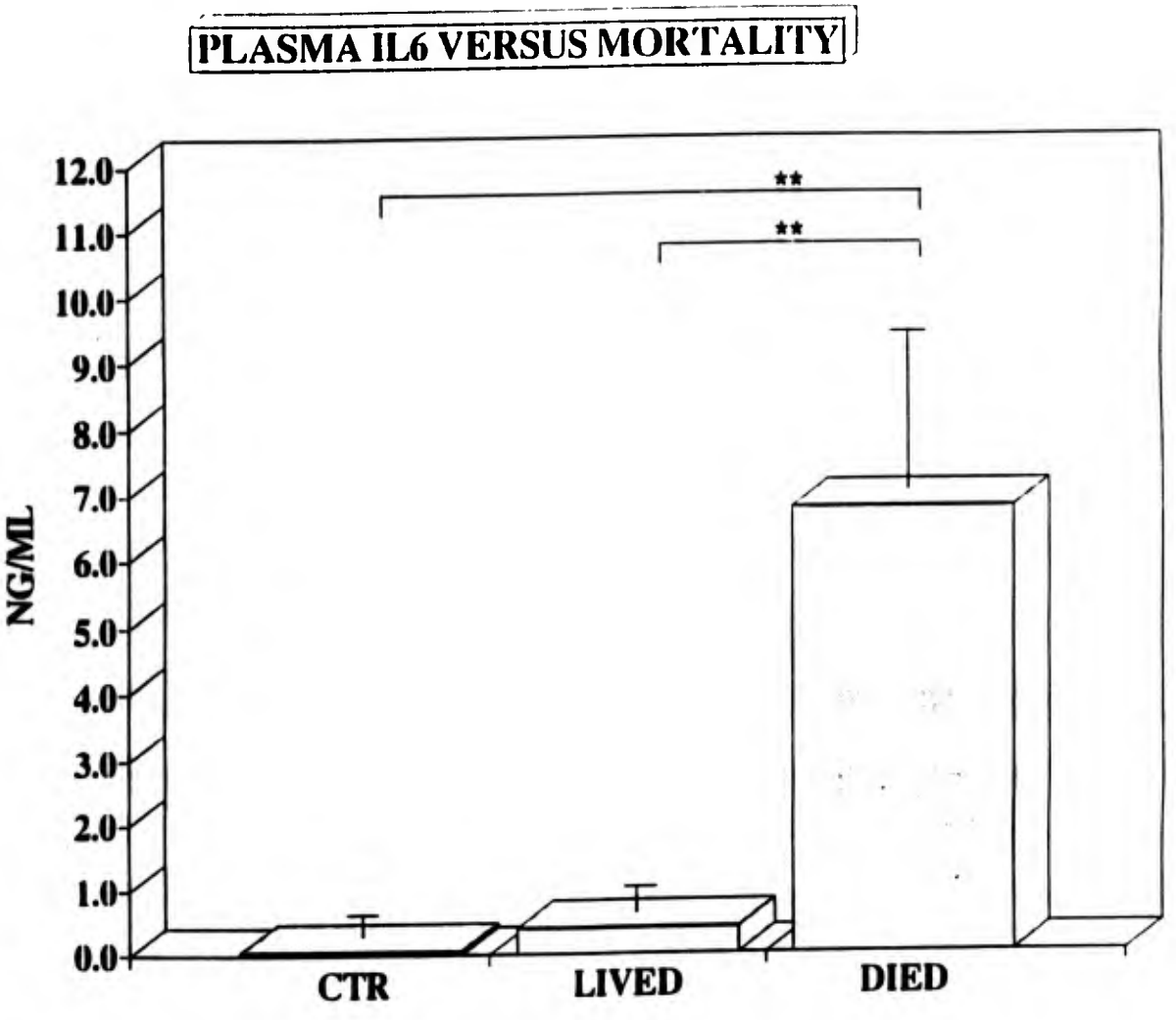


**FIGURE 6**: Plasma IL6 levels in dying and surviving burn patients as compared to controls. Average values + SEM are expressed. ** = $p < 0.01$.

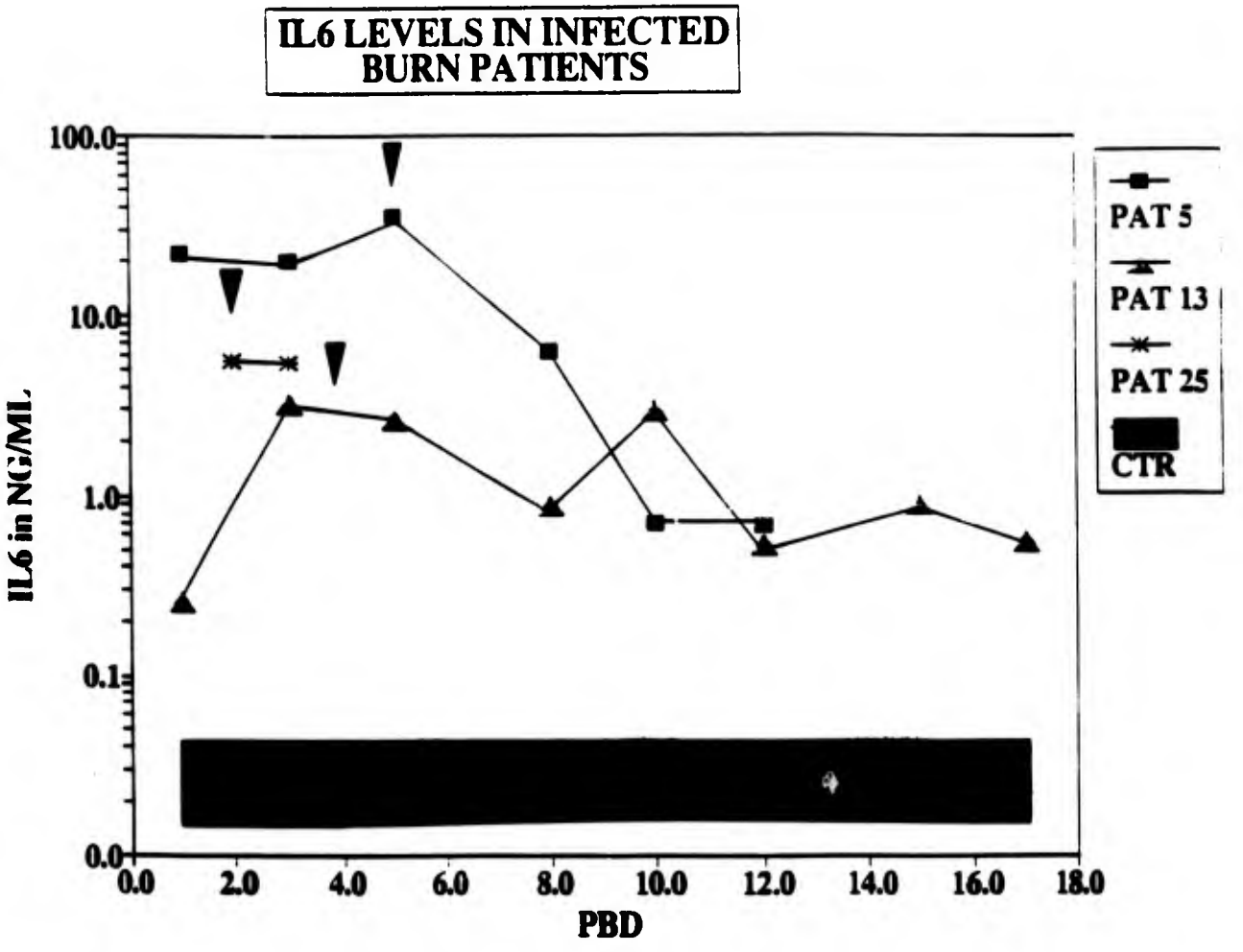


**FIGURE 7**: Cytokine profiles of 3 patients. The data are expressed as the log of the IL6 concentration. EXP refers to the expiration of the patient. Arrows indicate infection diagnosis. Controls are expressed as the average of 17 plasma samples from healthy laboratory personnel +/−SEM.

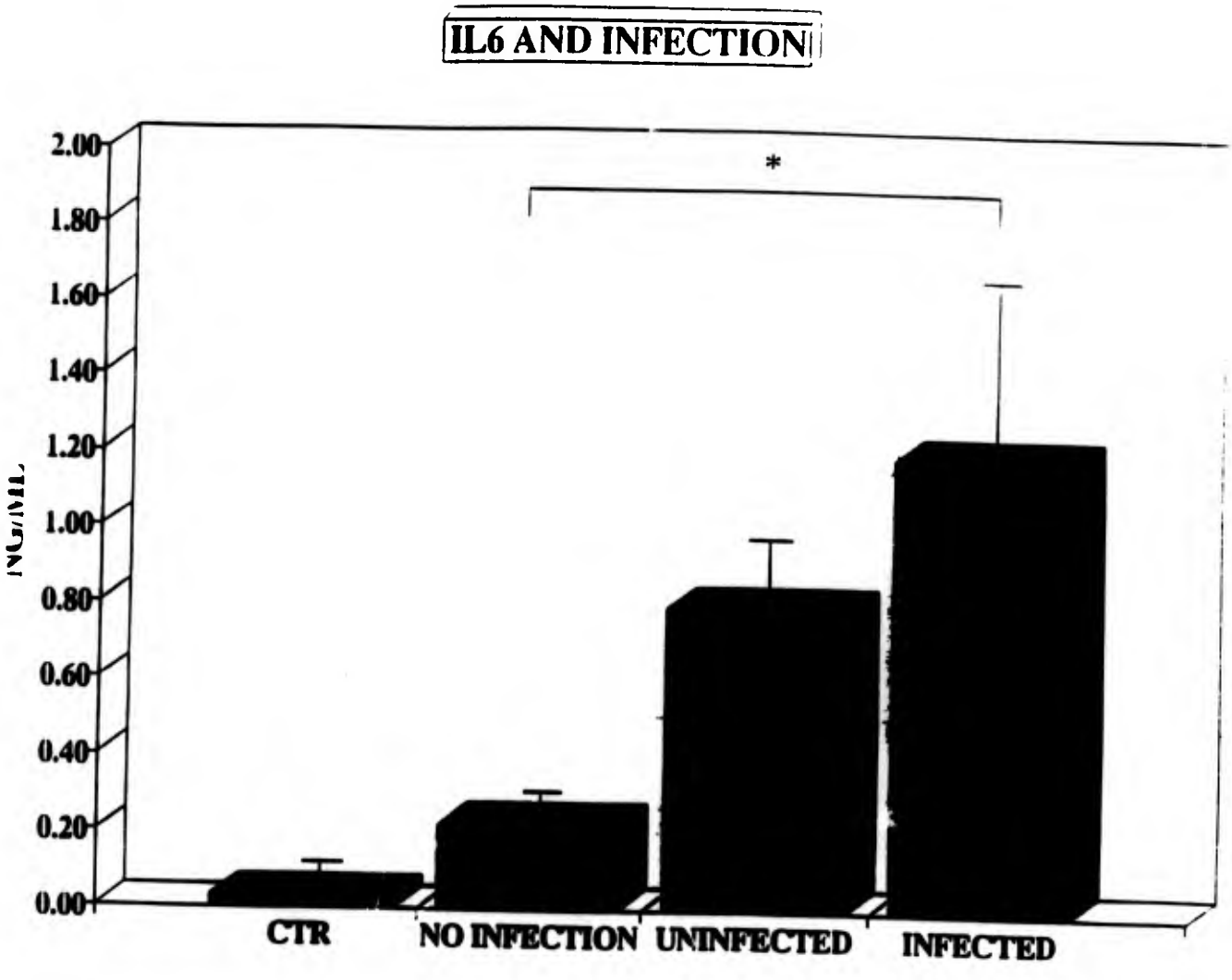


**FIGURE 8:** IL6 plasma levels in uninfected patients (NO INFECTION), infected patients outside the assigned infection window (UNINFECTED) and infected patients within the infection window (INFECTED) as compared to controls (CTR). Mean + SEM * = $p < 0.05$.

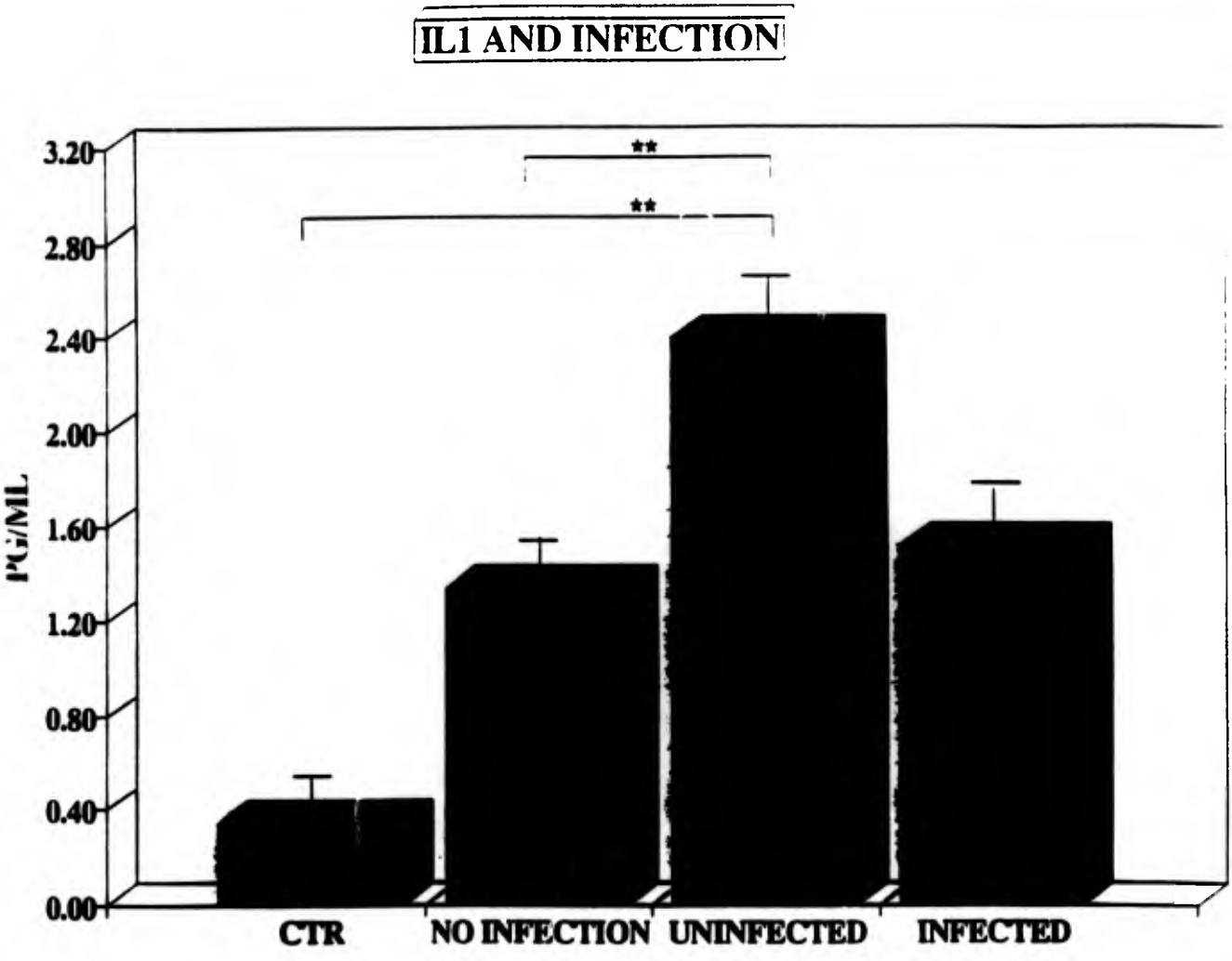


**FIGURE 9**: Plasma IL1ß levels in patients who stayed infection free (NO INFECTION), infected patients outside the assigned infection window (UNINFECTED) and infected patients within the infection window (INFECTED) as compared to controls (CTR). ** = $p < 0.01$. Mean + SEM.

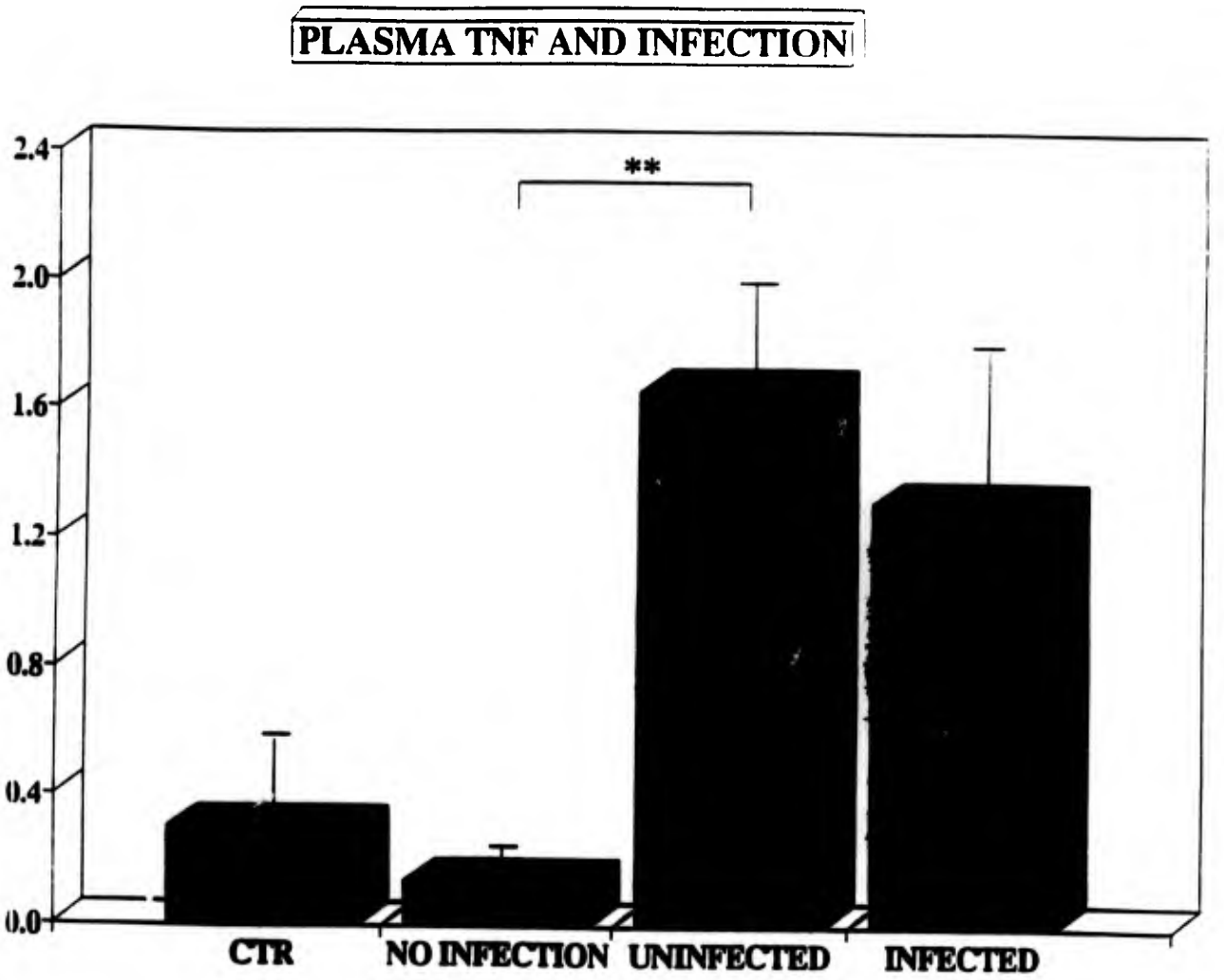


**FIGURE 10:** TNFα levels in patients who stayed infection free (NO INFECTION), infected patients ouside the assigned infection window (UNINFECTED) and infected patients within the infetion window (INFECTED) as compared to controls (CTR). Mean + SEM. ** = $p < 0.01$.

In this context it is important to note that the IL6 and TNFα measurements reflected relative levels of cytokine present in the plasma since plasma factors interfered with cytokine measurements. The amount of TNF determined using the standard curve made in BSA was about 50% higher than that determined using a standard curve made in control plasma. The level of IL6 determined from the BSA standard curve was approximately 100% greater than that in control plasma. All results were reported on the assumption that the interference was similar in control plasma and patient plasma.

There was a negative correlation between IL1ß levels and time postburn. The cytokine was significantly increased during the first two weeks postburn and decreased to almost normal levels by postburn week six. This corresponded to the time post injury that normal hypermetabolism, hormonal levels and positive nitrogen balance are seen in most patients. Another indication of a relationship between plasma IL1ß levels and the course of burn injury was the observation of increasing IL1ß levels with increasing burn size (Figure 5).

IL6 was increased during the first week postburn and returned to near normal levels thereafter. Most burn patients, regardless of burn size or age, had increased IL6 levels compared to healthy controls (IL6 was elevated in 24/27 patients). There was no correlation between IL6 and burn size, but IL6 was significantly increased in the three patients who died as compared to controls and patients who survived. In these patients (Figure 7), the plasma IL6 was higher early following the injury, decreasing before death. Several patients were diagnosed with infection shortly after high plasma IL6 concentrations were observed. This observation, together with the increase in IL6 within the infection window and the tendency to be lower outside the infection window, is strong evidence for a direct relationship between IL6 and infection.

IL1ß and TNFα were also increased in infected patients as compared to uninfected patients. In contrast to IL6, IL1ß and TNFα were elevated outside the infection window rather than inside the ten day infection window. Due to multiple infections in some patients, it was not always possible to separate the time before from the time after an infection. There are several reports describing the induction of IL6 by TNFα and IL1ß (25-27). The temporal pattern of increased IL1ß and TNFα prior to infection and the increase in IL6 during infection support these findings.

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| RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY | | | | 1. AGENCY ACCESSION DA313374 | 2. DATE OF SUMMARY 01 Oct 90 | REPORT CONTROL SYMBOL DD-DR&E(AR) 636 |
|---|---|---|---|---|---|---|
| 3. DATE PREV SUM'RY 01 Oct 89 | 4. KIND OF SUMMARY H. Term | 5. SUMMARY SCTY U | 6. WORK SECURITY U | 7. REGRADING | 8. DISB'N INSTR'N CX | 9. LEVEL OF SUM A WORK UNIT |

| 10. NO./CODES | PROGRAM ELEMENT | PROJECT NUMBER | TASK AREA NUMBER | WORK UNIT NUMBER |
|---|---|---|---|---|
| a. PRIMARY | 61102A | 3M161102BS14 | CK | 308 |
| b. CONTRIBUTING | | | | |
| c. CONTRIBUTING | | | | |

11. TITLE *(Precede with Security Classification Code)*
(U) Medium-Chain Triglyceride Utilization in the Thermally Injured Patient

12. SUBJECT AREAS
0605 Medicine and Medical Research

| 13. START DATE | 14. ESTIMATED COMPLETION DATE | 15. FUNDING ORGANIZATION | 16. PERFORMANCE METHOD |
|---|---|---|---|
| 8710 | 9010 | DA | C. In-House |

17. CONTRACT/GRANT MILITARY RELEVANCY CERTIFIED APPROVED BY Brad W. Pruitt
a. DATE EFFECTIVE
b. CONTRACT/GRANT NUMBER
c. TYPE
d. AMOUNT
e. KIND OF AWARD
f. CUM/TOTAL

18. RESOURCES ESTIMATE

| FISCAL YEARS | a. PROFESSIONAL WORKYEARS | b. FUNDS *(In thousands)* |
|---|---|---|
| 90 | 0.1 | 2 |
| 91 | 0.0 | 0 |

19. RESPONSIBLE DOD ORGANIZATION
a. NAME: US Army Institute of Surgical Research
b. ADDRESS *(include zip code)*: Fort Sam Houston, San Antonio, Texas 78234-5012
c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A
d. TELEPHONE NUMBER *(include area code)*: 512-221-2720

20. PERFORMING ORGANIZATION
a. NAME: US Army Institute of Surgical Research
b. ADDRESS: Fort Sam Houston, San Antonio, Texas 78234-5012
c. NAME OF PRINCIPAL INVESTIGATOR: CIOFFI, W G
d. TELEPHONE NUMBER *(include area code)*: 512-221-4440
f. NAME OF ASSOCIATE INVESTIGATOR *(if available)*: MILNER, E A
g. NAME OF ASSOCIATE INVESTIGATOR *(if available)*

21. GENERAL USE: FINA
MILITARY/CIVILIAN APPLICATION: M

22. KEYWORDS *(Precede EACH with Security Classification Code)* (U) Burns (Injuries); (U) Nutrition; (U) Metabolism; (U)Carbohydrates; (U) Therapy

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS *(Precede text of each with Security Classification Code)*

23/24. (U) The objective of this work is to examine the ability of thermally injured patients to use medium-chain triglycerides when administered in significant quantity as an effective energy source to maintain nitrogen balance. Resting energy expenditure will be measured on postburn day 10 and the patient started on enteral nutrition consisting of a carbohydrate load of 5 mg/kg/min with the residual caloric requirements administered as long-chain triglycerides. After a 3-day stabilization period, metabolic measurements will made. Finally, the patient will be changed to a nutrition regimen consisting of a carbohydrate load of 3.8 mg/kg/min and the remaining necessary calories will be administered as 90% medium-chain triglycerides and 10% long-chain triglycerides.

25. (U) 8710 - 8809. This project was approved by the USAISR Research Council and US Army Institute of Surgical Research Human Use Committee during the fourth quarter of fiscal year 1987.

(U) 8810 - 8909. Data from two study patients were analyzed. Intestinal intolerance prompted a search for an alternate medium-chain triglyceride preparation.

(U) 8910 - 9009. Because of intestinal intolerance to large amounts of medium-chain triglycerides, the search continued for an alternative medium-chain triglyceride preparation for parenteral use. However, it was found that no such formulation met with Food and Drug Administration approval. Therefore, the project was terminated.

**SUPPLEMENTAL WORK UNIT INFORMATION FOR THE PROJECT ENTITLED "MEDIUM-CHAIN TRIGLYCERIDE UTILIZATION IN THE THERMALLY INJURED PATIENT"**

**Subrecord/Linking Accession Number:** Not applicable.

**Search Control Data:** W6L42E/W6L43E, 20 October 1989

**Product Identification:** For technical reports, refer to the US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Years 1988-90.

**Unclassified Special Categories:** Volunteers: Adults; RA II

**ANNUAL RESEARCH PROGRESS REPORT**

**PROJECT NUMBER:** 3M161102BS14-00, RESEARCH

**PROJECT TITLE:** Medium-Chain Triglyceride Utilization in the Thermally Injured Patient

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

**1 October 1989 - 30 September 1990**

**INVESTIGATORS**

William G. Cioffi, Jr., MD, Major, MC
Theresa A. Graves, MD, Captain, MC
Dawn E. Carlson, RD, Major, SP
William F. McManus, MD, Colonel, MC
Basil A. Pruitt, Jr., MD, Colonel, MC

## ABSTRACT

**PROJECT NUMBER:** 3M161102BS14-00, RESEARCH

**PROJECT TITLE:** Medium-Chain Triglyceride Utilization in the Thermally Injured Patient

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

**PERIOD COVERED IN THIS REPORT:** 1 Oct 89 through 30 Sep 90

**INVESTIGATORS:** William G. Cioffi, Jr., MD, Major, MC
Theresa A. Graves, MD, Captain, MC
Dawn E. Carlson, RD, Major, SP
William F. McManus, MD, Colonel, MC
Basil A. Pruitt, Jr., MD, Colonel, MC

The optimal nutritional support program for the thermally injured patient has not been defined. The ability of burn patients to use fat, usually administered as long-chain triglycerides, as an effective energy source has been questioned. Few studies comparing the efficacy of medium-chain triglycerides to standard carbohydrate and long-chain triglyceride regimens have been performed. This study was designed to use medium-chain triglycerides, when administered in significant quantity, as an effective energy source to maintain nitrogen balance. Two patients out of a projected 10 have been enrolled and these patients have completed the study.

## MEDIUM-CHAIN TRIGLYCERIDE UTILIZATION IN THE THERMALLY INJURED PATIENT

Optimal nutritional support for thermally injured patients has yet to be defined. Various studies have questioned the ability of burn patients to use fat, usually administered as long-chain triglycerides (LCT), as an effective energy source (1-3). Few studies comparing the efficacy of medium-chain triglycerides (MCT) to standard carbohydrate and LCT regimens have been performed. The purpose of this study is to examine the ability of thermally injured patients to use MCT when administered in significant quantity as an effective energy source to maintain nitrogen balance.

Multiple studies have documented that carbohydrate is used preferentially over fat as an energy source when both are administered to injured animals and humans (4-7). However, there is a physiological limit to the amount of carbohydrate which can be effectively utilized by man. Burke et al. (8) has suggested that 5 mg/kg/min is the amount of carbohydrate which can be administered before excessive CO production occurs. Excess carbohydrate administered is first transformed to fat, a process which has a RQ of 8, meaning for every molecule of oxygen utilized, 8 molecules of CO are produced. The fat may then be used as an energy source after this conversion. In thermally injured patients, it is usually impossible to meet the increased kilocalorie requirements using carbohydrate alone if one adheres to this limit. Fat administered as LCT can be given to supplement the carbohydrate load and meet energy requirements even though the LCT may be utilized ineffectively.

Recent studies have suggested that MCT may be utilized more effectively than LCT by injured animals (2-3,9). In comparison to LCT, MCT are not stored in liver or fat deposits and are quickly oxidized when administered (10). Maiz et al. (3) has demonstrated in a rat burn model that MCT in combination with carbohydrate are just as effective as carbohydrate alone in maintaining nitrogen balance. However, Stein et al. (4) has demonstrated that when MCT are administered as the sole energy source, there is a negative nitrogen balance, decreased protein synthesis, and decreased peripheral fat stores as compared to carbohydrate alone.

On a cellular level, Goodwin et al. (1) has investigated the ability of hepatocyte mitochondria from burned rats to oxidize both MCT and LCT. Oxidation of LCT was decreased when compared to normals while beta oxidation of MCT was increased.

These studies suggest that MCT administered in combination with carbohydrate could serve as an effective energy source in thermally injured patients. The safety of administration of MCT to humans has been documented (10). No untoward effects of these compounds have been reported to date (11-12). Excessive ketone

production does not occur and ketoacidosis has not been a problem. The RQ has been noted to decrease after administration, suggesting utilization as an energy source.

The objective of this study is to determine the effects of MCT on the respiratory quotient and nitrogen balance as compared to LCT.

## MATERIALS AND METHODS

**Number of Patients.** Ten consecutive patients will be enrolled in the study. Two patients have been enrolled to date.

**Criteria for Admission into the Study.** Patients admitted to the US Army Institute of Surgical Research who require the use of enteral nutrition to meet all nutritional requirements are eligible for enrollment in this study.

**Patient Inclusion.** Ten patients meeting the following criteria will be considered for enrollment in the study:

1. Male or female patients > 18 yr of age. Female patients must be previously surgically sterilized or postmenopausal (> 45 yr of age and lack of menstrual periods > 1 yr) or have a negative pregnancy test.

2. Patients with burns > 30% of the total body surface area.

**Patient Exclusion.** Patients meeting the following criteria will be excluded from the study:

1. Patients < 18 yr of age.

2. Patients with burns < 30% of the total body surface area.

3. Any pregnant patient.

4. Patients who have clinical and/or laboratory indications of sepsis. Any patient who develops sepsis during participation in the study will also be excluded from the study at that time.

**Procedures During the Study Period.** On postburn day 10, each patient is transported to the Metabolic Room prior to the morning dressing changes. $VO_2$ and $VCO_2$ are measured utilizing the Horizon MMC Metabolic Cart™. The resting energy expenditure (REE) is calculated and baseline triglyceride, cholesterol, ketone, and insulin levels are obtained.

Each patient is started on enteral nutrition with energy requirements calculated as 1.2 X REE (13). Daily nitrogen requirements are calculated as 1 g/150 kcal. Carbohydrates are administered at a dose of 5 mg/kg/min. The remainder of the

kilocalories requirement is administered as LCT. Electrolyte composition is tailored according to each individual patient's needs. Each patient receives standard vitamins and trace minerals. Once the patient's intake has reached his/her projected requirement for 3 continuous days, the $VO_2$, $VCO_2$, REE, nitrogen balance calculated from a 24-h UUN, and RQ are measured. Triglyceride, cholesterol, insulin, and ketone levels are obtained from a blood sample.

The following calculation is then made: ME (energy metabolism in kcal/min) = (5.083 X $VO_2$) + (0.138 X $VCO_2$) - (0.125 X NM). NM equals nitrogen metabolized in grams per minute calculated from the 24-h UUN.

Grams per minute of carbohydrate, fat, and protein metabolized are then calculated. The formulae used as previously described by Weir depends upon the RQ of the patient at each time.

The enteral formula is then changed. Protein and carbohydrate concentrations remain the same. Fat is administered as 90% MCT and 10% LCT. After a 3-day stabilization period, the same measurements are repeated.

The enteral formula is again altered. Protein remains the same, carbohydrates are reduced to 3.8 mg/kg/min, and fats consisting of 90% MCT and 10% LCT are then administered in sufficient quantity to meet the REE. The total amount of fat does not exceed 3 g/kg/day. After a 3-day stabilization period, all measurements are again repeated.

Upon completion of the study, the patient's enteral formula is changed back to a conventional formulation with 5 mg/kg/min carbohydrate and the residual caloric needs as 50% LCT and 50% MCT.

**Statistical Analysis.** The RQ, nitrogen balance, and percentage of calories from carbohydrate, fat, and protein will be compared by ANOVA for each patient.

## RESULTS

Two patients have been enrolled in the study to date. These patients have both completed the study. Intestinal intolerance has prompted a search for an alternate MCT preparation.

## DISCUSSION

When the projected total of 10 patients have completed the study, the data will be analyzed as to the ability of medium-chain triglycerides to serve as an effective energy source in thermally injured patients.

## PRESENTATIONS/PUBLICATIONS

None.

## REFERENCES


1. Goodwin CW, Whitson JD, Mason AD Jr, et al: The study of metabolism and nutritional effects of burn injury in soldiers - metabolic effect of injury: Mitochondrial studies. In **US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Year 1983**. San Antonio: US Government Printing Office, 1984, pp 230-43.

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**RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY**

| 1. AGENCY ACCESSION | 2. DATE OF SUMMARY | REPORT CONTROL SYMBOL DD-DR&E(AR) 636 |
|---|---|---|
| DA313583 | 01 Oct 90 | |

| 3. DATE PREV SUM'RY | 4. KIND OF SUMMARY | 5. SUMMARY SCTY | 6. WORK SECURITY | 7. REGRADING | 8. DISB'N INSTR'N | 9. LEVEL OF SUM A. WORK UNIT |
|---|---|---|---|---|---|---|
| 01 Oct 89 | D. Chg | U | U | | CX | |

| 10. NO./CODES: | PROGRAM ELEMENT | PROJECT NUMBER | TASK AREA NUMBER | WORK UNIT NUMBER |
|---|---|---|---|---|
| a. PRIMARY | 61102A | 3M161102BS14 | CK | 309 |
| b. CONTRIBUTING | | | | |
| c. CONTRIBUTING | | | | |

11. TITLE *(Precede with Security Classification Code)*
(U) Caloric Requirements of Thermally Injured Children

12. SUBJECT AREAS
0605 Medicine and Medical Research

| 13. START DATE | 14. ESTIMATED COMPLETION DATE | 15. FUNDING ORGANIZATION | 16 PERFORMANCE METHOD |
|---|---|---|---|
| 8710 | 9109 | DA | C. In-House |

17. CONTRACT/GRANT MILITARY RELEVANCY CERTIFIED
a. DATE EFFECTIVE APPROVED BY
b. CONTRACT/GRANT NUMBER
c. TYPE d. AMOUNT
e. KIND OF AWARD f. CUM/TOTAL

18. RESOURCES ESTIMATE

| a. FISCAL YEARS | a. PROFESSIONAL WORKYEARS | b. FUNDS (In thousands) |
|---|---|---|
| 90 | 0.1 | 1 |
| 91 | 0.1 | 5 |

19. RESPONSIBLE DOD ORGANIZATION

a. NAME
US Army Institute of Surgical Research

b. ADDRESS *(include zip code)*
Fort Sam Houston
San Antonio, Texas 78234-5012

c. NAME OF RESPONSIBLE INDIVIDUAL
PRUITT, B A

d. TELEPHONE NUMBER *(include area code)*
512-221-2720

20. PERFORMING ORGANIZATION

a. NAME
US Army Institute of Surgical Research

b. ADDRESS
Fort Sam Houston
San Antonio, Texas 78234-5012

c. NAME OF PRINCIPAL INVESTIGATOR
CIOFFI, W G

d. TELEPHONE NUMBER *(include area code)*
512-221-4440

21. GENERAL USE
FINA

MILITARY/CIVILIAN APPLICATION: M

e. NAME OF ASSOCIATE INVESTIGATOR *(if available)*
MILNER, E A

g. NAME OF ASSOCIATE INVESTIGATOR *(if available)*
MC MANUS, W F

22. KEYWORDS *(Precede EACH with Security Classification Code)* (U) Burns (Injuries); (U) Nutrition; (U) Metabolism; (U) Therapy

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS *(Precede text of each with Security Classification Code)*

23/24. (U) The objective of this work is to determine optimal nutritional support for the burned child. On the fifth postburn day, the REE will be calculated as will the RQ. Baseline laboratory data will be collected and liver function and partial thromboplastin time tests will be performed. The patient will then be begun on alimentation that will be adjusted every 3 days until the caloric need is determined and met for two successive, 3-day cycle measurements as determined by a positive nitrogen balance, a RQ between 0.85 and 1.0, and a caloric intake equal to 1.25 X REE.

25. (U) 8710 - 8809. This project was approved by the USAISR Research Council, the US Army Institute of Surgical Research Human Use Committee, and The Surgeon General's Human Subjects Research Review Board during the fourth quarter of fiscal year 1987. No suitable pediatric patients were admitted to the Institute during this reporting period.

(U) 8810 - 8909. No suitable pediatric patients were admitted to the Institute during this reporting period.

(U) 8910 - 9009. No suitable pediatric patients were admitted to the Institute during this reporting period.

DD FORM 83 MAR 1498 EDITION OF MAR 88 IS OBSOLETE. * USGPO 1988 -491-003/50329

**SUPPLEMENTAL WORK UNIT INFORMATION FOR THE PROJECT ENTITLED "CALORIC REQUIREMENTS OF THERMALLY INJURED CHILDREN"**

**Subrecord/Linking Accession Number:** Not applicable.

**Search Control Data:** W6L57B/W6M03C, 20 October 1989

**Product Identification:** Not applicable.

**Unclassified Special Categories:** Volunteers: Children; RA II

**ANNUAL RESEARCH PROGRESS REPORT**

**PROJECT NUMBER:** 3M161102BS14-00, RESEARCH

**PROJECT TITLE:** Caloric Requirements of Thermally Injured Children

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

**1 October 1989 - 30 September 1990**

**INVESTIGATORS**

Teresa M. Buescher, MD, Captain, MC
William G. Cioffi, Jr., MD, Major, MC
Dawn E. Carlson, RD, Major, SP
William F. McManus, MD, Colonel, MC
Basil A. Pruitt, Jr., MD, Colonel, MC

## ABSTRACT

**PROJECT NUMBER:** 3M161102BS14-00, RESEARCH

**PROJECT TITLE:** Caloric Requirements of Thermally Injured Children

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

**PERIOD COVERED IN THIS REPORT:** 1 Oct 89 through 30 Sep 90

**INVESTIGATORS:** Teresa M. Buescher, MD, Captain, MC
William G. Cioffi, Jr., MD, Major, MC
Dawn E. Carlson, RD, Major, MS
William F. McManus, MD, Colonel, MC
Basil A. Pruitt, Jr., MD, Colonel, MC

This project was approved by the US Army Institute of Surgical Research Human Use Committee on 9 October 1987. No suitable pediatric patients were admitted to the Institute during this reporting period. Patients will be asked to enroll in the study as they become available.

## CALORIC REQUIREMENTS OF THERMALLY INJURED CHILDREN

The optimum nutritional support program for the thermally injured child has not been determined. The caloric requirements of a burned child are only marginally estimated by the existing formulas. The Curreri formulas, the Harris-Benedict Equations, and the Wilmore nomograms all differ in their estimation of the caloric requirements for children, e.g., a 2-yr-old girl weighing 12 kg (50th percentile) and measuring 86 cm in length (50th percentile) who has sustained a 40% total body surface area burn will have an estimated daily caloric requirement of 2,120 kcal by the original Curreri formula for children or 2,200 kcal by the Curreri "Junior" formula for 1- to 3-yr-olds, 1,839 kcal by the Harris-Benedict equation, and 1,600 kcal by the Wilmore nomograms. Determination of adequate nutritional support is important since inadequate caloric intake may result in protein wasting and malnutrition, whereas excess caloric intake can result in fatty infiltration of the liver, the fat intoxication syndrome, dehydration secondary to hyperglycemia and glucosuria, and excess carbon dioxide production with subsequent ventilator weaning failure. All of these potential problems could be avoided by the administration of the correct number of calories distributed between protein, fat, and carbohydrate.

Nitrogen requirements in thermally injured patients are increased over those in uninjured people. Numerous studies have demonstrated that injured, hypermetabolic patients demonstrate ineffective utilization of administered protein and have an optimum nitrogen to calorie ratio between 1:135 and 1:200 (grams nitrogen to nonprotein kilocalories). An optimum ratio of 1:150 has been recommended by Goodwin (3). Larger amounts of protein create a progressively more positive nitrogen balance but have not been shown to improve survival (7).

The role of fat as a source of nonprotein calories is dependent upon the extent of injury and the other nutrients administered. When diets lacking in protein are used, carbohydrate is more effective in sparing body protein than fat. However, when a "balanced" alimentation regimen containing protein, fat, and carbohydrate is devised, consideration is given to the administration of sufficient calories as fat not only to prevent essential fatty acid deficiency, but also to supply a large number of calories. Fat administration in excess of 3 g/kg/day in normal infants and 4 g/kg/day in normal adults can produce a fat overload syndrome (4). This has been described as consisting of hyperlipidemia, coagulopathy, fever, cholestatic jaundice, and gastrointestinal distress. This syndrome is believed to occur when the rate of infusion exceeds the maximum rate of peripheral clearance.

Studies comparing the utilization of fat and carbohydrate as energy sources have been undertaken in unburned surgical patients. A controlled study by MacFie et al. (6) demonstrated that the administration of as little as 17% of calories as fat can reduce the loss of lean body tissue and the accumulation of body fat which is seen when glucose is used as the sole nonprotein energy source. A positive nitrogen balance has been achieved in postoperative patients with regimens supplying 33-38% of nonprotein calories from intravenous fat (9). The fat infusions depressed the respiratory quotient and insulin levels and they elevated the serum fatty acid and ketone levels, whereas the glucose infusions elevated the respiratory quotient and the pyruvate, lactate, alanine, and insulin levels. A RQ > 1.0 indicates that lipogenesis is occurring and that some of the administered calories are being utilized to synthesize fat (10).

The amount of glucose which can be effectively utilized by a stressed, injured patient is also unknown. Based on adult burn patients, Burke et al. (1) have proposed that a value of 5 mg/kg/min is the maximum rate beyond which physiologically significant increase in protein synthesis and direct oxidation of glucose cannot be expected. At levels above this, there is increased carbon dioxide production and increased fatty infiltration of the liver. Looking at adult surgical patients, Hill and Church (5) have suggested a maximum rate of 7 mg/kg/min. However, neither of these studies addresses the situation of a burned child and the glucose administration ceiling remains unknown in this subpopulation of patients.

In a thermally injured child, these various formulas and recommendations create an impossible situation. Even when the lowest caloric estimate is used, the constraints of a 1:150 gram nitrogen to nonprotein kilocalories ratio, a maximum of 3 g/kg/day fat and a maximum of 5 mg/kg/min glucose are impossible to match. At least one of these recommendations must be ignored. The optimum nitrogen to kilocalorie ratio is well supported in the literature. The fat administration ceiling is well supported in unburned children but no data exist in burned children. The carbohydrate ceiling has also not been determined in burned children. For these reasons, the alimentation regimen which will be used as a starting point in this study will be based on the Wilmore nomograms for determination of the total caloric requirement. A 1:150 nitrogen to kilocalorie ratio will be maintained. The amount of fat will be initially limited to 3 g/kg/day and glucose will supply the remaining calories. It is expected that this glucose infusion rate may be > 5 mg/kg/min. If the patient is unable to tolerate the glucose infusion rate needed to deliver the calculated number of calories based on the initial estimate, the quantity of fat will be increased and the amount of carbohydrate decreased. This will continue until the total number of calories delivered equals that suggested in the initial estimate. The quantity of lipid administered will be kept below that which causes a serum

triglyceride level > 150 mg/dl. If it should prove to be impossible to reach the estimated caloric intake due to severe hyperglycemia and coexisting hyperlipidemia preventing further increase in both glucose and fat infusions, the oxygen consumption and carbon dioxide production will be determined at the maximum infusion rates which the patient will tolerate. These values shall be used as a starting point to calculate a more accurate measure of the caloric need. Further adjustments in the calories administered will follow these measurements and the respiratory quotient and resting energy expenditure determinations derived from these two values. The patient's caloric needs will be determined by measurements in the Metabolic Room using the Horizon™ metabolic cart and the nutritional support will be adjusted to administer kilocalories equal to 1.25 X REE (8), maintain the respiratory quotient between 0.85 and 1.00, and maintain a positive nitrogen balance. The amount of calories needed to comply with these restraints will be considered the patient's caloric requirement.

## MATERIALS AND METHODS

**Number of Patients.** Twenty patients will be enrolled in the study. Properly signed and witnessed voluntary agreement affidavits will be obtained for each patient prior to enrollment in the study.

**Inclusion Criteria.** Patients meeting the following criteria will be eligible for enrollment in the study:

1. Patients admitted to the US Army Institute of Surgical Research with burn injury.

2. Male or female patients < 13 yr of age.

3. Patients with burn wounds > 30% of the total body surface area.

**Exclusion Criteria.** Patients meeting the following criteria will be excluded from enrollment in the study:

1. Patients 13 yr of age and older.

2. Patients with burn wounds < 30% of the total body surface area.

3. Patients with electrical injury.

4. Patients with fractures or major associated injuries.

5. Patients with inhalation injury.

6. Patients who are wards of the state or any other agency, institution, or entity.

**Assent.** For children from 6–12 yr of age, judgment by the primary investigator and the attending surgeon will be made as to whether the child is capable of assent. In determining whether the child is capable of assenting, the primary investigator and the attending surgeon will take into account the age, maturity, and psychological state of the child involved. This judgment will be made for each child. If it is deemed that the child is capable of assent, then the research protocol will be explained to that child in terms that he/she will understand. The child will then be enrolled in the study if his/her assent is given and permission is obtained from the child's parent or legal guardian. If it is deemed that the child is not capable of assent or if the child is 5 years of age or younger, then permission will be obtained from the child's parent or legal guardian only.

**Study Procedures.** On the fifth postburn day, each patient will be transported to the Metabolic Room on Ward 14A prior to the morning dressing change. Oxygen consumption and carbon dioxide production will be measured using the Horizon™ metabolic cart. The environment temperature and humidity will be maintained constant throughout each patient's stay in the Metabolic Room. The REE will be calculated as will the RQ. Baseline laboratory data will include serum electrolytes, creatinine, cholesterol, triglycerides, platelet count, prothrombin time, ketone, and insulin values. Liver function and partial thromboplastin time tests will also be performed. These serum laboratory values will be repeated at the time of each subsequent trip to the Metabolic Room for further measurements. All measurements in the Metabolic Room will take place prior to the morning dressing change. The patient's height and baseline weight will be determined upon admission. Weights will be obtained on a daily basis.

The patient will then be begun on alimentation using either parenteral hyperalimentation or enteral feeding. If possible, enteral feedings will be used to supply the patient's nutrition. If the patient's gastrointestinal tract is not capable of tolerating enteral feedings for any reason, intravenous hyperalimentation will be employed. The total calorie requirement will be based upon the lowest estimated caloric need as calculated from the Wilmore nomograms, the Curreri formulas, and the Harris-Benedict equations. Nitrogen administration will be calculated to produce a 1 g of nitrogen to 150 nonprotein kcal ratio. Lipids will be administered at a rate of 3 g/kg/day. Electrolyte composition of the fluids will be adjusted to the patient's needs. Each patient will receive standard vitamin and mineral supplements.

Once the patient's intake has reached the projected requirements and has remained stable for 3 days, the patient will be transported to the Metabolic Room where oxygen consumption and carbon dioxide production will again be measured. A 24-h urine collection will be obtained on that day as well. From this data,

the RQ and REE will calculated. The grams of totally metabolized nitrogen, carbohydrate, and fat as well as the nitrogen balance will also be calculated.

Based on the new RQ, REE, and nitrogen balance measurements, the caloric requirements will be recalculated. If the RQ value is < 0.85, the total number of calories will be increased by 10%, maintaining the 1:150 gram of nitrogen to kilocalories ratio and the 3 g/kg/day lipid infusion rate. If the RQ is > 1.0, nitrogen, carbohydrate, and fat will be examined in an effort to determine which component or components (protein, carbohydrate, fat) should be reduced in order to decrease the total number of calories by 10% (2).

After a 3-day stabilization period, these metabolic measurements will be rechecked and again the caloric intake adjusted to bring the RQ to between 0.85 and 1.0 and to keep the nitrogen balance positive. This 3-day cycle will be repeated until the caloric need is determined and met for two successive, 3-day cycle measurements. This will be determined by a positive nitrogen balance, a RQ between 0.85 and 1.0, and a caloric intake equal to 1.25 X REE. Caloric needs shall be redetermined following any operative procedure after a 3-day stabilization period. During these days, alimentation will be maintained at the preoperative level.

## RESULTS

This project was approved by the US Army Institute of Surgical Research Human Use Committee on 9 October 1987. No suitable pediatric patients were admitted to the Institute during this reporting period. Patients will be asked to enroll in the study as they become available.

## DISCUSSION

When 20 patients have completed the study, the data will be analyzed to determine the optimum nutritional support program for the thermally injured child.

## PRESENTATIONS/PUBLICATIONS

None.

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**RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY**

| 1. AGENCY ACCESSION | 2. DATE OF SUMMARY | REPORT CONTROL SYMBOL |
|---|---|---|
| DA314706 | 01 Oct 90 | DD-DR&E(AR) 636 |

| 3. DATE PREV SUM'RY | 4. KIND OF SUMMARY | 5. SUMMARY SCTY | 6. WORK SECURITY | 7. REGRADING | 8. DISB'N INSTR'N | 9. LEVEL OF SUM A. WORK UNIT |
|---|---|---|---|---|---|---|
| 01 Oct 89 | D. Chg | U | U | | CX | |

| 10. NO./CODES | PROGRAM ELEMENT | PROJECT NUMBER | TASK AREA NUMBER | WORK UNIT NUMBER |
|---|---|---|---|---|
| a. PRIMARY | 61102A | 3M161102BS14 | AZ | 310 |
| b. CONTRIBUTING | | | | |
| c. CONTRIBUTING | | | | |

11. TITLE *(Precede with Security Classification Code)*
(U) Salt and Water Balance in the Thermally Injured Patient

12. SUBJECT AREAS
0605 Medicine and Medical Research

| 13. START DATE | 14. ESTIMATED COMPLETION DATE | 15. FUNDING ORGANIZATION | 16. PERFORMANCE METHOD |
|---|---|---|---|
| 8805 | 9109 | DA | C. In-House |

17. CONTRACT/GRANT MILITARY RELEVANCY CERTIFIED
a. DATE EFFECTIVE APPROVED BY
b. CONTRACT/GRANT NUMBER
c. TYPE
d. AMOUNT
e. KIND OF AWARD
f. CUM/TOTAL

18. RESOURCES ESTIMATE

| FISCAL YEARS | a. PROFESSIONAL WORKYEARS | b. FUNDS *(In thousands)* |
|---|---|---|
| 90 | 0.1 | 53 |
| 91 | 0.1 | 56 |

19. RESPONSIBLE DOD ORGANIZATION
a. NAME
US Army Institute of Surgical Research
b. ADDRESS *(include zip code)*
Fort Sam Houston
San Antonio, Texas 78234-5012
c. NAME OF RESPONSIBLE INDIVIDUAL
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d. TELEPHONE NUMBER *(include area code)*
512-221-2720

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VAUGHAN, G M
g. NAME OF ASSOCIATE INVESTIGATOR *(if available)*
HEIRONIMUS, J D

21. GENERAL USE
FINA
MILITARY/CIVILIAN APPLICATION
M

22. KEYWORDS *(Precede EACH with Security Classification Code)* (U) Burns (Injuries); (U) Hormones; (U) Peptides; (U) Renin; (U) Aldosterone; (U) Angiotensin; (U) Inulin;

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS *(Precede text of each with Security Classification Code)*

22. (Continued) (U) Blood Volume; (U) Resuscitation

23/24. (U) The objective of this work is to describe the alterations of plasma levels of ADH, atrial natriuretic peptide, and the renin-angiotensin-aldosterone axis following thermal injury as related to plasma volume, osmolality, and tonicity. Twenty consecutive patients will be entered into this study. On postburn days 2, 5, and 10, intravascular volume measurements will be made utilizing chromium-labeled RBCs to measure red cell volume. Also on postburn day 5, the glomerular filtration rate will be measured utilizing inulin and a radiopharmaceutical. Effective renal plasma flow will be measured using a colorimetric hippurate method. The two methods will then be compared.

25. (U) 8710 - 8809. This project was approved by the USAISR Research Council, the Brooke Army Medical Center Radiation Control Committee, the US Army Institute of Surgical Research Human Use Committee, and The Surgeon General's Human Subjects Research Review Board during the third quarter of fiscal year 1988.

(U) 8810 - 8909. An addendum to the study was approved by the USAISR Research Council, the Brooke Army Medical Center Radiation Control Committee, the US Army Institute of Surgical Research Human Use Committee, and The Surgeon General's Human Subjects Research Review Board.

DD FORM 1498 83 MAR EDITION OF MAR 68 IS OBSOLETE * USGPO 1988-491-003/50329

**CONTINUATION OF DD FORM 1498 FOR THE PROJECT ENTITLED "SALT AND WATER BALANCE IN THE THERMALLY INJURED PATIENT"**

(U) 8910 - 9009. Ten patients and 10 control subjects were enrolled in the study during this reporting period. Upon enrollment of 20 patients and 20 control subjects, the data will be analyzed to better define the neurohormonal and fluid volume alterations which occur following thermal injury.

**SUPPLEMENTAL WORK UNIT INFORMATION FOR THE PROJECT ENTITLED "SALT AND WATER BALANCE IN THE THERMALLY INJURED PATIENT"**

**Subrecord/Linking Accession Number:** Not applicable.

**Search Control Data:** W6M09B/W6M10A, 20 October 1989

**Product Identification:** For technical reports, refer to the US Army Institute of Surgical Research Annual Research Progress Report for fiscal years 1988-90.

**Unclassified Special Categories:** Volunteers: Adults; RA II

**ANNUAL RESEARCH PROGRESS REPORT**

**PROJECT NUMBER:** 3M161102BS14-00, RESEARCH

**PROJECT TITLE:** Salt and Water Balance in the Thermally Injured Patient

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

**1 October 1989 - 30 September 1990**

**INVESTIGATORS**

William G. Cioffi, Jr., MD, Major, MC
George M. Vaughan, MD, Colonel, MC
J. D. Heironimus

## ABSTRACT

**PROJECT NUMBER:** 3M161102BS14-00, RESEARCH

**PROJECT TITLE:** Salt and Water Balance in the Thermally Injured Patient

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

**PERIOD COVERED IN THIS REPORT:** 1 Oct 89 through 30 Sep 90

**INVESTIGATORS:** William G. Cioffi, Jr., MD, Major, MC
George M. Vaughan, MD, Colonel, MC
J.D. Heironimus

Control of solute and water retention following resuscitation for thermal injury has been thought to depend upon a resetting of hormonal control mechanisms. We measured serum sodium, plasma renin activity (PRA), arginine vasopressin (AVP), cardiac index (CI), glomerular filtration rate (GFR, $^{99m}$Tc-DTPA), effective renal plasma flow (ERPF, $^{131}$I-hippurate), and total blood volume (BV) in four burned patients (mean age, total burn size, and postburn day - 33.6 years, 60.6%, and 10 days) and most of these variables in five control subjects (mean age 25 years). Hematocrit was 28 ± 0.5% in the patients. Cardiac index, GFR and ERPF were significantly elevated in the patients. Despite findings related to increased flow, BV (based on $^{51}$Cr-labelled red cells) was not elevated. PRA and AVP (elevated) were altered in the direction expected from reduced effective volume. Because of vasodilation even in areas outside the wound, indices of organ perfusion may not reflect BV or effective volume at hormonal control sites. The dissociation of organ flow and hormonal response indicates that simultaneous direct volume measurements are necessary before valid claims can be made for altered neural set-points to explain hormonal changes in the flow phase of injury.

# SALT AND WATER BALANCE IN THE THERMALLY INJURED PATIENT

## INTRODUCTION

Factors responsible for sodium and volume regulation following injury are not clearly understood. Several authors have interpreted their data to imply that resetting of hormonal control mechanisms occurs following thermal injury, and that this is a stress response and not sodium and volume-dependent (1-2). Although various studies have examined one or two factors responsible for sodium and volume regulation following thermal injury, no one has studied the system as a whole. In normal man, antidiuretic hormone (ADH), atrial natriuretic peptide (ANP), and the renin-angiotensin-aldosterone loop are responsible for salt and water balance. How these systems interact following thermal injury is unknown.

The ADH response following thermal injury has been recently examined (2-4). Morgan et al. have concluded that ADH levels are elevated postburn and remain so for seven to ten days. In addition, the increased ADH levels appear to have little relation to the serum osmolality and do not affect urine output. There is no satisfactory explanation for this at present. None of these studies have measured blood or plasma volume simultaneously with the measurements of ADH.

The renin-angiotensin-aldosterone axis has been examined in thermal injury (1). Shirani et al. suggested that the elevated plasma levels of renin, angiotensin I, angiotensin II, and aldosterone following thermal injury reflect a resetting of hormonal control and are not dependent upon an effective plasma volume deficit. No volume measurements were made in this study. In this group of patients, combinations of these hormones did remain volume-responsive as verified by saline-loading tests.

ANP, a potent natriuretic and diuretic as well as a vasorelaxant agent, is present in mammalian cardiac atria (5). Central hypervolemia and increased blood pressure have been postulated as factors promoting ANP secretion (6). The vasorelaxant properties of ANP appear to be mediated by an increased level of intracellular cyclic guanosine monophosphate (GMP) which antagonizes the vasopressor effects of angiotensin II an norepinephrine (6). Infusion of ANP in humans effects a profound natriuresis with an accompanying diuresis (7). The mechanism by which ANP causes these changes is unclear. In animal models, infusion of ANP causes an increase in the glomerular filtration rate (GFR) as well as sodium excretion (8). It appears, at least in part, that the mechanism responsible for natriuresis is the increase in GFR. No proximal tubular effect of ANP has been documented. The renal effects of ANP can be blocked by calcium channel blockers, suggesting that its effects are calcium dependent (8).

ANP also has an effect on ADH release and the renin-angiotensin-aldosterone axis. In isolated rat posterior pituitary lobes, ANP causes a massive release of ADH (9). Other investigators have indicated that in isolated hypothalamic hypophyseal preparations, ANP induces a decrease in ADH. ANP induction of ADH release may serve as a negative feedback loop regulating the actions of ANP. The effects of ANP on the renin-angiotensin-aldosterone axis appear to be more constant. Infusion of ANP causes a decrease of plasma renin activity and renin excretion (10,12) and at the same time blunts aldosterone release stimulated by angiotensin II (11-12).

A syndrome of inappropriately low plasma aldosterone levels in the presence of elevated plasma renin activity has been identified in a subset of critically ill patients and was associated with a higher mortality during critical illness (13). The nature of this abnormality has not been elucidated. Elevated ANP could explain this dissociation during critical illness, with its ability to decrease aldosterone levels in the face of an activated renin system (14).

The effects of thermal injury on plasma ANP level and how it in turn affects salt and water balance have not been described.

**Study Objective.** The purpose of this study will be to describe the alterations of plasma levels of ADH, ANP, and the renin-angiotensin-aldosterone axis following thermal injury as related to plasma volume, osmolality, and tonicity. The information generated will permit refinement of fluid resuscitation regimens for severely burned and critically ill patients.

## MATERIALS AND METHODS

1. Number of Patients: Twenty consecutive patients admitted to the US Army Institute of Surgical Research will be eligible for entry into this study.

2. Selection of Patients:

a. Inclusion Criteria: Patients meeting the following criteria will be eligible for entry into the study:

(1) Male or female patients older than 18 years of age. Female patients must have been surgically sterilized, be postmenopausal (over 45 years of age and lack of menstrual periods for at least one year), or have a negative pregnancy test prior to entry into the study.

(2) Patients with burns between 30 and 80 percent of the total body surface area.

(3) Patients admitted to the U.S. Army Institute of Surgical Research within 24 hours of the time of injury.

b. Exclusion Criteria: Patients meeting the following criteria will be excluded from the study:

(1) Patients younger than 18 years of age.

(2) Any pregnant patient.

(3) Patients with burns of less than 30 percent or more than 80 percent of the total body surface area.

(4) Patients admitted to the United States Army Institute of Surgical Research more than 24 hours postburn.

(5) Patients with a history of diabetes mellitus or congestive heart failure.

(6) Patients with a history of treatment for hypertension within the past month.

(7) Patients with concomitant central nervous system injury.

(8) Patients with sepsis or who develop sepsis during the study period.

(9) Patients with acute renal failure or who develop acute renal failure during the study period (defined as an acute rise in serum creatinine to a level greater than 1.5).

3. Patient Procedure During the Study Period:

a. Part I. Upon enrollment into the study, the following data will be collected each day for each patient on postburn days 2 through 10:

(1) Percentage of the total body surface area burned.

(2) Medication administered.

(3) Body weight.

(4) Total intake of water and salt.

(5) Urine and nasogastric output, to include volume as well as sodium and potassium content.

(6) Serum concentrations of sodium, potassium, chloride, glucose, phosphate, uric acid, urea nitrogen, creatinine, and b2-microglobulin.

(7) Serum and urine osmolality.

(8) Urine concentrations of creatinine, urea nitrogen, phosphate, total protein, b2--microglobulin, and aldosterone from a 24 hour urine sample.

From this data, the endogenous GFR, osmolal clearance, $H_2O(CH_2O)$ clearance, and fractional excretion of sodium will be calculated.

At 0700 hours each morning at the time of the routine blood drawing, blood will be obtained for ADH, ANP, plasma renin activity, and aldosterone assays.

b. Part II. On postburn days 2, 5, and 10, intravascular volume measurements will be made utilizing chromium-labeled red blood cells to measure red cell volume (see Appendix A). Total blood volume will than be calculated after measuring a central hematocrit.

On postburn day five, a Swan-Ganz catheter, if not already in place, will be inserted through the central line which the patient will already have for clinical care and readings of cardiac output and pulmonary artery occlusion pressures will be recorded. Systemic vascular resistance will be calculated from the appropriate variables.

Also on postburn day 5, GFR will be measured utilizing both the inulin technique and radioisotopes (See Appendix B). Effective renal plasma flow will be measured using a calorimetric hippurate study as well as a radioisotope (see Appendix C). Inulin clearance will be repeated for any patient who demonstrates a subsequent decrease in renal function during the hospital course.

NOTE: All tests involving radioactivity will be performed in conjunction with the Nuclear Medicine Department, Brooke Army Medical Center.

## RESULTS

Ten patients and ten normal volunteers have been entered into this protocol during this reporting period. Complete data from four of the burn patients and five of the controls are available. The mean age, total burn size, and postburn day of study of the burn patients were 33.6 years, 60.6%, and 10 days respectively. The mean age of the five control subjects was 25 years. Table 1 contains the mean data and standard error of the mean for serum sodium, plasma renin activity, vasopressin level, cardiac index, glomerular filtration rate, effective renal plasma flow, and blood volume. Cardiac index, glomerular filtration rate, and effective renal plasma flow were significantly elevated in the patients as compared to the controls. In addition, plasma renin activity and

vasopressin levels were markedly elevated in the patients. Blood volume measurements in the patients were 87% of predicted.

**TABLE 1**

| | Controls | Patients |
|---|---|---|
| Serum Na+ (mM) | 138 ± 1.2 | 138 ± 2.3 |
| PRA (ng/ml/h) | 1.08 ± 0.26 | 30.5 ± 13.0** |
| AVP (pg/ml) | < 1.0 | 7.25 ± 4.3** |
| CI ($L/min/m^2$) | Normal: 2.3-4.1 | 8.5 ± 0.7 |
| GFR ($ml/min/1.73\ m^2$) | 102 ± 8.1 | 165 ± 11** |
| ERPF ($ml/mn/1.73\ m^2$) | 490 ± 23 | 847 ± 106** |
| BV (% predicted) | Normal: 100 | 87 ± 7 |

[The table gives means ± SE (**$p < 0.01$)]

## DISCUSSION

Despite findings related to increased flow, blood volume based on 51 chromium labelled red cells was not elevated in this group of patients. Plasma renin activity and arginine vasopressin levels were elevated in the direction expected from reduced effective volume. Because of vasodilatation even in areas outside the wound, indices of organ perfusion may not reflect blood volume or effective volume at hormonal control sites. The dissociation of organ flow and hormone response indicates that blood volume may be a stronger and more important determinant than blood flow in determining levels of hormones responsible for salt and water balance. Analysis of data from all enrolled patients and the remaining five control subjects will be necessary before firm conclusions can be reached.

## PRESENTATIONS/PUBLICATIONS

None.

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| RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY | 1. AGENCY ACCESSION | 2. DATE OF SUMMARY | REPORT CONTROL SYMBOL |
|---|---|---|---|
| | DA315349 | 01 Oct 90 | DD-DR&E(AR) 636 |

| 3. DATE PREV SUM'RY | 4. KIND OF SUMMARY | 5. SUMMARY SCTY | 6. WORK SECURITY | 7. REGRADING | 8. DISB'N INSTR'N | 9. LEVEL OF SUM A. WORK UNIT |
|---|---|---|---|---|---|---|
| 01 Oct 89 | D. Chg | U | U | | CX | |

| 10. NO./CODES: | PROGRAM ELEMENT | PROJECT NUMBER | TASK AREA NUMBER | WORK UNIT NUMBER |
|---|---|---|---|---|
| a. PRIMARY | 61102A | 3M161102BS14 | EA | 311 |
| b. CONTRIBUTING | | | | |
| c. CONTRIBUTING | | | | |

11. TITLE *(Precede with Security Classification Code)* (U) Effect of Growth Factors on the Healing of Partial-Thickness Scald Wounds in the Guinea Pig

12. SUBJECT AREAS
0604 Anatomy and Physiology 0605 Medicine and Medical Research

| 13. START DATE | 14. ESTIMATED COMPLETION DATE | 15. FUNDING ORGANIZATION | 16. PERFORMANCE METHOD |
|---|---|---|---|
| 8701 | 9109 | DA | C. In-House |

17. CONTRACT/GRANT MILITARY RELEVANCY CERTIFIED
a. DATE EFFECTIVE APPROVED BY
b. CONTRACT/GRANT NUMBER
c. TYPE d. AMOUNT
e. KIND OF AWARD f. CUM/TOTAL

18. RESOURCES ESTIMATE

| FISCAL YEARS | a. PROFESSIONAL WORKYEARS | b. FUNDS *(In thousands)* |
|---|---|---|
| 90 | 0.1 | 57 |
| 91 | 0.1 | 60 |

19. RESPONSIBLE DOD ORGANIZATION

a. NAME
US Army Institute of Surgical Research

b. ADDRESS *(include zip code)*
Fort Sam Houston
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c. NAME OF RESPONSIBLE INDIVIDUAL
PRUITT, B A

d. TELEPHONE NUMBER *(include area code)*
512-221-2720

20. PERFORMING ORGANIZATION

a. NAME
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d. TELEPHONE NUMBER *(include area code)*
512-221-4440

21. GENERAL USE
FINA

MILITARY/CIVILIAN APPLICATION: M

e. NAME OF ASSOCIATE INVESTIGATOR *(if available)*

g. NAME OF ASSOCIATE INVESTIGATOR *(if available)*

22. KEYWORDS *(Precede EACH with Security Classification Code)* (U) Burns (Injuries); (U) Contraction; (U) Healing; (U) Therapy; (U) Histology

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS *(Precede text of each with Security Classification Code)*

23/24. (U) The objective of this work is to determine whether infusion of a recently described copolymer will enhance burn epithelization in a deep partial-thickness burn wound in the guinea pig. After receiving deep partial-thickness burns, male guinea pigs will receive either saline or the copolymer. In the first phase, guinea pigs will be sacrificed at 72 h and the effects of the copolymer on the zone-of-stasis studied histologically. In the second phase, guinea pigs will be sacrificed at 5, 10, and 20 days following injury and the effects of the copolymer on reepithelization and hair follicle survival will be measured. The extent of healing by contraction will be assessed by planimetry and the extent of reepithelization will be assessed histologically.

25. (U) 8710 - 8809. This project was approved by the USAISR Research Council and US Army Institute of Surgical Research Animal Care and Use Committee during the second quarter of fiscal year 1987.

(U) 8810 - 8909. An addendum was approved by the USAISR Research Council and US Army Institute of Surgical Research Animal Care and Use Committee during this reporting period. Initial studies revalidating the deep partial-thickness burn wound model in guinea pigs was initiated.

(U) 8910 - 9009. A deep partial-thickness burn wound model in guinea pigs has been revalidated. Preliminary studies comparing the copolymer to saline following thermal injury have indicated no effect on wound histology

DD FORM 83 MAR 1498 EDITION OF MAR 68 IS OBSOLETE. * USGPO 1988-491-003/50329

at 72 h following injury. Second phase studies have been undertaken and the data are currently being analyzed.

**SUPPLEMENTAL WORK UNIT INFORMATION FOR THE PROJECT ENTITLED "EFFECT OF GROWTH FACTORS ON THE HEALING OF PARTIAL-THICKNESS SCALD WOUNDS IN THE GUINEA PIG"**

**Subrecord/Linking Accession Number:** DA312335

**Search Control Data:** W6M16F/W6M17E, 20 October 1989

**Product Identification:** For technical reports, refer to the US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Years 1987-90.

**Unclassified Special Categories:** Lab Animals: Guinea Pigs; RA II

**ANNUAL RESEARCH PROGRESS REPORT**

**PROJECT NUMBER:** 3M161102BS14-00, RESEARCH

**PROJECT TITLE:** Effect of Growth Factors on the Healing of Partial-Thickness Scald Wounds in the Guinea Pig

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

**1 October 1989 - 30 September 1990**

**INVESTIGATORS**

William G. Cioffi, Jr., MD, Major, MC

## ABSTRACT

**PROJECT NUMBER:** 3M161102BS14-00, RESEARCH

**PROJECT TITLE:** Effect of Growth Factors on the Healing of Partial-Thickness Scald Wounds in the Guinea Pig

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

**PERIOD COVERED IN THIS REPORT:** 1 Oct 89 through 30 Sep 90

**INVESTIGATORS:** William G. Cioffi, Jr., MD, Major, MC

RheothRx is a copolymer which has been widely used as an emulsifying agent in food, oral drugs, and cosmetics for many years. Preliminary data have indicated this copolymer may prevent vascular occlusion in the zone of stasis in deep, partial-thickness burn wounds in rats. We have investigated the effect of this copolymer on the healing of partial-thickness burn wounds in guinea pigs. Animals received deep, partial-thickness burn wounds and then were treated with saline or the copolymer for 48 hours following injury. Animals were sacrificed at 10 and 20 days postburn, and the following data collected: the percent re-epithelialization of the wound, percent wound contracture, and histologic grading of the wound to include evidence of follicular damage, inflammation, hemorrhagic epithelium and muscle damage. Comparison of the treated and untreated animals at postburn days 10 and 20 revealed no difference in the histologic appearance of the wounds, no difference in the extent of re-epithelialization of the wound, and no difference in the percent wound contracture. We conclude from this data that RheothRx copolymer has no effect on the healing of partial-thickness scald wounds in the guinea pig at the dosage employed in this study.

**EFFECT OF GROWTH FACTORS ON THE HEALING OF PARTIAL-THICKNESS SCALD WOUNDS IN THE GUINEA PIG**

RheothRx is a copolymer which has been widely used as an emulsifying agent in food, oral drugs, and cosmetics for many years. Preliminary data from Emory University have indicated that this copolymer may prevent vascular occlusion in the zone of stasis in deep, partial-thickness burn wounds in rats. Animals treated with the drug went on to heal their wounds, while those receiving placebo converted their wounds to full-thickness injuries.

Preliminary experiments performed at this Institution evaluated the histologic appearance of a deep, partial-thickness guinea pig scald wound 72 hours following injury. Animals were randomized to receive copolymer or saline for the 72 hour period. No discernable differences in dermal injury, inflammatory response, hemorrhage, red cell stasis, vascular damage, or muscle damage could be delineated by light microscopy between the two groups. The current experiments were carried out to assess whether RheothRx copolymer affects the rate of healing in this deep, partial-thickness burn wound model.

## METHODS

Male guinea pigs weighing 500 grams were used throughout the experiment. On the day of burning, animals were anesthetized the phenobarbital (35 mg/kg body weight IP). The dorsal surface was shaved, and a 20% partial-thickness scald injury produced by exposing the dorsal surface to 78°C water for 10 seconds in a specially designed template. Upon completion of burning, the wound edges were tattooed and the animals allowed to recover from the anesthesia.

Forty animals were randomized to two groups. Group 1 received a 50 mg/kg bolus of RheothRx copolymer intraperitoneally immediately following injury. The animals then received 2 cc of a 5% solution intraperitoneally every six hours for six doses. The control group received saline. Following injury, photographs and wound tracings were obtained. Photographs and wound tracings were then repeated on postburn days 10 and 20.

On postburn days 10 and 20, one-half of the animals in each group were sacrificed and the wounds examined grossly and histologically using the criteria previously stated. Repeat wound tracings were obtained, and the difference in tracing weight between the postburn day 0 and the postburn day of study were obtained to document the extent of wound contracture. An estimation of the percent of wound epithelialization was made at this time.

## RESULTS

There were no discernable differences between the two groups of animals on postburn day 10 or 20. (See Tables) Of note, there was no difference in the extent of wound contracture at either of the study dates. The percent of re-epithelialization was identical between the two groups with approximately 50 to 75% of the wounds re-epithelialized at 10 and 20 days. There were no histologic differences noted between those animals receiving saline and those receiving RheothRx copolymer.

**TABLE 1. Histologic Grading on Postburn Day**

| | RheothRx | CONTROL |
|---|---|---|
| FOLLICLES | 2.1 ± 3.9 | 2.5 ± 3.8 |
| INFLAMMATION | 1.67 ± .33 | 1.7 ± .26 |
| HEMORRHAGE | 1.44 ± .18 | 1.3 ± .21 |
| EPITHELIUM | 1.3 ± .53 | 1.5 ± .45 |
| MUSCLE | 0.11 ± .11 | 0.5 ± .22 |

TABLE 1: Histologic Grading of Wounds at Postburn Day 10. All indices are graded on a score of 1 to 4, with 1 being minimal damage and 4 being severe damage. There were no statistical differences between the two groups. Data are expressed as the mean ± standard deviation.

**TABLE 2. Percent Re-epithelialization on Postburn Day 10**

| | RheothRx | CONTROL |
|---|---|---|
| SCORE | 2.55 ± .24 | 2.4 ± .116 |

TABLE 2: Percent Re-epithelialization on Postburn Day 10. A score of 1 indicates 25% re-epithelialization, 2 - 50% re-epithelialization, 3 - 75% re-epithelialization, and 4, complete re-epithelialization of the wound. There were no differences in percent re-epithelialization on postburn day 10 between the two groups.

**TABLE 3. Wound Contracture on Postburn Day 10**

| | RheothRx | CONTROL |
|---|---|---|
| DIFFERENCE | .104 ± .006 | .105 ± .009 |

TABLE 3: Wound Contraction. Wound tracings were obtained on postburn day 0 and postburn day 10. The difference in tracing weight was used to index the degree of wound contracture. No difference was noted between the two groups.

**TABLE 4. Histologic Grading on Postburn Day 20**

| | RheothRx | CONTROL |
|---|---|---|
| FOLLICLES | 3.0 ± .24 | 3.4 ± .27 |
| INFLAMMATION | 1.2 ± .15 | 1.6 ± .16 |
| HEMORRHAGE | 0.89 ± .26 | 0.70 ± .21 |
| EPITHELIUM | 1.3 ± .6 | 2.4 ± .6 |
| MUSCLE | 0.89 ± .26 | 1.1 ± .31 |

TABLE 4: Histologic Classification on Postburn Day 20. The scoring system is the same as in Table 1. No differences were noted between the two groups. Data is expressed as mean ± standard deviation.

**TABLE 5. Percent Re-epithelialization on Postburn Day 20**

| | RheothRx | CONTROL |
|---|---|---|
| SCORE | 2.4 ± .33 | 3.0 ± .39 |

TABLE 5: Percent Re-epithelialization at 20 days. No difference was noted between the two groups.

**TABLE 6. Wound Contracture on Postburn Day 20**

| | RheothRx | CONTROL |
|---|---|---|
| DIFFERENCE | .161 ± .005 | .153 ± .009 |

TABLE 6: Percent Wound Contracture Postburn Day 20. No differences noted between the two groups. Data are expressed as mean + standard deviation.

## DISCUSSION

Despite previous findings that RheothRx copolymer prevented vascular occlusion in the zone of stasis in deep partial-thickness burn wounds in rats, our results indicate no benefit from the administration of this copolymer immediately following injury. Our previous data, examining the wounds at 72 hours following injury, showed no difference in the histologic appearance of the wounds to indicate that the zone of stasis had been altered. These further studies, investigating the wounds at postburn days 10 and 20, confirm our earlier results. Animals receiving saline or RheothRx copolymer exhibited the same percentage of wound contracture and re-epithelialization, while having no discernable differences in the histologic appearance of their wounds. From these data we conclude that in the guinea pig deep partial scald model, RheothRx copolymer, applied at the doses used in this experiment, had no effect on wound healing.

## PRESENTATIONS/PUBLICATIONS

None.

## REFERENCES

None.

**RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY**

| 1. AGENCY ACCESSION | 2. DATE OF SUMMARY | REPORT CONTROL SYMBOL |
|---|---|---|
| DA315350 | 01 Oct 90 | DD-DR&E(AR) 636 |

| 3. DATE PREV SUM'RY | 4. KIND OF SUMMARY | 5. SUMMARY SCTY | 6. WORK SECURITY | 7. REGRADING | 8. DISP'N INSTR'N | 9. LEVEL OF SUM A. WORK UNIT |
|---|---|---|---|---|---|---|
| 01 Oct 89 | D. Chg | U | U | | CX | |

| 10. NO./CODES: | PROGRAM ELEMENT | PROJECT NUMBER | TASK AREA NUMBER | WORK UNIT NUMBER |
|---|---|---|---|---|
| a. PRIMARY | 61102A | 3M161102BS14 | CH | 312 |
| b. CONTRIBUTING | | | | |
| c. CONTRIBUTING | | | | |

11. TITLE (Precede with Security Classification Code) (U) Cellular Host Defense Function After Thermal Injury: Assessment by Flow Cytometry of Peripheral Blood Cells

12. SUBJECT AREAS
0601 Biochemistry 0605 Medicine and Medical Research

| 13. START DATE | 14. ESTIMATED COMPLETION DATE | 15. FUNDING ORGANIZATION | 16. PERFORMANCE METHOD |
|---|---|---|---|
| 8605 | 9109 | DA | C. In-House |

17. CONTRACT/GRANT MILITARY RELEVANCY CERTIFIED
a. DATE EFFECTIVE APPROVED BY
b. CONTRACT/GRANT NUMBER
c. TYPE
d. AMOUNT
e. KIND OF AWARD
f. CUM/TOTAL

18. RESOURCES ESTIMATE

| FISCAL YEARS | a. PROFESSIONAL WORK YEARS | b. FUNDS (In thousands) |
|---|---|---|
| 90 | 0.1 | 241 |
| 91 | 0.1 | 253 |

19. RESPONSIBLE DOD ORGANIZATION

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MASON, A D

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21. GENERAL USE
FINA

MILITARY/CIVILIAN APPLICATION: M

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Burns (Injuries); (U) Lymphocytes; (U) Immunosuppression; (U) Morbidity

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

23/24. (U) The objective of this work is to analyze the complex leukocyte mixtures seen in the blood of burned patients, quantitate the changes that occur, and correlate those changes with changes in cell function as well as clinical outcome. The immune status of burn patients will be assessed in terms of lymphocyte subpopulation composition and function using flow cytometry to differentiate the subpopulations. Data will be correlated with patient morbidity and compared to data from unburned control subjects.

25. (U) 8710 - 8809. Sixty-nine burn patients were enrolled in the study during this reporting period. Data indicate the proportion of T lymphocytes, including helper and suppressor subsets, decreased in burn patients as compared to control subjects. The proportion of B lymphocytes increased, but there was no change in NK cells. The ratio of helper to suppressor lymphocytes was also unchanged in burn patients. Nonsurviving patients had decreased proportions of helper and suppressor lymphocytes, yet the proportion of T lymphocytes was unchanged as compared to survivors. Nonsurviving patients also had decreased T lymphocyte function as compared to survivors. This decrease in function was specific for T lymphocytes as B lymphocyte function remained unchanged or increased.

(U) 8810 - 8909. Seventeen burn patients and 15 control subjects were enrolled in the study during this reporting period. The function of T lymphocyte subsets was measured as the expression of IL 2 receptor after mitogen stimulation. The expression of IL 2 receptor by helper and suppressor T lymphocyte subsets after mitogen stimulation was decreased in

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burned patients as compared to controls. The ability of B lymphocytes and NK cells to express the IL 2 receptor appeared to be unimpaired by thermal injury.

(U) 8910 - 9009. Thirty-three patients and 33 control subjects were enrolled in the study during this reporting period. The expression of IL 2 receptor after mitogen stimulation was used as a measure of immunocompetence. Although resting levels of IL 2 receptor expression were increased in freshly isolated lymphocytes from burn patients as compared to control subjects, mitogen-induced IL 2 receptor expression was decreased immediately after burn injury and for up to 8 wk postburn. This suppressed response was similar for both CD4 (helper) and CD8 (suppressor/cytotoxic) subsets. NK cells (CD16) from burn patients also expressed increased levels of IL 2 receptor when freshly isolated, but the induction of IL 2 receptors by mitogen in these cells was not impaired at any postburn time measured.

**SUPPLEMENTAL WORK UNIT INFORMATION FOR THE PROJECT ENTITLED "CELLULAR HOST DEFENSE FUNCTION AFTER THERMAL INJURY: ASSESSMENT BY FLOW CYTOMETRY OF PERIPHERAL BLOOD CELLS"**

**Subrecord/Linking Accession Number:** DA311488

**Search Control Data:** W6M25D/W6M27E, 20 October 1989

**Product Identification:** For technical reports, refer to the US Army Institute of Surgical Research Annual Research Progress Report for fiscal years 1986-90.

**Unclassified Special Categories:** Volunteers: Adults; RA II

**ANNUAL RESEARCH PROGRESS REPORT**

**PROJECT NUMBER:** 3M161102BS14-00, 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**
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**1 October 1989 - 30 Sepeptember 1990**

**INVESTIGATORS**
David G. Burleson, PhD, Lieutenant Colonel, MS
Karen Wolcott
A.D. Mason, Jr., MD
Basil A. Pruitt, Jr., MD, Colonel, MC

## ABSTRACT

**PROJECT NUMBER:** 3M161102BS14-00, 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 1989 through 30 Sep 90

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

The best predictors of mortality in burned patients are burn size and age. Other conditions such as inhalation injury are additional risk factors, but burn size and age correlate best with mortality. The manner in which various components of host defense change in relation to mortality predictors may indicate their relationship to the infection susceptibility seen after thermal injury. We have analyzed Ficoll-Hypaque purified peripheral blood lymphocyte subpopulations in 103 burned patients. The patients were monitored twice weekly for up to eight weeks postburn. The mortality predictor was developed from experience at this burn center using burn size and age as variables. The proportion of CD8 positive cells was sharply decreased soon after injury and gradually returned to unburned control levels by the seventh post-burn week. During the first few weeks when CD8 positive cells were lowest, there was a negative correlation between the proportion of CD8 positive cells and predicted mortality i.e., the more severe injuries led to smaller proportions of positive cells. In contrast, CD4 positive cells were decreased below control in the first week postburn but returned to normal rapidly and remained there for most of the postburn period. As with CD8 positive cells, there was a negative correlation between predicted mortality and the proportion of CD4 positive cells. However, the correlation was unchanged for the full eight week observation period. The proportion of CD16 positive cells decreased soon after injury and returned to unburned control levels in a manner similar to CD8 positive cells. In contrast to CD8 and CD4, there was a positive correlation between predicted mortality and the proportion of CD16 positive cells. This correlation was only evident during the latter part of the postburn recovery period. Changes in lymphocyte subpopulation level after thermal injury did not appear to be passive responses to the insult, but varied independently of each other with respect to injury severity and time postburn.

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

## INTRODUCTION

The survival of patients with severe thermal injury has improved dramatically in recent years, but burn size and age remain the best predictors of mortality. The predictor equation developed by this institute (1-2) relates the severity of thermal injury (as measured by burn size and age) to survival. In vitro measurements that purport to indicate the clinical status of patients should show a relationship to this predictor to establish whether the indicator is related to the thermal injury or reflects other environmental influences on the patient. We have measured the lymphocyte subpopulations in burned patients and have tested their correlation with the mortality predictor. The variation of these measurements was quite large from patient to patient, but correlations between several lymphocyte subpopulations and the predictor were found.

## MATERIALS AND METHODS

**Patient Data.** One hundred three burned patients admitted to the USAISR over a four year period were included in the study. All patients were entered within five days of injury. The patient's expected mortality was determined from burn size and age by the following equation derived from previous mortality experience at our institute:

$(e^y/ (1 + e^y) ) X 100$ where

$y=(.113 \times \%BURN+(.00582 \times (AGE^2))-.203 \times AGE-(.0000361 \times (AGE^3))-4.36)$

The range of predicted mortality was from 1% to 96% for this group of patients. The average burn size was 43% and the average age was 41.

**Cell preparation.** Heparinized blood samples were obtained from patients twice weekly for up to eight weeks postburn. Lymphocytes were isolated on Ficoll-Hypaque gradients. After washing, a portion of the cells were used for staining with monoclonal antibodies and preparation of a slide to determine the extent of non-lymphocyte contamination.

**Cell staining.** Cells were stained with monoclonal antibodies (Becton Dickinson) bound to either phycoerythrin, fluorescein isothiocyanate or biotin. Allophycocyanin was bound to biotin labelled primary antibodies by subsequent addition of a streptavidin-allophycocyanin conjugate. Anti-Leu-2 (CD8), anti-Leu-3 (CD4), and anti-Leu-11 (CD16) were used to identify subpopulations. IgG1 conjugated with the appropriate dye marker

was employed as an isotypic control. The staining procedure followed that specified by the manufacturer of the monoclonal antibody. Cells were fixed immediately after staining in 1% paraformaldehyde.

**Flow Cytometry Analysis.** Subpopulations were analyzed by flow cytometry using a FACSTAR+ (Becton Dickinson) or an EPICS model 753 flow cytometer. Electronic gates were set on forward angle and side scatter intensity using normal human peripheral blood lymphocytes. Non-lymphoid cell contamination was monitored by observing the level of anti-Leu-M3 positives (anti-Leu-M3 binds monocytes and weakly binds granulocytes). The positive cutoff was set at a point that defined 1% or less of the electronically gated isotypic control sample as positive.

## RESULTS

Lymphocytes were isolated by Ficoll-Hypaque gradients and stained with the appropriate monoclonal antibodies. The temporal relationship of the CD8, CD16 and CD4 subpopulations in this patient population is depicted in Figures 1-3. The mean proportion of each cell subpopulation for all patients in the study is depicted in the line graph and the range for unburned controls is shown by the shaded bar. The proportion of CD4 positive cells (Figure 1) was decreased by the first week after injury and remained below controls for most of the rest of the postburn period. The proportion of circulating CD8 positive lymphocytes (Figure 2) dropped dramatically after injury and returned to normal levels approximately 7 weeks later. Like CD8 positive cells, the proportion of CD16 positive cells (natural killer cells) was low after injury and returned to normal and above late in the recovery period. This illustrates that subpopulations changed with time after injury and that each subpopulation had its own particular pattern of change.

Predicted mortalities were calculated for each patient using the total (partial and full-thickness) burn size and age of the patient in the equation described in methods. The calculated mortality probability was compared to the mean lymphocyte subpopulation for each patient over the time of observation. When all postburn day values were used, only the proportion of CD4 positive cells was correlated with predicted mortality (Figure 4). The correlation of the CD4 positive cells with predicted mortality was negative ($r=.2795$, $p. <.0001$).

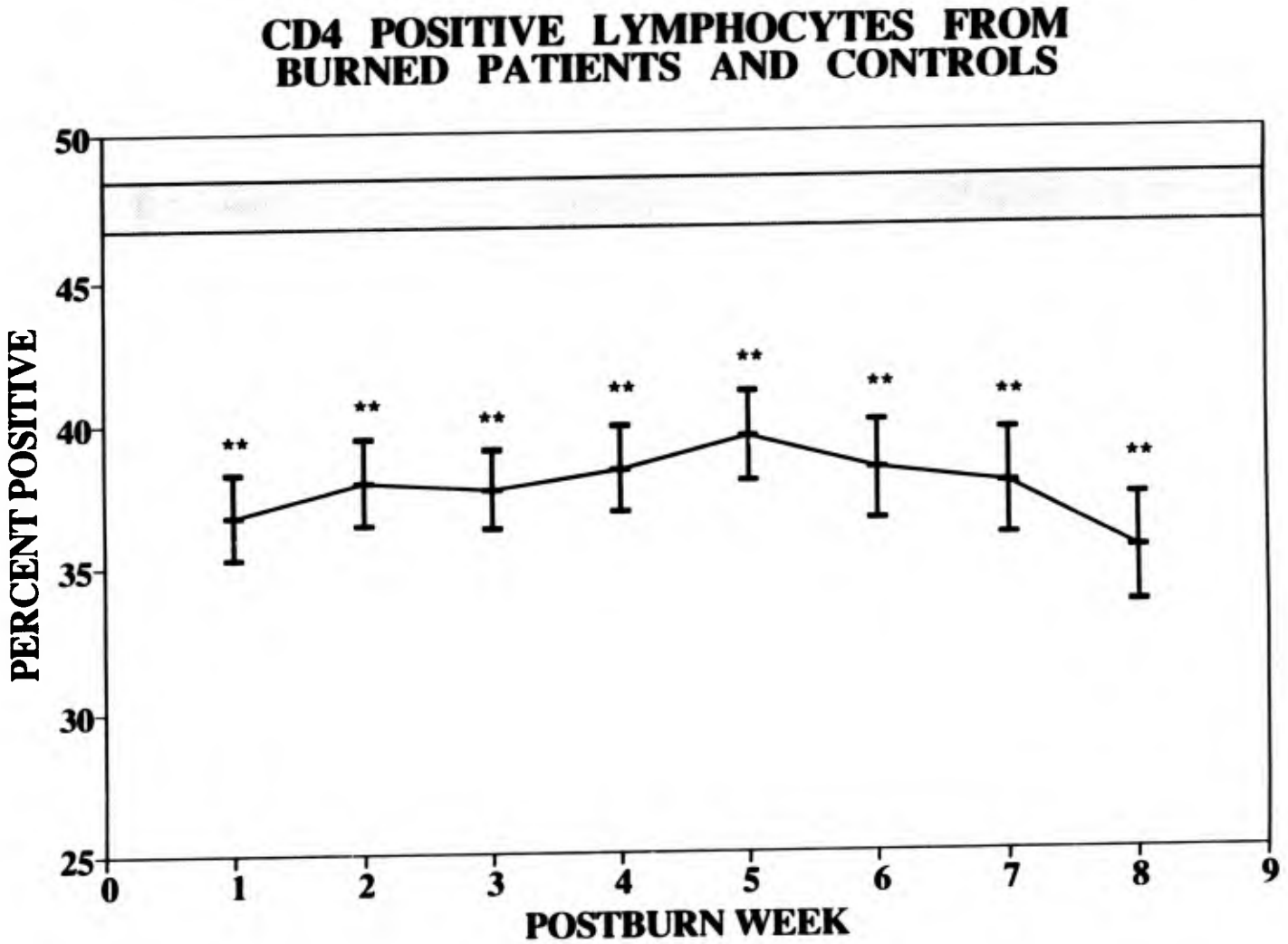


**FIGURE 1.** CD4 positive lymphocyte subpopulations from burned patients and controls. Ficoll-Hypaque purified lymphocytes were stained with the appropriate monoclonal antibodies. Mean values for the controls +/- SEM are represented by the shaded area. Mean values +/- SEM for the patient are represented by the curve. P<.0001

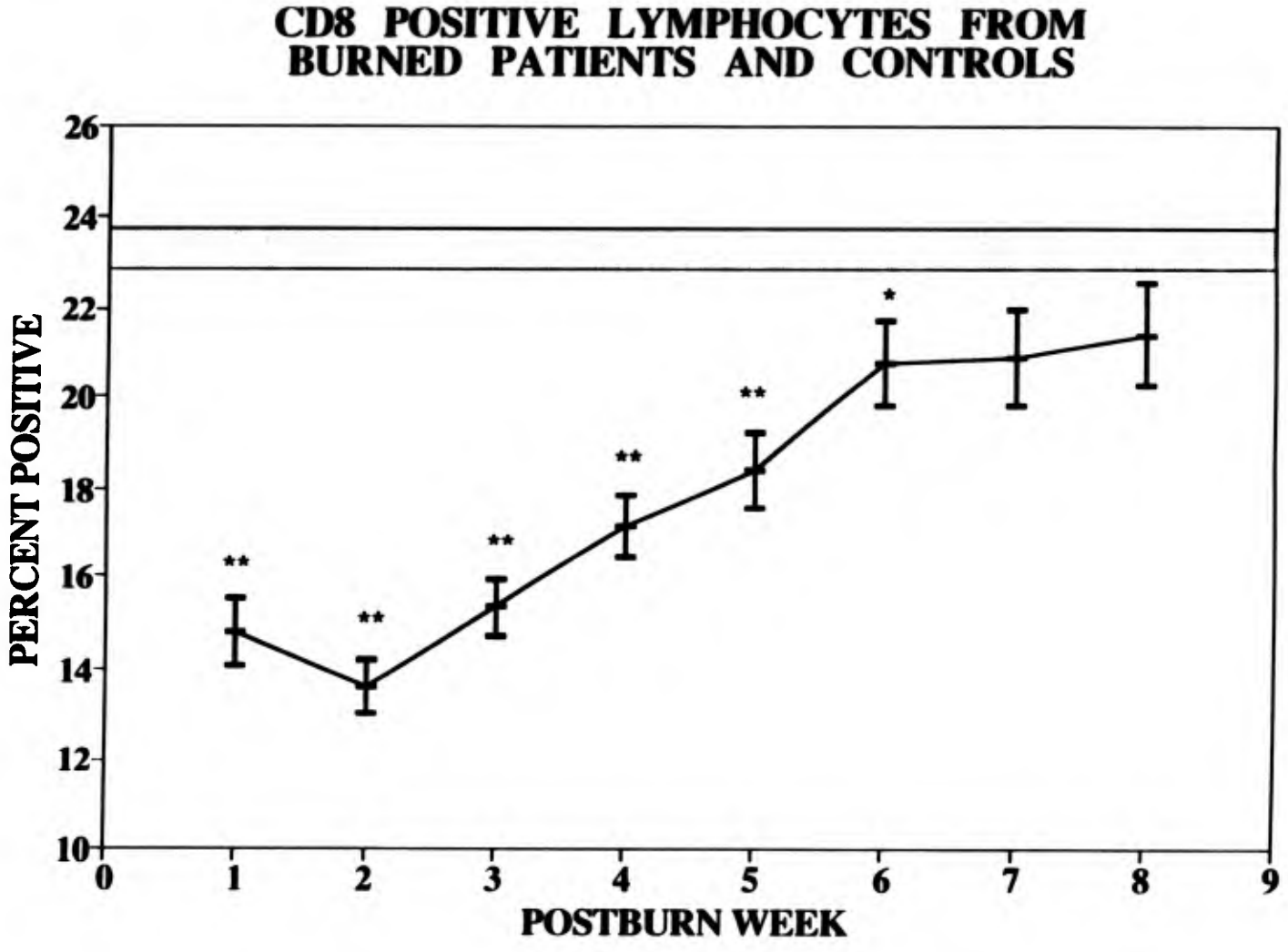


**FIGURE 2.** CD8 positive lymphocyte subpopulations from burned patients and controls. Conditions are the same as Figure 1.

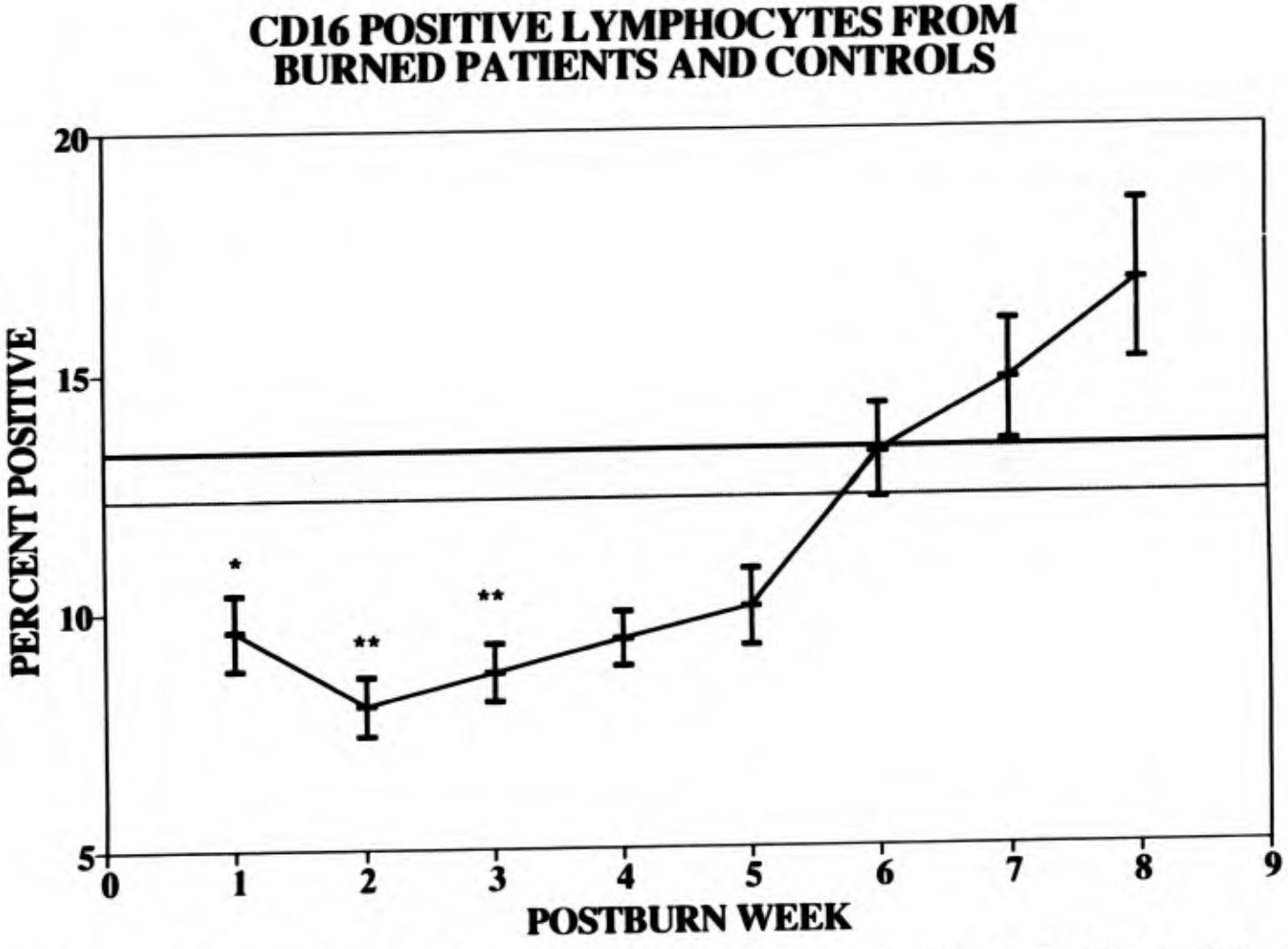


**FIGURE 3.** CD16 positive lymphocyte subpopulations from burned patients and controls. Conditions are the same as Figure 1. *=P<.01; *=P<.05.

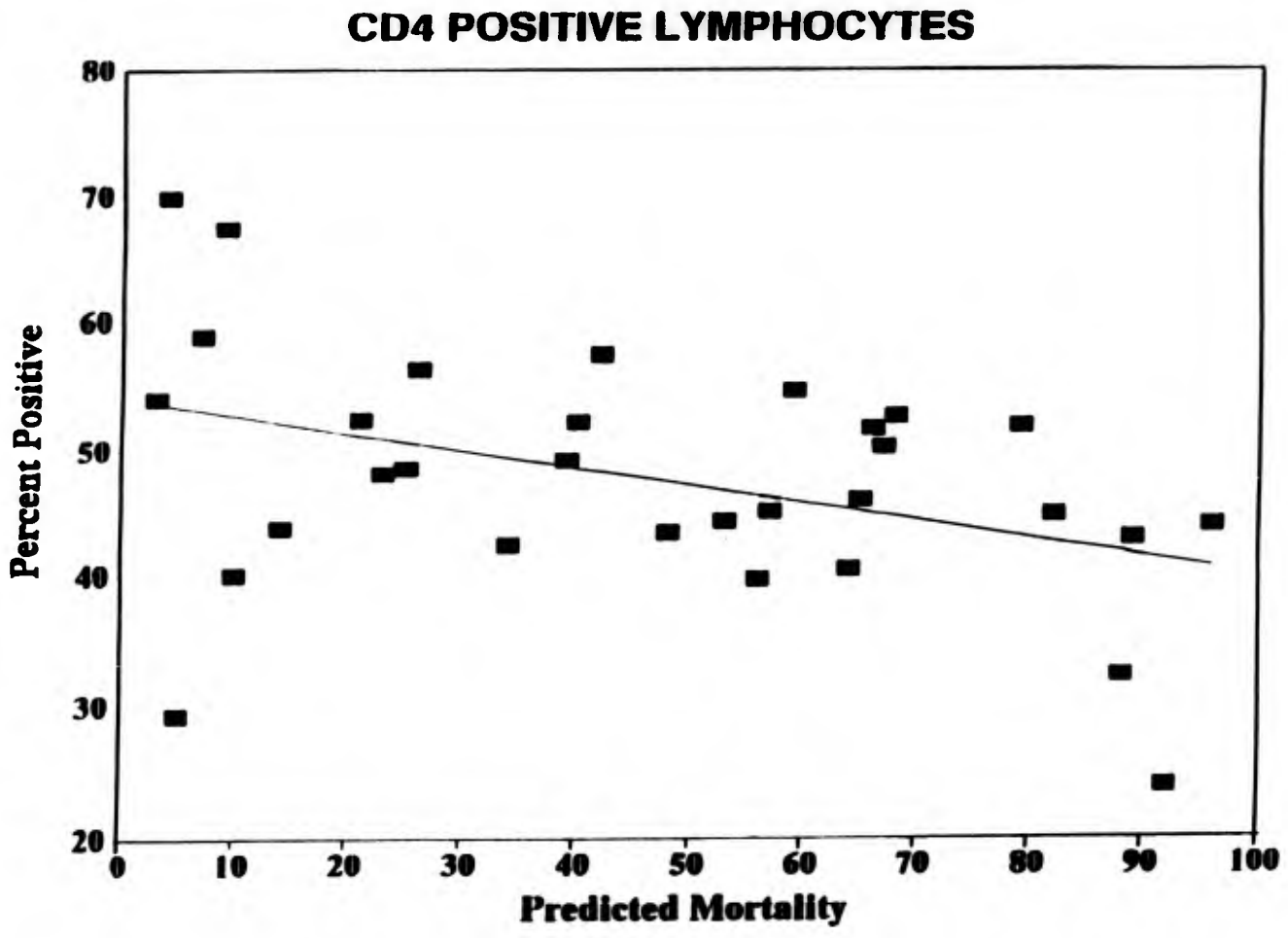


**FIGURE 4.** Correlation of CD4 positive lymphocyte subpopulations to the severity of thermal injury. The proportion of each subpopulation determined for each patient was correlated with the predicted mortality for that patient. Symbols plotted on the graph are the mean subpopulation percentages for patients with each predicted mortality value shown. The calculated regression line for the analysis is also shown on the graph. CD4 data were averaged for samples collected from all post burn days. R=.2795, P<.0001;

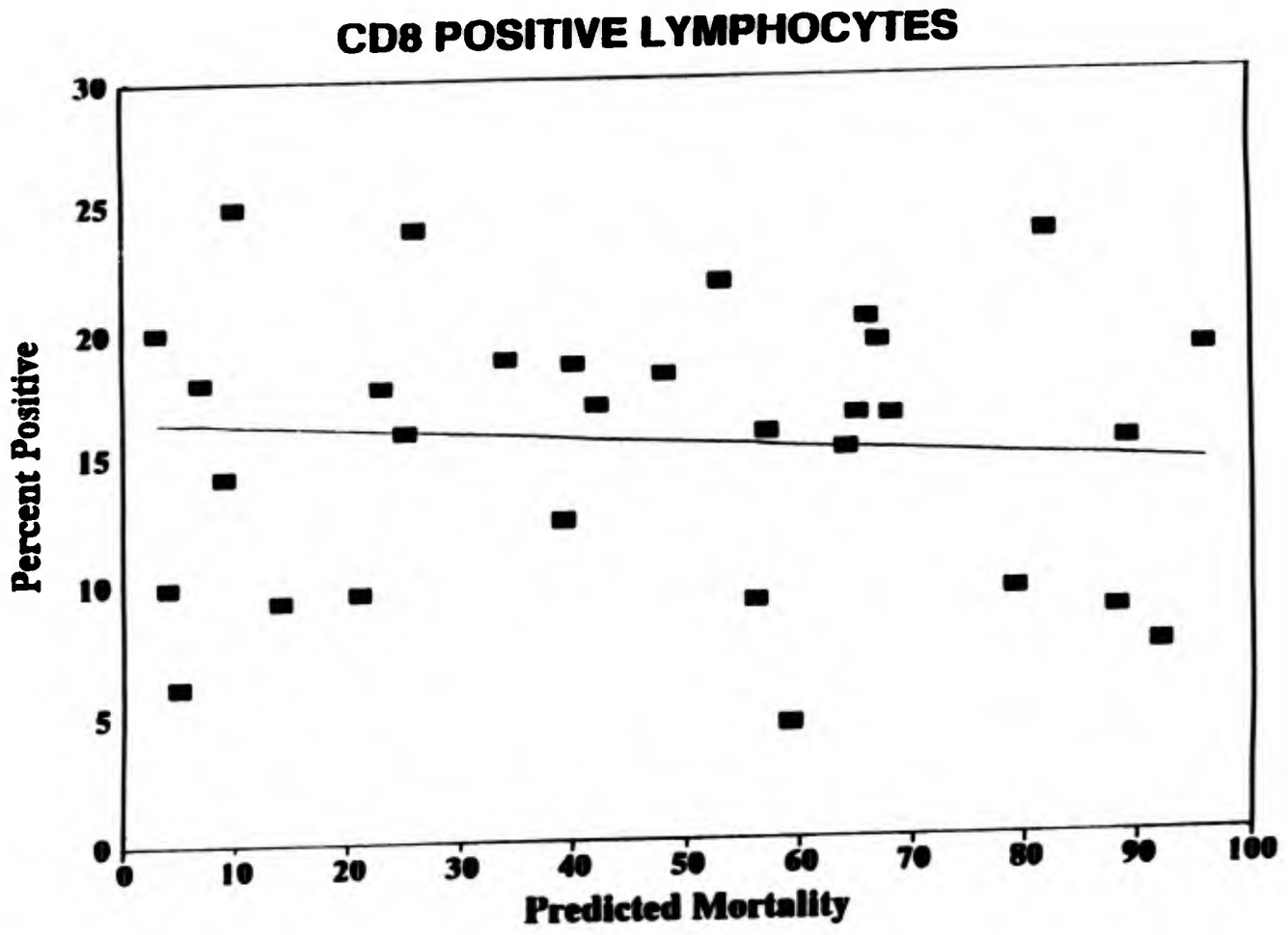


**FIGURE 5.** Correlation of the CD8 positive lymphocyte subpopulations to the severity of thermal injury. Data on CD8 positive cells were from samples taken at postburn day 26 or less. R=.1930, P=.003. Conditions are the same as in Figure 4.

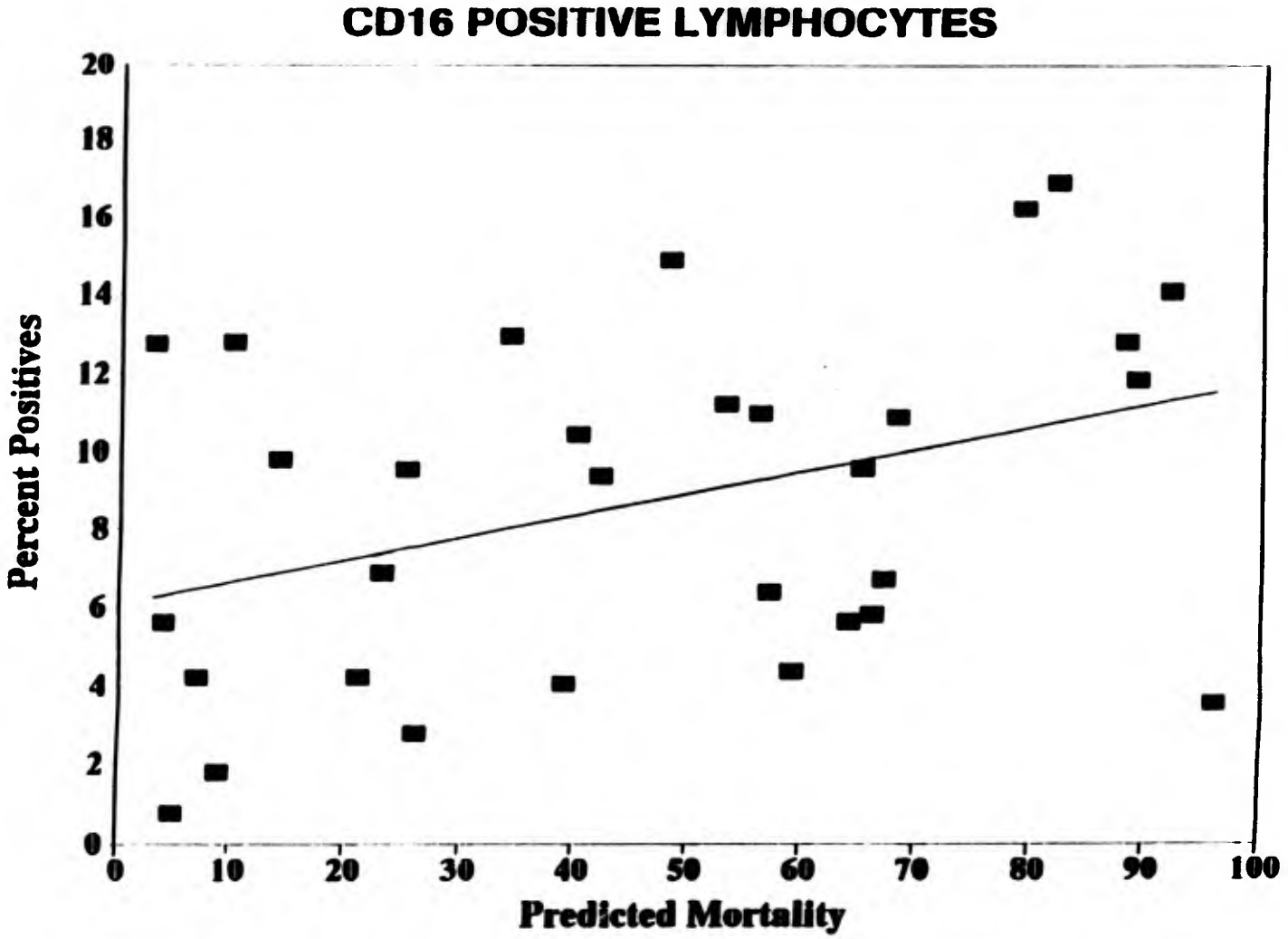


**FIGURE 6.** Correlation of CD16 positive lymphocyte subpopulations to the severity of thermal injury. Data for CD16 positive cells from samples taken on postburn days greater than 26 were used. R=.1437, P=.05. Other conditions are the same as in Figure 4.

The proportion of CD4 cells varies relatively less over time than CD8 and CD16 populations (Figures 1-3). Since this variation over time might obscure a significant correlation with predicted mortality, the point in time that separated the data into two equal groups (26 days) was used to classify the measurements into early and late postburn periods for these two subpopulations. The mean proportions measured during each time period was determined for each patient.

The mean proportions of the CD8 positive cells taken from the first 26 days postburn (Figure 5) were negatively correlated with predicted mortality (r=.1930 p.=.003). This is the period of time when the proportion of CD8 cells is severely decreased below unburned control levels. The mean proportions of CD16 positive cells (Figure 6) taken from the later postburn period ( > than 26 days) were positively correlated with predicted mortality. During this time the proportion of CD16 cells is normal or increased above unburned patient control levels (r=.1347, p.=05). Values for the other time periods were not correlated with predicted mortality.

## CONCLUSIONS

There is a large change in circulating leukocyte populations after burn injury. The proportion and absolute number of polymorphonuclear leukocytes increase dramatically while the absolute number and proportion of lymphocytes decrease after thermal injury. Similarly, we have shown that there are changes in the proportions of lymphocyte subpopulations as well. These changes occur independently in that they do not follow the same trend in terms of either time or relation to severity of injury.

Although it is not clear how these subpopulations changes affect the patients ability to resist infection, they should relate to severity of injury if they are important. Increased patient mortality is related to increased susceptibility to infection.

As a case in point, the relationship between age and infection susceptibility seems clear (3,4). Age related changes in the immune system may make older individuals more susceptible to infections in many circumstances. Age is clearly correlated with burned patient outcome, and seems to confer increased infection susceptibility on burned patients as well. For any test of a relationship between a measurable change in the immune system and infection susceptibility to have clinical utility, it must also correlate with patient outcome.

The correlations of the proportions of lymphocyte subpopulations with predicted mortality reported here were not strong. There could be several reasons for this. First, flow cytometry data are quite variable from patient to patient. Normal ranges from healthy individuals are quite large in the absence of detectable disease (5-11).

Second, subpopulation measurements are rather broad measures of immune system capability. Lymphocyte subpopulations are quite complex, composed of many subtypes. The function of many of these subtypes is unknown or not clearly defined by subpopulation markers. For instance, suppressor lymphocytes are known to reside in both the CD4 and CD8 subpopulations (12-20). Additionally, it is not clear whether the number of circulating lymphocytes or their relative subpopulation proportions have any relation to the protection of the host against infection. In that case, decreases in circulating lymphocytes may be more a refection of changes in lymphocyte compartments than immunodeficiency.

Third, subpopulation measurement does not measure cell function. Full numbers of circulating but tolerant lymphocytes might be more detrimental than small numbers of fully functional cells. Measurement of function is difficult, as the interaction of cells in the host defense process is complex. It is frequently difficult to distinguish suppressive activity from stimulatory activity. One classic measure of lymphocyte function is Con A stimulation of tritiated thymidine incorporation. However, stimulations of both suppressor and helper calls are included in this measurement (12,21,22).

It is clear from this study that circulating levels of lymphoid subpopulations change during the time when infections are a major clinical concern. The subpopulations showed distinct individually regulated changes with time post injury and some were moderately correlated with severity of injury as well.

## REFERENCES


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| RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY | | | | 1. AGENCY ACCESSION | 2. DATE OF SUMMARY | REPORT CONTROL SYMBOL |
|---|---|---|---|---|---|---|
| | | | | DA315351 | 01 Oct 90 | DD-DR&E(AR) 636 |
| 3. DATE PREV SUM'RY | 4. KIND OF SUMMARY | 5. SUMMARY SCTY | 6. WORK SECURITY | 7. REGRADING | 8. DISB'N INSTR'N | 9. LEVEL OF SUM A. WORK UNIT |
| 01 Oct 89 | D. Chg | U | U | | CX | |

| 10. NO./CODES | PROGRAM ELEMENT | PROJECT NUMBER | TASK AREA NUMBER | WORK UNIT NUMBER |
|---|---|---|---|---|
| a. PRIMARY | 61102A | 3M161102BS14 | AZ | 313 |
| b. CONTRIBUTING | | | | |
| c. CONTRIBUTING | | | | |

11. TITLE *(Precede with Security Classification Code)* (U) A Study of Biochemical Changes in the Cellular Environment of Tissue of the in vivo Partial-Thickness Rat Burn Wound

12. SUBJECT AREAS
0601 Biochemistry 0604 Anatomy and Physiology

| 13. START DATE | 14. ESTIMATED COMPLETION DATE | 15. FUNDING ORGANIZATION | 16 PERFORMANCE METHOD |
|---|---|---|---|
| 8609 | 9109 | DA | C. In-House |

17. CONTRACT/GRANT — MILITARY RELEVANCY CERTIFIED APPROVED BY [illegible]

| a. DATE EFFECTIVE | | b. RESOURCES ESTIMATE | | |
|---|---|---|---|---|
| b. CONTRACT/GRANT NUMBER | | FISCAL YEARS | a. PROFESSIONAL WORK YEARS | b. FUNDS *(In thousands)* |
| c. TYPE | a. AMOUNT | 90 | 0.1 | 131 |
| e. KIND OF AWARD | f. CUM/TOTAL | 91 | 0.1 | 137 |

| 19. RESPONSIBLE DOD ORGANIZATION | 20. PERFORMING ORGANIZATION |
|---|---|
| a. NAME: US Army Institute of Surgical Research | a. NAME: US Army Institute of Surgical Research |
| b. ADDRESS *(include zip code)*: Fort Sam Houston, San Antonio, Texas 78234-5012 | b. ADDRESS: Fort Sam Houston, San Antonio, Texas 78234-5012 |
| c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A | c. NAME OF PRINCIPAL INVESTIGATOR: BROWN, W L |
| d. TELEPHONE NUMBER *(include area code)*: 512-221-2720 | d. TELEPHONE NUMBER *(include area code)*: 512-221-4652 |
| 21. GENERAL USE: FINA<br>MILITARY/CIVILIAN APPLICATION: M | f. NAME OF ASSOCIATE INVESTIGATOR *(if available)*: MASON, A D<br>g. NAME OF ASSOCIATE INVESTIGATOR *(if available)* |

22. KEYWORDS *(Precede EACH with Security Classification Code)* (U) Burns (Injuries); (U) Wounds and Injuries; (U) Metabolism; (U) Metabolites

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS *(Precede text of each with Security Classification Code)*

23/24. (U) The objective of this work is to determine the biochemical and metabolic changes that occur in the in vivo partial-thickness rat burn wound during the early postburn period and identify criteria of reversibility. The data generated may identify means to block or reverse the metabolic changes and limit progression in the extent and severity of injury in wounds of burned soldiers. Microelectrodes will be used to measure changes in extracellular potassium 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 using enzymatic methods. Cells and subcellular organelles will be isolated for measurement of changes in function with time postburn.

25. (U) 8710 - 8809. A series of measurements of water content, dry weight, and pH of 20% sham and partial-thickness scald burn wounds from 1-72 h postburn were completed.

(U) 8810 - 8909. A variety of preparation procedures for cell suspensions from rat skin that would be suitable for measurement of cellular function in partial-thickness burn wounds were tested. The best yield of viable cells (trypan-blue impermeable) was obtained from minced tissue incubated first in trypsin solution followed by washing and subsequent incubation in a solution of collagenase and hyaluronidase. Biopsy samples of wound tissue were obtained from areas adjacent to the area in which microelectrode measurements were made and blood samples were obtained for

DD FORM 1498 83 MAR — EDITION OF MAR 68 IS OBSOLETE. — * USGPO 1988-491-003/50329

**CONTINUATION OF DD FORM 1498 FOR THE PROJECT ENTITLED "A STUDY OF BIOCHEMICAL CHANGES IN THE CELLULAR ENVIRONMENT OF TISSUE OF THE IN VIVO PARTIAL-THICKNESS RAT BURN WOUND"**

determination of changes in the levels of selected metabolites with time postburn.

(U) 8910 - 9009. Enzymatic procedures were used to analyze ATP and lactate in extracts of blood and wound tissue taken from sham and burn rats from 1-48 h postburn. ATP content of blood from burned rats varied from 85-115% of sham values over 48 h postburn. Blood lactate ranged from 60-103% of sham values during that time. Burn wound ATP decreased after 1 h postburn to its lowest value of 18% of sham at 24 h postburn. Burn wound lactate content reached approximately 180% of sham at 9 h postburn and 150% at 18 h postburn. Burn wound lactate decreased to approximately 65% of sham value at 24 and 48 h postburn, a time at which burn tissue pH had returned to normal values.

**SUPPLEMENTAL WORK UNIT INFORMATION FOR THE PROJECT ENTITLED "A STUDY OF BIOCHEMICAL CHANGES IN THE CELLULAR ENVIRONMENT OF TISSUE OF THE IN VIVO PARTIAL-THICKNESS RAT BURN WOUND"**

**Subrecord/Linking Accession Number:** DA311489

**Search Control Data:** W6M37B/W6N22D, 20 October 1989

**Product Identification:** For technical reports, refer to the US Army Institute of Surgical Research Annual Research Progress Report for fiscal years 1986-90.

**Unclassified Special Categories:** Lab Animals: Rats; RA II

**ANNUAL RESEARCH PROGRESS REPORT**

**PROJECT NUMBER:** 3M161102BS14-00 RESEARCH

**PROJECT TITLE:** A Study of Biochemical Changes in the Cellular Environment of Tissue of the <u>In Vivo</u> Partial-Thickness Rat Burn Wound

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

**1 October 1989 - 30 September 1990**

**INVESTIGATORS**

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

## ABSTRACT

**PROJECT NUMBER:** 3M161102BS14-00, 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 89 through 30 Sep 90

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

Enzymatic procedures were used to analyze ATP and lactate in extracts of blood and wound tissue taken from sham and burned rats from 1 - 48 hours postburn (PB). ATP content of blood from burned rats varied from 85 - 115% of sham values over 48 hours PB. Blood lactate ranged from 60 - 103% of sham values during that time. Burn wound ATP decreased after one hour PB to its lowest value of 18% of sham at 24 hours PB. Burn wound lactate content reached approximately 180% of sham at nine hours PB and was 150% of sham at 18 hours PB. Burn wound lactate then decreased to approximately 65% of sham value at 24 and 48 hours PB, a time at which burn tissue pH had returned to normal values.

# A STUDY OF BIOCHEMICAL CHANGES IN THE CELLULAR ENVIRONMENT OF TISSUE OF THE *IN VIVO* PARTIAL-THICKNESS RAT BURN WOUND

The objective of this study is to determine *in vivo* biochemical and metabolic changes in partial-thickness rat burn wounds during the early postburn period and to identify those changes which either foster or impede recovery of cellular function in such wounds.

In a previous report (1) we showed that both the quantity and the time course of edema accumulation were similar in partial-thickness and full-thickness burn wounds in rats during the first 24 hours PB. Edema volume, however, decreased more rapidly in the partial-thickness injuries. Burn wound pH decreased during the period of maximal edema, suggesting an accumulation of acidic metabolites in the wound. In this report we present preliminary measurements of ATP and lactate in the blood and in wound tissue of rats with sham or partial-thickness burns.

## MATERIALS AND METHODS

Sprague-Dawley rats weighing 180-200g were anesthetized with alpha-chloralose solution (5.5mg/100g, intraperitoneally). 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 total body surface area (TBSA). Immersion of this exposed area in water at 80°C for eight seconds produced a partial-thickness burn. Sham controls were anesthetized, clipped, placed in the protective mold, and an equivalent area on the dorsum was outlined in ink. The rats were housed in individual cages and permitted free access to food and water. No parenteral fluids were given. At selected times PB, groups of sham and burned rats were reanesthetized with alpha-chloralose before *in vivo* testing or collection of samples. After these studies, the rats were euthanized by injecting a lethal dose of alpha-chloralose, without having been allowed to awaken.

Blood and tissue samples were taken from groups of sham and burned rats at 1, 3, 6, 9, 18, 24, and 48 hours PB for analysis of ATP and lactate. Blood obtained by cardiac puncture was placed in a tube containing ACD solution that had been evaporated to dryness. After mixing, an aliquot was immediately added to an equal volume of cold 12% trichloroacetic acid (TCA) (2). The tubes were tightly capped, kept in ice for five minutes to assure protein precipitation and then quick frozen with liquid nitrogen before being stored in a freezer at -80°C. Just before analysis, the samples were thawed in ice water and centrifuged at 4°C at 1800g for 30 minutes. Supernates were decanted and kept in tubes in an ice-salt bath while being prepared for analysis.

Tissue samples consisting of skin and subcutaneous tissue down to fascia were obtained from the wounds using a hollow-bore 4mm

stainless steel drill tip driven by a Model 950 high-speed pneumatic biopsy drill (Alko Diagnostic Corp., Holliston, MA) connected to a thermos bottle containing a beaker of chilled 2-methylbutane (-150°C) immersed in liquid nitrogen. Continuous vacuum drew the biopsy through the drill into the 2-methylbutane, reducing to a minimum the delay between cutting and freezing. Samples were rapidly taken from 8 to 10 sites on each wound and pooled. The frozen samples were transferred to tubes chilled in an isopropyl alcohol-dry ice bath, tightly capped, transferred to liquid nitrogen, and then stored in a freezer at -80°C until processed.

Each weighed tissue sample was transferred to a tube containing 2ml cold 6% (w/v) perchloric acid (PCA) containing 1mM/1 EDTA and was kept ice-cold through all of the extraction procedure (3). The tissues were homogenized using a Polytron PT 10/35 homogenizer (Brinkmann Instruments, Inc., Westbury, NY) operated at speed setting 8 for two 15 second runs separated by a 2-3 minute chilling period. The tubes were allowed to stand for 30 minutes in an ice-salt bath before they were centrifuged at 1800g for 30 minutes at 2°C. The supernates were decanted and an aliquot neutralized by mixing with a predetermined volume of 2 N KOH with 0.4 M imidazole base and 0.4 M KCl. The tubes were kept in an ice bath for 10 minutes to allow crystallization of potassium chlorate before they were centrifuged at 1800g for 15 minutes at 2°C. The supernates were decanted into tubes, tightly capped, immediately frozen in liquid nitrogen, and stored in a freezer at -80°C until analysis. At that time the tissue extracts were thawed in chilled water and the tubes were kept in an ice-salt bath until the analyses were completed. Typically, no more tubes than could be analyzed in one hour were thawed at one time.

Enzymatic methods using commercially prepared reagent kits (Sigma Chemical Co., St. Louis, MO) were used to measure ATP and lactate in both the blood and tissue extracts. ATP was determined using the coupled phosphoglycerate kinase/glyceraldehyde phosphate dehydrogenase reaction. The decrease in absorbance at 340nm that results when NADH is oxidized to NAD is proportional to the amount of ATP originally present in the extract (2). Lactate was determined using lactate dehydrogenase in the presence of excess NAD and trapping the pyruvate formed with hydrazine to force the reaction to completion. The increased absorbance at 340nm due to NADH formation is proportional to the amount of lactate originally present (4).

Tissue pH was measured using a needle pH microelectrode paired with a micro reference electrode with glass barrel (Microelectrodes, Inc., Londonderry, NH) 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 EA 940 microprocessor controlled IonAnalyzer which was

equipped with a Model GLP printer (Orion Research,Inc., Boston, MA).

## RESULTS AND DISCUSSION

Preliminary results of a study of changes in ATP and lactate content of blood of rats with sham and partial-thickness burns are shown in Table 1. The ATP content of blood of burned rats was greater than that of sham rats at one hour PB, equal to sham at six and nine hours PB, and then decreased to a level slightly lower than sham at 48 hours PB. These differences were not very large and may have been due, at least in part, to changes in hemo-concentration that occur in burned rats during the early PB period. Lactate content of blood of burned rats was lower than that of sham rats except at six and 48 hours PB when the values for the two groups were essentially equal.

**TABLE 1**

**ATP AND LACTATE IN BLOOD OF RATS WITH PARTIAL-THICKNESS BURN WOUNDS**

| HOUR POSTBURN | ATP PERCENT OF SHAM VALUES | LACTATE |
|---|---|---|
| 1 | 115 | 59 |
| 3 | 115 | 78 |
| 6 | 101 | 104 |
| 9 | 100 | 93 |
| 18 | 94 | 71 |
| 24 | 92 | 63 |
| 48 | 86 | 103 |

ATP content of burn wound was equal to that of sham wounds at one hour PB, but then decreased at each time measured to reach its lowest value at 24 hours PB before increasing slightly at 48 hours PB. (Table 2) Burn wound lactate content increased rapidly to a maximum almost twice that of sham wound at nine hours PB and decreased only a small amount at 18 hours PB. At 24 and 48 hours PB, burn wound lactate content was down to about two-thirds that of sham wound.

The decrease in pH in burn wound correlated temporally with the decrease in ATP and increase in lactate content of the burn wound through 18 hours PB. (Table 2) At 24 hours and 48 hours PB, burn wound pH was normal (Sham = 7.09).

**TABLE 2**

**ATP AND LACTATE IN PARTIAL-THICKNESS RAT BURN WOUNDS**

| HOUR POSTBURN | BURN WOUND pH | ATP PERCENT OF SHAM VALUES | LACTATE |
|---|---|---|---|
| 1 | 6.95 | 98 | 105 |
| 3 | 6.82 | 79 | 120 |
| 6 | 6.75 | 87 | 128 |
| 9 | 6.85 | 49 | 179 |
| 18 | 6.92 | 28 | 152 |
| 24 | 7.10 | 19 | 63 |
| 48 | 7.10 | 30 | 65 |

Changes similar to these occur, but on a time scale of minutes rather than hours, in myocardium made ischemic by partial occlusion of the coronary artery (5). In addition, in ischemic myocardium there was a marked increase in intramural $Pco_2$ that we did not find in rat burn wound at the few times we made measurements. It is possible that increased $CO_2$ was formed in the burn wound, but was not retained by the burned skin. More measurements are needed to confirm this difference.

Khuri et al. compared simultaneous biochemical and histological changes in ischemic myocardium and concluded that the decrease in pH and increase in intramural $Pco_2$ are the most reliable indicators of the severity of cellular injury (6). It is not known how long a tissue can be exposed to such conditions and be able to recover cellular function. We are currently extending our sampling times to include intervals up to seven days PB and we will attempt to correlate simultaneous changes in ATP, lactate, pH, and $Pco_2$ content of burn wound with histological changes determined by both light and electron microscopy.

## REFERENCES


1. Brown WL, Mason AD, Jr., Pruitt BA, Jr.: A study of biochemical changes in the cellular environment of tissue of the in vivo partial-thickness rat burn wound. **USAISR Annual Research Progress Report for FY87**, p 255-263.

2. **Sigma Chemical Co. Procedure No. 366-UV.** Adenosine-5'-triphosphate (ATP) quantitative, enzymatic determination in blood at blood at 340nm, March 1989.

3. Williamson JR, Corkey BE: Assay of citric acid cycle intermediates and related compounds - update with tissue levels and intracellular distribution. In **Methods in Enzymology**. Fleischer S. and Packer K. (eds). Academic Press, New York, 1979, Vol. LV, p 201-202.

4. **Sigma Chemical Co. Procedure No. 826-UV**. Lactate quantitative, enzymatic determination in whole blood at 340nm, March, 1989.

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**RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY**

| 1. AGENCY ACCESSION | 2. DATE OF SUMMARY | REPORT CONTROL SYMBOL |
|---|---|---|
| DA315353 | 01 Oct 90 | DD-DR&E(AR) 636 |

| 3. DATE PREV SUM'RY | 4. KIND OF SUMMARY | 5. SUMMARY SCTY | 6. WORK SECURITY | 7. REGRADING | 8. DISS'N INSTR'N | 9. LEVEL OF SUM / A. WORK UNIT |
|---|---|---|---|---|---|---|
| 01 Oct 89 | D. Chg | U | U | | CX | |

| 10. NO./CODES | PROGRAM ELEMENT | PROJECT NUMBER | TASK AREA NUMBER | WORK UNIT NUMBER |
|---|---|---|---|---|
| a. PRIMARY | 61102A | 3M161102BS14 | CH | 314 |
| b. CONTRIBUTING | | | | |
| c. CONTRIBUTING | | | | |

11. TITLE *(Precede with Security Classification Code)* (U) The Effect of Interleukin 2 Administration on Mortality to Rats with Pseudomonas Burn Wound Sepsis

12. SUBJECT AREAS
0604 Anatomy and Physiology 0605 Medicine and Medical Research

| 13. START DATE | 14. ESTIMATED COMPLETION DATE | 15. FUNDING ORGANIZATION | 16. PERFORMANCE METHOD |
|---|---|---|---|
| 8710 | 9109 | DA | C. In-House |

17. CONTRACT/GRANT — MILITARY RELEVANCY CERTIFIED — APPROVED

| a. DATE EFFECTIVE | b. CONTRACT/GRANT NUMBER | c. TYPE | d. AMO | e. KIND OF AWARD | f. CUM/TOTAL |
|---|---|---|---|---|---|
| | | | | | |

18. RESOURCES ESTIMATE

| FISCAL YEARS | a. PROFESSIONAL WORK YEARS | b. FUNDS *(In thousands)* |
|---|---|---|
| 90 | 0.1 | 63 |
| 91 | 0.1 | 66 |

19. RESPONSIBLE DOD ORGANIZATION

a. NAME: US Army Institute of Surgical Research

b. ADDRESS *(include zip code)*: Fort Sam Houston, San Antonio, Texas 78234-5012

c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A

d. TELEPHONE NUMBER *(include area code)*: 512-221-2720

20. PERFORMING ORGANIZATION

a. NAME: US Army Institute of Surgical Research

b. ADDRESS: Fort Sam Houston, San Antonio, Texas 78234-5012

c. NAME OF PRINCIPAL INVESTIGATOR: CIOFFI, W G

d. TELEPHONE NUMBER *(include area code)*: 512-221-4440

e. NAME OF ASSOCIATE INVESTIGATOR *(if available)*

f. NAME OF ASSOCIATE INVESTIGATOR *(if available)*

21. GENERAL USE: FINA

MILITARY/CIVILIAN APPLICATION: M

22. KEYWORDS *(Precede EACH with Security Classification Code)* (U) Burns (Injuries); (U) Septicemia; (U) Prostaglandin; (U) Cells; (U) Pseudomonas

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS *(Precede text of each with Security Classification Code)*

23/24. (U) The objective of this work is to study the effects of exogenous IL 2 administration following burn wound infection in a rodent model. Due to the failure of IL 2 to improve survival in this infection model, the study has been extended to evaluate the effects of indomethacin, gamma interferon, and other cytokines on IL 2 receptor expression. The ability to enhance IL 2 receptor expression may allow one to reverse T cell abnormalities with the exogenous administration of IL 2.

25. (U) 8710 - 8809. The optimal dose which could be tolerated by the septic animal was identified. In LD100 and LD50 models, IL 2 administration concomitant with burning and seeding of the burn wound with Pseudomonas failed to improve survival. To increase IL 2 receptor expression, both high doses (5 mg/kg) and low doses (0.5 mg/kg) of indomethacin were administered with the IL 2. This failed to improve survival in the LD100 model.

(U) 8810 - 8909. The protocol for measuring IL 2 receptor expression in rodent splenocytes and peripheral blood mononuclear cells was developed.

(U) 8910 - 9009. Addenda to the protocol were approved during this reporting period. The ability of indomethacin, prostaglandin E2, and gamma interferon to improve IL2 receptor expression will be studied during the next fiscal year.

DD FORM 1498 83 MAR — EDITION OF MAR 68 IS OBSOLETE. — * USGPO. 1988 -491-003/50329

**SUPPLEMENTAL WORK UNIT INFORMATION FOR THE PROJECT ENTITLED "THE EFFECT OF INTERLEUKIN 2 ADMINISTRATION ON MORTALITY TO RATS WITH PSEUDOMONAS BURN WOUND SEPSIS"**

**Subrecord/Linking Accession Number:** DA313322

**Search Control Data:** W6N47A/W6N48A, 20 October 1989

**Product Identification:** For technical reports, refer to the US Army Institute of Surgical Research Annual Research Progress Report for fiscal years 1987-90.

**Unclassified Special Categories:** Lab Animals: Rats; RA II

**ANNUAL RESEARCH PROGRESS REPORT**

**PROJECT NUMBER:** 3M161102BS14-00, RESEARCH

**PROJECT TITLE:** The Effect of Interleukin-2 Administration in the Mortality of Rats with Pseudomonas Burn Wound Sepsis

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

**1 October 1989 - 30 September 1990**

**INVESTIGATORS**

William G. Cioffi, Jr., MD, Major, MC

## ABSTRACT

**PROJECT NUMBER:** 3M161102BS14-00, RESEARCH

**PROJECT TITLE:** The Effect of Interleukin 2 Administration on Mortality to Rats with Pseudomonas Burn Wound Sepsis

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

**PERIOD COVERED IN THIS REPORT:** 1 Oct 89 through 30 Sep 90

**INVESTIGATORS:** William G. Cioffi, Jr., MD, Major, MC

Addenda to the protocol were approved during this reporting period. The ability of indomethacin, prostaglandin E2, and gamma interferon to improve IL2 receptor expression will be studied during the next fiscal year.

**THE EFFECT OF INTERLEUKIN 2 ADMINISTRATION ON MORTALITY TO RATS WITH PSEUDOMONAS BURN WOUND SEPSIS**

No work was done on this protocol during fiscal year 1990, and therefore no report is submitted.

**RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY**

| 1. AGENCY ACCESSION | 2. DATE OF SUMMARY | REPORT CONTROL SYMBOL |
|---|---|---|
| DA315354 | 13 Jun 90 | DD-DR&E(AR) 636 |

| 3. DATE PREV SUM'RY | 4. KIND OF SUMMARY | 5. SUMMARY SCTY | 6. WORK SECURITY | 7. REGRADING | 8. DISB'N INSTR'N | 9. LEVEL OF SUM A. WORK UNIT |
|---|---|---|---|---|---|---|
| 1 Oct 89 | H. Term | U | U | | CX | |

| 10. NO./CODES: | PROGRAM ELEMENT | PROJECT NUMBER | TASK AREA NUMBER | WORK UNIT NUMBER |
|---|---|---|---|---|
| a. PRIMARY | 61102A | 3M161102BS14 | CK | 315 |
| b. CONTRIBUTING | | | | |
| c. CONTRIBUTING | | | | |

11. TITLE *(Precede with Security Classification Code)* (U) Development of Thermal Ionization Mass Spectrometry Methodology for the Study of Calcium Metabolism

12. SUBJECT AREAS
0601 Biochemistry 0604 Anatomy and Physiology

| 13. START DATE | 14. ESTIMATED COMPLETION DATE | 15. FUNDING ORGANIZATION | 16. PERFORMANCE METHOD |
|---|---|---|---|
| 8805 | 9006 | DA | C. In-House |

17. CONTRACT/GRANT MILITARY RELEVANCY CERTIFIED
a. DATE EFFECTIVE APPROVED BY [signature]
b. CONTRACT/GRANT NUMBER
c. TYPE
d. AMOUNT
e. KIND OF AWARD
f. CUM/TOTAL

18. RESOURCES ESTIMATE

| FISCAL YEARS | a. PROFESSIONAL WORK YEARS | b. FUNDS *(In thousands)* |
|---|---|---|
| 89 | 0.3 | 21 |
| 90 | 0.3 | 22 |

19. RESPONSIBLE DOD ORGANIZATION
a. NAME: US Army Institute of Surgical Research
b. ADDRESS *(Include zip code)*: Fort Sam Houston, San Antonio, Texas 78234-5012
c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A
d. TELEPHONE NUMBER *(Include area code)*: 512-221-2720

20. PERFORMING ORGANIZATION
a. NAME: US Army Institute of Surgical Research
b. ADDRESS: Fort Sam Houston, San Antonio, Texas 78234-5012
c. NAME OF PRINCIPAL INVESTIGATOR: SHIPPEE, R L
d. TELEPHONE NUMBER *(Include area code)*: 512-221-7138
f. NAME OF ASSOCIATE INVESTIGATOR *(If available)*: VAUGHAN, G M
g. NAME OF ASSOCIATE INVESTIGATOR *(If available)*: OKERBERG, C V

21. GENERAL USE: FINA
MILITARY/CIVILIAN APPLICATION: M

22. KEYWORDS *(Precede EACH with Security Classification Code)* (U) Burns (Injuries); (U) Mass Spectrometry; (U) Metabolism; (U) Homeostasis; (U) Lab Animals; (U) Rats; (U) RA II.

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS *(Precede text of each with Security Classification Code)*

23. (U) A DTIC literature search was conducted under DTIC request number W6N55C dated 20 October 1989 for the technical report database and request number W6N56B dated 20 October 1989 for the work unit database. The proposed effort will not result in the duplication of effort. The objective of this work is to develop thermal ionization mass spectrometry techniques to investigate the homeostatic mechanisms of calcium regulation after thermal injury. Methodology developed from this study will be applied to clinical studies of calcium metabolism in thermally injured soldiers.

24. (U) Male Sprague-Dawley rats will be used as a burn model to study the effect of burn injury on calcium homeostasis. Stable isotopes of calcium will be used to perform calcium kinetic analyses after burn injury. Total fecal and urine excretion will be collected to perform calcium balance calculations. The combined data from the kinetic analyses and the balance calculations will be analyzed using a mathematical modeling computer program to determine the effect of burn injury on the various aspects of calcium metabolism.

25. (U) 8805 - 9006. Despite modifications of the mass spectrometer, we were unable to reach the precision necessary (< 0.05%) to detect stable isotopes of calcium in serum. A mass spectrometer specifically designed to detect stable isotopes of heavy metals by dual filament, thermal ionization would be needed to support this project. For technical reports, refer to the US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Year 1990.

DD FORM 83 MAR 1498 EDITION OF MAR 88 IS OBSOLETE. * USGPO 1988-491-003/50329

**ANNUAL RESEARCH PROGRESS REPORT**

**PROJECT NUMBER:** 3M161102BS14-00, RESEARCH

**PROJECT TITLE:** Development of Thermal Ionization Mass Spectrometry (TIMS) Methodology for the Study of Calcium Metabolism

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

**1 October 1989 - 13 June 1990**

**INVESTIGATORS**

Ronald L. Shippee, PhD, Major, MS
George M. Vaughan, MD, Colonel, MC
Carlin V. Okerberg, DVM, PhD, Major, DVM
Avery A. Johnson, BS
Basil A. Pruitt, Jr., MD, Colonel, MC

## ABSTRACT

**PROJECT NUMBER:** 3M161102BS14-00, RESEARCH

**PROJECT TITLE:** Development of Thermal Ionization Mass Spectrometry (TIMS) Methodology for the Study of Calcium Metabolism

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

**PERIOD COVERED IN THIS REPORT:** 1 Oct 89 through 13 Jun 90

**INVESTIGATORS:** Ronald L. Shippee, PhD, Major, MS
George M. Vaughan, MD, Colonel, MC
Carlin V. Okerberg, DVM, PhD, Major, VC
Avery A. Johnson, BS
Basil A. Pruitt, Jr., MD, Colonel, MC

## DEVELOPMENT OF THERMAL IONIZATION MASS SPECTROMETRY (TIMS) METHODOLOGY FOR THE STUDY OF CALCIUM METABOLISM

Hypocalcemia (6-7 mg/100 ml) is a consistent observation in patients with burns > 30% of the total body surface area (1). This sequel may be due to a number of reasons. Lennquist et al. (2) reported low serum calcium in burn patients which correlated with serum albumin; serum calcium/albumin ratios were always within normal range. Szyfelbein et al. (3) found good correlation of total serum calcium to total protein. Ionized calcium was depressed and showed a poor correlation to total protein. Ionized calcium remained depressed even when serum calcium and total protein were restored to normal levels. Intercellular calcium sequestering in burned muscle tissue has been reported by a number of investigators (4-6). Transeschar calcium leaching, which may occur in patients treated with silver nitrate soaks, has been reported to lower the serum calcium level to a point where symptoms occur (7). Abnormalities may exist in the secretion and metabolic function of calcium-regulating hormones, calcitonin and parathyroid hormone, in the postburn period. Loven et al. (8) and Lennquist et al. (2) have reported that low serum ionized calcium levels persist in burn patients, even though serum concentrations of calcitonin are elevated.

Despite numerous reports concerning abnormalities in calcium metabolism, the mechanisms involved are ill-defined. Recent technological advances in TIMS have made it practical to use stable isotope methodology to study the dynamics of calcium metabolism in biological systems (9-10).

### MATERIALS AND METHODS

**Study Design.** Thirty-six male Sprague-Dawley rats weighing 250-300 g will be individual housed in stainless steel hanging cages and observed for 1 wk prior to entry into the study to exclude the presence of any preexisting diseases. Twelve animals will be used to develop techniques. The remaining 24 animals will be divided into two groups (n=12), control group and burn group. A semipurified diet designed to meet all known nutrient requirements of the adult rat and distilled deionized water will be fed ad libitum. After a 2-wk equilibration period on this diet, the animals will be anesthetized with sodium pentobarbital (35 mg/kg IP), the dorsal surface will be shaved, and a 30% total body surface area scald or sham burn will be administered. For animals in the burn group, the dorsal area will be exposed to 100°C water for 10 sec. Animals in the control group will be handled in the same manner as those in the burn group, but they will exposed to water at room temperature. All animals will be placed back in their cages and allowed to recover from anesthesia without resuscitation. A pair-feeding regimen will be used from the day of burn injury until final disposition of the animals. Control animals will be fed the amount consumed by a weight-matched burned

rat during the previous 24-h period. Blood samples will be taken daily for 3 days from the tail vein with no anesthesia while the animals are restrained in a tube-type restraint device. Ten days postburn, the animals will be administered two isotopes of calcium, one given orally ($^{44}Ca$, 0.5 mg/kg) and the other intravenously ($^{42}Ca$, 0.1 mg/kg). Blood samples (300 $\mu$l) will be collected at 0.5, 1, 2, 4, 8, 12, 24, and 48 h following isotope administration. At 72 h, the animals will be reanesthetized with sodium pentobarbital (35 mg/kg IP). A ventral laparotomy will be performed and the animal will be exsanguinated via the caudal vena cava. Calcium will be extracted from the serum using ammonium oxalate precipitation, and the ratios of the calcium isotopes will be measured using TIMS (10). Feces and urine will be collected over the total 72-h study period. Serum samples collected at 72 h will be analyzed for total calcium, albumin, total protein, calcitonin, and parathyroid hormone. Aliquots of the feces and urine collections will be analyzed for total calcium and for the ratio of the stable calcium isotopes. Mathematical modeling procedures will be accomplished as outlined by Moore et al. (9). The experimental design allows for determination of net endogenous calcium fecal excretion, net dietary calcium absorption, calcium balance, and bone calcium deposition and resorption.

**Determination of Number of Animals Required.** Twelve animals will be necessary for refinement of a number of techniques associated with the TIMS. Twenty-four animals will be needed for the study, 12 for the control group and 12 for the burn group.

**Data Analysis Plan.** Significant differences between the control and burn groups for the various parameters will be accomplished using the student's t test ($P < 0.05$).

## RESULTS/DISCUSSION

Modifications of the mass spectrometer have been completed. Methods have been developed to determine natural abundance of calcium stable isotopes in standard solutions. Work will proceed upon delivery of enriched standards of stable calcium isotopes that will enable us to spike standards and biological samples.

## PRESENTATIONS/PUBLICATIONS

None.

## REFERENCES

1. Merrill RH, Myers WD, and Jacobson HR: Evaluation of calcium metabolism in burned troops. In **US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Year 1976**, p 292-.

2. Lennquist S, Lindell B, Nordstrom H, et al: Hypophosphatemia in severe burns: a prospective study. **Acta Chir Scand** 145:1-6, 1979.

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5. Baar S: The effect of thermal injury on the loss of calcium from calcium loaded red cells: its relationship to red cell function and patient survival. **Clin Chim Acta** 126:25-39, 1982.

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7. Pruitt BA Jr: Other complications of burn injury. In Artz CP, Moncrief JA, and Pruitt BA Jr (eds), **Burns, A Team Approach**. Philadelphia: WB Saunders Company, 1979, Chapter 36, p 523-52.

8. Loven L, Nordstrom H, and Lennquist S: Changes in calcium and phosphate and their regulating hormones in patients with severe burn injuries. **Scand J Plast Reconstr Surg** 18:49-53, 1984.

9. Moore LJ, Machlan LA, Lim MO, et al: Dynamics of calcium metabolism in infancy and childhood. I. Methodology and quantification in the infant. **Pediatr Res** 19:329-34, 1985.

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11. Walker HL and Mason AD Jr: A standard animal burn. **J Trauma** 8:1049-51, 1968.

| RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY | | | | 1. AGENCY ACCESSION DA315355 | 2. DATE OF SUMMARY 01 Oct 90 | REPORT CONTROL SYMBOL DD-DR&E(AR) 636 |
|---|---|---|---|---|---|---|
| 3. DATE PREV SUM'RY 01 Oct 89 | 4. KIND OF SUMMARY D. Chg | 5. SUMMARY SCTY U | 6. WORK SECURITY U | 7. REGRADING | 8. DISS'N INSTR'N CX | 9. LEVEL OF SUM A. WORK UNIT |
| 10. NO./CODES | PROGRAM ELEMENT | PROJECT NUMBER | | TASK AREA NUMBER | WORK UNIT NUMBER | |
| a. PRIMARY | 61102A | 3M161102BS14 | | CA | 316 | |
| b. CONTRIBUTING | | | | | | |
| c. CONTRIBUTING | | | | | | |

11. TITLE (Precede with Security Classification Code) (U) The Effect of High Frequency Ventilation on VA/Q in Sheep with Inhalation Injury

12. SUBJECT AREAS
0604 Anatomy and Physiology 0605 Medicine and Medical Research

| 13. START DATE | 14. ESTIMATED COMPLETION DATE | 15. FUNDING ORGANIZATION | 16. PERFORMANCE METHOD |
|---|---|---|---|
| 8701 | 9109 | DA | C. In-House |

17. CONTRACT/GRANT MILITARY RELEVANCY CERTIFIED
a. DATE EFFECTIVE APPROVED BY Paul A. Trimble
b. CONTRACT/GRANT NUMBER
c. TYPE
d. AMOUNT
e. KIND OF AWARD
f. CUM/TOTAL

18. RESOURCES ESTIMATE

| FISCAL YEARS | a. PROFESSIONAL WORK YEARS | b. FUNDS (In thousands) |
|---|---|---|
| 90 | 0.1 | 77 |
| 91 | 0.1 | 81 |

19. RESPONSIBLE DOD ORGANIZATION
a. NAME: US Army Institute of Surgical Research
b. ADDRESS (include zip code): Fort Sam Houston, San Antonio, Texas 78234-5012
c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A
d. TELEPHONE NUMBER (include area code): 512-221-2720

20. PERFORMING ORGANIZATION
a. NAME: US Army Institute of Surgical Research
b. ADDRESS: Fort Sam Houston, San Antonio, Texas 78234-5012
c. NAME OF PRINCIPAL INVESTIGATOR: CIOFFI, W G
d. TELEPHONE NUMBER (include area code): 512-221-4440
f. NAME OF ASSOCIATE INVESTIGATOR (if available)
g. NAME OF ASSOCIATE INVESTIGATOR (if available)

21. GENERAL USE: FINA
MILITARY/CIVILIAN APPLICATION: M

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Burns (Injuries); (U) Inhalation; (U) Respirations; (U) Pulmonary Function; (U) Therapy

23 TECHNICAL OBJECTIVE 24 APPROACH 25 PROGRESS (Precede text of each with Security Classification Code)

23/24. (U) The objective of this work is to compare the effects of volumetric diffusive ventilation and conventional ventilation on pulmonary and hemodynamic indices which are altered in an ovine inhalation injury model. Inhalation injury will be induced using the standard ovine smoke inhalation model developed at this Institute. Animals will be randomized to treatment with either conventional or high frequency ventilation. Changes in VA/Q as well as other pulmonary and physiologic measurements will be compared between groups.

25. (U) 8710 - 8809. Due to technical problems with the newly acquired mass spectrophotometer, the multiple inert gas elimination technique was not operational.

(U) 8810 - 8909. The multiple inert gas elimination technique was revalidated and 8 animals with inhalation injury and 6 normal control animals were studied.

(U) 8910 - 9009. High frequency flow interruption was found to be inferior to conventional ventilation when instituted 24 h following smoke injury in an ovine model. High frequency ventilation resulted in a further shift of the VA/Q curve to the left, indicating additional VA/Q mismatching.

DD FORM 1498 83 MAR — EDITION OF MAR 68 IS OBSOLETE — * USGPO 1988-491-003/50326

**SUPPLEMENTAL WORK UNIT INFORMATION FOR THE PROJECT ENTITLED "THE EFFECT OF HIGH FREQENCY VENTILATION ON VA/Q IN SHEEP WITH INHALATION INJURY"**

**Subrecord/Linking Accession Number:** DA312336

**Search Control Data:** W6003E/W6006A, 20 October 1989

**Product Identification:** For technical reports, refer to the US Army Institute of Surgical Research Annual Research Progress Report for fiscal years 1987-90.

**Unclassified Special Categories:** Lab Animals: Sheep; RA II

**ANNUAL RESEARCH PROGRESS REPORT**

**PROJECT NUMBER:** 3M161102BS14-00, RESEARCH

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

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

**1 October 1989 - 30 September 1990**

**INVESTIGATORS**

William G. Cioffi, Jr., MD, Major, MC

## ABSTRACT

**PROJECT NUMBER:** 3M161102BS14-00, RESEARCH

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

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

**INVESTIGATORS:** William G. Cioffi, Jr., MD, Major, MC

High frequency flow interruption was found to be inferior to conventional ventilation when instituted 24 h following smoke injury in an ovine model. High frequency ventilation resulted in a further shift of the VA/Q curve to the left, indicating additional VA/Q mismatching.

**THE EFFECT OF HIGH FREQUENCY VENTILATION ON VA/Q IN SHEEP WITH INHALATION INJURY**

## INTRODUCTION

Severe inhalation injury has been shown to cause hypoxia, hypercarbia and a shift of VA/Q to the left (i.e., increase in segments with VA/Q greater than 0 but less than 1). Attempts to alter these derangements with conventional ventilation utilizing positive end expiratory pressure (PEEP) resulted in an increased dead space ventilation but had no significant effect on shunt or low VA/Q compartments. The study was designed to investigate the effects of high frequency percussive ventilation on these changes.

High frequency ventilation has been proposed as a means of increasing ventilation of the low VA/Q compartments. In a dog model using methacholine to induce low VQ compartments, Wagner was unable to demonstrate a beneficial effect of high frequency oscillation/ventilation (3). This ventilator, however, was relatively inefficient in terms of gas exchange. Recent availability of the high frequency oscillatory ventilator with an active exhalation phase has made it possible to study the effect of this form of high frequency ventilation in the ovine smoke injury model. Our previous work, utilizing a high frequency flow interrupter with passive exhalation, showed a lack of benefit and actual detriment in some animals as compared to conventional ventilation following smoke injury. The addition of active exhalation should allow for ventilation at lower peak airway pressures while at the same time allowing maintenance of higher mean airway pressures to improve oxygenation.

The purpose of this study is to compare high frequency oscillatory ventilation with conventional ventilation and changes in the pulmonary and hemodynamic parameters which are altered in an ovine inhalation injury model.

## MATERIALS AND METHODS

Neutered male sheep weighing between 25 and 45 kg will be utilized throughout the study. Each sheep will be housed in a conventional outdoor run and have access to commercial feed and water ad libitum. Sheep will be dewormed two weeks prior to use. Inhalation injury will be induced using the standard ovine smoke inhalation model developed at the United States Army Institute of Surgical Research.

Sheep will be exposed to a moderate smoke injury as previously described. Twenty-four hours following injury the sheep will be reintubated and ventilated by three techniques with three different ventilators; conventional ventilation, high frequency flow interruption, and high frequency oscillatory ventilation. In the first experiment, six sheep will be studied with standard pulmonary

and hemodynamic measurements made. On the day of the experiment a peripheral venous catheter, central venous pressure catheter, a balloon directed thermodilution pulmonary artery catheter (7 French, Swan-Ganz catheter, American Edwards Company), and a femoral arterial catheter will be placed following general anesthesia and intubation. Anesthesia will be maintained with chloralose (0.05 grams/kg body weight) and the animals will be paralyzed with pancuronium bromide. After placement of all catheters, animals will be positioned prone and conventional mechanical ventilation will be continued with a volume limited ventilator (Bear 2, Bear Medical Systems, Inc.). Ventilator settings will be altered to maintain an arterial Ph between 7.35 and 7.40, and a $PO_2$ between 80 and 100 millimeters of mercury. Lactated ringer's will be constantly infused at the rate of 1 ml/kg/hr. Central venous pressure and pulmonary artery repair will be monitored by Stathan P23 DB transducers and systemic artery pressures by Hewlett-Packard 1290A Quartz Transducer. Heart rate, blood pressure, central venous pressure, pulmonary artery pressures, cardiac output, arterial blood pressures, and arterial blood gases will be measured every thirty minutes. Once the ventilator settings are maximized the animals will be switched to one of two forms of high frequency ventilation. Upon stabilization of the animal on this form of ventilation and data measurements, the animal will then be switched to the second form of high frequency ventilation. Repeat cardiopulmonary parameters will be measured following stabilization on this ventilator. If high frequency oscillatory ventilation improves oxygenation in moderately smoke-injured animals, then an additional set of animals will be studied in which VA/Q will be measured using the multiple inert gas illumination technique (MIGET). In this set of experiments, 21 animals will be studied. Seven animals will receive a mild smoke injury, seven animals a moderate smoke injury, and seven animals a severe smoke injury. VA/Q distribution will be measured 24 hours following injury on each of the three types of ventilation. In this set of experiments, the Ringer's lactate infusion will be replaced with a Ringer's lactate solution containing six inert gases (sulphur hexachloride, krypton, cyclopropane, halothane, ether and acetone) which will be infused at a rate of 0.1 ml/kg/ min. After a 30 minute stabilization period, arterial and mixed venous blood will be drawn anaerobically in a preweighed heparinized syringe (30 ml matched glass) simultaneously. Blood samples will be analyzed immediately by gas chromatography mass spectrophotometer. The animals will then be switched to an alternate form of ventilation and six gas measurements repeated. The MIGET data will be stored in a data quantified by software program on the VAX computer system.

## RESULTS

During this reporting period, four animals were studied in Phase I of the protocol. Following moderate smoke injury, the animals were easily supported using conventional ventilatory

techniques at 24 hours following injury with arterial $PO_2$s ranging from 80 to 100 millimeters of mercury. Transition to the new high frequency oscillatory ventilator resulted in a decrement of pulmonary function. Airway pressures exceeding 40 centimeters of water were necessary to ventilate and oxygenate the animals adequately. The high frequency oscillator was unable to deliver sufficient sized tidal breaths to support the animal on a pure form of high frequency oscillatory ventilation. Prior to continuing this protocol, benchwork utilizing this ventilator in an attempt to increase volume output will be necessary.

## DISCUSSION

None.

## PRESENTATIONS/PUBLICATIONS

None.

## REFERENCES


1. Shimazu T, Tetouo Y, Hubbard GB, et al: Inequality of VA/Q ratios following smoke inhalation injury and the effect of angiotensin analogues. In **Annual Research Progress Report, US Army Institute of Surgical Research**, Fort Sam Houston, Texas, Washington, DC, US Army Research and Development Command, 1985.

2. Shimazu T: Acute effects of positive end-expiratory pressure (PEEP) on cardiopulmonary indices, including VA/Q ratios, unpublished data.

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5. Cioffi WG, Jordan BS, Johnson AA: The effect of high frequency ventilation on VA/Q in sheep with inhalation injury. **Annual Research Progress Report, US Army Institute of Surgical Research**, Fort Sam Houston, Texas, 1989.

| RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY | | | | 1. AGENCY ACCESSION: DA315356 | 2. DATE OF SUMMARY: 01 Oct 90 | REPORT CONTROL SYMBOL: DD-DR&E(AR) 636 |
|---|---|---|---|---|---|---|
| 3. DATE PREV SUM'RY: 01 Oct 89 | 4. KIND OF SUMMARY: D. Chg | 5. SUMMARY SCTY: U | 6. WORK SECURITY: U | 7. REGRADING: | 8. DISB'N INSTR'N: CX | 9. LEVEL OF SUM A. WORK UNIT |

| 10. NO./CODES: | PROGRAM ELEMENT | PROJECT NUMBER | TASK AREA NUMBER | WORK UNIT NUMBER |
|---|---|---|---|---|
| a. PRIMARY | 61102A | 3M161102BS14 | AZ | 317 |
| b. CONTRIBUTING | | | | |
| c. CONTRIBUTING | | | | |

11. TITLE *(Precede with Security Classification Code)* (U) Effects of Replacement Therapy on Hemodynamic Parameters in an Ovine Model of Controlled Pure Plasma Loss

12. SUBJECT AREAS
0604 Anatomy and Physiology 0605 Medicine and Medical Research

| 13. START DATE | 14. ESTIMATED COMPLETION DATE | 15. FUNDING ORGANIZATION | 16. PERFORMANCE METHOD |
|---|---|---|---|
| 8701 | 9109 | DA | C. In-House |

17. CONTRACT/GRANT — MILITARY RELEVANCY CERTIFIED APPROVED BY [signature]
a. DATE EFFECTIVE
b. CONTRACT/GRANT NUMBER
c. TYPE — d. AMOUNT
e. KIND OF AWARD — f. CUM/TOTAL

18. RESOURCES ESTIMATE

| FISCAL YEARS | a. PROFESSIONAL WORKYEARS | b. FUNDS *(In thousands)* |
|---|---|---|
| 90 | 0.1 | 57 |
| 91 | 0.1 | 60 |

| 19. RESPONSIBLE DOD ORGANIZATION | 20. PERFORMING ORGANIZATION |
|---|---|
| a. NAME: US Army Institute of Surgical Research | a. NAME: US Army Institute of Surgical Research |
| b. ADDRESS *(include zip code)*: Fort Sam Houston, San Antonio, Texas 78234-5012 | b. ADDRESS: Fort Sam Houston, San Antonio, Texas 78234-5012 |
| c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A | c. NAME OF PRINCIPAL INVESTIGATOR: CIOFFI, W G |
| d. TELEPHONE NUMBER *(include area code)*: 512-221-2720 | d. TELEPHONE NUMBER *(include area code)*: 512-221-4440 |
| 21. GENERAL USE: FINA<br>MILITARY/CIVILIAN APPLICATION: M | e. NAME OF ASSOCIATE INVESTIGATOR *(if available)*: MASON, A D<br>g. NAME OF ASSOCIATE INVESTIGATOR *(if available)*: OKERBERG, C V |

22. KEYWORDS *(Precede EACH with Security Classification Code)* (U) Burns (Injuries); (U) Albumins; (U) Shock (Pathology); (U) Resuscitation

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS *(Precede text of each with Security Classification Code)*

23/24. (U) The objective of this work is to determine the hemodynamic consequences of controlled pure plasma loss in sheep using a method to simulate the acute burn. A plasmapheresis filter will be used to produce intravascular plasma loss similar to that caused by burn injury. This device selectively removes plasma while leaving the formed elements of blood in the vascular system.

25. (U) 8710 - 8809. Eight animals were studied and the model was established. Animals subjected to pure plasma loss were resuscitated with several resuscitation schema, including crystalloid and colloid fluids.

(U) 8810 - 8909. Eight animals were studied.

(U) 8910 - 9009. Analyses of the data from the animals studied indicate that pure plasma volume loss can be replaced with either plasma or crystalloid solutions. The volume of crystalloid fluid required to achieve replacement is greater than the volume of colloid. These changes will now be validated in a 50% burn model.

DD FORM 83 MAR 1498 EDITION OF MAR 68 IS OBSOLETE © USGPO 1986-491-003/50325

**SUPPLEMENTAL WORK UNIT INFORMATION FOR THE PROJECT ENTITLED "EFFECTS OF REPLACEMENT THERAPY ON HEMODYNAMIC PARAMETERS IN AN OVINE MODEL OF CONTROLLED PURE PLASMA LOSS"**

**Subrecord/Linking Accession Number:** DA312334

**Search Control Data:** W6O21F/W6023D, 20 October 1989

**Product Identification:** For technical reports, refer to the US Army Institute of Surgical Research Annual Research Progress Report for fiscal years 1987-90.

**Unclassified Special Categories:** Lab Animals: Sheep; RA II

**ANNUAL RESEARCH PROGRESS REPORT**

**PROJECT NUMBER:** 3M161102BS14-00, RESEARCH

**PROJECT TITLE:** The Effects of Replacement Therapy on Hemodynamic Parameters in an Ovine Model Controlled Pure Plasma Loss

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

**1 October 1989 - 30 September 1990**

**INVESTIGATORS**

William G. Cioffi, Jr., MD, Major, MC
Arthur D. Mason, Jr., MD
Carlin V. Okerberg, DVM, Lieutenant Colonel, VC

## ABSTRACT

**PROJECT NUMBER:** 3M161102BS14-00, RESEARCH

**PROJECT TITLE:** Effects of Replacement Therapy on Hemodynamic Parameters in an Ovine Model of Controlled Pure Plasma Loss

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

**PERIOD COVERED IN THIS REPORT:** 1 Oct 89 through 30 Sep 90

**INVESTIGATORS:** William G. Cioffi, Jr., MD, Major, MC
Arthur D. Mason, Jr., MD
Carlin V. Okerberg, DVM, Lieutenant Colonel, VC

Analyses of the data from the animals studied indicate that pure plasma volume loss can be replaced with either plasma or crystalloid solutions. The volume of crystalloid fluid required to achieve replacement is greater that the volume of colloid. These changes will now be validated in a 50% burn model.

**EFFECTS OF REPLACEMENT THERAPY ON HEMODYNAMIC PARAMETERS IN AN OVINE MODEL OF CONTROLLED PURE PLASMA LOSS**

No work was done on this protocol during fiscal year 1990, therefore, no report is submitted.

| RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY | | | | 1. AGENCY ACCESSION | 2. DATE OF SUMMARY | REPORT CONTROL SYMBOL |
|---|---|---|---|---|---|---|
| | | | | DA315357 | 01 Oct 90 | DD-DRA&IAR) 636 |
| 3. DATE PREV SUM'RY | 4. KIND OF SUMMARY | 5. SUMMARY SCTY | 6. WORK SECURITY | 7. REGRADING | 8. DISB'N INSTR'N | 9. LEVEL OF SUM A. WORK UNIT |
| 01 Oct 89 | D. Chg | U | U | | CX | |

| 10. NO./CODES | PROGRAM ELEMENT | PROJECT NUMBER | TASK AREA NUMBER | WORK UNIT NUMBER |
|---|---|---|---|---|
| a. PRIMARY | 61102A | 3M161102BS14 | EA | 318 |
| b. CONTRIBUTING | | | | |
| c. CONTRIBUTING | | | | |

11. TITLE (Precede with Security Classification Code) (U) Antibacterial and Wound Healing Effects of Silver-Nylon Electrodes with Weak Direct Current

12. SUBJECT AREAS
0601 Biochemistry 0605 Medicine and Medical Research

| 13. START DATE | 14. ESTIMATED COMPLETION DATE | 15. FUNDING ORGANIZATION | 16. PERFORMANCE METHOD |
|---|---|---|---|
| 8808 | 9109 | DA | C. In-House |

17. CONTRACT/GRANT MILITARY RELEVANCY CERTIFIED APPROVED BY [signature]
a. DATE EFFECTIVE
b. CONTRACT/GRANT NUMBER
c. TYPE
d. AMOUNT
e. KIND OF AWARD
f. CUM/TOTAL

18. RESOURCES ESTIMATE

| a. FISCAL YEARS | a. PROFESSIONAL WORKYEARS | b. FUNDS (In thousands) |
|---|---|---|
| 90 | 1.0 | 99 |
| 91 | 1.0 | 104 |

19. RESPONSIBLE DOD ORGANIZATION

a. NAME
US Army Institute of Surgical Research

b. ADDRESS (Include zip code)
Fort Sam Houston
San Antonio, Texas 78234-5012

c. NAME OF RESPONSIBLE INDIVIDUAL
PRUITT, B A

d. TELEPHONE NUMBER (Include area code)
512-221-2720

20. PERFORMING ORGANIZATION

a. NAME
US Army Institute of Surgical Research

b. ADDRESS
Fort Sam Houston
San Antonio, Texas 78234-5012

c. NAME OF PRINCIPAL INVESTIGATOR
CHU, C S

d. TELEPHONE NUMBER (Include area code)
512-221-3411

e. NAME OF ASSOCIATE INVESTIGATOR (If available)
MC MANUS, A T

g. NAME OF ASSOCIATE INVESTIGATOR (If available)
OKERBERG, C V

21. GENERAL USE
FINA

MILITARY/CIVILIAN APPLICATION: M

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Burns (Injuries); (U) Wounds and Injuries; (U) Healing; (U) Skin Grafts; (U) Direct Current; (U) Therapy

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

23/24. (U) The objective of this work is to determine the antimicrobial and wound healing effects of weak direct current and to measure silver concentrations of burn eschars and underlying tissue as a function of time and direct current. The guinea pig will be used to study healing in deep second degree burns, third degree burns, donor site wounds, and skin grafts with and without stimulation with weak direct current.

25. (U) 8710 - 8809. This project was approved by the USAISR Research Council and US Army Institute of Surgical Research Animal Care and Use Committee during the fourth quarter of fiscal year 1988.

(U) 8810 - 8909. In addition to effective topical antimicrobial action, direct current-conducting silver-nylon dressings were found to improve wound healing. Using a guinea pig model of scald injury, application of low amperage current reduced scar formation, reduced wound contraction, and improved hair follicle survival. Similar treatment following excision of partial-thickness scalds and autografting improved graft healing time and reduced scarring.

(U) 8910 - 9009. Direct current treatment using silver-nylon dressings enhanced the speed and quality of healing of partial-thickness scald burns in the guinea pig. Dorsal scald wounds treated with direct current reepithelized by 12 days postburn. Split-thickness grafts taken from healed scald wounds treated with direct current showed more rapid

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revascularization, decreased contraction, improved hair survival, and decreased dermal fibrosis when compared to control animals ($P < 0.05$).

**SUPPLEMENTAL WORK UNIT INFORMATION FOR THE PROJECT ENTITLED "ANTIBACTERIAL AND WOUND HEALING EFFECTS OF SILVER-NYLON ELECTRODES WITH WEAK DIRECT CURRENT"**

**Subrecord/Linking Accession Number:** Not applicable.

**Search Control Data:** W6O35C/W6O36B, 20 October 1989

**Product Identification:** For technical reports, refer to the US Army Institute of Surgical Research Annual Research Progress Report for fiscal years 1988-90.

**Unclassified Special Categories:** Lab Animals: Guinea Pigs; Rats; RAII

**ANNUAL RESEARCH PROGRESS REPORT**

**PROJECT NUMBER:** 3M161102BS14-00, RESEARCH

**PROJECT TITLE:** Antibacterial and Wound Healing Effects of Silver-Nylon Electrodes with Weak Direct Current

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

**1 October 1989 - 30 September 1990**

**INVESTIGATORS**

Chi-Sing Chu, MD
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Carlin V. Okerberg, DVM, Lieutenant Colonel, VC
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## ABSTRACT

**PROJECT NUMBER:** 3M161102BS14-00, RESEARCH

**PROJECT TITLE:** Antibacterial and Wound Healing Effects of Silver-Nylon Electrodes with Weak Direct Current

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

**PERIOD COVERED IN THIS REPORT:** 1 Oct 89 through 30 Sep 90

**INVESTIGATORS:** Chi-Sing Chu, MD
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We have examined the effects of direct current conducted through silver-nylon dressings on the healing time and morphologic maturation of split-thickness grafts placed on tangentially excised deep second degree burn wounds. Male guinea pigs (n=120) were used as the experimental hosts. DC treated animals required two days for complete revascularization of their grafts; control animals required seven days ($p<0.01$). DC treated animals had increased epithelial proliferation at the graft-wound interface when compared to controls ($P<.01$). Grafts from DC treated animals were firmly adherent in four days, while graft adherence in controls was weak prior to seven days post-grafting (PG). At three months PG, control grafts had mild contraction with moderate hair loss and thick sub-epidermal fibrosis; the grafts in treated animals expanded with the growth of the animals, and had abundant hair growth and significantly reduced dermal fibrosis ($p<0.01$).

## INTRODUCTION

The practice of excision and autografting of full-thickness burn wounds to effect wound closure has been a major advance in burn care. Excision of deep partial-thickness burns to the level of intact microcirculation (tangential excision) followed by partial-thickness autografting is also commonly thought to result in improved outcome through preservation of viable dermal and nervous tissue and shortened wound closure time. A major complication of both procedures is graft loss due to physical damage or infection during the time required to establish the grafts.

We have previously shown that DC anodal silver-nylon dressings are effective as topical antimicrobial therapy, and shorten healing time with an improved quality of healing for partial-thickness donor sites taken from areas of healed partial-thickness burns (1,2). In the present study we have examined the effect of DC anodal silver-nylon dressings on the take and growth of split-thickness autografts placed on tangentially excised deep partial-thickness burn wounds.

## MATERIALS AND METHODS

**Standard Guinea Pig Burn.** One hundred twenty male Hartley guinea pigs, weighing an average of 400±25 grams, were used. The animals were anesthetized by intraperitoneal injection of 36 mg/kg of sodium pentobarbital. The hair on the dorsal trunk was clipped and a depilatory cream (Nair™) was applied for 15 minutes. The residual hair roots were then gently washed out with warm tap water. Deep partial-thickness scalds were inflicted, using a 10 second exposure to 78°C water. A Walker-Mason burn template with a 4.5 x 5.5 $cm^2$ window was used to restrict the wound to the cephalad portion of the back (3). The resulting scald wounds covered 8-9% of the total body surface.

**Tangential Excision and Autografting:** One hour after scalding, a 5 cm transverse incision was placed 1 cm cephalad and parallel to the burn wound edge. A 25 cm x 5 cm, smooth, surgical steel plate was inserted 12 cm into the subpannicular space under the scald wound. To make the skin tense, four towel clips were applied to the skin at the corners of the metal plate. A 0.022 inch-thick skin graft (4.5 x 11 cm) was harvested from both the scalded wound and the normal depilated area caudad to the burn wound, using a Brown electro-dermatome. This excision depth resulted in a minimally bleeding wound bed. A partial-thickness graft was prepared by discarding the scalded portion of the excised tissue and fitted to the excised scald wound. As shown in Figure 1, each animal had a cephalad autografted excised scald wound and a caudad ungrafted donor site.

**Experimental Groups.** As shown in Table I, the animals were divided into control and DC treatment groups. The wounds were covered with silver-nylon (SN) dressings (Swift Textile Metalizing Corporation, Hartford, Connecticut), and sutured in place (4). The SN dressings were covered with a layer of sponge and three layers of gauze and fixed in place with flexible tubular bandage. The SN dressing in the DC treated animals was connected as an anode in a DC circuit, as previously described (2). A constant 40 μA current was applied for two days, followed by 20 μA for three days, using a constant direct current generator (5). The dressings of the DC treated animals and controls were moistened once or twice daily with 2-3 ml of saline through irrigation tubes. All dressings were removed on the seventh post-grafting (PG) day, or as indicated below.

**TABLE 1. Experimental Groups**

| GROUP | DESCRIPTION | NO OF ANIMALS |
|---|---|---|
| Control | Graft + Donor Site + SN | 60 |
| Treatment | Graft + Donor Site + SN + 40 μA (2 Days)+ 20 μA (3 Days) | 60 |

**Evaluation of Graft Healing.** Grafted wounds were examined daily for three weeks and then weekly for three months post-grafting. Graft adherence, hair growth, percentage of graft take and wound contraction were recorded and photographed at selected times. Necropsy samples of the grafted wounds were collected for microscopic examination on selected days. Two animals were sacrificed and examined from each group at 1, 3, 5, 30 and 60 days PG and ten animals at 2, 4, 7, 14 and 90 days PG. All specimens were obtained from the central areas of the grafted wounds.

Estimation of circulatory integrity in the grafts was made by microscopic examination for the presence of carbon particles in the capillaries after perfusion with 40 ml of Pelikan ink (Pelikan AG D3000, Hannover, West Germany). After the animals were anesthetized with sodium pentobarbital, the ink was perfused via a small polyethylene cannula placed in the superior mesenteric artery. The perfusion speed (38.2 ml/min) was regulated with an infusion-withdrawal pump (Harvard Apparatus). Immediately after infusion, the animals were sacrificed and sections were prepared for microscopic examination. Photomicrographs were taken with a low power objective lens (8X). Morphometric measurements of fixed tissue were made using a digital image analyzer (Vidas, Carl Zeiss Inc., NY).

## RESULTS

Control animal grafts were not adherent to the wound bed 1, 2 or 4 days PG and commonly separated from the wound bed with removal of the dressing. Partial adherence was noted by PG day 7 and grafts were firmly adherent at 14 days. Grafts on DC treated animals were partially adherent on PG day 1, adherent on day 2, and firmly adherent by PG day 4. Data comparing the effect of DC treatment on the re-establishment of graft microcirculation, as evidenced by histological demonstration of carbon black in graft vessels, are presented in Table 2. Intact microcirculation was observed in only 1 of 4 control animals on PG day 2, and in all on PG day 7 (Fig. 2). Grafts in control animals on PG day 1 separated from the underlying tissue during fixation and could not be examined. DC treatment enhanced the re-establishment of graft-host microcirculation; by PG day 2, carbon black was present in graft vessels in 7 of 8 treated animals (Fig. 3a).

**TABLE 2. Frequency of India Ink Carbon in Grafts (Revascularization) at Selected Times after Grafting**

| DAYS PG. | 1 | 2 | 4 | 5 | 7 |
|---|---|---|---|---|---|
| CONTROL | –[1] | 1/4 | 2/4 | 6/8 | 9/9 |
| DC TREATED | 0/1 | 7/8* | 11/11* | 6/6 | –[2] |

[1] Grafts were not adherent

[2] Grafts were not perfused

* $p<.01$

Graft epithelization and the development of fibrosis were markedly altered by DC treatment. Epithelial growth was distinctly stimulated by DC. Hair follicle epithelium in treated animals proliferated actively as early as 2 days PG (Fig. 3b). A comparison of epithelial proliferation at the graft-wound interfaces of control and treated animals is presented in Table 3. DC treatment resulted in earlier, more extensive epithelial growth. By PG day 4 in treated animals, an epithelial layer had formed between the hair follicles at the graft-wound interface and there was further evidence of expanding microcirculation (Fig. 4). The hyperplastic epithelial layer was not seen in control animals and resolved in treated animals between 7 and 14 days PG, as dermal and graft follicles joined (Fig. 5). A comparison of control and treated animals 14 days PG is presented in Figure 6. Grossly, control animals appeared to have mild contraction (Fig. 6a) compared to treated animals (Fig. 6b). Control animals had a significant amount of fibrosis in the upper dermis. A mature stratum corneum was observed in hair follicle canals and there was minimal subepithelial fibrosis in the treated animals.

**TABLE 3. Epithelial Proliferation from Hair Follicles at the Graft-Wound Interface at Various Times after Grafting**

| Days | 2 | 3 | 4 | 5 | 7 | 14 | 90 |
|---|---|---|---|---|---|---|---|
| Control | 0/6[1] | 0/3 | 1/8 | 2/8 | 0/9 | 4/10 | 0/9 |
| DC Treated | 3/8 | 3/3 | 6/10 | 4/5 | 3/5[2] | 0/9 | 0/10 |

[1] Number of animals having epithelial proliferation over number of the animals examined.

[2] Increased frequency of sub-graft epithelial proliferation with D.C. treatment for days 2-7 ($P<.01$).

The appearance of control and treated grafts 90 days PG is presented in Figure 7. Grossly, the grafts of treated animals had more hair and, unlike the grafts of control animals (Fig. 7a), appeared to have grown with the animals (Fig. 7c). Histologic examination at 90 days PG showed treated animals to have nearly normal skin structure, with the exception of mild subepidermal fibrosis and a minimal decrease in hair follicle density (Fig. 7d). Control animals showed a markedly wider zone of fibrosis and loss of many hair follicles (Fig. 7b). Morphometric comparisons of the thickness of subepithelial fibrosis, dermal thickness measured from the deepest subepithelial fibrosis to the panniculus muscle, and the dimensions of the residual grafts were made at 90 days PG. Data are presented in Tables 4 and 5 respectively. DC treatment resulted in a significant decrease in healed graft thickness and a significant increase in graft expansion.

**TABLE 4. Morphometric Comparison of Dermis Profile in Skin at 3 Months Post-Graft**

| | THICKNESS OF SUBEPIDERMAL FIBROSIS (MM) MEAN±S.E.M. | THICKNESS OF LOWER DERMIS (MM) MEAN±S.E.M. |
|---|---|---|
| Control (n=9) | 0.619±0.016 | 1.736±0.024 |
| DC Treated (n=10) | 0.435±0.007* | 1.518±0.015* |

*P<0.01

**TABLE 5. Contraction and Expansion of Grafted Wounds Treated with and without Direct Current**

| | 14 DAYS POSTGRAFTING | | 3 MONTHS POST GRAFTING | |
|---|---|---|---|---|
| | TREATMENT (n = 10) | CONTROL (n = 7) | TREATMENT (n = 10) | CONTROL (n = 10) |
| WOUND SIZE ($cm^2$) | | | | |
| MEAN | 16.00 | 15.58 | 29.07* | 14.61 |
| S.E.M. | (0.731) | (1.245) | (2.306) | (1.212) |

* $P < 0.0001$


**FIGURE 1:** A cephalad autografted wound (9% TBS) and a caudad donor site (9% TBS) soon after tangential excision, harvesting and grafting.

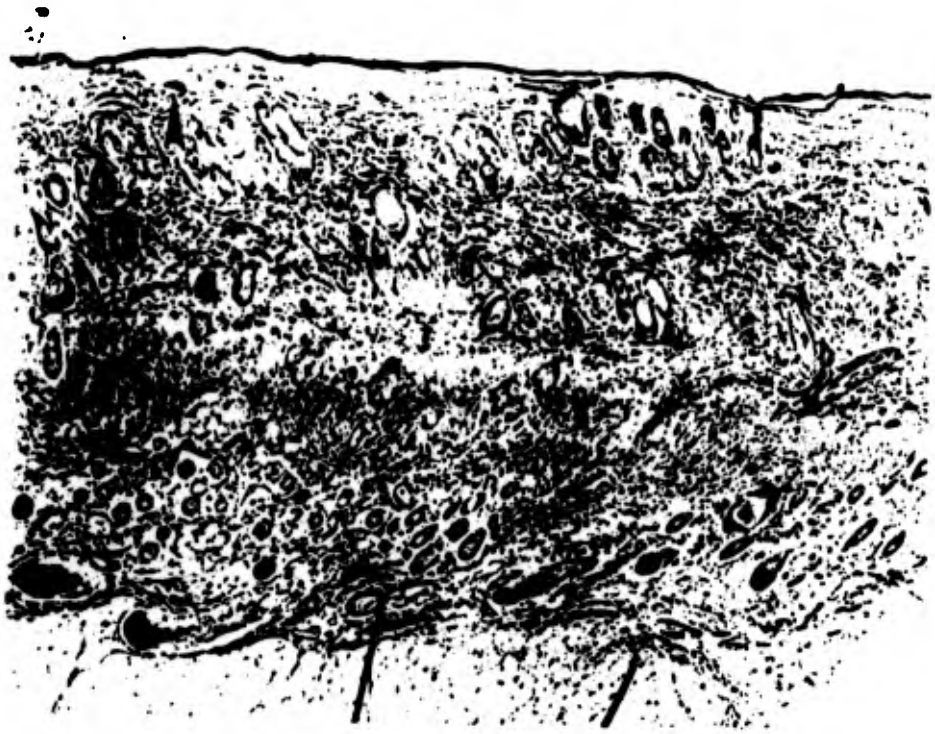

**FIGURE 2**: Control animals at seven days PG. Example of the complete re-establishment of graft microcirculation showing ink particles (arrow) in subepidermal capillaries (H and E, 8X).

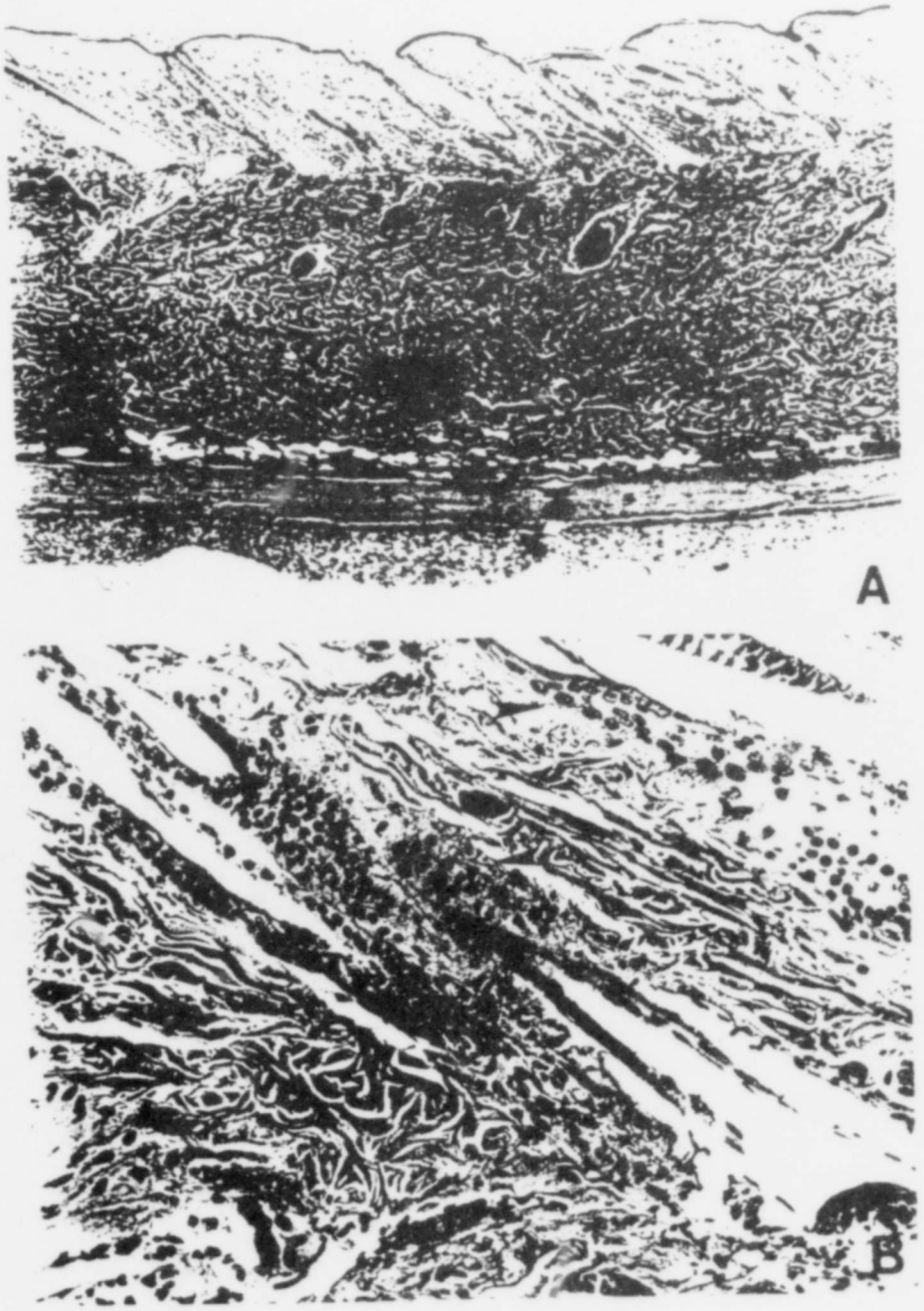


**FIGURE 3**: Two days after grafting and starting DC treatment. (A) Carbon from infused Pelikan ink is evident in the whole layer of graft tissue. (B) Proliferation of epithelium (arrows) is beginning in hair follicles (H and E, 8X and 50X).

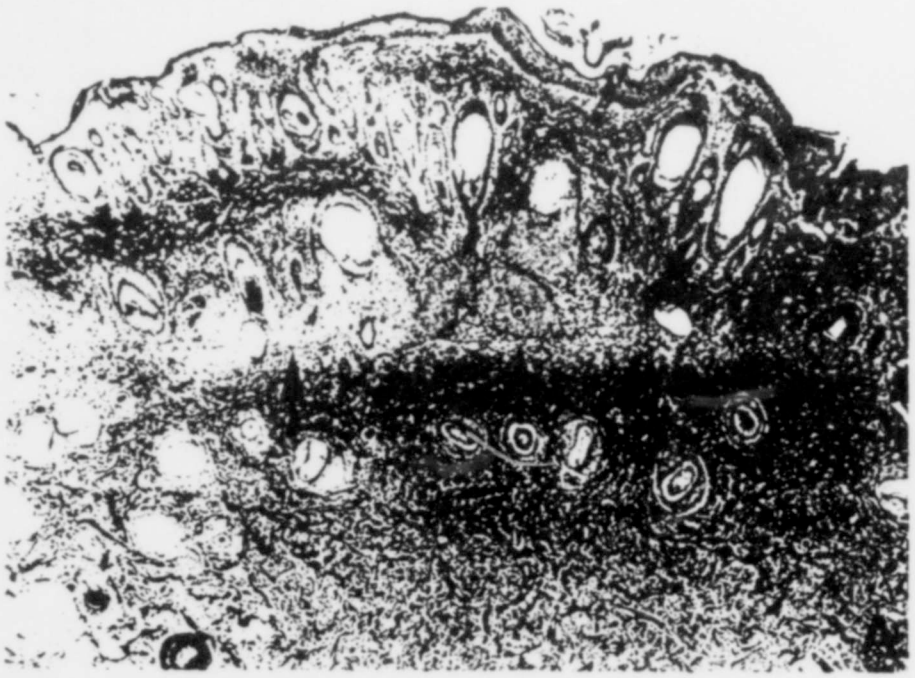

**FIGURE 4**: A portion of the graft from a DC treated animal at 4 days PG. An incomplete epithelial layer is present at the graft-wound interface (arrows)(H and E, 8X).

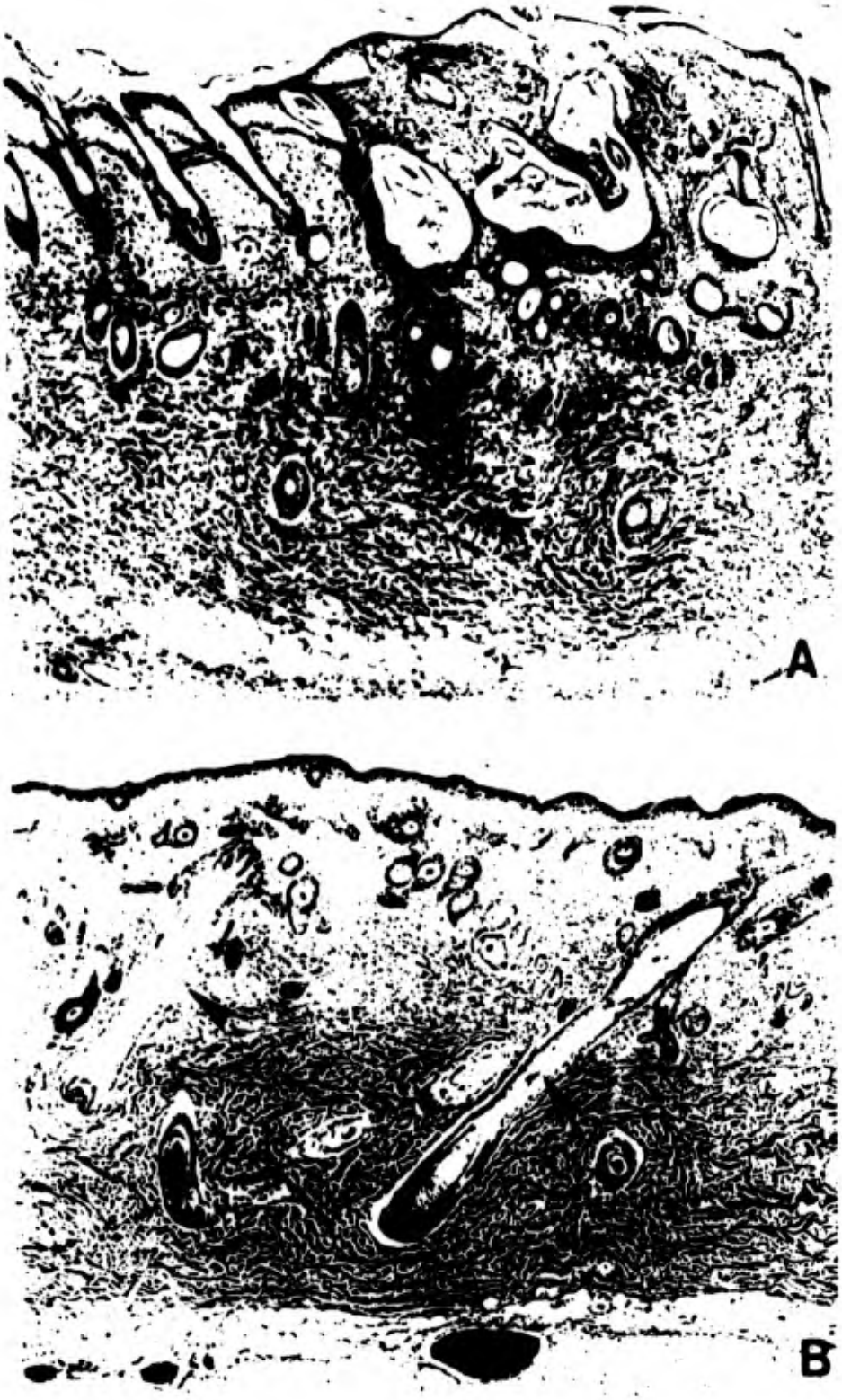


**FIGURE 5**: Example of a DC treated wound seven days after grafting. (A) The incomplete epithelial layer at the graft-wound interface is much less prominent than at 4 days (see Figure 4). Hair follicles are dilated and contain debris and hair shafts. (B) Hair shafts in the dermis and graft have connected (arrows) (H and E, 8X).

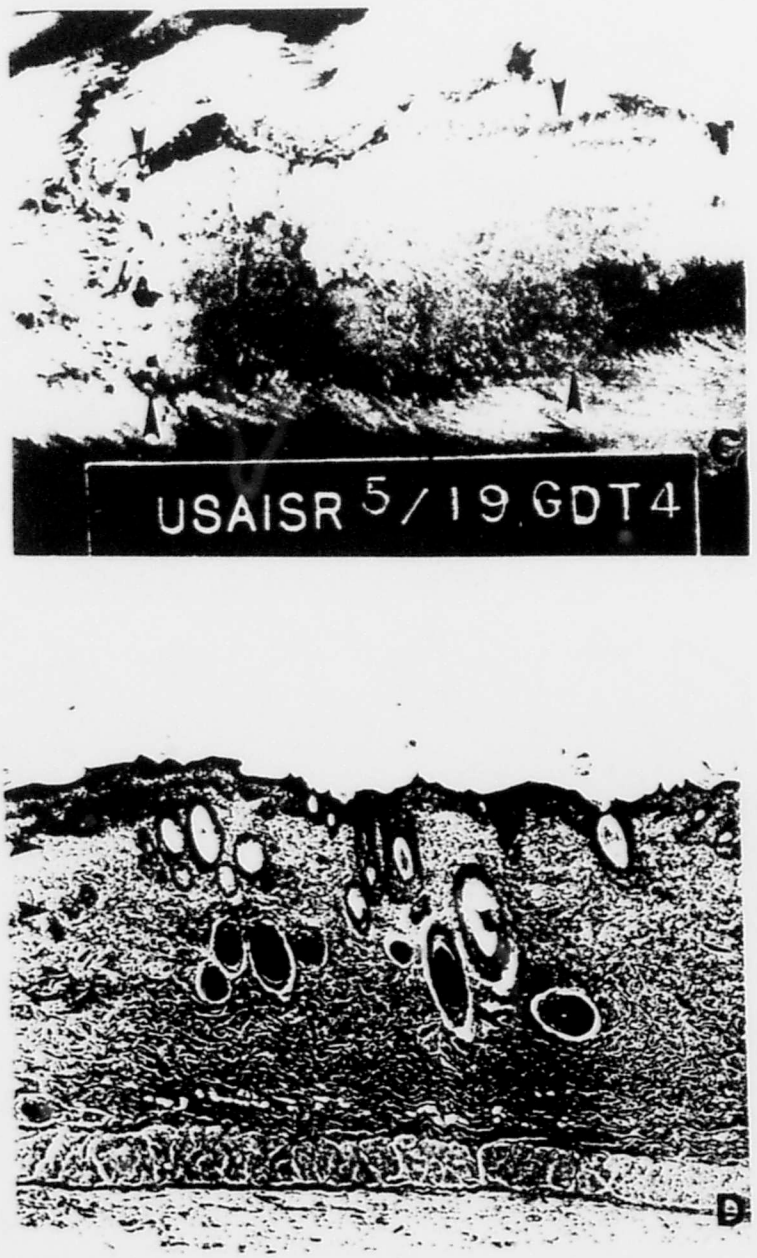


**FIGURE 6:** (C) Photograph shows smoothly adherent graft (arrows) with short hair growth of a treated animal. (D) Photomicrograph of a treated graft shows thin layer of fibrosis in the upper dermis (arrow heads) (H and E, 8X).


**FIGURE 6:** (C) Photograph shows smoothly adherent graft (arrows) with short hair growth of a treated animal. (D) Photomicrograph of a treated graft shows thin layer of fibrosis in the upper dermis (arrow heads) (H and E, 8X).

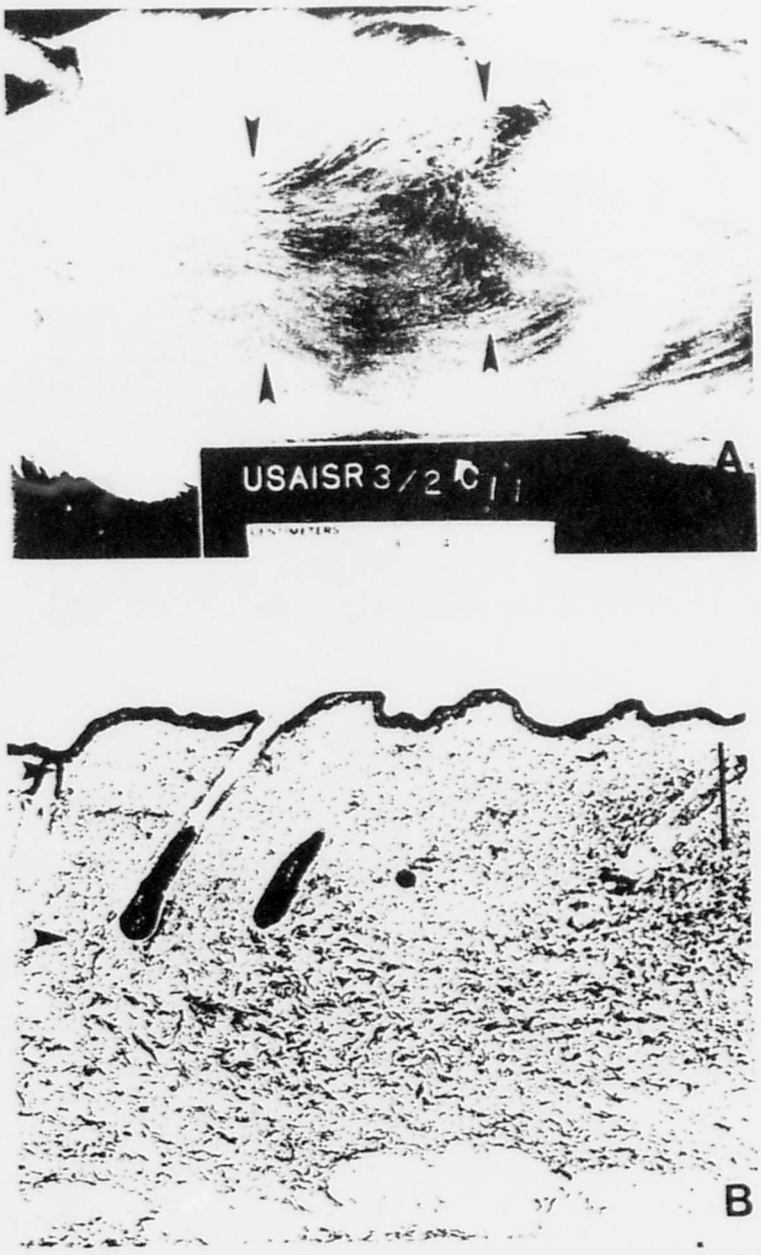


**FIGURE 7:** Comparison of treated and control animals 3 months PG. (A) Gross appearance of grafted wound (arrows) from a control animal which healed with mild-to-moderate contraction and moderate hair growth. (B) Microscopic section of control graft shows a subepidermal layer of fibrosis (arrow heads). The right portion of the section has fewer hair follicles (H and E, 8X, Bar=500 microns). (FIGURE 7 CONTINUED)

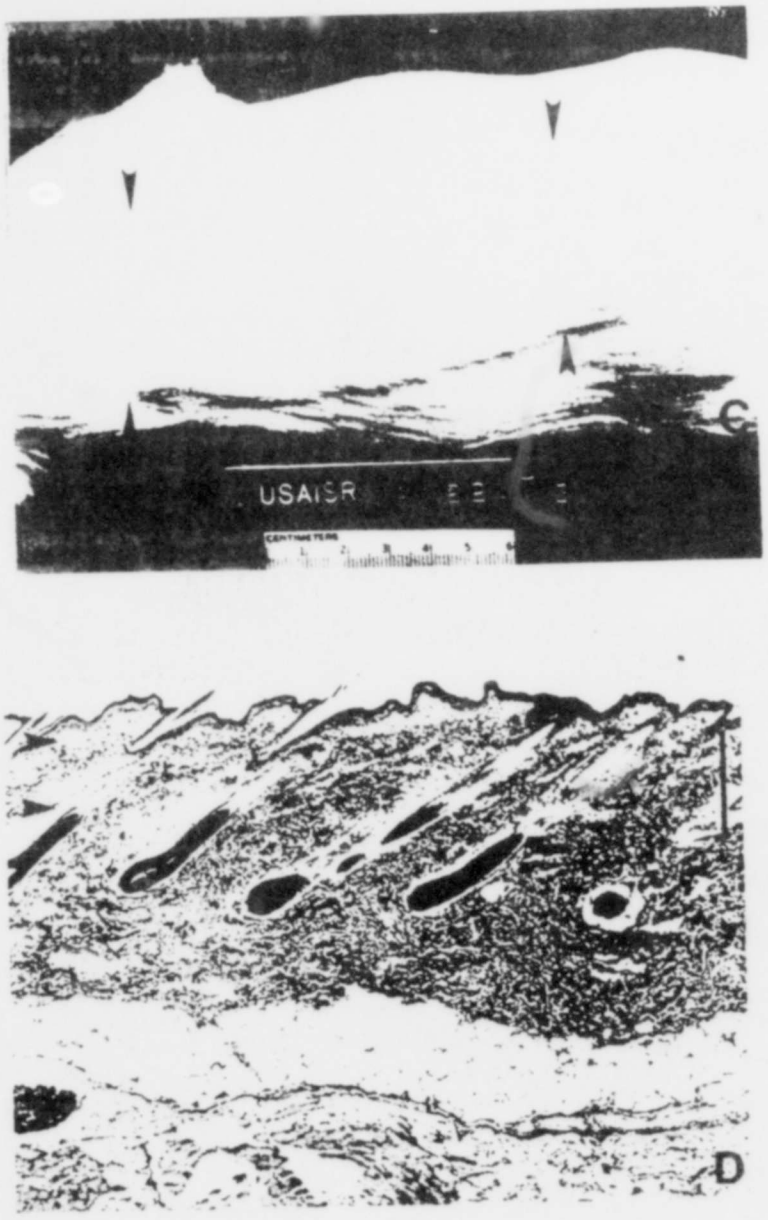


**FIGURE 7:** (C) Gross photograph of a treated graft (arrows) shows no contraction and abundant hair growth. (D) Photomicrograph from the grafted wound of a treated animal shows nearly normal skin except for a slight decrease in the number of hair follicles and thin subepidermal fibrosis (arrow heads) (H and E, 8X, Bar=500 microns).

## DISCUSSION

The final depth of circulatory impairment after thermal injury defines the extent of tissue destruction and, therefore, the possibility of wound healing. This depth of tissue destruction is not always immediately obvious, and wounds that appear to be deep second degree burns may, during subsequent days, undergo further ischemia or become infected with resulting loss of initially viable deep epidermal elements, and are said to have converted to full-thickness injuries (6-8). These complications are, in part, the basis for the practice of early tangential excision and autografting of deep partial-thickness burns. Such procedures remove the infection-prone nonviable tissue and, with successful grafting, preserve the underlying microcirculation and reduce the inflammation and subsequent scarring associated with tissue slough.

The survival of an autograft depends on early establishment of the circulation for nutrient supply and disposal of metabolic waste products. Delayed healing, severe wound contraction and failure of graft adherence are serious complications in deep second degree burn wounds after tangential excision and split-thickness autografting. In 1972, Shepard suggested that such complications are due to the time required for adherence between the graft and wound bed and re-establishment of the graft's circulation. He studied the viability of split-thickness autografts on their donor sites and stated that normal, non-neoplastic epithelium will not migrate through viable tissue. This inability of epithelium to migrate down through a viable split-thickness graft was thought to reduce adherence of the graft to the wound bed for up to 10 days PG (9).

In this study, we found that the transient epithelial cell proliferation occurring between viable host tissue and a graft, at the graft-wound interface, was exaggerated by DC treatment. This hyperplastic epithelium was present by PG day two, and appeared to be derived from dermal hair follicles in the wound bed. The hyperplasia was maximal at one week and resolved by 14 days, by what appeared to be extension of the epithelial cells into connections made with adjacent viable hair canals in the graft. This phenomenon may be an example of the previously reported effects of direct current on epithelial growth (10-13).

Although the mechanisms underlying the overall improvement in healing in DC treated animals in this study are not clear, the results suggest a possible mechanistic explanation. There was more rapid return of graft blood supply in the DC treated group than in the control group. In most DC treated animals, graft microcirculation was re-established by the second postgraft day, while grafts in control animals required seven days to achieve this extent of circulatory restoration. This rapid re-establishment of the graft's microcirculation is compatible with reduced circulatory stasis in the wound bed (2,6,7). Any reduction in severity or

duration of graft and wound bed ischemia would be expected to result in a reduced inflammatory response and, in turn, less fibroblast replacement and wound contraction. Early re-establishment of graft circulation in the DC treated animals appears to have provided the nutrients needed for more rapid take of the grafts and either prevented the accumulation of toxic metabolic products or accelerated their elimination to reduce the inflammatory response and improve the quality of healing of both the wound and the graft.


## ACKNOWLEDGEMENT

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| RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY | | | | 1. AGENCY ACCESSION DA318558 | 2. DATE OF SUMMARY 30 Apr 90 | REPORT CONTROL SYMBOL DD-DRA&E(AR) 636 |
|---|---|---|---|---|---|---|
| 3. DATE PREV SUM'RY 1 Oct 89 | 4. KIND OF SUMMARY K. Comp | 5. SUMMARY SCTY U | 6. WORK SECURITY U | 7. REGRADING | 8. DISB'N INSTR'N CX | 9. LEVEL OF SUM A. WORK UNIT |

| 10. NO./CODES: | PROGRAM ELEMENT | PROJECT NUMBER | TASK AREA NUMBER | WORK UNIT NUMBER |
|---|---|---|---|---|
| a. PRIMARY | 61102A | 3M161102BS14 | CA | 320 |
| b. CONTRIBUTING | | | | |
| c. CONTRIBUTING | | | | |

11. TITLE (Precede with Security Classification Code) (U) The Effect of Cutaneous Burn and Inhalation Injury on Pulmonary Function in Rabbits

12. SUBJECT AREAS
0604 Anatomy and Physiology

| 13. START DATE | 14. ESTIMATED COMPLETION DATE | 15. FUNDING ORGANIZATION | 16. PERFORMANCE METHOD |
|---|---|---|---|
| 8812 | 9004 | DA | C. In-House |

17. CONTRACT/GRANT MILITARY RELEVANCY CERTIFIED BY Brig Gen Pruitt
a. DATE EFFECTIVE APPROVED BY
b. CONTRACT/GRANT NUMBER
c. TYPE d. AMOUNT
e. KIND OF AWARD f. CUM/TOTAL

18. RESOURCES ESTIMATE

| a. FISCAL YEARS | a. PROFESSIONAL WORKYEARS | b FUNDS (In thousands) |
|---|---|---|
| 89 | 1.0 | 33 |
| 90 | 1.0 | 34 |

| 19. RESPONSIBLE DOD ORGANIZATION | 20 PERFORMING ORGANIZATION |
|---|---|
| a. NAME US Army Institute of Surgical Research | a. NAME US Army Institute of Surgical Research |
| b. ADDRESS (include zip code) Fort Sam Houston San Antonio, Texas 78234-5012 | b. ADDRESS Fort Sam Houston San Antonio, Texas 78234-5012 |
| c. NAME OF RESPONSIBLE INDIVIDUAL PRUITT, B A | c. NAME OF PRINCIPAL INVESTIGATOR SAKANO, T |
| d. TELEPHONE NUMBER (include area code) 512-221-2720 | d. TELEPHONE NUMBER (include area code) 512-221-7832 |
| 21. GENERAL USE FINA<br>MILITARY/CIVILIAN APPLICATION M | f. NAME OF ASSOCIATE INVESTIGATOR (if available) MASON, A D<br>g. NAME OF ASSOCIATE INVESTIGATOR (if available) |

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Burn Injury; (U) Inhalation Injury; (U) Pulmonary Function; (U) Prostanoid; (U) Lipid Peroxide; (U) Lab Animals; (U) Rabbits; (U) RA II

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

23. (U) A DTIC literature search was conducted under DTIC request number W6048D dated 20 October 1989 for the technical report database and request number W6049C dated 20 October 1989 for the work unit database. The proposed effort will not result in the duplication of effort. The objective of this work was to identify the pulmonary changes in a rabbit model following smoke inhalation, cutaneous burns, and a combination of both.

24. (U) Twenty-percent full-thickness scald burns and/or various severities of smoke inhalation were produced in a rabbit model. Histologic evaluation was performed and carboxyhemoglobin, arterial oxygen pressure, extravascular lung water, etc., were be used to estimate an interaction between burn and inhalation injury.

25. (U) 8812 - 9004. A rabbit model with smoke inhalation injury and/or cutaneous burn injury was developed and observed for 2 days. However, lung injury, as measured by decreased arterial oxygen pressure and lung water accumulation, was less in the group with both injuries than in the group with inhalation injury only. For technical reports, refer to the US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Year 1990.

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**ANNUAL RESEARCH PROGRESS REPORT**

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**PROJECT TITLE:** The Effect of Cutaneous Burn and Inhalation Injury on Pulmonary Function in Rabbits

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

**1 October 1989 - 30 April 1990**

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**ABSTRACT**

**PROJECT NUMBER:** 3M161102BS14-00, RESEARCH

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

**PERIOD COVERED BY THIS REPORT:** 1 Oct 89 through 30 Apr 90

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In the course of developing a model of inhalation injury, the relationship between the severity of pulmonary injury and specific techniques and doses of smoke exposure was examined in pairs of rabbits simultaneously exposed to smoke. In group I (5 pairs), one animal in each pair was exposed to smoke with a breath hold (BH) at the end of each exposure; the second animal received an exposure producing the same level of carboxyhemoglobin without BH. In group II (6 pairs), both animals were exposed to 25 units of smoke simultaneously, with BH. In group III (3 pairs), one animal received a 20 unit and the other a 25 unit exposure, both with BH. In group IV, 9 animals received 25 unit exposures with BH and were observed for four days. Groups V and VI served as controls.

Smoke exposure with BH regularly produced severe injury in terms of decreased $PaO_2$ and histopathology, while exposure without BH did not, despite high levels of carboxyhemoglobin after smoke inhalation. The mean differences in percent residual $PaO_2$ ($PaO_2$ at 48 hrs X 100/pre-injury $PaO_2$) and in extravascular lung water (EVLW) at 48 hrs within pairs of animals receiving 25 units with BH were 12.3±5.33%, and 0.271±0.157 ml/g, respectively. Histological findings such as necrotic tracheobronchitis with pseudomembrane were consistently present. No differences were observed between animals receiving exposures of 20 and 25 units. During the four days of observation, three animals in Group IV died. $PaO_2$ was lowest on the second day and rose thereafter in all surviving animals except in one that had massive pneumonia. EVLW was still elevated on the fourth day after injury. Histologically, the destroyed surface epithelium in the airway was covered by a nonciliated epithelium, and focal pneumonia was found frequently in the pulmonary parenchyma.

These results indicate an advantage of the extended exposure afforded by BH in creating consistent, severe injury and the

important part played by pneumonia in determining prognosis beyond the second post injury day. The model appears useful for evaluating the effects of inhalation injury with concurrent cutaneous burn or wound infection, and for assessing various regimens for the treatment of inhalation injury.

**THE EFFECT OF CUTANEOUS BURN AND INHALATION INJURY ON PULMONARY FUNCTION IN RABBITS**

In recent years, great advances in clinical management and the prevention of burn wound infection have enhanced the survival of severely burned patients. The prognosis of large cutaneous burns is still compromised, however, when complicated by concurrent smoke inhalation injury. Inhalation injury, especially when combined with pneumonia, remains a major contributor to the morbidity and mortality of burn injury (20). Several animal models (12,16,19, 23,24) have been developed to address the pathophysiology of inhalation injury. We selected the rabbit as the experimental animal for this model for several reasons. First, the species lends itself to studies of the interaction between inhalation injury and cutaneous burn, since its size facilitates the use of immersion in hot water to produce burn injuries of precise size and depth. Second, the animal is large enough to permit successive blood samples, which are necessary for the analysis of the time course of physiological response. Finally, the animals are easy to maintain and handle, and their cost is not high.

Inhalation injuries are usually produced by gaseous or particulate products of incomplete combustion. Particles in smoke may be coated with irritating chemical agents, such as aldehydes and hydrogen chloride, and thus carry these irritants as far as the alveoli (5). Incorporation of a 0.5μm pore-size filter significantly blunts the changes in lung mechanics observed after smoke exposure (3). Generation of such toxic elements depends on conditions of combustion such as oxygen supply, temperature and heating rate in the fire (21). Since each fire generates its own variety of smoke and associated toxic materials, the severity of experimental inhalation injury is not completely controllable.

In pilot studies, we found that administration of smoke to rabbits in the usual manner did not produce consistent injury, although carboxyhemoglobin levels in arterial blood taken immediately after smoke exposure were between 60 and 75%. A spectrum of severity of injury was observed at constant doses of smoke generated from the same material.

For these reasons, we studied the effects of a breath hold at the end of each smoke inhalation on the consistency of severity of injury. To assess the variance inherent in this model, we exposed pairs of rabbits to the same or different doses of smoke simultaneously. Finally, we studied the longer term effects of smoke exposure in these animals, assessing mortality, morbidity and histopathologic changes.

## MATERIALS AND METHODS

**Animals**. Forty-seven male New Zealand white rabbits (mean weight 3050 ± 380 g) were used in this study. The animals were housed singly in stainless steel cages and were studied in an unanesthetized state for two or four days with food and water provided ad libitum. Intravenous fluid was not administered during the experiments.

Six groups of rabbits were studied. In Group I (5 pairs), pairs of animals were simultaneously exposed to smoke. One received 25 units of smoke with a breath hold (BHU) at the end of each smoke insufflation. One unit of smoke required 27 seconds to administer and consisted of three successive insufflations of smoke (1 sec) with a breath hold of four seconds, followed by 12 successive ventilations with air (Fig. 1A). The other member of the pair received eight units of smoke without breath hold, each unit consisting of 15 successive insufflations with smoke, followed by 12 ventilations with air (Fig. 1B). Tidal volume was fixed at 12 ml/Kg for all ventilations. In Group II (6 pairs), members of a pair of animals were simultaneously exposed to 25 BHU and observed for two days. In Group III (3 pairs), one animal in each pair was exposed to 20 BHU and the other to 25 BHU. In Group IV (n=9), animals were individually exposed to 25 BHU and pulmonary changes were observed for four days. Groups V (n=5) and VI (n=5) served as sham-operated controls and were observed for two and four days, respectively.

## METHODS

A 24 gauge catheter was placed in the middle artery of the ear a day before an experiment. Animals were anesthetized with ketamine (40-45 mg/Kg) administered intramuscularly, followed by an additional dose of pentobarbital (10-20 mg/Kg), and intubated. Muscle relaxant (pancuronium bromide 0.01 mg/Kg) was administered before smoke exposure. Blood gas levels were determined (BGElectrolytes, Instrumentation Laboratory) before the experiment and every 24 hours after injury. Carboxyhemoglobin concentrations (CO-Hb) were measured in arterial blood taken immediately after smoke exposure, using a Co-Oximeter (Model 282, Instrumentation Laboratory). After sacrifice, the right lung was removed rapidly for measurement of extravascular lung water (EVLW) by a modification of the gravimetric method described by Pearce *et al*. (13). Lung homogenate was dried in a microwave oven (Model MDS-81D, CEM Corporation) by the method of Peterson *et al*. (15). The left lung was fixed for histological examination.

**Intubation Technique**. For accurate and reliable endotracheal intubation, a string was used to guide a tube from mouth to trachea. A one-inch midline skin incision was made on the ventral surface of the neck and the trachea was exposed. The glottis was anesthetized, using 0.1 ml of 4% Xylocaine injected through an 18

gauge needle inserted into the trachea 1 cm below the thyroid cartilage. A guide wire with 4-0 suture was passed cephalad from the needle hole in the trachea into the mouth.

This suture was tied to a suture attached to the tip of a 3-0 cuffed endotracheal tube. The tube was then drawn caudad by traction on the lower suture until correct placement in the trachea was reached. If resistance was encountered at the larynx, the tube was rotated gently, or the position of the head was changed to permit the bevel of the tube to pass between the vocal folds. After confirmation of hemostasis, the incision was then closed.

**Smoke Inhalation.** Smoke was produced in a generator by burning seven and a half commercially available disposable pads (Stanford Professional Products Corporation) made of cellulose (83.4% by weight), polyethylene (8.8%), polypropylene, etc. Smoke was collected in a Douglas Bag (50 L, Harvard) through a smoke delivery system and cooled to room temperature. Carbon monoxide (CO) concentration was measured in the collected smoke (CO 101, Neotronics), and if the level was outside a range of 1 to 2%, the process was repeated until smoke having the desired concentration was obtained. A volume-adjustable syringe was used for alternate insufflation of smoke or air.

**Statistical Analysis.** The data are presented as means ± standard deviation. Statistical differences in the change of $PaO_2$ within and between groups were evaluated using the Tukey studentized range method, after a two-factor ANOVA with repeated measures on one factor. The differences in EVLW on the second and fourth days post injury were evaluated using the Tukey studentized range method after one-factor ANOVA. Null hypotheses were rejected at $p<0.05$.

## RESULTS

**Group I.** One animal died 26 hours after 25 units of smoke exposure with breath hold and this pair was excluded from analysis. The average immediate post-exposure CO-Hb concentrations in rabbits receiving eight units without BH and 25 units with BH were 68.1±2.58 (SE) and 69.0±4.10%, respectively. Changes of $PaO_2$ in each group are shown in Fig. 2. Only rabbits exposed to smoke with breath hold had significantly lower $PaO_2$ than the control at 24 or 48 hours. EVLW increased in rabbits exposed to smoke with or without breath hold; there was no statistical difference between the two (Fig.3). Smoke exposure with BH consistently produced necrotic tracheobronchitis and bronchiolitis with pseudomembrane formation. Inflammation of surrounding tissue, including edema and increased accumulation of neutrophils, was also present. Histological changes were more severe in the trachea and major bronchi than in the distal bronchioles or parenchyma. Most of the rabbits exposed to smoke without BH had only minimal focal damage.

**Group II.** The average of CO-Hb levels after smoke exposure in all rabbits was 75.4±2.98%. The mean difference of CO-Hb levels within a pair was 2.67±1.2%. The average $PaO_2$ in all animals before, 24 and 48 hours after smoke exposure was 80.9±6.67, 67.5±8.56 and 57.0±10.2 mm Hg, respectively. Figure 4 shows the paired differences in percent residual $PaO_2$ ($PaO_2$X100/pre-injury $PaO_2$) at 24 and 48 hours after injury; the mean differences within a pair were 11.1±10.1 and 12.3±5.33%, respectively. The average of EVLW in all rabbits was 3.655±0.229 ml/g; the mean difference of EVLW within a pair was 271±0.157 ml/g.

**Group III.** The percent residual $PaO_2$ at 48 hours after injury in paired rabbits receiving 20 and 25 BHU is depicted in Fig. 5. One rabbit did not experience any decrease of $PaO_2$ and in one pair 20 units of smoke exposure produced more histologic evidence of damage than 25 units. No consistent difference in severity of injury was identified in this group.

**Group IV.** The average of CO-Hb levels in all rabbits immediately after smoke exposure was 72.5±6.83%. Three of nine animals died during the four day period of observation (Fig. 6). In those animals that died, wheezing was always present, and death was sudden. In all the surviving animals, $PaO_2$ decreased gradually and was lowest on the second day, rising thereafter except in one rabbit with severe bilateral pneumonia (Fig. 7). EVLW at the fourth day was 3.533±0.371 ml/g and was still high in the animals exposed to smoke compared to EVLW of the control animals (Fig. 8). Histologically, the injured tracheobronchial surface was covered by a nonciliated, stratified epithelium, with some areas still covered with pseudomembrane. The extent of subepithelial edema and the numbers of infiltrating inflammatory cells on day four was markedly less than on the second post injury day. In contrast to the reparative processes in the trachea and bronchi, scattered focal areas of pneumonia were frequently found in the parenchyma.

(A) One unit of smoke with a breath hold

Smoke (15 seconds) 1, hold, 2, hold, 3, hold

Air (12 seconds) 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12.

(B) One unit of smoke without a breath hold

Smoke (15 seconds) 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15.

Air (12 seconds) 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12.

FIG. 3 The "Wright" [illegible] smoke

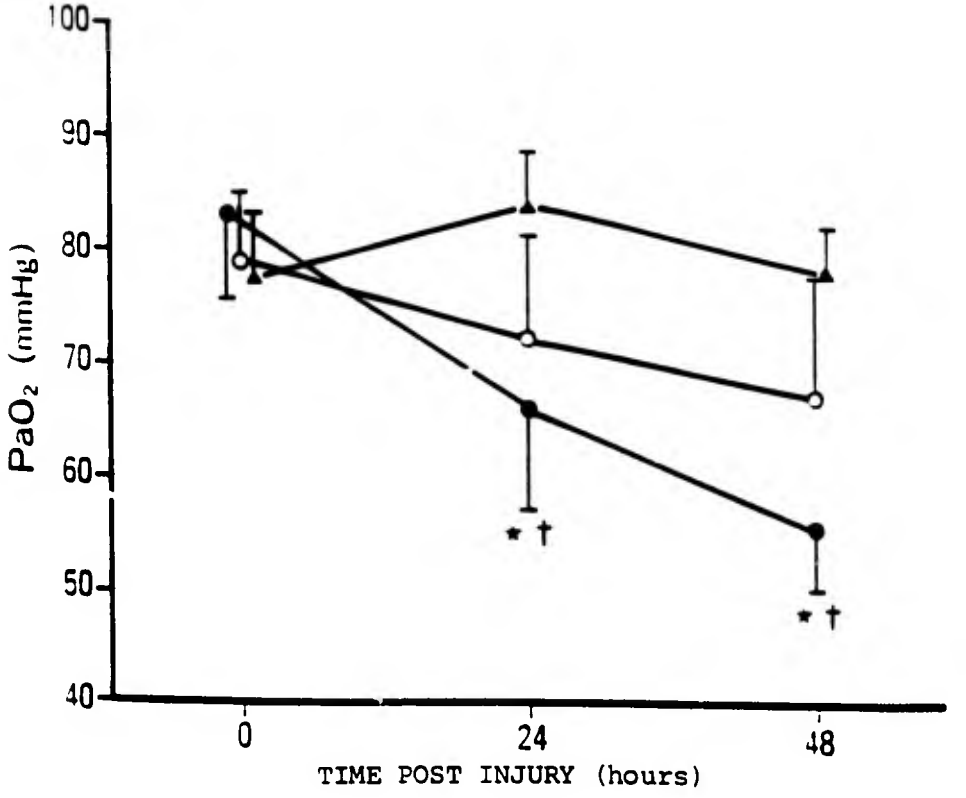


**FIGURE 2.** Time course of change in $PaO_2$ for 48 hours after injury. Closed triangles: control. Open circles: animals exposed without breath hold. Closed circles: animals exposed with breath hold. * Significantly different ($p<0.05$ or more) from control values. + Significantly different from pre-injury values.

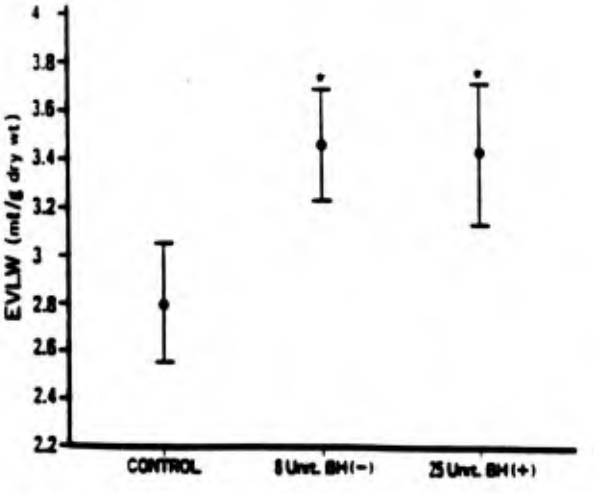


**FIGURE 3.** Extravascular lung water at 48 hours after injury. *Significantly different ($p<0.05$ or more) from control values.

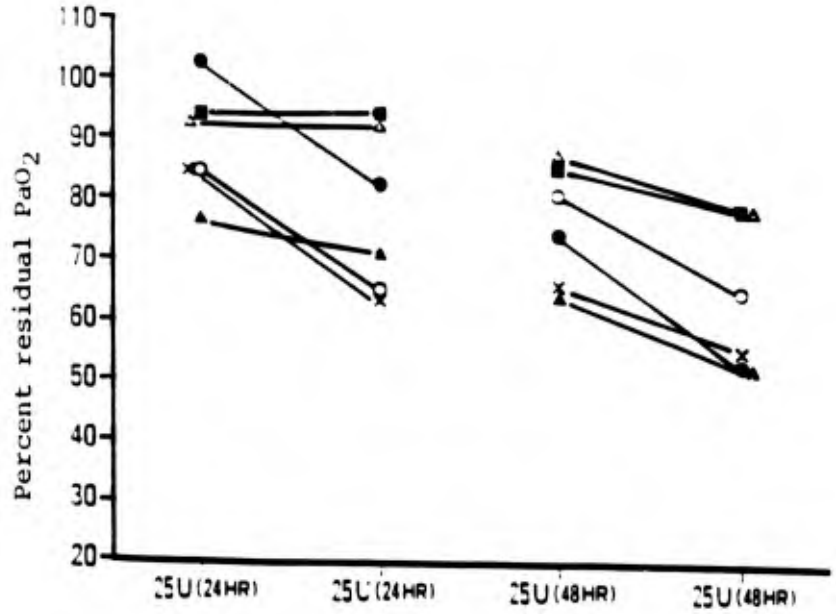


**FIGURE 4.** Percent residual $PaO_2$ in paired animals at 24 and 48 hours after 25 BHU. Lines connect pairs.

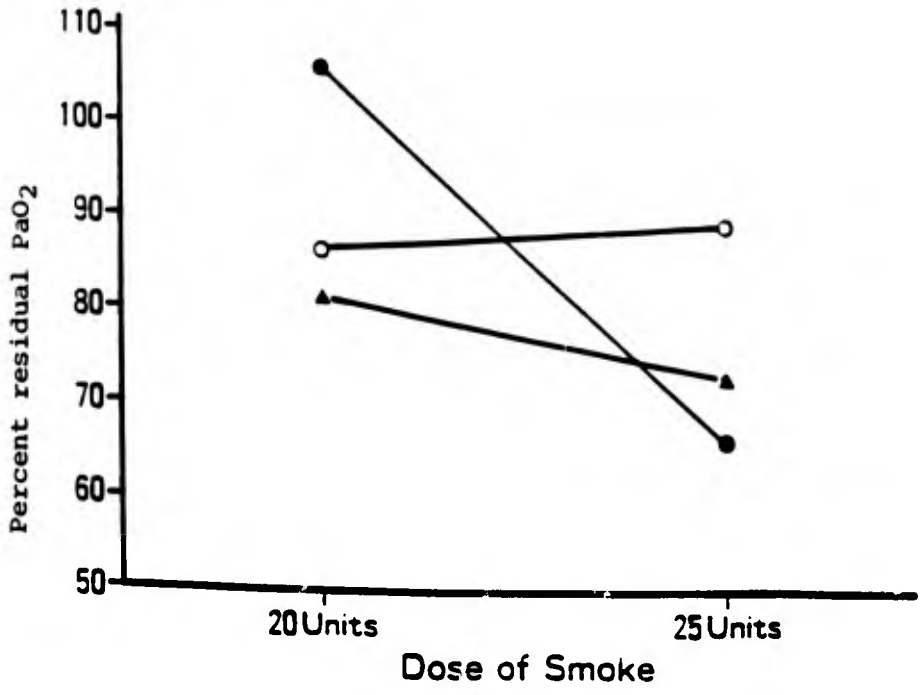


**FIGURE 5.** Percent residual $PaO_2$ at 48 hours in paired animals exposed to 20 and 25 BHU. Lines connect pairs.

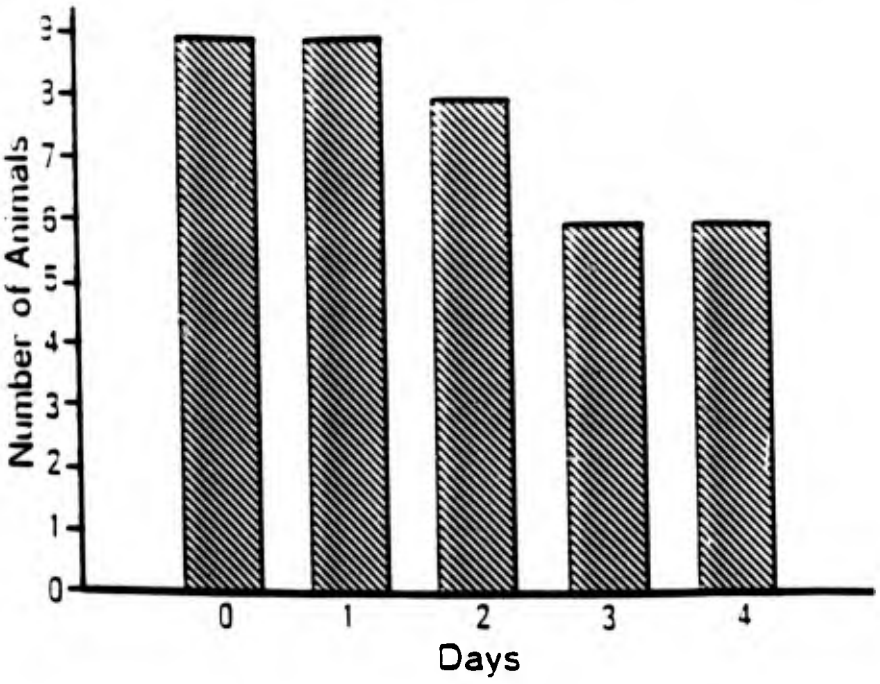


**FIGURE 6.** Survival during four days after 25 units of smoke exposure.

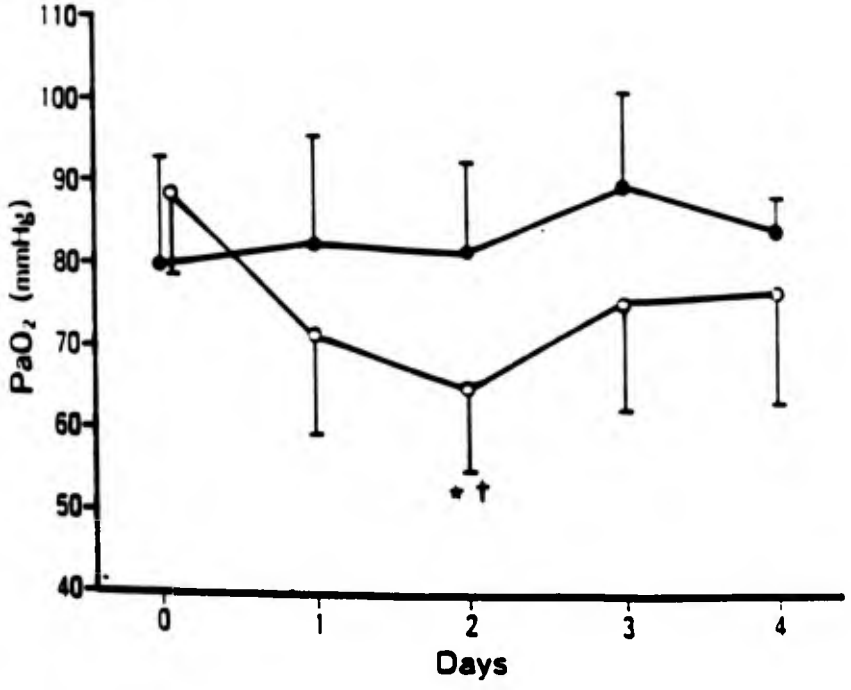


**FIGURE 7.** Time course of change in $PaO_2$ for four days after injury. Closed circles: control. Open circles: animals exposed to 25 BHU. *Significantly different ($p<0.05$) from control values. + Significantly different ($p<0$ from pre-injury values.

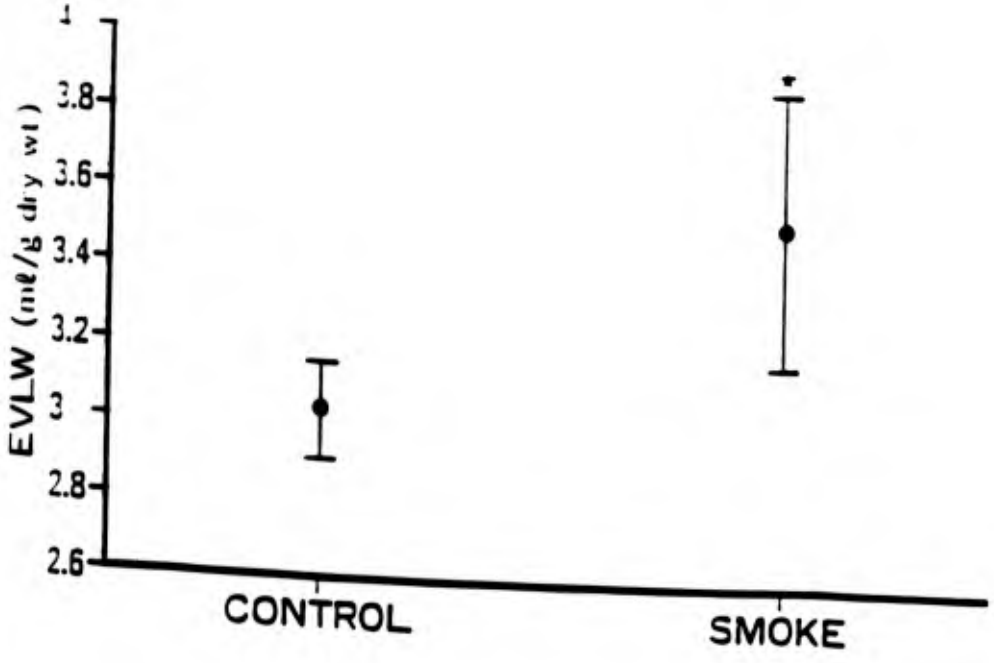


**FIGURE 8.** Extravascular lung water at four days after injury. *Significantly different ($p<0.05$) from control values.

## DISCUSSION

In human and experimental studies of inhalation injury (8,9), histological changes in the lung characteristically include loss of cilia and respiratory epithelium. Sloughing necrotic tissue and inflammatory exudate lead to pseudomembrane formation and airway obstruction with resultant atelectasis, congestion, and edema. Coincident with these changes, progressive hypoxia develops and the risk of life-threatening bacterial infection is increased (4). Rabbits exposed to smoke in this study showed similar changes.

The use of rabbits in inhalation studies is not widespread, partly because of difficulty in maintaining a patent airway and adequate levels of anesthesia. The susceptibility of rabbits to anesthesia varies widely and the margin between surgical anesthesia and death is quite narrow (11). Pentobarbital is the most commonly used intravenous anesthetic agent for rabbits, and has the serious disadvantage of depressing respiration; this is the principal cause of death (18). The small glottis and larynx hidden behind the tongue are major obstacles to rapid intubation. The intubation

technique used in this study was simple to perform without injury to the glottis and required minimal anesthetic doses. No complications attributable to anesthesia were encountered.

Potkin et al. (16) observed severe hypoxemia and pathological changes in rabbits after 40 minutes exposure to white pine wood smoke diluted by air, with a resultant mean CO-Hb level of 39.6%. Long-term exposure to smoke with low CO concentration will permit severe injury without high CO-Hb levels. We used smoke with relatively high concentrations of CO to render exposure time as short as possible because, in a pilot study, smoke with high CO levels produced more severe injury than smoke with low CO concentration, even when the same dose was administered. Severity of injury did not appear to be better controlled by long-term exposure, due to the technical difficulty in generating and administering smoke of constant quantity and quality.

Breath hold has been used in only one previous study of inhalation injury (19) and its effect has not been well defined. In the present study, severe pulmonary damage was not produced consistently without breath hold, and even with similar levels of CO-Hb, exposure to smoke with BH produced more severe injury in terms of oxygenation and histology. The total number of smoke insufflations was smaller in rabbits receiving 25 units with a hold than in those exposed to eight units without a hold (75 vs 120), while exposure time was longer in the former animals (375 vs 120 seconds). This result suggests that almost all CO in smoke may be absorbed during the early phase of a breath hold, while other combustion products may continue to be deposited in the pulmonary tree during the later phase of the hold, resulting in increased absorption of other combustion products and greater toxicity to the exposed tissue.

The factors necessitating this manner of smoke exposure, which may differ from those affecting clinical patients, are not known. The material burned in this study was mostly cellulose, whose principal decomposition products are acrolein and other aldehydes (17). These products are present in high concentration in smoke from the combustion of household materials and are known to cause acute pulmonary injury (25). Although we did not measure combustion products from the pads, it is unlikely that smoke from this material is in any sense uniquely benign or unlikely to produce severe injury. Differences in the velocity of CO-Hb saturation due to differences in blood volume, cardiac output, or affinity of rabbit hemoglobin for CO, or different sensitivity of the respiratory system to combustion products may offer possible explanations. It is possible that patients exposed to smoke with high CO levels do sustain only slight injury if they survive and that the observed clinical correlation between CO-Hb levels and severity of inhalation injury pertains only to patients exposed to smoke having relatively low CO concentrations.

Despite the considerable differences observed in $PaO_2$ and histology, EVLW was equally elevated in animals exposed to smoke with and without breath hold, suggesting that the etiologies of these indices of injury may differ. In a sheep model of inhalation injury, the increase of lung lymph flow has been reported to correlate well with CO-Hb level after smoke exposure, while diminution in the ratio of the $PaO_2$ to the fraction of inspired $O_2$ ($PaO_2/FiO_2$) does not (10). It has been reported that rabbits exposed to carbon monoxide, with resultant CO-Hb level of 63%, show widespread areas of both epithelial and endothelial swelling and marked interstitial edema without an increase in the alveolar/arterial $O_2$ difference (6). Our results are consistent with these findings and suggest that carbon monoxide in smoke may be related to edema formation.

In a pair of animals receiving the same dose of smoke simultaneously, small differences of CO-Hb were detected and were probably attributable to individual differences in cardiac output, blood volume, etc. The average differences in residual percent $PaO_2$ and EVLW at 48 hours post injury were 12.3±5.33% and 0.271±0.157 ml/g, respectively. These differences are probably related to individual biological variation, since heterogeneity in the smoke itself was excluded by pairing and other problems, such as technique and apparatus are regarded as negligible due to small differences in CO-Hb levels within a pair. Beeley et al. (1) recognized marked variability in the extent of histologic changes in the lungs of rabbits despite uniform exposure to acrolein in terms of time and concentration. Even with the addition of a uniform inhaled volume we still found modest differences in $PaO_2$ and EVLW within pairs. In a sheep model of inhalation injury (19), the curve of physiologic response with increasing dose is sigmoid in shape; beyond a certain dose of smoke, deterioration of pulmonary function accelerates rapidly toward lethality. The difficulty in precise control of severity may be a consequence of this characteristic of the physiological response.

Animals died between 24 and 72 hours; no deaths occurred before or after this period. It appeared that the immediate cause of death was not hypoxia due to impairment of alveolar structure, but partial obstruction of the trachea or major bronchi. Wheezing was recognized in all animals before death and the animals' condition deteriorated suddenly just prior to death. Massive sloughing casts in the trachea may have separated and occluded a lower airway already narrowed by pseudomembrane formation. Although we could not demonstrate complete obstruction of the trachea, compromised animals with little pulmonary reserve may die of acute partial obstruction. $PaO_2$ fell progressively and was lowest on the second day; it rose thereafter in all animals except one that had extensive pneumonia. Pathological changes in trachea, bronchi and bronchioles also improved by the fourth post injury day. Peitzman et al. (14) have shown lung water accumulation in patients with inhalation injury, whereas Tranbaugh et al. (22) could detect edema

only after the patients became septic. Recent studies support an increase of lung water during the acute phase of injury (2,7). Our results confirm an increase in lung water two days after injury, with edema observed on the fourth day. This finding is consistent with human studies in which patients recovered during the first three days except for elevated EVLW (7).

In the experimental model of inhalation injury described here, the differences in $PaO_2$ and EVLW within a pair were small and, as long as massive pneumonia did not occur, the time course of $PaO_2$ showed consistent improvement beyond the second day. This model appears useful for the evaluation of the effects of various treatments on inhalation injury, and for the assessment of the consequences of interactions among burns, infection and inhalation injury.

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| RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY | | | | 1. AGENCY ACCESSION | 2. DATE OF SUMMARY | REPORT CONTROL SYMBOL |
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| | | | | DA318371 | 01 Oct 90 | DD-DR&E(AR) 636 |
| 3. DATE PREV SUM'RY | 4. KIND OF SUMMARY | 5. SUMMARY SCTY | 6. WORK SECURITY | 7. REGRADING | 8. DISP'N INSTR'N | 9. LEVEL OF SUM / A. WORK UNIT |
| 01 Oct 89 | D. Chg | U | U | | CX | |

| 10. NO./CODES: | PROGRAM ELEMENT | PROJECT NUMBER | TASK AREA NUMBER | WORK UNIT NUMBER |
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| a. PRIMARY | 61102A | 3M161102BS14 | AZ | 321 |
| b. CONTRIBUTING | | | | |
| c. CONTRIBUTING | | | | |

11. TITLE *(Precede with Security Classification Code)* (U) Effect of Resuscitation Fluid on Hepatic Blood Flow and High Energy Phosphate Production in a Swine Hemorrhagic Shock Model

12. SUBJECT AREAS
0605 Medicine and Medical Research

| 13. START DATE | 14. ESTIMATED COMPLETION DATE | 15. FUNDING ORGANIZATION | 16. PERFORMANCE METHOD |
|---|---|---|---|
| 8905 | 9109 | DA | C. In-House |

17. CONTRACT/GRANT MILITARY RELEVANCY CERTIFIED
a. DATE EFFECTIVE APPROVED BY David Trunkey
b. CONTRACT/GRANT NUMBER
c. TYPE
d. AMOUNT
e. KIND OF AWARD
f. CUM/TOTAL

18. RESOURCES ESTIMATE

| FISCAL YEARS | a. PROFESSIONAL WORKYEARS | b. FUNDS *(In thousands)* |
|---|---|---|
| 90 | 0.1 | 74 |
| 91 | 0.1 | 77 |

19. RESPONSIBLE DOD ORGANIZATION
a. NAME: US Army Institute of Surgical Research
b. ADDRESS *(Include zip code)*: Fort Sam Houston, San Antonio, Texas 78234-5012
c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A
d. TELEPHONE NUMBER *(Include area code)*: 512-221-2720
21. GENERAL USE: FINA
MILITARY/CIVILIAN APPLICATION: M

20. PERFORMING ORGANIZATION
a. NAME: US Army Institute of Surgical Research
b. ADDRESS: Fort Sam Houston, San Antonio, Texas 78234-5012
c. NAME OF PRINCIPAL INVESTIGATOR: BECKER, W K
d. TELEPHONE NUMBER *(Include area code)*: 512-221-5703
f. NAME OF ASSOCIATE INVESTIGATOR *(If available)*: MASON, A D
g. NAME OF ASSOCIATE INVESTIGATOR *(If available)*:

22. KEYWORDS *(Precede EACH with Security Classification Code)* (U) Burns (Injuries); (U) Hemorrhage; (U) Hemorrhagic Shock; (U) Trauma; (U) Resuscitation; (U) Liver;

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS *(Precede text of each with Security Classification Code)*

22. (Continued) (U) Blood; (U) Colloids

23/24. (U) The objective of this work is to evaluate the effects of various resuscitation formulae on hepatic blood flow and hepatic high energy phosphate production following hemorrhagic shock. The effective use of resuscitation fluids is vital to the management of battle casualties. Liver high energy phosphate levels, hepatic blood flow, and oxygen delivery will be measured in swine at baseline, after 25% and 50% hemorrhage, and after administration of various resuscitative fluids.

25. (U) 8710 - 8809. Not applicable.

(U) 8810 - 8909. This project was approved by the USAISR Research Council and US Army Institute of Surgical Research Animal Care and Use Committee during the third quarter of fiscal year 1988.

(U) 8910 - 9009. Studies have been completed in 50 animals. Results indicate that hypertonic saline-dextran administration following hemorrhage results in a period of organ support that is comparable to Ringer's lactate resuscitation. The protocol has been amended to investigate the usefulness of hypertonic saline following dehydration.

DD FORM 1498 83 MAR — EDITION OF MAR 68 IS OBSOLETE. * USGPO 1986-491-003/50327

**SUPPLEMENTAL WORK UNIT INFORMATION FOR THE PROJECT ENTITLED "EFFECT OF RESUSCITATION FLUID ON HEPATIC BLOOD FLOW AND HIGH ENERGY PHOSPHATE PRODUCTION IN A SWINE HEMORRHAGIC SHOCK MODEL"**

**Subrecord/Linking Accession Number:** Not applicable.

**Search Control Data:** W6055C/W6056F, 20 October 1989

**Product Identification:** For technical reports, refer to the US Army Institute of Surgical Research Annual Research Progress Report for fiscal years 1989-90.

**Unclassified Special Categories:** Lab Animals: Swine; RA II

**ANNUAL RESEARCH REPORT**

**PROJECT NUMBER:** 3M161102BS14-00, RESEARCH

**PROJECT TITLE:** Effect of Resuscitation Fluid on Hepatic Blood Flow and Hepatic High Energy Phosphate Production in a Swine Model of Hemorrhagic Shock

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

**1 October 1989 - 30 September 1990**

**INVESTIGATORS**

William K. Becker, MD, Lieutenant Colonel, MC
Teresa M. Buescher, MD, Major, MC
William G. Cioffi, Jr., MD, Major, MC
Arthur D. Mason, Jr., MD

## ABSTRACT

**PROJECT NUMBER:** 3M161102BS14-00, RESEARCH

**PROJECT TITLE:** Effect of Resuscitation Fluid on Hepatic Blood Flow and Hepatic High Energy Phosphate Production in a Swine Model of Hemorrhagic Shock

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

**PERIOD COVERED IN THIS REPORT:** 1 Oct 89 through 30 Sep 90

**INVESTIGATORS:** William K. Becker, MD, Lieutenant Colonel, MC
Teresa M. Buescher, MD, Major, MC
William G. Cioffi, Jr., MD, Major, MC
Arthur D. Mason, Jr., MD

The role of small volume hypertonic saline-dextran (HSD) resuscitation following hemorrhage is unclear. Improvement in hemodynamic parameters may be at the expense of cellular function due to shift of intracellular water. Following a 35% hemorrhage, the ability of small volume (ml/kg) HSD to restore hemodynamic indices and hepatic adenosine triphosphate (ATP) was compared to Ringer's lactate (RL, 3ml/ml shed blood) and no resuscitation (NR) in immature swine fitted with arterial and venous catheters and hepatic artery and portal venous ultrasonic flow probes. Resuscitation began 30 minutes following a 35% hemorrhage which decreased cardiac output (CO 2.28±.56 to 1.26±.4 L/min. $p<.001$), hepatic blood flow (HBF 345±134 to 229±108 ml/min. $p<.02$), oxygen delivery ($O_2$ DEL 278±64 to 130±40 ml/min., $p<.001$) hepatic ATP (3.7±1.9 to 1.5±.4 µmole/gm, $p<.001$) and mean blood pressure, (BP, 76±23 to 39±8 mm/Hg, $p<.001$). One hour post resuscitation:

**TABLE 1**

| | NR | RL | HSD |
|---|---|---|---|
| CO | 1.2 ± 6 | 2.7 ± .83 | 1.9 ± .5 |
| HBF | 189 ± 103 | 331 ± 182 | 362 ± 101 |
| $O_2$ DEL | 134 ± 55 | 247 ± 80 | 184 ± 69 |
| ATP | 1.3 ± .3 | 2.5 ± .6* | 3.5 ± 1.2* |
| BP | 42 ± 13 | 60 ± 13 | 58 ± 5 |

*$p<.05$ compared to NR (A=6/group)

## CONCLUSION

While HSD was less effective than RL in restoring CO it was equally effective in restoring visceral blood flow, $O_2$ DEL and hepatic ATP production. HSD may provide a brief period of organ support when standard resuscitation measures are impractical.

**EFFECT OF RESUSCITATION FLUID ON HEPATIC BLOOD FLOW AND HEPATIC HIGH ENERGY PHOSPHATE PRODUCTION IN A SWINE MODEL OF HEMORRHAGIC SHOCK**

The role of small volume resuscitation fluids such as hypertonic saline or hypertonic saline dextran in the clinical setting following acute hemorrhage is unclear. Potential benefits associated with this form of therapy include decreased tissue edema and a reduction in pulmonary complications related to administration of large volumes of resuscitation fluid. Small volume resuscitation fluids are easier to store and administer when compared to standard solutions such as Ringer's Lactate. Because hypertonic saline solution must "borrow" water from the extravascular and intracellular spaces to achieve restoration of effective circulating volume, it is possible that the intracellular dehydration caused by the use of these fluids may be detrimental to organ function. Little is actually known about the effect of these fluids on organ function following resuscitation from hemorrhagic shock. The following experiments were performed to develop a model in which to explore the effects of resuscitation fluids on hepatic blood flow and hepatic high energy phosphate levels following shock and resuscitation.

Immature swine of either sex were used in this model, weighing 1825 kgs. Animals were sedated, intubated and placed on a volume cycled animal ventilator. Anesthesia was maintained by halothane inhalation (0.51% halothane). A Swan Ganz catheter was placed into the pulmonary artery and a femoral arterial line was also placed. A midline abdominal incision was performed and ultrasonic flow probes (Transonic, Ithaca NY) were placed on the portal vein and hepatic artery. Pilot studies with this model indicated that the animals wound not tolerate greater than a 35% total blood volume hemorrhage, so this level of hemorrhage was chosen for these experiments. Animals were bled over 30 minutes and resuscitation was begun 30 minutes after the completion of the bleed. Three resuscitation regimes were used:

1) No resuscitation

2) Lactated Ringers solution (RL) 3 ml/ml shed blood

3) Hypertonic saline dextran (HSD, 7.5% NaCl) 4 ml/kg body weight.

The resuscitation fluid was administered over five minutes. Hemodynamic indices were measured preshock, post shock and one hour post resuscitation. In addition, hepatic tissue sample were obtained and analyzed for hepatic ATP content. The results are listed below:

**TABLE 2**

| | NR | RL | HSD |
|---|---|---|---|
| CO | 1.2 ± 6 | 2.7 ± .83 | 1.9 ± .5 |
| HBF | 189 ± 103 | 331 ± 182 | 362 ± 101 |
| $O_2$ DEL | 134 ± 55 | 247 ± 80 | 184 ± 69 |
| ATP | 1.3 ± .3 | 2.5 ± .6* | 3.5 ± 1.2* |
| BP (mean) | 42 ± 13 | 601 ± 3 | 58 ± 5 |

*p<.05 compared to NR

Based on these results it appears that hypertonic saline-dextran (HSD) is a relatively effective resuscitation fluid for acute hemorrhagic shock. HSD was as effective as RL in restoring hepatic blood flow and hepatic ATP, although less effective than RL in restoring cardiac output. We conclude that HSD may provide a period of organ support when other resuscitation practices are impractical.

To further evaluate HSD, it was elected to switch to an unanesthetized swine model. Pilot studies in this model have been performed. The model includes a preliminary procedure under general anesthesia. A splenectomy is performed and flow probes are placed on the portal vein and hepatic artery. An arterial catheter is placed in the aorta through the sacral artery, and catheters are placed in the portal and hepatic veins and a Swan Ganz catheter in the pulmonary artery. Catheters are tunneled subcutaneously, protected by Velcro patches and flushed daily with a heparin-saline solution. Approximately 5 days after this procedure the animals are placed, awake, into a modified Pavlon sling for the shock experiment. Pilot studies determined that a 40% total blood volume hemorrhage had an LD80 at 24 hours, and allowed for completion of the experimental protocol. A 45% hemorrhage resulted in an LD 100 at 24 hours with many animals dying before resuscitation could begin. Therefore, a 40% hemorrhage was chosen for this model. Additional experiments using this model have been performed, but results are not yet available for analysis.

**RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY**

| 1. AGENCY ACCESSION | 2. DATE OF SUMMARY | REPORT CONTROL SYMBOL |
|---|---|---|
| DA346176 | 01 Oct 90 | DD-DR&E(AR) 636 |

| 3. DATE PREV SUM'RY | 4. KIND OF SUMMARY | 5. SUMMARY SCTY | 6. WORK SECURITY | 7. REGRADING | 8. DISP'N INSTR'N | 9. LEVEL OF SUM A. WORK UNIT |
|---|---|---|---|---|---|---|
| 01 Oct 89 | D. Chg | U | U | | CX | |

| 10. NO./CODES | PROGRAM ELEMENT | PROJECT NUMBER | TASK AREA NUMBER | WORK UNIT NUMBER |
|---|---|---|---|---|
| a. PRIMARY | 61102A | 3M161102BS14 | CK | 323 |
| b. CONTRIBUTING | | | | |
| c. CONTRIBUTING | | | | |

11. TITLE *(Precede with Security Classification Code)* (U) Correlation of Plasma Amino Acid and Pyridoxal-5'-Phosphate (PLP) Levels in Thermally Injured Patients

12. SUBJECT AREAS
0601 Biochemistry 0605 Medicine and Medical Research

| 13. START DATE | 14. ESTIMATED COMPLETION DATE | 15. FUNDING ORGANIZATION | 16. PERFORMANCE METHOD |
|---|---|---|---|
| 8906 | 9309 | DA | C. In-House |

17. CONTRACT/GRANT MILITARY RELEVANCY CERTIFIED
a. DATE EFFECTIVE APPROVED BY [signature]
b. CONTRACT/GRANT NUMBER
c. TYPE
d. AMOUNT
e. KIND OF AWARD
f. CUM/TOTAL

18. RESOURCES ESTIMATE:

| FISCAL YEARS | a. PROFESSIONAL WORK YEARS | b. FUNDS *(In thousands)* |
|---|---|---|
| 90 | 0.1 | 104 |
| 91 | 0.1 | 109 |

19. RESPONSIBLE DOD ORGANIZATION

a. NAME: US Army Institute of Surgical Research
b. ADDRESS *(include zip code)*: Fort Sam Houston, San Antonio, Texas 78234-5012
c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A
d. TELEPHONE NUMBER *(include area code)*: 512-221-2720

20. PERFORMING ORGANIZATION

a. NAME: US Army Institute of Surgical Research
b. ADDRESS: Fort Sam Houston, San Antonio, Texas 78234-5012
c. NAME OF PRINCIPAL INVESTIGATOR: BECKER, W K
d. TELEPHONE NUMBER *(include area code)*: 512-221-5703
f. NAME OF ASSOCIATE INVESTIGATOR *(if available)*
g. NAME OF ASSOCIATE INVESTIGATOR *(if available)*

21. GENERAL USE: FINA
MILITARY/CIVILIAN APPLICATION: M

22. KEYWORDS *(Precede EACH with Security Classification Code)* (U) Burns (Injuries); (U) Trauma; (U) Septicemia; (U) Morbidity; (U) Amino Acids; (U) Enzymes; (U) Metabolism

23. TECHNICAL OBJECTIVE 24 APPROACH 25 PROGRESS *(Precede text of each with Security Classification Code)*

**23/24. (U) The objective of this work is to measure PLP levels in thermally injured patients and correlate those with abnormalities in amino acid metabolism. One hundred burn patients will have plasma PLP and amino acid profiles drawn on admission, weekly, and when indicated by a change in clinical status. Burn size, presence of inhalation injury, morbidity, mortality, liver function test results, nitrogen balance, calories predicted and received, usage of aminoglycosides, theophylline, and/or digoxin, and the amount of vitamin B-6 supplementation received in tube feedings or hyperalimentation will be recorded. Multiple regression will be used to detect relationships between the various independent variables which are measured and the dependent variables.**

25. (U) 8710 - 8809. Not applicable.

(U) 8810 - 8909. This project was approved by the USAISR Research Council and US Army Institute of Surgical Research Human Use Committee during the fourth quarter of fiscal year 1989.

(U) 8910 - 9009. Ten patients were entered into this study during this reporting period. Upon completion of enrollment, the data will be analyzed as indicated.

DD FORM 83 MAR 1498 EDITION OF MAR 68 IS OBSOLETE

**SUPPLEMENTAL WORK UNIT INFORMATION FOR THE PROJECT ENTITLED "CORRELATION OF PLASMA AMINO ACID AND PYRIDOXAL-5'-PHOSPHATE (PLP) LEVELS IN THERMALLY INJURED PATIENTS"**

**Subrecord/Linking Accession Number:** Not applicable.

**Search Control Data:** W6R20M/W6R22N, 29 May 1990

**Product Identification:** For technical reports, refer to the US Army Institute of Surgical Research Annual Research Progress Report for fiscal years 1989-90.

**Unclassified Special Categories:** Volunteers: Adults; RA II

**ANNUAL RESEARCH REPORT**

**PROJECT NUMBER:** 3M161102BS14-00, RESEARCH

**PROJECT TITLE:** Correlation of Plasma Amino Acid and Pyridoxal-5'-Phosphate (PLP) Levels in Thermally Injured Patients

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

**1 October 1989 - 30 September 1990**

**INVESTIGATORS**

William K. Becker, MD, Lieutenant Colonel, MC
Teresa M. Buescher, MD, Major, MC
William G. Cioffi, Jr., MD, Major, MC
Bryan S. Jordan, RN
J. Enriquez, Sr.*
*Department of Clinical Investigations, WBMAC, El Paso, TX

## ABSTRACT

**PROJECT NUMBER:** 3M161102BS14-00, RESEARCH

**PROJECT TITLE:** Correlation of Plasma Amino Acid and Pyridoxal-5'-Phosphate(PLP) Levels in Thermally Injured Patients

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

**PERIOD COVERED IN THIS REPORT:** 1 Oct 89 through 30 Sep 90

**INVESTIGATORS:** William K. Becker, MD, Lieutenant Colonel, MC
Teresa M. Buescher, MD, Major, MC
William G. Cioffi, Jr., MD, Major, MC
Bryan S. Jordan, RN
J. Enriquez, Sr.*

*Department of Clinical Investigations, WBMAC, El Paso, TX

Pyridoxal 5'phosphate (PLP), the active form of vitamin B6, is a cofactor for over 100 enzymes, many of which involve amino acid metabolism. Although B6 deficiency is unusual in the general population, the hypermetabolism associated with major injury may predispose this subset of individuals to B6 deficiency. Un-deproteinized PLP and plasma amino acids were measured in 20 patients following thermal injury (age 39±13, total body burn size 48±19%, inhalation injury 50%, mortality 50%). Normal values of PLP range from 35-150 nM/L. Results (x±SD):

| POSTBURN WEEK | 1 | 3 | 5 |
|---|---|---|---|
| PLP nM/L | 18.3±8.8 | 18.5±3.8 | 16.1±10.3 |
| Alanine µM/L | 360±232 | 232±77 | 316±94 |
| Valine | 165±43 | 162±29 | 158±25 |
| Glutamine | 450±209 | 374±60 | 490±70 |
| Phenylalanine | 91±26 | 70±27 | 63±19 |

PLP was uniformly depressed to levels indicative of vitamin deficiency. No correlation between PLP and burn size, outcome or amino acid levels was noted. These results may indicate an increased requirement for vitamin B6 following major thermal injury. An altemate explanation is a shift of PLP to intracellular locations following thermal injury.

## CORRELATION OF PLASMA AMINO ACID AND PYRIDOXAL-5'-PHOSPHATE (PLP) LEVELS IN THERMALLY INJURED PATIENTS

PLP is the active form of vitamin B6, which is a cofactor for more than 100 enzymes. Many of these enzymes are important in amino acid metabolism. Deficiency of PLP is unusual in individuals ingesting a normal diet, but has been observed in critically ill patients, during pregnancy and following acute myocardial infarction. It is unclear whether the depression in PLP observed in these conditions is clinically important. To further investigate PLP in critically ill patients the following study was performed.

### PATIENTS

Twenty-five patients were entered into this study. All patients had thermal injury and were admitted to the USAISR. Partial data are available on 20 patients and form the results listed below. Weekly levels of underproteinized PLP and plasma amino acids were determined in these patients and patient demographics were collected. Representative values of amino acid levels and PLP levels for post burn weeks 1,3,5 are listed in Table 1. Demographics for this group of patients are: Age, 39±13; total burn size, 48±19%; inhalation injury, 50%, mortality 50%.

In this group of patients with severe thermal injury there was an immediate and sustained fall in PLP levels. No correlation between PLP levels, outcome, burn size or amino acid levels was noted. PLP levels were in range generally considered indicative of vitamin deficiency (PLP< 35 nM/L). It is unclear whether this represents a true deficiency or, possibly a shift of PLP to intracellular sites associated with an increased requirement for enzymes (and co-factors) needed to metabolize the increased flux of amino acids associated with major burn injury. Further studies to measure intracellular PLP levels and enzyme activity will be performed to answer this question.

**TABLE I**

| POSTBURN WEEK | 1 | 3 | 5 |
|---|---|---|---|
| PLP nM/L | 18.3 ±8.8* | 8.5 ±3.8 | 16.1 ±10.3 |
| Alanine μM/L | 360 ±232 | 232 ±77 | 316 ±94 |
| Valine | 165 ±43 | 162 ±29 | 158 ±25 |
| Glutamine | 450 ±209 | 374 ±60 | 490 ±70 |
| Phenylalanine | 91 ±26 | 70 ±27 | 63 ±19 |

*(X±SD)

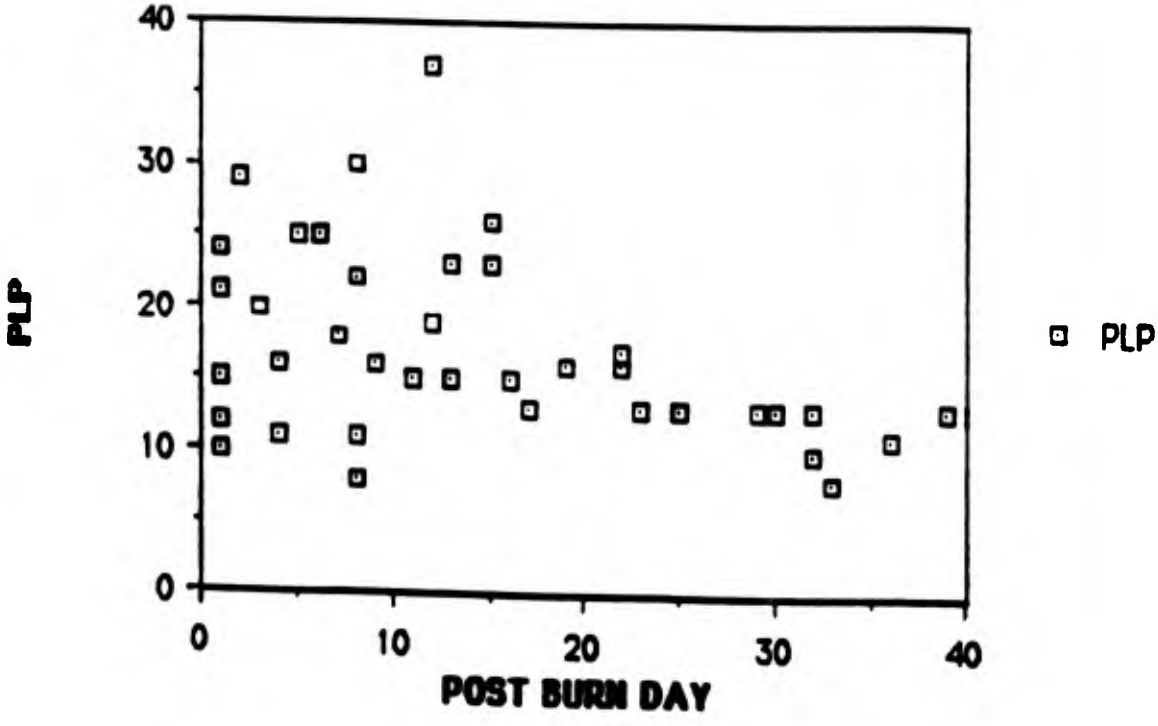


**FIGURE 1:** Plasma PLP (nM/L in thermally injured patients.

| RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY | 1. AGENCY ACCESSION | 2. DATE OF SUMMARY | REPORT CONTROL SYMBOL |
|---|---|---|---|
| | DA346164 | 01 Oct 90 | DD-DR&E(AR) 636 |

| 3. DATE PREV SUM'RY | 4. KIND OF SUMMARY | 5. SUMMARY SCTY | 6. WORK SECURITY | 7. REGRADING | 8. DISB'N INSTR'N | 9. LEVEL OF SUM A WORK UNIT |
|---|---|---|---|---|---|---|
| 07 Feb 90 | D. Chg | U | U | | CX | |

| 10. NO./CODES. | PROGRAM ELEMENT | PROJECT NUMBER | TASK AREA NUMBER | WORK UNIT NUMBER |
|---|---|---|---|---|
| a. PRIMARY | 61102A | 3M161102BS14 | CK | 324 |
| b. CONTRIBUTING | | | | |
| c. CONTRIBUTING | | | | |

11. TITLE (Precede with Security Classification Code) (U) Effect of Arginine Deprivation on the Response to Thermal Injury in Burn Wound Infection in the Rat - A Pilot Study

12. SUBJECT AREAS
0605 Medicine and Medical Research

| 13. START DATE | 14. ESTIMATED COMPLETION DATE | 15. FUNDING ORGANIZATION | 16. PERFORMANCE METHOD |
|---|---|---|---|
| 9002 | 9109 | DA | C. In-House |

17. CONTRACT/GRANT MILITARY RELEVANCY CERTIFIED APPROVED BY Basil A. Pruitt

| a. DATE EFFECTIVE | b. CONTRACT/GRANT NUMBER | c. TYPE | d. AMOUNT | e. KIND OF AWARD | f. CUM/TOTAL |
|---|---|---|---|---|---|
| | | | | | |

18. RESOURCES ESTIMATE

| a. FISCAL YEARS | a. PROFESSIONAL WORKYEARS | b. FUNDS (In thousands) |
|---|---|---|
| 90 | 0.1 | 83 |
| 91 | 0.1 | 88 |

| 19. RESPONSIBLE DOD ORGANIZATION | 20 PERFORMING ORGANIZATION |
|---|---|
| a. NAME: US Army Institute of Surgical Research | a. NAME: US Army Institute of Surgical Research |
| b. ADDRESS (include zip code): Fort Sam Houston, San Antonio, Texas 78234-5012 | b. ADDRESS: Fort Sam Houston, San Antonio, Texas 78234-5012 |
| c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A | c. NAME OF PRINCIPAL INVESTIGATOR: BECKER, W K |
| d. TELEPHONE NUMBER (include area code): 512-221-2720 | d. TELEPHONE NUMBER (include area code): 512-221-5703 |
| 21. GENERAL USE: FINA<br>MILITARY/CIVILIAN APPLICATION: M | f. NAME OF ASSOCIATE INVESTIGATOR (if available): CIOFFI, W G<br>g. NAME OF ASSOCIATE INVESTIGATOR (if available): MC MANUS, A T |

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Burns (Injuries); (U) Nutrition; (U) Metabolism; (U) Therapy

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

23/24. (U) The objective of this work is to determine the effect of a diet deficient in arginine on the response to thermal injury and burn wound infection. Sprague-Dawley rats will be fed regular, arginine-deficient, or arginine-sufficient diets for 5 weeks. Weekly weight gain and urinary orotic acid and polyamine excretion will be measured. At the end of 5 weeks, the animals will be administered 30% scald or sham wounds. One-half of these animals will be inoculated with Pseudomonas. Blood and urine samples will be obtained from any animal not surviving to 15 days and surviving animals sacrificed at 15 days for plasma amino acid analysis, BUN determination, and urine orotic acid and polyamine secretion. Mortality will be compared by life-table analysis. Differences in biochemical parameters will be studied by ANOVA, and where warranted, intergroup significance determined by the Scheffe' technique.

25. (U) 8710 - 8809. Not applicable.

(U) 8810 - 8909. Not applicable.

(U) 8910 - 9009. This project was approved by the USAISR Research Council and US Army Institute of Surgical Research Animal Care and Use Committee during the second quarter of fiscal year 1990. Studies have been conducted in 200 animals and results indicate that arginine deprivation results in alterations in growth, T cell function, and orotic acid excretion. The protocol has been amended to include other urea cycle amino

DD FORM 83 MAR 1498 EDITION OF MAR 68 IS OBSOLETE. * USGPO 1986-491-003/50329

**CONTINUATION OF THE DD FORM 1498 FOR THE PROJECT ENTITLED "EFFECT OF ARGININE DEPRIVATION ON THE RESPONSE TO THERMAL INJURY IN BURN WOUND INFECTION IN THE RAT - A PILOT STUDY"**

acids in the study diets. Survival studies did not yield a significant difference with any of the diets following burn or burn wound infection.

**SUPPLEMENTAL WORK UNIT INFORMATION FOR THE PROJECT ENTITLED "EFFECT OF ARGININE DEPRIVATION ON THE RESPONSE TO THERMAL INJURY IN BURN WOUND INFECTION IN THE RAT - A PILOT STUDY"**

**Subrecord/Linking Accession Number:** Not applicable.

**Search Control Data:** W6M22E/W6M25A, 8 January 1990

**Product Identification:** For technical reports, refer to the US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Year 1990.

**Unclassified Special Categories:** Lab Animals: Rats; RA II

**ANNUAL RESEARCH PROGRESS REPORT**

**PROJECT NUMBER:** 3M161102BS14-00, RESEARCH

**PROJECT TITLE:** Effect of Arginine Deprivation on the Response to Thermal Injury in Burn Wound Infection in the Rat - A Pilot Study

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

**1 October 1989 - 30 September 1990**

**INVESTIGATORS**

William K. Becker, MD, Lieutenant Colonel, MC
William G. Cioffi, Jr., MD, Major, MC
Albert T. McManus, PhD

## ABSTRACT

**PROJECT NUMBER:** 3M161102BS14-00, RESEARCH

**PROJECT TITLE:** Effect of Arginine Deprivation on the Response to Thermal Injury in Burn Wound Infection in the Rat - a Pilot Study

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

**PERIOD COVERED IN THIS REPORT:** 1 Oct 89 through 30 Sep 90

**INVESTIGATORS:** William K. Becker, MD, Lieutenant Colonel, MC
William G. Cioffi, Jr., MD, Major, MC
Albert T. McManus, PhD

Arginine is an amino acid involved in protein synthesis and in urea production. It is generally thought to be nonessential and can be produced de novo from other sources in most tissues. Recent reports indicate a possible role for arginine in wound healing and recovery from trauma. These reports suggest that arginine may become conditionally essential in growing animals, and following trauma and sepsis. The studies also suggest that there may be an optimal level of arginine in dietary supplements given following trauma or sepsis. The following experiments were performed to evaluate the role of arginine and the other urea cycle amino acids on growth, immunologic function and survival in rats following thermal injury and burn wound infection.

## STUDIES

Initial studies were performed in male Sprague-Dawley rats (growth studies) or inbred Lewis rats (immunologic studies). Rats weighed between 180-200 grams at the start of the studies, and were fed isocaloric, isonitrogenous defined amino acid diets (Teklad, Madison Wisconsin) deficient (Arg-) in arginine (0.9/kg diet) or sufficient in arginine (Arg+) (12.1 gm/kg diet), or diets in which arginine was replaced by ornithine (ORN) or citrulline (CIT).

Weight gain was monitored over four weeks:

| Diet (NS 10/group) | Weight Gain (gms) |
|---|---|
| Arg+ | 79.4 ± 13.9 |
| Arg- | 14.8 ± 7.4* |
| Orn | 40.1 ± 5.9* |
| Cit | 98.8 ± 8.9 |

X±SD
*p<.05 vs Arg+ (ANOVA)

In addition the urinary output of orotic acid, an alternate metabolic product of carbamyl phosphate, a urea cycle intermediate, and peripheral blood T cell markers were measured in Lewis rats on the Arg- and Arg+ diets. At five days the t cell helper-suppressor ratio was significantly decreased in Arg- rats (Arg+ 3.04±.51, Arg- 2.24±.17, p<.05) and at fourteen days orotic acid secretion was markedly increased in Arg- rats (Arg+ .08±.02 µg/mg creatinine, Arg- 10.9±5.1, P<.01).

Additional studies were performed to assess the effect of alterations of arginine in diet on the response to burn wound infection. Sprague-Dawley rats weighing 180-200 g were subjected to a standard 20% full-thickness burn and inoculated with various concentrations of Pseudomonas aeruginosa postburn. Diet was normal rat chow preburn and the indicated study diet postburn.

| DIET | INOCULUM (organisms/ml) | SURVIVAL (Days) | p |
|---|---|---|---|
| Arg- | $10^6$ | 7.63 ± .33 | |
| Arg+ | $10^6$ | 8.45 ± .42 | NS |
| Arg- | $10^4$ | 11.52 ± .58 | |
| Arg+ | $10^4$ | 12.85 ± .59 | NS |

n=20 group
X±SE
Survival at 15 days

Additional groups of animals (N=20/group) were fed an Arg- or an Arg+ diet for four weeks prior to burn injury and wound inoculation. Survival in this group was:

| Diet | Inoculum | Survival | p |
|---|---|---|---|
| Arg+ | $10^4$ | 10.7 ± .13 | |
| Arg- | $10^4$ | 7.7 ± .48 | <.01 |

Additional metabolic studies, including plasma amino acid levels, orotic acid excretion and polyamine excretion are pending.

In conclusion, alterations in the intake of a single amino acid involved in the urea cycle affect growth, intermediary metabolism and the response to trauma. Further investigations will be performed to assess the mechanisms of these effects.

**RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY**

| 1. AGENCY ACCESSION | 2. DATE OF SUMMARY | REPORT CONTROL SYMBOL |
|---|---|---|
| DA346163 | 01 Oct 90 | DD-DR&E(AR) 636 |

| 3. DATE PREV SUM'RY | 4. KIND OF SUMMARY | 5. SUMMARY SCTY | 6. WORK SECURITY | 7. REGRADING | 8. DISP'N INSTR'N | 9. LEVEL OF SUM A. WORK UNIT |
|---|---|---|---|---|---|---|
| 07 Feb 90 | D. Cha | U | U | | CX | |

| 10. NO./CODES | PROGRAM ELEMENT | PROJECT NUMBER | TASK AREA NUMBER | WORK UNIT NUMBER |
|---|---|---|---|---|
| a. PRIMARY | 61102A | 3M161102BS14 | FB | 325 |
| b. CONTRIBUTING | | | | |
| c. CONTRIBUTING | | | | |

11. TITLE *(Precede with Security Classification Code)* (U) Development of an Aspergillus Rat Burn Wound Infection Model - A Pilot Study

12. SUBJECT AREAS
0604 Anatomy and Physiology 0605 Medicine and Medical Research

| 13. START DATE | 14. ESTIMATED COMPLETION DATE | 15. FUNDING ORGANIZATION | 16. PERFORMANCE METHOD |
|---|---|---|---|
| 9002 | 9109 | DA | C. In-House |

17. CONTRACT/GRANT MILITARY RELEVANCY CERTIFIED
a. DATE EFFECTIVE APPROVED BY Basil Pruitt
b. CONTRACT/GRANT NUMBER
c. TYPE | d. AMOUNT
e. KIND OF AWARD | f. CUM TOTAL

18. RESOURCES ESTIMATE

| a. FISCAL YEARS | a. PROFESSIONAL WORK YEARS | b. FUNDS *(In thousands)* |
|---|---|---|
| 90 | 0.1 | 58 |
| 91 | 0.1 | 61 |

19. RESPONSIBLE DOD ORGANIZATION
a. NAME: US Army Institute of Surgical Research
b. ADDRESS *(Include zip code)*: Fort Sam Houston, San Antonio, Texas 78234-5012
c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A
d. TELEPHONE NUMBER *(Include area code)*: 512-221-2720

20. PERFORMING ORGANIZATION
a. NAME: US Army Institute of Surgical Research
b. ADDRESS: Fort Sam Houston, San Antonio, Texas 78234-5012
c. NAME OF PRINCIPAL INVESTIGATOR: BECKER, W K
d. TELEPHONE NUMBER *(Include area code)*: 512-221-5703
f. NAME OF ASSOCIATE INVESTIGATOR *(If available)*: MC MANUS, A T
g. NAME OF ASSOCIATE INVESTIGATOR *(If available)*: KIM, S H

21. GENERAL USE: FINA
MILITARY/CIVILIAN APPLICATION: M

22. KEYWORDS *(Precede EACH with Security Classification Code)* (U) Burns (Injuries); (U) Fungi; (U) Histopathology

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS *(Precede text of each with Security Classification Code)*

23/24. (U) The objective of this work is to develop an animal model of Aspergillus burn wound infection. Sprague-Dawley rats will be administered 30% scald wounds and inoculated with Aspergillus flavus at various times postburn. Group 1 will serve as the control group, Group 2 will receive streptozocin 10 days prior to injury, Group 3 will receive cortisone acetate 24 h prior to injury, and Group 4 will receive cyclosporine 24 h prior to injury. Wounds will be examined daily and the appearance recorded. Any animal not surviving to 15 days and surviving animals sacrificed at 15 days will undergo necropsy and histopathologic examination. Results of wound appearance, mortality, wound culture, and histopathology and any evidence of disseminated infection will be recorded.

25. (U) 8710 - 8809. Not applicable.

(U) 8810 - 8909. Not applicable.

(U) 8910 - 9009. This project was approved by the USAISR Research Council and US Army Institute of Surgical Research Animal Care and Use Committee during the second quarter of fiscal year 1990. Studies have been conducted in 250 animals. Results indicate that cyclosporine- and streptozocin-treated animals have a deeper level of colonization of fungus than control animals; however, invasive infection was observed in < 10% of treated animals. The protocol has been amended to add another species (Beige mouse) with an inbred T cell disorder in an attempt to increase the number of animals that develop fungal burn wound infection.

DD FORM 1498 83 MAR — EDITION OF MAR 69 IS OBSOLETE — * USGPO 1988 -491-003/50329

**SUPPLEMENTAL WORK UNIT INFORMATION FOR THE PROJECT ENTITLED "DEVELOPMENT OF AN ASPERGILLUS RAT BURN WOUND INFECTION MODEL - A PILOT STUDY"**

**Subrecord/Linking Accession Number:** Not applicable.

**Search Control Data:** W6L16M/W6L18M, 9 January 1990

**Product Identification:** For technical reports, refer to the US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Year 1990.

**Unclassified Special Categories:** Lab Animals: Rats; Mice; RA II

**ANNUAL RESEARCH REPORT**

**PROJECT NUMBER:** 3M161102BS14-00, RESEARCH

**PROJECT TITLE:** Development of an Aspergillus Burn Wound Infection Model - A Pilot Study

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

**1 October 1989 - 30 September 1990**

**INVESTIGATORS**

William K. Becker, MD, Lieutenant Colonel, MC
William G. Cioffi, Jr., MD, Major, MC
Albert T. McManus, PhD

## ABSTRACT

**PROJECT NUMBER:** 3M161102BS14-00, RESEARCH

**PROJECT TITLE:** Development of an Aspergillus Burn Wound Infection Model - A Pilot Study

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

**PERIOD COVERED IN THIS REPORT:** 1 Oct 89 through 30 Sep 90

**INVESTIGATORS:** William K. Becker, MD, Lieutenant Colonel, MC
William G. Cioffi, Jr., MD, Major, MC
Albert T. McManus, PhD

Fungal infection has replaced bacterial infection as the most common type of burn wound infection. There is no reliable animal model of fungal burn wound infection. This study was performed in an attempt to develop a reliable model of fungal burn wound infection (FBWI). Previous reports indicated that immunologically unmodified rodents were resistant to development of FBWI. In this study Sprague-Dawley rats were immunologically modified by a) cyclosporine, b) steroids, or c) streptozotocin induced diabetes, and were inoculated with Aspergillus following a standard burn injury. Despite these immunologic modifications the development of FBWI in these animals was rare (<2%), though colonization of the burn wound by Aspergillus was common. These experiments (burn wound and Aspergillus inoculation) were repeated in a small group of immunologically deficient mice, again with no FBWI developing. These results suggest that rodents are resistant to the development of FBWI and that model development should proceed in other species.

**DEVELOPMENT OF AN ASPERGILLUS BURN WOUND INFECTION MODEL - A PILOT STUDY**

This study was performed in an attempt to develop a reliable model of FBWI in small rodents. FBWI has replaced bacterial burn wound infection (BBWI) as the most common form of burn wound infection in patients at this Institute. The development of a small animal model of BBWI was useful in evaluating treatment strategies for this problem. Previous work on the development of models of FBWI suggested that rodents (rats, mice) were relatively resistant to the development of FBWI. A program was therefore designed to modify resistance in these animals in an attempt to increase the likelihood of the development of FBWI. The following experiments were performed:

Sprague-Dawley rats underwent a standard 20% body surface area burn (BSAB) under pentobarbital anesthesia. The following groups were studied:

1. Control

2. Cyclosporine 10 mg/kg IP starting 24 hours before burn injury and continuing for five days after burn injury.

3. Steroids - Cortisone acetate 5 mg subcutaneous 24 hours prior to injury.

4. Streptozotocin diabetes - 50 mg/kg streptozotocin IP 10 days prior to burn injury.

$10^6$ organisms of Aspergillus were inoculated at times 0 or 24 or 48 hours postburn injury. There were ten animals per group.

**RESULTS**

FBWI developed in less than 2% of animals in this study. Aspergillus colonized the wounds easily and was recovered by culture of the wounds in animals from each group. Colonization of the wound with fungus at levels Ib and Ic was observed in each group but no group had a statistically significant occurrence of FBWI. In those few animals that did develop focal FBWI, no systemic organ involvement with fungus was noted at autopsy.

Thirty beige mice, who have an inbred T cell dysfunction, were also subjected to 20% BSAB and Aspergillus inoculation. No evidence of FBWI was noted at that time of histopathologic examination of the burn wound.

## SUMMARY

Small rodents are remarkably resistant to the development of FBWI, in contrast to their susceptibility to BBWI. Therefore, model development should proceed in other species.

**RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY**

| 1. AGENCY ACCESSION | 2. DATE OF SUMMARY | REPORT CONTROL SYMBOL |
|---|---|---|
| DA346177 | 01 Oct 90 | DD-DR&E(AR) 636 |

| 3. DATE PREV SUM'RY | 4. KIND OF SUMMARY | 5. SUMMARY SCTY | 6. WORK SECURITY | 7. REGRADING | 8. DISB'N INSTR'N | 9. LEVEL OF SUM A. WORK UNIT |
|---|---|---|---|---|---|---|
| 07 Feb 90 | D. Chg | U | U | | CX | |

| 10. NO./CODES | PROGRAM ELEMENT | PROJECT NUMBER | TASK AREA NUMBER | WORK UNIT NUMBER |
|---|---|---|---|---|
| a. PRIMARY | 61102A | 3M161102BS14 | CA | 326 |
| b. CONTRIBUTING | | | | |
| c. CONTRIBUTING | | | | |

11. TITLE *(Precede with Security Classification Code)* (U) Effect of Surfactant Replacement on VA/Q in Sheep with Inhalation Injury

12. SUBJECT AREAS
0605 Medicine and Medical Research

| 13. START DATE | 14. ESTIMATED COMPLETION DATE | 15. FUNDING ORGANIZATION | 16. PERFORMANCE METHOD |
|---|---|---|---|
| 9002 | 9309 | DA | C. In-House |

17. CONTRACT/GRANT MILITARY RELEVANCY CERTIFIED
a. DATE EFFECTIVE APPROVED BY
b. CONTRACT/GRANT NUMBER
c. TYPE
d. AMOUNT
e. KIND OF AWARD
f. CUM/TOTAL

18. RESOURCES ESTIMATE

| FISCAL YEARS | a. PROFESSIONAL WORKYEARS | b. FUNDS *(In thousands)* |
|---|---|---|
| 90 | 0.1 | 58 |
| 91 | 0.1 | 61 |

19. RESPONSIBLE DOD ORGANIZATION

a. NAME
US Army Institute of Surgical Research

b. ADDRESS *(Include zip code)*
Fort Sam Houston
San Antonio, Texas 78234-5012

c. NAME OF RESPONSIBLE INDIVIDUAL
PRUITT, B A

d. TELEPHONE NUMBER *(Include area code)*
512-221-2720

20. PERFORMING ORGANIZATION

a. NAME
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b. ADDRESS
Fort Sam Houston
San Antonio, Texas 78234-5012

c. NAME OF PRINCIPAL INVESTIGATOR
CIOFFI, W G

d. TELEPHONE NUMBER *(Include area code)*
512-221-4440

21. GENERAL USE
FINA

MILITARY/CIVILIAN APPLICATION: M

f. NAME OF ASSOCIATE INVESTIGATOR *(If available)*
JORDAN, B S

g. NAME OF ASSOCIATE INVESTIGATOR *(If available)*
MASON, A D

22. KEYWORDS *(Precede EACH with Security Classification Code)* (U) Burns (Injuries); (U) Morbidity; (U) Inhalation; (U) Therapy

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS *(Precede text of each with Security Classification Code)*

23/24. (U) The objective of this work is to examine the effect of exogenously administered surfactant on VA/Q changes seen following inhalation injury in an ovine model. Forty-two sheep will be randomized to one of three groups. Group I will be exposed to a moderate smoke injury and administered surfactant 24 h following injury. Groups II and III will be exposed to a severe smoke injury. Group II will be administered surfactant or saline immediately following injury and every 12 h for 24 h. Group III will be administered surfactant or saline continuously during the first 24 h after injury. Group I data obtained following surfactant replacement will be indexed to pretreatment data and compared by the student's t test. Group II and III data will be compiled and, after analysis of mean and standard deviations, compared utilizing the student's t test and ANOVA.

25. (U) 8710 - 8809. Not applicable.

(U) 8810 - 8909. Not applicable.

(U) 8910 - 9009. This project was approved by the USAISR Research Council and US Army Institute of Surgical Research Animal Care and Use Committee during the second quarter of fiscal year 1990. Ten animals were used to validate the model and to begin the assessment of the efficacy of surfactant replacement in an ovine model of smoke injury.

DD FORM 83 MAR 1498 EDITION OF MAR 88 IS OBSOLETE * USGPO 1988-491-003/50324

**SUPPLEMENTAL WORK UNIT INFORMATION FOR THE PROJECT ENTITLED "EFFECT OF SURFACTANT REPLACEMENT ON VA/Q IN SHEEP WITH INHALATION INJURY"**

**Subrecord/Linking Accession Number:** Not applicable.

**Search Control Data:** W6015A/W6015D, 30 May 1990

**Product Identification:** For technical reports, refer to the US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Year 1990.

**Unclassified Special Categories:** Lab Animals: Sheep; RA II

**ANNUAL RESEARCH PROGRESS REPORT**

**PROJECT NUMBER:** 3M161102BS14-00, RESEARCH

**PROJECT TITLE:** Effect of Surfactant Replacement on VA/Q in Sheep with Inhalation Injury.

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

**1 October 1989 - 30 September 1990**

**INVESTIGATORS**

William G. Cioffi, Jr., MD, Major, MC
Bryan S. Jordan, RN
Arthur D. Mason, Jr., MD

**ABSTRACT**

**PROJECT NUMBER:** 3M161102BS14-00, RESEARCH

**PROJECT TITLE:** Effect of Surfactant Replacement on VA/Q in Sheep with Inhalation Injury

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

**PERIOD COVERED IN THIS REPORT:** 1 Oct 89 through 30 Sep 90

**INVESTIGATORS:** William G. Cioffi, Jr., MD, Major, MC
Bryan S. Jordan, RN
Arthur D. Mason, Jr., MD

This project was approved by the USAISR Research Council and US Army Institute of Surgical Research Animal Care and Use Committee during the second quarter of FY1990. Ten animals were used to validate the model and to begin the assessment of the efficacy of surfactant replacement in an ovine model of smoke injury.

# EFFECT OF SURFACTANT REPLACEMENT ON VA/Q IN SHEEP WITH INHALATION INJURY

## INTRODUCTION

The effect of inhalation injury on VA/Q, utilizing the multiple inert gas elimination technique (MIGET), and on cardiopulmonary parameters has been well described in an ovine model (1). Moderate to severe injury causes hypoxia, hypercarbia, and a shift of VA/Q to the left, i.e., an increase in lung segments with VA/Q > 0 but < 1. In addition, smoke-exposed animals show increased perfusion to shunt and low VA/Q lung segments. Attempts to alter these derangements with conventional ventilation utilizing PEEP resulted in increased dead space ventilation but had no effect on shunt or low VA/Q compartments (2).

It has been previously shown in a canine model that inhalation injury is associated with a marked increase in minimum surface tension in bronchoalveolar lavage samples, indicating that surfactant is no longer active or that less surfactant is available (3). Additionally, repeated exposure of rats to cigarette smoke is followed by a significant reduction in the recovery of pulmonary surfactant. The degree of reduction in surfactant recovery was dose-dependent (4).

This loss of surfactant following smoke exposure may partially explain the atelectasis and marked instability of alveolar walls seen following injury. The recent availability of synthetic surfactant has led to the suggestion that surfactant replacement may have a therapeutic effect in respiratory distress syndromes, including that caused by inhalation injury. Severe respiratory insufficiency induced by repeated lung lavage in the guinea pig model was significantly reversed by the administration of exogenous surfactant (5). Nonrandomized trials in infants with neonatal respiratory distress syndrome have shown increased compliance and improved blood gases following surfactant administration (6). In a recently completed randomized controlled trial, human surfactant was administered endotracheally at birth to very premature infants. The surfactant-treated group had significantly fewer deaths than the control group, with fewer cases of bronchopulmonary interstitial emphysema and pneumothorax (7). Prophylactic treatment with surfactant also substantially reduced the period of neonatal intensive care. These authors concluded that treatment with human surfactant offered promise of improving the survival of very premature infants and of reducing the pulmonary sequelae of the respiratory distress syndrome.

## MATERIALS AND METHODS

a. Experimental Design: Forty-two neutered male sheep weighing 25-45 kg will be utilized throughout this study. Inhalation injury will be induced using the standard ovine smoke

inhalation model developed at this Institute. The experiments will be divided into three phases. In Phase I of the experiment, 10 sheep will receive a moderate smoke injury which should result in a carboxyhemoglobin level of approximately 70% at the completion of the smoke exposure. Twenty-four hours following injury, the animals will be intubated and instrumented. Heart rate, blood pressure, central venous pressure, pulmonary artery pressure, cardiac output, arterial blood gases, tidal volume, flow rates, transpulmonary pressures, and static and dynamic compliance will be measured every 30 min. Once the ventilator settings are maximized, the animals will be allowed to stabilize for one hour. VA/Q distributions will then be measured using multiple inert gas elimination technique. Upon completion of two measurements, each animal will then receive a liquid bolus of EXOSURF (5 ml/kg) via the endotracheal tube. Thirty minutes and one hour following surfactant administration, repeat cardiopulmonary parameters, arterial and mixed venous blood gases, and VA/Q will be measured. In Phase II of the experiment, animals will receive a severe inhalation injury with carboxyhemoglobins of 80-90% following smoke exposure. The animals will remain intubated and paralyzed post smoke exposure. Immediately following injury and q12h, each animal will receive a liquid bolus of either EXOSURF or EXOSURF vehicle in the amount of 5 ml/kg via the endotracheal tube. After the third dose or 24 hours following smoke exposure, each animal will then have VA/Q measured. During the initial 24 hours, ventilator settings will be a tidal volume of 15-18 ml/kg, a ventilatory rate of 10-12 breaths per minute, an $FIO_2$ of .21, and a PEEP of 5. These will not be altered during the experimental period. Sixteen animals will be used in this experiment, eight receiving EXOSURF, and six EXOSURF vehicle only. In the third set of experiments, animals will be subjected to a severe smoke injury, and remain intubated and paralyzed post injury. Animals will receive aerosolized EXOSURF or EXOSURF vehicle beginning immediately following injury and continuing for 24 hours. At the end of 24 hours, VA/Q will be measured. Sixteen animals will be used in this experiment, eight receiving EXOSURF, and seven EXOSURF vehicle.

b. Animal Procedures:

(1) Methods for Appropriate Alleviation of Pain and Distress: All animals will be anesthetized with alpha-chloralose (0.5 g/kg) prior to manipulation.

(2) Exceptions to Alleviation of Pain: None.

(3) Description of Procedures: Forty-two neutered male sheep weighing 25-45 g will be utilized throughout this study. Each sheep will be housed in a conventional outdoor run and have access to food and water ad libitum. Sheep will be dewormed with injectable ivermectin (Ivomec®, Merck and Co., Rahway, NJ 07065) two weeks prior to use. Inhalation injury will be induced using the standard ovine smoke inhalation model developed at this

Institute. Ten sheep will be exposed to a moderate to severe smoke injury with a carboxyhemoglobin of approximately 70% at completion of the smoke exposure. The remaining 32 sheep will be subjected to a severe smoke inhalation with a carboxyhemoglobin of 80-90% at completion of their smoke exposure. Animals will be studied as previously described.

For animals in Phase I of the experiment, the following protocol will be used. On the day of the study, a peripheral venous catheter, a central venous pressure catheter, a balloon-directed thermodilution pulmonary artery catheter (7F, American Edwards Company, Irvine, California), and a femoral artery catheter will be placed. Anesthesia will be maintained with alpha-chloralose (0.05 g/kg). Animals will be paralyzed with pancuronium bromide (0.03-0.04 mg/kg, Pavulon$^{R}$, Organon Pharmaceuticals, West Orange, NJ). After placement of all catheters, animals will be positioned prone and conventional mechanical ventilation will be continued with a volume-limited ventilator (Bear II$^{TM}$, Bear Medical Systems, Inc., Riverside, CA). Ventilator settings will be altered to maintain a pH between 7.35-7.40. The animals will be ventilated with an $FIO_2$ of .21 and 0 PEEP. Lactated Ringer's will be constantly infused at a rate of 1 ml/kg/h. Central venous and pulmonary artery pressures will be monitored with Statham P23Db transducers (Statham Instruments, Oxnard, CA) and systemic artery pressures with a Hewlett-Packard 1290A quartz transducer (Hewlett-Packard Company, Waltham, MA).

Heart rate, blood pressure, central venous pressure, pulmonary artery pressure, cardiac output, arterial blood gases, tidal volume, flow rates, transpulmonary pressures, and static and dynamic compliance will be measured every 30 min until the end of the experiment. Once the ventilator settings are maximized to maintain a pH of 7.35-7.40, the animal will be allowed to stabilize for one hour. VA/Q distributions will then be measured utilizing MIGET. After stabilization, the Ringer's lactate infusion will be replaced with a lactated Ringer's solution containing six inert gases (sulphur hexafluoride, krypton, cyclopropane, halothane, ether, and acetone) which will be infused at the rate of 0.1 ml/kg/min. After 30 minutes, arterial and mixed venous gases will be drawn anaerobically into preweighed heparinized syringes (30 ml, matched, glass) simultaneously. Mixed expired gas will be obtained from a temperature-controlled copper coil (outside diameter = 3.49 cm, length = 64 cm) one minute after obtaining the blood samples. Blood and expired gas samples will be immediately analyzed by a gas chromatography mass spectrophotometer (sulphur hexafluoride by Hewlett-Packard GC-MDS, Model 5970, and other five gases by Hewlett-Packard GC, Model 5890). Repeat cardiopulmonary parameters will then be measured. MIGET data will be stored and quantified by a software program on the Hewlett-Packard Chem Station computer system. After 30 minutes, a second set of gases will be obtained.

At this point, the animal will receive exogenous synthetic surfactant obtained from the Burroughs Wellcome Company (Research Triangle Park, NC). In this experiment, the surfactant will be administered as a 5 ml/kg liquid bolus via the endotracheal tube. Thirty minutes and one hour following surfactant administration, arterial and mixed venous blood will be drawn and immediately analyzed. Expired gas samples will be obtained with each blood sample. Cardiopulmonary parameters will be re-measured at these times.

In the next two experiments, the following protocol will be used. Thirty sheep will be exposed to a severe smoke injury with a carboxyhemoglobin of 80-90% at the completion of smoke exposure. Post smoke exposure, the animals will remain intubated, paralyzed, anaesthetized and ventilated. Ventilator settings will be a tidal volume of 15-18 ml/kg, a ventilatory rate of 10-12 breaths per min, an $FIO_2$ of 0.21, and a PEEP of 5. The animals will be positioned prone and ventilated with the volume-limited ventilator (Bear II™, Bear Medical Systems, Inc., Riverside, CA). Pulmonary and hemodynamic parameters, as in the first experiment, will be measured in these animals every 30 min for the duration of the experiment. Surfactant will be administered to these animals in one of two ways. In the first group of animals, surfactant will be administered as a liquid bolus (5 ml/kg) q12h via the endotracheal tube. Eight animals will receive EXOSURF, and seven will receive a .1 N normal sodium chloride. During the initial 24 hours, each animal will receive lactated Ringer's infused at a rate of 1 ml/kg/hr. At the end of 24 hours, this solution will be switched to one which contains six inert gases as previously described. VA/Q will then be measured using the previously described protocol. An additional 15 animals will receive aerosolized EXOSURF or .1 N normal sodium chloride on a continuous basis following smoke exposure. After 24 hours of aerosolization, VA/Q will then be measured.

In the second and third experiments, each animal will undergo bronchoscopy and bronchoalveolar lavage following measurement of VA/Q. Tracheal fluid will be obtained for measurement of surface activity, phospholipid content, and surfactant lipoprotein content and will be performed by the Burroughs Wellcome Company. At the end of each experiment, each animal will then be euthanized and necropsied. Representative sections of each lung will then be obtained for histologic study.

## RESULTS

During this fiscal year ten animals were studied in this protocol. Revalidation of the smoke injury model, allowing for prolonged support of the animals, was necessary. Long term anesthesia and paralysis is not possible in the ovine model. The experimental design has been altered to study the sheep awake, and tracheotomized, with arterial $PCO_2$ maintained at 25 torr or less.

This enables the investigators to control ventilation entirely. Phase III of the trial will be initiated during the upcoming fiscal year.

## CONCLUSIONS

None.

## PRESENTATIONS/PUBLICATIONS

None.

## REFERENCES


1. Shimazu T, Yukioka T, Hubbard GB, et al: Inequality of VA/Q ratios following smoke inhalation injury and the effect of angiotensin analogues. In **US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Year 1985**. Davis CC (ed). San Antonio: Fort Sam Houston, pp 425-442

2. Shimazu T: Acute effects of positive end-expiratory pressure (PEEP) on cardiopulmonary indices including VA/Q ratios (unpublished data).

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4. LeMesurier SM, Lykke AW, and Stewart BW: Reduced yield of pulmonary surfactant: patterns of response following administration of chemicals to rats by inhalation. **Toxicol Lett** 5:89-93, 1980.

5. Berggren P, Lachmann B, Curstedt T, et al: Gas exchange and lung morphology after surfactant replacement in experimental adult respiratory distress syndrome induced by repeated lung lavage. **Acta Anaesthesiol Scand** 30:321-328, 1986.

6. Taeusch HW Jr, Clements J, and Benson B: Exogenous surfactant for human lung disease. Joint Program in Neonatolgoy, Harvard Medical School, Boston MA.

7. Merritt TA, Hallman M, Bloom BT, et al: Prophylactic treatment of very premature infants with human surfactant. **New Engl J Med** 315(13):785-790, 1986.

**RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY**

| 1. AGENCY ACCESSION | 2. DATE OF SUMMARY | REPORT CONTROL SYMBOL |
|---|---|---|
| DA346160 | 01 Oct 90 | DD-DR&E(AR) 636 |

| 3. DATE PREV SUM'RY | 4. KIND OF SUMMARY | 5. SUMMARY SCTY | 6. WORK SECURITY | 7. REGRADING | 8. DISP'N INSTR'N | 9. LEVEL OF SUM A. WORK UNIT |
|---|---|---|---|---|---|---|
| ? Mar 90 | D. Chg | U | U | | CX | |

| 10. NO./CODES: | PROGRAM ELEMENT | PROJECT NUMBER | TASK AREA NUMBER | WORK UNIT NUMBER |
|---|---|---|---|---|
| a. PRIMARY | 61102A | 3M161102BS14 | CG | 327 |
| b. CONTRIBUTING | | | | |
| c. CONTRIBUTING | | | | |

11. TITLE *(Precede with Security Classification Code)*
(U) Intestinal Permeability Following Thermal Injury

12. SUBJECT AREAS
0605 Medicine and Medical Research

| 13. START DATE | 14. ESTIMATED COMPLETION DATE | 15. FUNDING ORGANIZATION | 16. PERFORMANCE METHOD |
|---|---|---|---|
| 9003 | 9209 | DA | C. In-House |

17. CONTRACT/GRANT MILITARY RELEVANCY CERTIFIED
a. DATE EFFECTIVE APPROVED BY Paul Pruitt
b. CONTRACT/GRANT NUMBER
c. TYPE d. AMOUNT
e. KIND OF AWARD f. CUM/TOTAL

18. RESOURCES ESTIMATE

| a. FISCAL YEARS | a. PROFESSIONAL WORKYEARS | b. FUNDS *(In thousands)* |
|---|---|---|
| 90 | 0.1 | 49 |
| 91 | 0.1 | 52 |

19. RESPONSIBLE DOD ORGANIZATION

a. NAME
US Army Institute of Surgical Research

b. ADDRESS *(Include zip code)*
Fort Sam Houston
San Antonio, Texas 78234-5012

c. NAME OF RESPONSIBLE INDIVIDUAL
PRUITT, B A

d. TELEPHONE NUMBER *(Include area code)*
512-221-2720

20. PERFORMING ORGANIZATION

a. NAME
US Army Institute of Surgical Research

b. ADDRESS
Fort Sam Houston
San Antonio, Texas 78234-5012

c. NAME OF PRINCIPAL INVESTIGATOR
CIOFFI, W G

d. TELEPHONE NUMBER *(Include area code)*
512-221-4440

21. GENERAL USE
FINA

MILITARY/CIVILIAN APPLICATION: M

e. NAME OF ASSOCIATE INVESTIGATOR *(If available)*
MC MANUS, W F

g. NAME OF ASSOCIATE INVESTIGATOR *(If available)*

22. KEYWORDS *(Precede EACH with Security Classification Code)* (U) Burns (Injuries); (U) Septicemia; (U) Intestines; (U) Bacteria; (U) Mannitol

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS *(Precede text of each with Security Classification Code)*

23/24. (U) The objective of this work is to assess alterations in intestinal permeability in the acute phase of thermal injury. Twenty consecutive patients will have intestinal permeability measured on postburn days 2, 4, 6, 8, 10, and 12 and 10 healthy volunteers will be individually studied for 2 consecutive days. Intestinal permeability will be measured by administering lactulose and mannitol, two different molecular weight sugars which are absorbed via different mechanisms in the gastrointestinal tract. Lactulose/mannitol ratios will be subjected to repeated measures ANOVA and multiple regression analysis to determine variation of lactulose excretion with burn size and postburn day.

25. (U) 8710 - 8809. Not applicable.

(U) 8810 - 8909. Not applicable.

(U) 8910 - 9009. This project was approved by the USAISR Research Council and US Army Institute of Surgical Research Human Use Committee during the second quarter of fiscal year 1990. Twelve burn patients and 5 control subjects were enrolled in the study during this reporting period. Preliminary analysis of the data indicates significant alteration in intestinal permeability to the markers following thermal injury.

DD FORM 83 MAR 1498 EDITION OF MAR 68 IS OBSOLETE. ★ U.S.G.P.O. 1988-491-003/50339

**SUPPLEMENTAL WORK UNIT INFORMATION FOR THE PROJECT ENTITLED "INTESTINAL PERMEABILITY FOLLOWING THERMAL INJURY"**

**Subrecord/Linking Accession Number:** Not applicable.

**Search Control Data:** W6L38M/W6L39L, 9 January 1990

**Product Identification:** For technical reports, refer to the US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Year 1990.

**Unclassified Special Categories:** Volunteers: Adults; RA II

**ANNUAL RESEARCH PROGRESS REPORT**

**PROJECT NUMBER:** 3M161102BS14-00, RESEARCH

**PROJECT TITLE:** Intestinal Permeability Following Thermal Injury

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

**1 October 1989 - 30 September 1990**

**INVESTIGATORS**

William G. Cioffi, Jr., MD, Major, MC
William F. McManus, MD, Colonel, MC

## ABSTRACT

**PROJECT NUMBER:** 3M161102BS14-00, RESEARCH

**PROJECT TITLE:** Intestinal Permeability Following thermal Injury

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

**PERIOD COVERED IN THIS REPORT:** 1 Oct 89 through 30 Sep 90

**INVESTIGATORS:** William G. Cioffi, Jr., MD, Major, MC
William F. McManus, MD, Colonel, MC

This project was approved by the USAISR Research Council and US Army Institute of Surgical Research Human Use Committee during the second quarter of FY1990. Twelve patients and five normal volunteers were enrolled in the study during this reporting period. Preliminary analysis of the data indicates significant alteration in intestinal permeability to the markers following thermal injury.

# INTESTINAL PERMEABILITY FOLLOWING THERMAL INJURY

## INTRODUCTION

Alteration in gastrointestinal mucosal permeability leading to translocation of bacteria and endotoxin has been proposed as an etiology of occult sepsis and multiple organ failure. Normally, the large numbers of pathogenic flora residing in the human intestines and their products are prevented from gaining systemic entrance by the intestinal mucosa. Berg (1) has proposed that this epithelial barrier may be damaged and that the translocation of indigenous bacteria may occur when intestinal permeability is altered secondary to specific disease states, the normal pre-existing bacterial relationships are altered such that one subpopulation experiences a tremendous population surge, or the host immune system is compromised such as that seen following thermal injury.

The effect of thermal injury on intestinal permeability in humans has been studied by Ziegler et al. (2) who demonstrated that intestinal permeability was increased in infected burn patients but not in patients with thermal injury alone. However, due to the design of the study, it could not be ascertained whether the infections were caused by increased intestinal permeability or whether the infections promoted increased intestinal permeability. This study also failed to address the status of intestinal permeability changes during the acute phase of thermal injury. Deitch et al. (3) have shown that when rats are exposed to a significant thermal injury (>40% of the total body surface area), translocation of gastrointestinal bacteria may occur as early as two days post injury.

## MATERIALS AND METHODS

a. Design: Twenty consecutive patients meeting admission criteria will be entered into the study. Intestinal permeability will be measured on postburn days 2,4,6,8,10, and 12. A control group of 10 healthy volunteers will be individually studied on two consecutive days. Intestinal permeability will be measured by administering two different molecular weight sugars which are absorbed via different mechanisms in the gastrointestinal tract (4). Lactulose is a disaccharide which is absorbed paracellularly via extrusion zones at villous tips or via tight junctions. If intestinal permeability is abnormal, an increased amount of lactulose will cross the tight junctions between mucosal enterocytes. Following absorption, the lactulose is not metabolized and is excreted unchanged in the urine. Thus, the amount of lactulose excreted per unit time can be used as a marker of intestinal permeability. Because lactulose excretion may be influenced by extrinsic factors such as gastric emptying, intestinal transit time, mucosal surface area, renal function, cardiac output, and intestinal blood flow, a second sugar such as

mannitol is used to index lactulose excretion. Small sugars such as mannitol are absorbed via the water-filled pores in the enterocyte membrane. alterations in intestinal permeability should not affect absorption of this type of sugar. Indexing lactulose excretion as a percentage of mannitol excretion will allow one to account for the effect of the above factors.

b. Description of Procedures:

(1) Burn Patients: On the day of the study, the test solution will be instilled via the patient's nasogastric tube. The solution will consist of 10 g of lactulose and 5 g of mannitol mixed in 60 ml of distilled water. The test solution will be instilled through the nasogastric tube and flushed with 10 cc of distilled water to insure completeness of dosing. All urine will be collected for 6 h and refrigerated. Once the urine collection is completed, the urine will be divided into aliquots and frozen at -20°C prior to analyses. Urinary lactulose will be measured according to the method described by Behrens (5). Prior to enzymatic degradation, the urine samples will be incubated with glucose oxidase to remove any urinary glucose which might affect the results. Urinary mannitol will be measured as described by Neihaus and Dilts (6) utilizing mannitol dehydrogenase. Data will then be expressed as total lactulose excretion indexed to mannitol excretion.

(2) Control Patients: On the day of the study, the test solution will be given to the patient to drink. The solution will consist of 10 g of lactulose and 5 g of mannitol mixed in 60 ml of distilled water. All urine will be collected for 6 h and refrigerated. Once the urine collection is completed, the urine will be divided into aliquots and frozen at -20°C prior to analyses as indicated above for burn patients.

(3) Patient Inclusion:

(a) Male or female patients 18 years of age and older. Female patients must have been surgically sterilized, be postmenopausal (over 45 years of age and the lack of menstrual periods for at least on year), or have a negative pregnancy test prior to initiation into the study.

(b) Patients admitted to the U.S. Army Institute of Surgical Research within the first 48 hours postburn.

(c) Patients with burns >20% of the total body surface area (the presence of an inhalation injury not being exclusionary).

(4) Patient Exclusion:

(a) Patients under 18 years of age.

(b) Patients not admitted to the U.S. Army Institute of Surgical Research within the first 48 hours postburn.

(c) Patients with burns <20% of the total body surface area.

(d) Patients who are pregnant.

(e) Patients with preexisting renal dysfunction as indexed by a serum creatinine >2.

(f) Patients with a history of gastrointestinal disease such as inflammatory bowel disease, malabsorption syndromes, or any previous history of bowel resection.

(g) Patients with a history of chronic alcohol abuse.

(h) Patients with a history of diabetes.

(i) Patients with toxic epidermal necrolysis (TEN).

c. Determination of Number of Subjects Required: This is a preliminary study to determine if any gross alterations in intestinal permeability occur following thermal injury. If this study shows significant alterations, then a larger study will be performed.

d. Data Collection: The burn size and medical history and the results of urine analyses will be recorded for each patient. A medical history will be obtained from each control subject and the results of urine analyses will be recorded for each control subject.

e. Data Analysis Plan: Patient data will be analyzed to assess whether changes in permeability occur over time following thermal injury. In addition, all patient data will be pooled and compared to control population date.

## RESULTS

During fiscal year 1990 twelve patients were enrolled in this protocol. In addition, five volunteer subjects from the USAISR were studied. Prior to performance of the mannitol and lactulose assays, new assays were developed by the Biochemistry Section for measurement of these sugars. Previously reported assays were not suitable for the measurement of mannitol and lactulose in the urine, due to the numerous interfering substances found in the urine of thermally injured patients. Development of the new assays was completed during the last quarter of fiscal year 1990.

Preliminary review of data from the burned patients entered so far reveals an approximately 10-fold increase in the lactulose/

mannitol ratio, indicating a marked increase in intestinal permeability to lactulose in thermally injured patients. This increase in permeability persisted throughout the two week study period.

Upon enrollment of twenty patients and ten normal volunteers during the first quarter of fiscal year 1991, the data will be completely analyzed.

## DISCUSSION

None.

## PRESENTATIONS/PUBLICATIONS

None.

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**ANNUAL RESEARCH PROGRESS REPORT**

**PROJECT NUMBER:** 3M161102BS14-00, RESEARCH

**PROJECT TITLE:** INTESTINAL PERMEABILITY FOLLOWING THERMAL INJURY: Simultaneous Determination of Lactulose and Mannitol in the Urine of Burn Patients by Gas-Liquid Chromatography

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

**1 October 1989 - 30 September 1990**

**INVESTIGATORS**

Ronald L. Shippee, PhD, Major, MS
Avery A. Johnson
William G. Cioffi, Jr., MD, Major, MC
James Lasko, Sergeant First Class
Thomas E. LeVoyer, MD, Captain, MC
Bryan S. Jordan, RN

## ABSTRACT

**PROJECT NUMBER:** 3M161102BS14-00, RESEARCH

**PROJECT TITLE:** INTESTINAL PERMEABILITY FOLLOWING THERMAL INJURY: Simultaneous Determination of Lactulose and Mannitol in the Urine of Burn Patients by Gas Liquid Chromatography

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

**PERIOD COVERED IN THIS REPORT:** 1 Oct 89 through 30 Sep 90

**INVESTIGATORS:** Ronald L. Shippee, PhD, Major, MS
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Lactulose/mannitol urine excretion ratios have been used to assess the extent of intestinal permeability in various disease and trauma states. Reported studies have used the technique to correlate altered gastrointestinal mucosal permeability with translocation of bacteria and endotoxin and, in turn, occult sepsis in burned patients. Enzymatic methods of analysis for urine concentrations of mannitol and lactulose were used in these studies. We have found that urine from patients with severe burns frequently contains compounds that interfere with these enzymatic methods. We describe a method of using gas-liquid chromatography to determine mannitol and lactulose simultaneously in the urine of burned patients. To avoid the occurrence of multiple peaks caused by the anomeric forms of the reducing sugars during pre-column trimethylsilyl derivatization, we converted the sugars to oximes prior to the silylation step. The method gave good recoveries after spiking urine from burned patients with mannitol and lactulose. Unlike the enzymatic methods, gas-liquid chromatography eliminates the effect of interfering compounds and allows for the simultaneous determination of both sugars in urine samples.

# SIMULTANEOUS DETERMINATION OF LACTULOSE AND MANNITOL IN THE URINE OF BURN PATIENTS BY GAS LIQUID CHROMATOGRAPHY

## INTRODUCTION

A number of investigators have proposed that thermal injury alters gastrointestinal mucosal permeability, permitting translocation of bacteria and endotoxin and leading to occult sepsis and multiple organ failure (1-3). Lactulose/mannitol urine excretion ratios have been used to monitor changes in intestinal permeability in various disease conditions (4,5). This report describes the application of gas-liquid chromatography to simultaneous determinations of lactulose and mannitol in the urine of burn patients.

## METHODS AND MATERIALS

**Subjects.** Six male patients with a mean burn size of 58% of total body surface area (range 31% to 78%) were used in this preliminary study to develop the assay technique, and were from a larger study designed to assess perturbations of intestinal permeability after burn injury. On the fourth postburn day, each patient drank a solution containing 5g of mannitol and 10g of lactulose mixed with 100ml of deionized water. A six-hour urine collection followed after ingestion of the test solution. One milliliter of a 20% chlorhexidine solution was added to the total urine collected. Aliquots of the urine samples were frozen at -20°C until analyses were performed.

**Sample Preparation.** One hundred and twenty-five microliters of 1mM Methyl-mannopyranoside was added as an internal standard to 50µl of diluted (1:5 with deionized water) urine. The sample and internal standard were dried under nitrogen in a heating block at 75°C. After cooling, 100µl of oxime solution (25mg Hydroxylamine hydrochloride/ml pyridine) was added. The tubes were then capped and incubated at 75°C for 30 minutes. After the samples cooled to room temperature, 100µl of n-Trimethylsilyl Imidazole (TMSI) reagent (Pierce, P.O. Box 17, Rockford, IL 61105-9976) was added followed by a 15 min. incubation period at 75°C.

**Standard Preparation.** Stock solutions of 5mM mannitol and 1mM lactulose were made in deionized water. From the stock mannitol, solution 20, 40, 60, 80, and 100µl were pipetted into disposable injection vials to give standards containing 100, 200, 300, 400, and 500 nmoles of mannitol. From the stock lactulose solution, 10, 20, 30, 40, and 50µl were pipetted into disposable injection vials to give standards containing 10, 20, 30, 40, and 50nmoles of lactulose. One hundred twenty-five microliters of 1mM methyl-mannopyranoside was added to all standards. Standards were converted to oximes and silylated as described above.

**GLC Analysis.** Two microliters of prepared sample were injected into a DB-5, 15 m X 0.53 mm I.D. capillary column (J&W Scientific, Folsom, CA) installed in a Hewlett Packard Gas-Liquid Chromatograph 5890 Series II (FID detector, HP7673 Autoinjector, HP 3396A Integrater). The injection temperature was set at 220°C with a detection temperature of 300°C and a flow rate of 9.7 to 9.9 ml/min. Optimal performance was achieved by setting the initial oven temperature at 150°C for six minutes, then ramping the temperature to 220°C at 10°C/min. followed by an additional ramp to 300°C at 15°C/min. Program was terminated at 20 min.

## RESULTS

The chromatogram of a standard solution containing mannitol, fructose, galactose, glucose and lactulose is shown in Figure 1. None of the three monosaccharides caused interfering peaks with mannitol or lactulose.

Linearities of the standard curves for mannitol and lactulose are shown in Figures 2 and 3. Mannitol and lactulose were linear from 5? to 500 and 5 to 50 nmoles respectively. We determined that the minimum detectable concentrations for mannitol and lactulose in urine were 5nmoles/L and 1nmole/L respectively.

Urine samples from three burn patients were spiked with 50, 100, and 150nmol of mannitol and 5, 10, and 15nmol of lactulose. Recovery data from these samples are presented in Table 1.

**TABLE 1. Recovery of Mannitol and Lactulose Added to Urine from Burn Patients**

| | Mannitol Added | Lactulose Added | Recovery | | | |
|---|---|---|---|---|---|---|
| | | | Mannitol | | Lactulose | |
| | | | nmoles | % | nmoles | % |
| Sample 1 | 0 | 0 | 73.9 | - | 3.2 | - |
| | 50 | 5 | 125.4 | 101 | 8.9 | 106 |
| | 100 | 10 | 184.3 | 106 | 13.4 | 101 |
| | 150 | 15 | 228.1 | 102 | 20.1 | 110 |
| Sample 2 | 0 | 0 | 48.6 | - | 2.5 | - |
| | 50 | 5 | 101.6 | 103 | 7.9 | 104 |
| | 100 | 10 | 134.9 | 91 | 12.3 | 98 |
| | 150 | 15 | 195.5 | 98 | 17.7 | 101 |
| Sample 3 | 0 | 0 | 65.5 | - | 10.7 | - |
| | 50 | 5 | 121.3 | 105 | 17.6 | 112 |
| | 100 | 10 | 165.0 | 100 | 22.3 | 108 |
| | 150 | 15 | 208.5 | 97 | 30.0 | 117 |

Urine from one of the burn patients was used to test the precision of the assay. Six samples were prepared daily for five consecutive days. The mean within day precision for lactulose was found to be 4.66% and for mannitol, 0.52% (Table 2). The between day coefficient of variation was 1.9% and 0.4% for lactulose and mannitol respectively.

**TABLE 2. Within and Between Run Precision for Urine From a Single Burn Patient**

| | Mannitol (nmoles) | Lactulose (nmoles) |
|---|---|---|
| Day | | |
| 1 (n=10) | 79.6±1.0 (1.2)[1] | 3.5±0.2 (5.5) |
| 2 (n=10) | 78.8±0.2 (0.2) | 3.6±0.2 (4.5) |
| 3 (n=10) | 78.3±0.3 (0.3) | 3.5±0.1 (3.5) |
| 4 (n=10) | 78.6±0.4 (0.5) | 3.4±0.2 (4.6) |
| 5 (n=10) | 78.3±0.3 (0.4) | 3.5±0.2 (5.2) |
| Between Day | 78.7±0.3 (0.4) | 3.5±0.1 (1.9) |

[1] Mean ± STD (%C.V)

Data from the six burn and six control subjects are shown in Figure 4. There was no significant difference in urine mannitol concentration between the control and burn subjects ($p=0.44$). Both the lactulose concentration and the ratio of lactulose/mannitol were significantly different at $p>0.01$.

## DISCUSSION

Urine samples from patients with severe burns present an analytical challenge to the clinical chemist. Our experience has been that many analytical assays that are suitable for measurement in urine from normal or sick subjects can not be applied to urine from burn patients. We have found the enzymatic method of assay for urinary lactulose to be a prime example of this. The method outlined by Behrens (6) involves an enzymatic hydrolysis of lactulose to fructose and galactose followed by the conversion of fructose to glucose-6-phosphate. Lactulose is measured indirectly by converting G-6-P to gluconate-6-phosphate and monitoring the reduction of NADP as the absorbance at 340 nm.

Ziegler et al. (1) have measured lactulose and mannitol enzymatically in the urine of burn patients in an attempt to determine perturbations in intestinal absorption. We have found that specimens of urine from patients with severe burns frequently contain compounds that interfere with the enzymatic assays used to determine lactulose in urine. Filtration of the urine through a

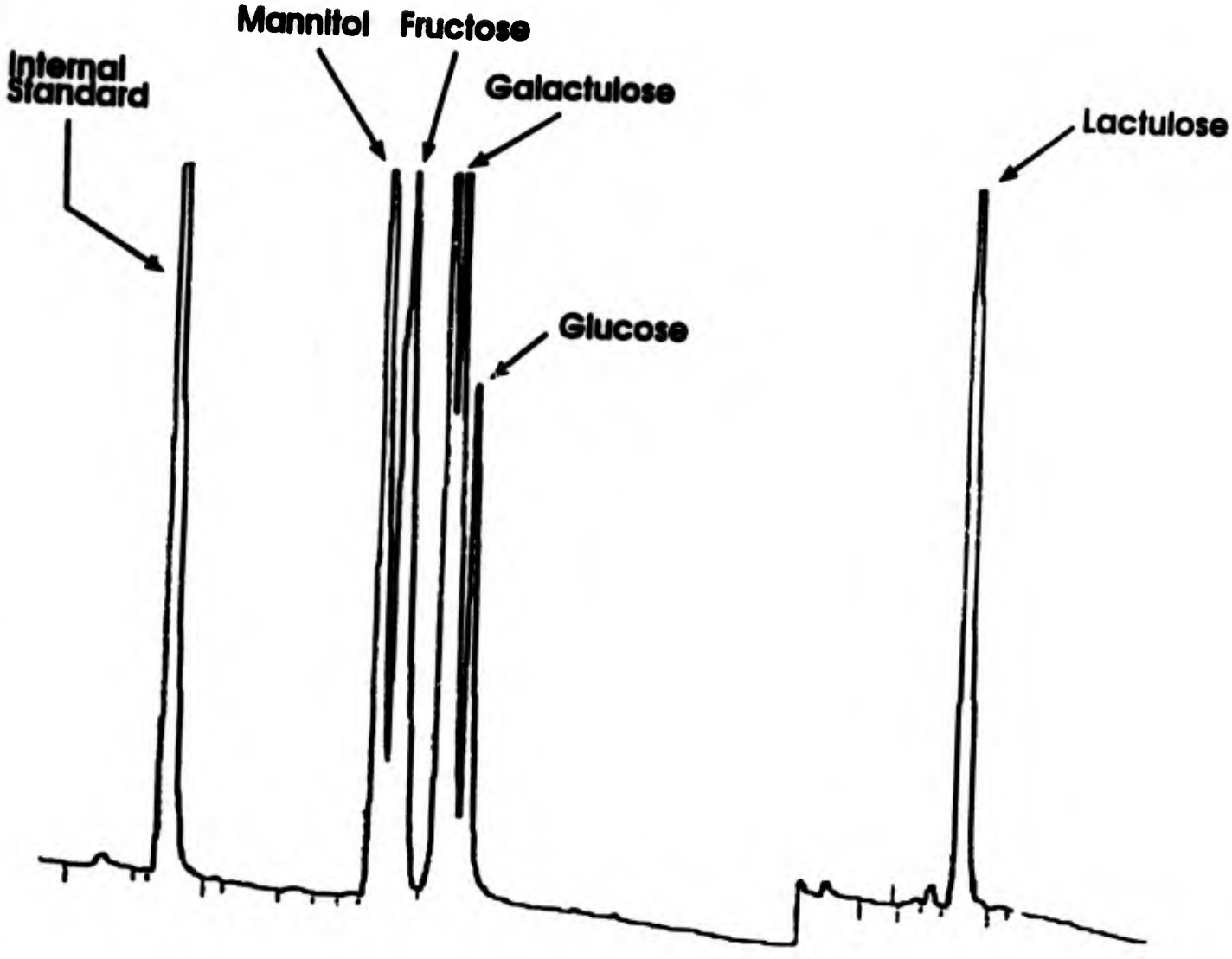


**FIGURE 1.** Chromatogram of a standard containing 100 nmoles of mannitol lactulose, spiked with 100 nmoles of fructose, galactulose and glucose.

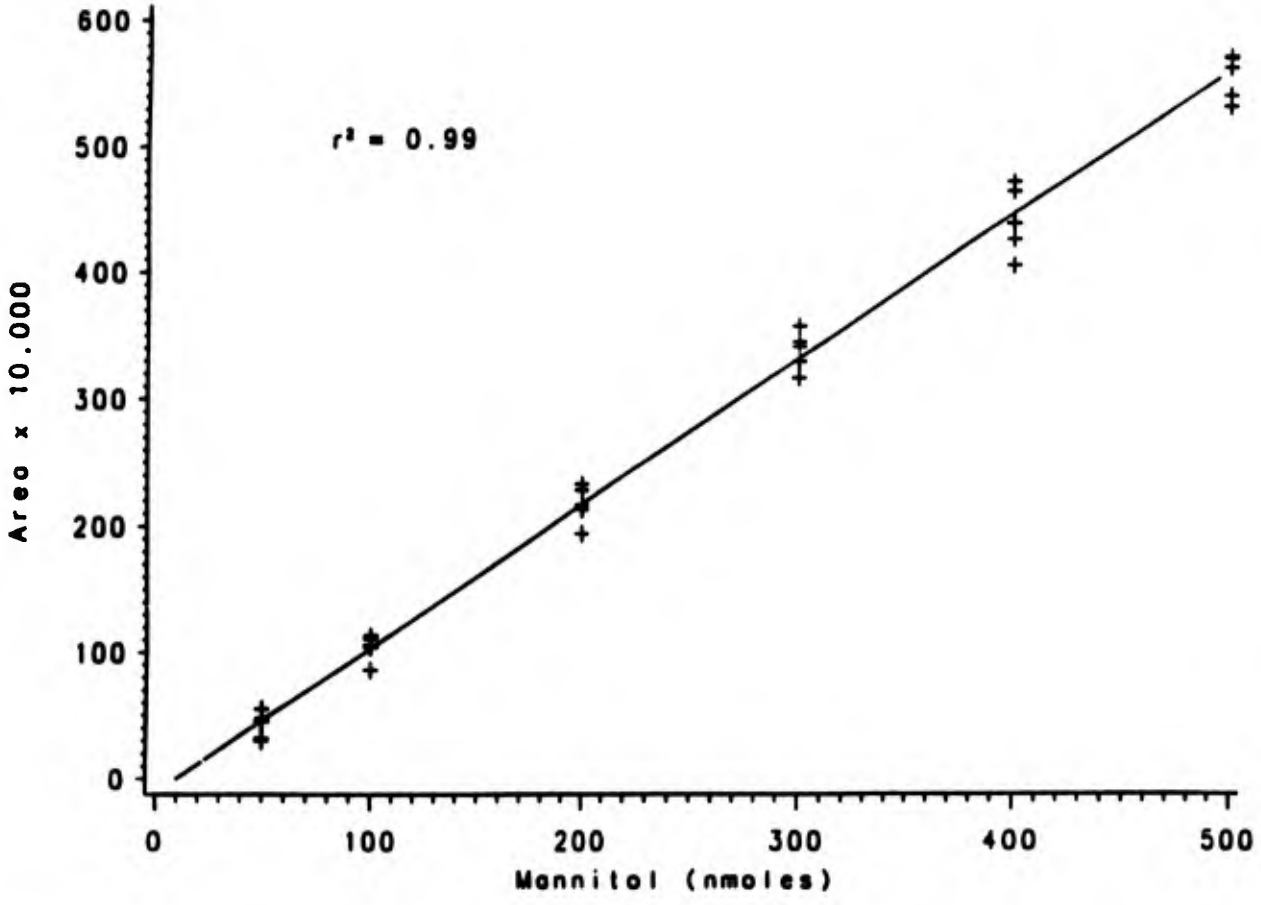


**FIGURE 2.** Standard curve for mannitol.

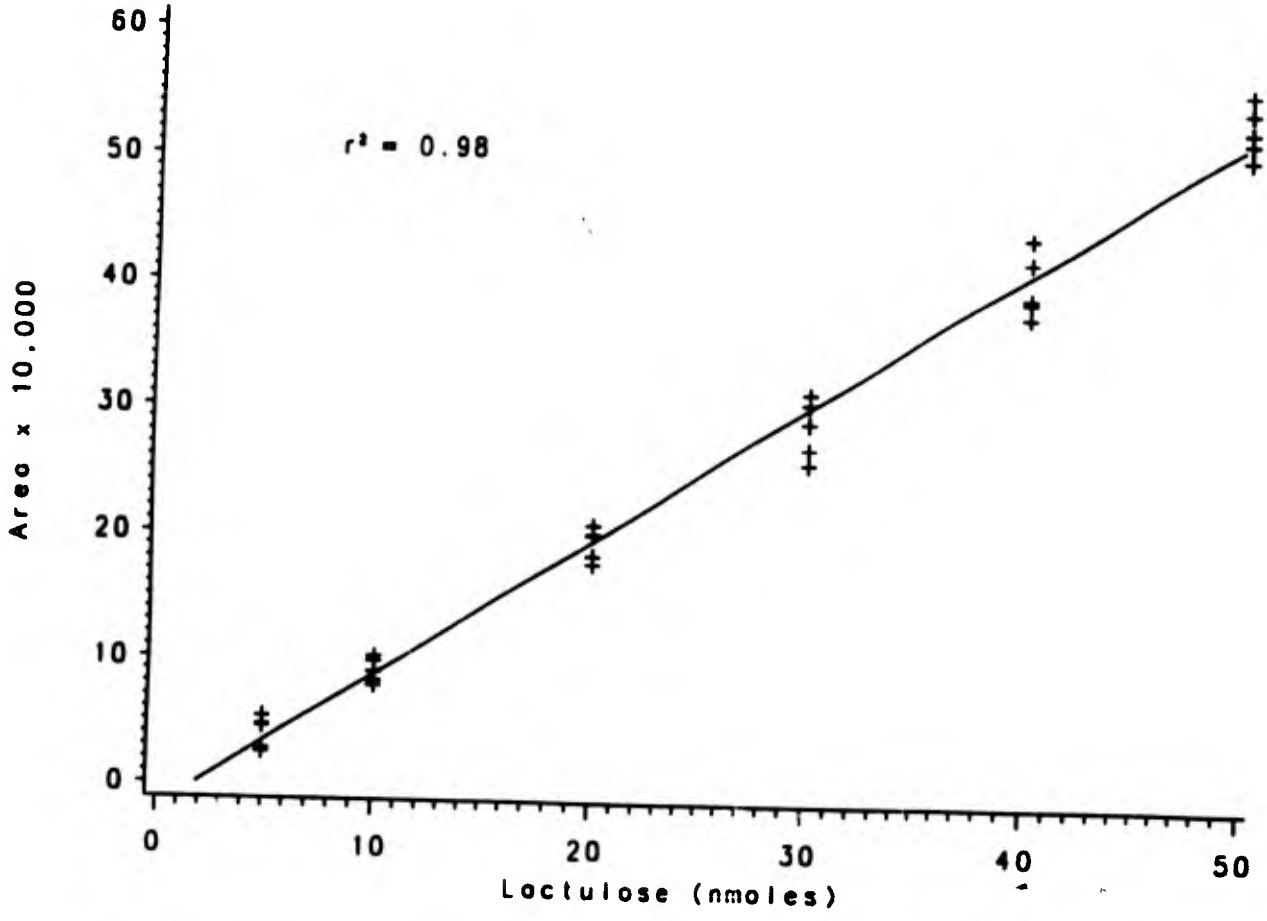


**FIGURE 3.** Standard curve for lactulose.

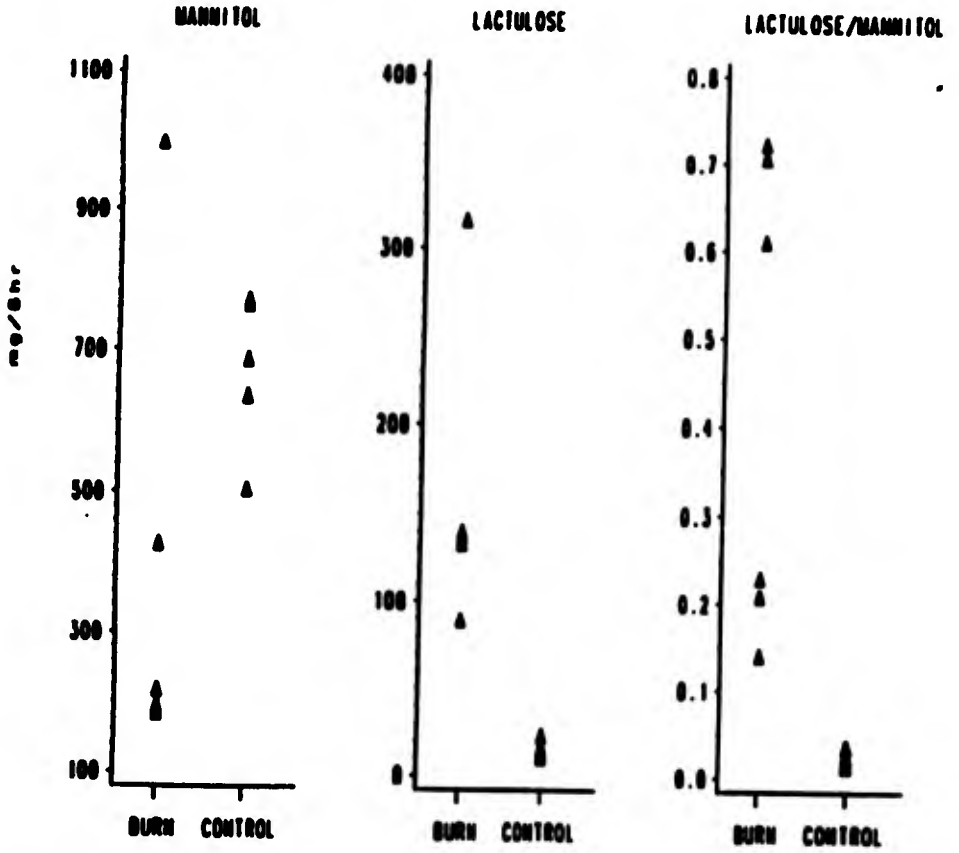


**FIGURE 4.** (Left) Amount of mannitol (mg) excreted in the urine of six burn patients (mean=566, SEM=205) and six control subjects (mean=748, SEM=86) six hours after dosage. (Center) Amount of lactulose (mg) excreted in the urine of six burn patients (mean=158, SEM=80) and six control subjects (mean=15, SEM=5) six hours after dosage. (Right) Lactulose/Mannitol ratio of six burn patients (mean=0.53, SEM=0.18) and six control subjects (mean=4, SEM=2).

cation exchange column removes these compounds; this is time consuming and increases the chance of dilution error. The enzymatic assay for mannitol requires the synthesis of mannitol dehydrogenase, which is not available commercially.

Fleming et al. (7) have described a method using HPLC with pulsed amperometric detection for simultaneously determining lactulose and mannitol concentration levels in urine. Unlike gas chromatography, HPLC does not require pre- or post-column derivatization when used with an anion-exchange column. They compared HPLC-PAD to gas-liquid chromatography, concluding that the HPLC-PAD method demonstrated better accuracy for lactulose. The authors attributed the problems with the gas-liquid chromatography to possible incomplete recovery of the anomers of lactulose.

Laker et al. (8) have described the estimation of disaccharides in plasma and urine by gas-liquid chromatography. The method involves pre-column trimethylsilyl derivatization of the carbohydrates. A disadvantage of the procedure as described by Laker is that the anomeric forms of the reducing sugars cause multiple peaks in the chromatogram.

Our use of an oxime solution described in the Methods Section for Gas Chromatography (Pierce Chemical, Handbook and General Catalog, 1989) eliminates the problem of multiple peaks caused by the anomeric forms. Mannitol and lactulose are first converted to oximes prior to silylation with trimethysilylimidazole. The results demonstrate that the problem with the anomers can be corrected when initial conversion to oximes is performed prior to silylation. Furthermore, the procedure using gas-liquid chromatography does not require de-salting with ion-exchange resin, a necessary step for the HPLC method.

The mean of 14.8±6.3 mg/6 hr for the control subjects is in good agreement with the estimate by Behrens et al. (6) of 21.2±6.1 mg/6 hr using 13 control subjects. The results from the burn patients support the conclusion by Ziegler et al. (1), that burn injury appears to cause increased gastrointestinal mucosal permeability.

Gas-liquid chromatography appears to be valid method of simultaneously determining lactulose and mannitol levels in the urine of normal and burn patients.

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| RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY | | | | 1. AGENCY ACCESSION DA346161 | 2. DATE OF SUMMARY 01 Oct 90 | REPORT CONTROL SYMBOL DD-DR&E(AR) 636 |
|---|---|---|---|---|---|---|
| 3. DATE PREV SUM'RY 09 Apr 90 | 4. KIND OF SUMMARY D. Chg | 5. SUMMARY SCTY U | 6. WORK SECURITY U | 7. REGRADING | 8. DISB'N INSTR'N CX | 9. LEVEL OF SUM A. WORK UNIT |

| 10. NO./CODES | PROGRAM ELEMENT | PROJECT NUMBER | TASK AREA NUMBER | WORK UNIT NUMBER |
|---|---|---|---|---|
| a. PRIMARY | 61102A | 3M161102BS14 | CK | 328 |
| b. CONTRIBUTING | | | | |
| c. CONTRIBUTING | | | | |

11. TITLE (Precede with Security Classification Code) (U) Mineral Absorption and Metabolism in a Burned Rat Model Using the Everted Gut Sacs Technique

12. SUBJECT AREAS
0601 Biochemistry 0605 Medicine and Medical Research

| 13. START DATE | 14. ESTIMATED COMPLETION DATE | 15. FUNDING ORGANIZATION | 16. PERFORMANCE METHOD |
|---|---|---|---|
| 9004 | 9109 | DA | C. In-House |

17. CONTRACT/GRANT MILITARY RELEVANCY CERTIFIED
a. DATE EFFECTIVE APPROVED BY
b. CONTRACT/GRANT NUMBER
c. TYPE
d. AMOUNT
e. KIND OF AWARD
f. CUM/TOTAL

18. RESOURCES ESTIMATE

| a. FISCAL YEARS | a. PROFESSIONAL WORKYEARS | b. FUNDS (In thousands) |
|---|---|---|
| 90 | 0.1 | 58 |
| 91 | 0.1 | 61 |

| 19. RESPONSIBLE DOD ORGANIZATION | 20. PERFORMING ORGANIZATION |
|---|---|
| a. NAME: US Army Institute of Surgical Research | a. NAME: US Army Institute of Surgical Research |
| b. ADDRESS (include zip code): Fort Sam Houston, San Antonio, Texas 78234-5012 | b. ADDRESS: Fort Sam Houston, San Antonio, Texas 78234-5012 |
| c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A | c. NAME OF PRINCIPAL INVESTIGATOR: SHIPPEE, R L |
| d. TELEPHONE NUMBER (include area code): 512-221-2720 | d. TELEPHONE NUMBER (include area code): 512-221-7138 |
| 21. GENERAL USE: FINA<br>MILITARY/CIVILIAN APPLICATION: M | f. NAME OF ASSOCIATE INVESTIGATOR (if available): YOUNG, E<br>g. NAME OF ASSOCIATE INVESTIGATOR (if available): OKERBERG, C V |

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Burns (Injuries); (U) Metabolism; (U) Nutrition; (U) Isotopes

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

23/24. (U) The objective of this work is to use the everted gut sac technique to investigate the effect of burn injury on mineral absorption in a burned rat model. Lewis rats will be administered either a 30% full-thickness scald or sham burn. At either 24 h or 5 days after burn injury, the rats will be sacrificed and the small intestine will be removed. The intestinal absorption of four radioactive-labelled nutrients will then be assessed.

25. (U) 8710 - 8809. Not applicable.

(U) 8810 - 8909. Not applicable.

(U) 8910 - 9009. This project was approved by the USAISR Research Council, US Army Institute of Surgical Research Animal Care and Use Committee, and Brooke Army Medical Center Radiation Control Committee during the second quarter of fiscal year 1990. Work on this project was delayed due to a lack of the necessary disposal system. However, a disposal contractor was recently located and work will be initiated upon receipt of the isotopes.

DD FORM 83 MAR 1498 EDITION OF MAR 68 IS OBSOLETE. * U.S.G.P.O. 1988-491-003/50329

**SUPPLEMENTAL WORK UNIT INFORMATION FOR THE PROJECT ENTITLED "MINERAL ABSORPTION AND METABOLISM IN A BURNED RAT MODEL USING THE EVERTED GUT SACS TECHNIQUE"**

**Subrecord/Linking Accession Number:** Not applicable.

**Search Control Data:** W6R28L/W6R30I, 9 January 1990

**Product Identification:** For technical reports, refer to the US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Year 1990.

**Unclassified Special Categories:** Lab Animals: Rats; RA II

**ANNUAL RESEARCH PROGRESS REPORT**

**PROJECT NUMBER:** 3M161102BS14-00, RESEARCH

**PROJECT TITLE:** Mineral Absorption and Metabolism in a Burned Rat Model Using the Everted Gut Sacs Technique

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

**1 October 1989 - 30 September 1990**

**INVESTIGATORS**

Ronald L. Shippee, PhD, Captain, MS
Selene R. Watiwat, Staff Sergeant

## ABSTRACT

**PROJECT NUMBER:** 3M161102BS14 RESEARCH

**PROJECT TITLE:** Mineral Absorption and Metabolism in a Burned Rat Model Using the Everted Gut Sacs Technique

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

**PERIOD COVERED IN THIS REPORT:** 1 Oct 89 through 30 Sep 90

**INVESTIGATORS:** Ronald L. Shippee, PhD, Captain, MS
Selene R. Watiwat, Staff Sergeant

Considering the hypermetabolic state of patients with severe burns, it would appear that above normal supplementation of essential minerals is warranted. However, little information is available to support a rationale for aggressive mineral supplementation. The present study used the everted gut sac technique to determine the effect of burn injury on zinc absorption in a rat model. Although burn injury caused decreased absorption on days one and four post injury, absorption reached normal levels by day eight. The data are consistent with previous results and the hypothesis that during burn injury absorption of zinc is unobstructed and that liver zinc stores are increased to insure adequate availability for this important trace element.

# MINERAL ABSORPTION AND METABOLISM IN A BURNED RAT MODEL USING THE EVERTED GUT SACS TECHNIQUE

## INTRODUCTION

Considering the hypermetabolic state of patients with severe burns, it would appear that above normal supplementation of essential minerals is warranted. However, little information is available to support the rationale for aggressive mineral supplementation. Some mix of parenteral and enteral feeding modalities is usually needed to meet the increased caloric requirements of patients with major burn injuries. The goal is usually to taper the parenteral nutrition as soon as practical, with concomitant increase in enteral alimentation. There is a paucity of research concerning the effect of burn injury on gut absorption of essential minerals. This report describes the results of initial experiments involving the use of the everted gut sac technique to study zinc absorption during recovery from a burn injury in a rat model.

## METHODS AND MATERIALS

Male Sprague-Dawley rats weighing between 250 and 275 grams were maintained in single stainless steel cages and kept on a 12hr on/off light cycle. Rats were fed Purina Chow and deionized water ad libitum throughout the experimental period.

The first experiment was designed to determine the area of maximum zinc absorption along the small intestine. Four animals were anesthetized and sacrificed by exsanguination. The small intestine was removed and placed in cold buffer (125 mM NaCl, 10 mM Fructose, 30 mM Tris, (pH 7.4 at 37°C), 0.5 mM $CaCl_2$, 1.2 mM MgCl). Starting at the pylorus, four 10 cm lengths of small intestine were excised. Each segment was everted by inserting a small crochet hook through the segment and pulling a suture through the segment. One end was tied off and the segment everted by pulling the tied end gently through the segment. The sac was filled with 1ml of buffer. A piece of polyethylene tubing with an inside diameter about the size of a 25 gauge needle was inserted into the sac and immobilized with suture.

The prepared gut sac was inserted into a 25 ml Ehrlenmeyer flask with 8 ml of buffer containing 1 nM Zinc (ZnCl) and 0.05 µCi Zn-65/ml. The Ehrlenmeyer flask was placed in a shaker bath set at 37°C for 90 minutes. Contents of the sac were removed by aspiration through the plastic tube with a tuberculin syringe. The volume removed was recorded and expelled into a plastic test tube and placed in a gamma counter. The sac was placed on a pre-weighed plastic boat and placed in a vacuum drying oven at 78°C for four hours to determine dry tissue weight.

After it was determined which 10 cm segment gave the maximum zinc absorption, eighteen male Sprague-Dawley rats were divided equally into burn and sham burn treatment groups. The burn used was a 30% total body surface (TBS) full-thickness scald burn (100°C, 10 sec). On days 1,4, and 8 postburn, three animals from each group were sacrificed and small intestine removed and prepared as described above.

## RESULTS AND DISCUSSION

Figure 1 shows the zinc absorption from the four gut segments from the four control, sham burned animals. Maximum absorption occurred in the second 10 cm segment. This is in agreement with similar studies in rats reported by Kowarski et al. (1). Maximum zinc absorption occurs in the upper portion of the small intestine.

Table 1 shows the zinc absorption (cpm/ml/gr dry tissue) for days 1, 4 and 8 postburn. Although burn injury inhibited zinc absorption on days one and four postburn, by day 8 absorption was comparable to the control animals.

This is in agreement with our early reports concerning zinc metabolism in burned rats (2). We analyzed the protein bound zinc in mucosal intestinal and hepatic cytosol fractions, using gel column chromatography. On day 10 postburn there was a dramatic increase in zinc bound to the zinc storage protein, metallothionein, in liver tissue. However, no increase in zinc bound to metallothionein was seen in cytosol preparations from mucosal tissue.

Cousins (3) has proposed a role for intestinal metallothionein in the excretion of zinc that is in excess of metabolic requirements. Intestinal metallothionein is induced in response to zinc loading, binds excess zinc and accumulates in the mucosal cells. Subsequently, the zinc bound to metallothionein is lost when cells are sloughed into the lumen, thereby increasing fecal endogenous zinc excretion. Consistent with this suggested role of intestinal metallothionein, it could be hypothesized that the lack of an increase in metallothionein binding of zinc in the intestinal cytosol of the burned rats would ensure unobstructed zinc absorption and decrease obligatory loss of fecal zinc. Our early results (2) support this hypothesis in that total endogenous fecal zinc excretion for the 10 days postburn did not differ significantly between the burned rats and the sham control group. The present study showing a return to normal absorption by day eight postburn gives further support to the hypothesis.

Our burned rat model is the first reported instance of a differential induction of zinc binding to metallothionein in liver and mucosal tissue. Models using excess zinc or cadmium always cause increased metallothionein induction in both tissue types. Early studies by Pekarek and Evans (4) showed that intraperitoneal

injection (IP) of a crude preparation of heat inactivated leukocytic endogenous mediator (LEM) in rats caused increased absorption of Zn-65 and increased uptake of Zn-65 in liver tissue. LEM has been purified and well characterized in the past few years and is now know to be the cytokine, interleukin-1 (IL-1). IL-1 has been shown to alter metallothionein gene expression in liver tissue and affect zinc metabolism (5). Burn injury is know to cause elevated IL-1 levels in both humans and animals. Hempe et al. (6) recently reported that rat intestinal tissue was completely refractory to IL-1 induction of metallothionein.

Our studies using the burn rat model will continue to focus on the effects of severe burns on zinc metabolism, adding to the data base and eventually leading to a rationale for optimal supplementation of trace minerals in burned patients.

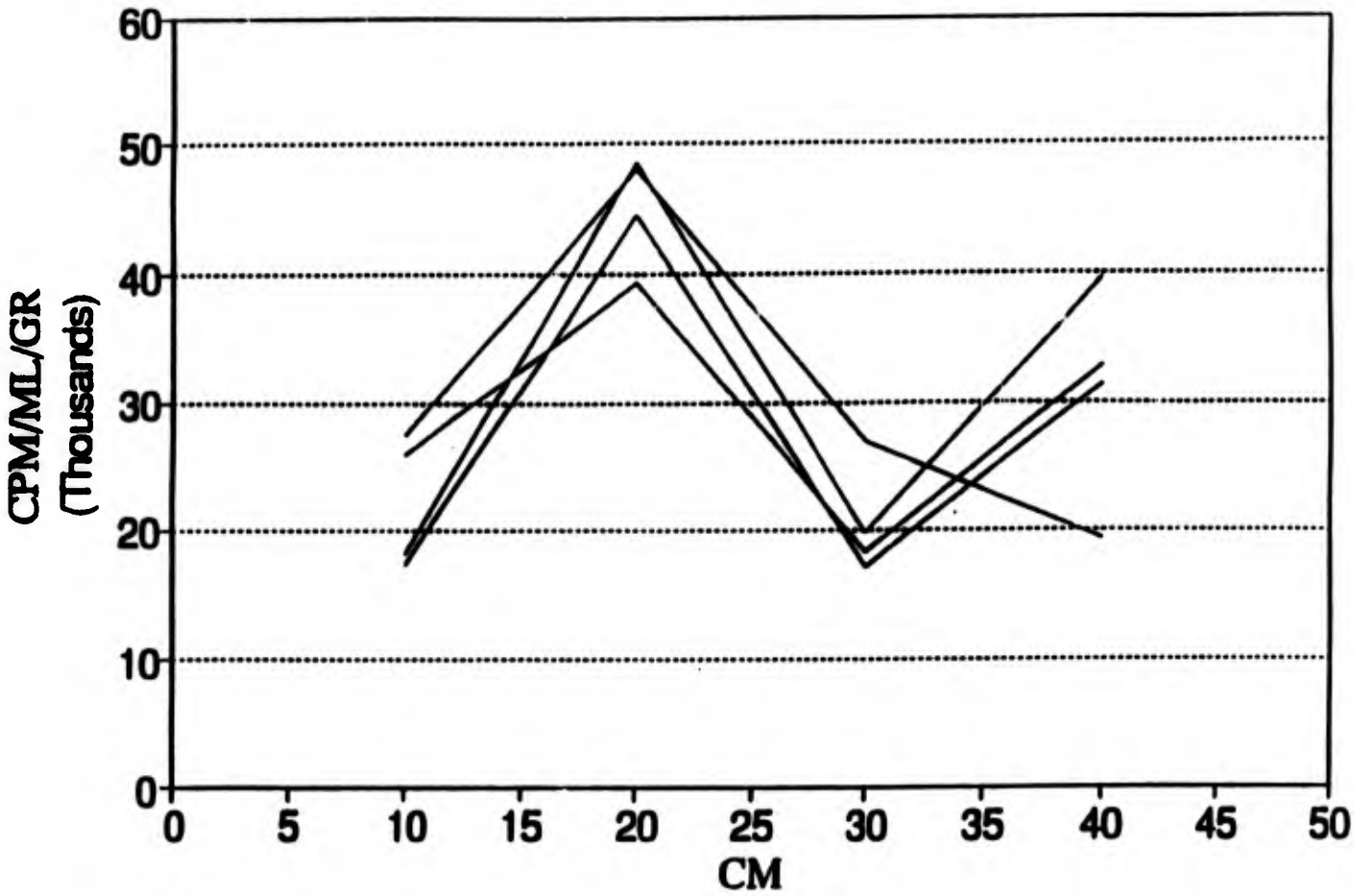


**FIGURE 1:** Zm-65 absorption from four sham-burn rats using four 10 cm segments of small intestine.

**TABLE 1**

| | | Day Post Burn (DPM/ml/gr dry tissue) 1 | 4 | 8 |
|---|---|---|---|---|
| Control | | 13353 | 15851 | 16097 |
| | | 9423 | 10882 | 17367 |
| | | 36207 | 17194 | 28491 |
| | Mean | 19661 | 14642 | 20652 |
| | SEM | 6818 | 1567 | 3214 |
| Burn | | 5765 | 9977 | 17907 |
| | | 14569 | 4860 | 36062 |
| | | 17209 | 8890 | 23678 |
| | Mean | 12514 | 7909 | 25882 |
| | SEM | 2825 | 1271 | 4373 |

## REFERENCES


1. Kowarski S, Blair-Stanek C, Schachter D: Active transport of zinc and identification of zinc-binding protein in rat jejuna mucosa. **Amer J Phys** 226:401-407, 1974.

2. Shippee RL, Mason AD Jr, Burleson DG: The effect of burn injury and zinc nutriture on fecal endogenous zinc, tissue zinc distribution, and T-lymphocyte subset distribution using a murine model. **Proceedings of the Society for Experimental Biology and Medicine** 189:31-38, 1988.

3. Cousins RJ: Mechanism of Zinc Absorption, IN: **Clinical Biochemical and Nutritional Aspects of Trace Elements**, Alan R. Liss Inc., New York, 1982, pp117-128.

4. Pekarek RS, Evans GW: Effect of leukocytic endogenous mediator (LEM) on zinc absorption in the rat. Proceedings of the **Society for Experimental Biology and Medicine** 152:573-575, 1976.

5. Huber KL, Cousins RJ: Maternal zinc deprivation and interleukin-1 influence metallothionein gene expression and zinc metabolism of rats. **J Nutr** 118:1570-1576, 1988.

6. Hempe JM, Carlson JM, Cousins RJ: Tissue specific metallothionein gene expression in liver and intestine by dexamethasone, Interleukin-1 and elevated zinc status. **FASEB Journal** 4:A649 Abstract 2222, 1990.

**RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY**

| 1. AGENCY ACCESSION | 2. DATE OF SUMMARY | REPORT CONTROL SYMBOL DD-DR&E(AR) 636 |
|---|---|---|
| DA305424 | 01 Oct 90 | |

| 3. DATE PREV SUM'RY | 4. KIND OF SUMMARY | 5. SUMMARY SCTY | 6. WORK SECURITY | 7. REGRADING | 8. DISS'N INSTR'N | 9. LEVEL OF SUM A. WORK UNIT |
|---|---|---|---|---|---|---|
| 09 Jul 90 | D. Chg | U | U | | CX | |

| 10. NO./CODES: | PROGRAM ELEMENT | PROJECT NUMBER | TASK AREA NUMBER | WORK UNIT NUMBER |
|---|---|---|---|---|
| a. PRIMARY | 61102A | 3M161102BS14 | CH | 330 |
| b. CONTRIBUTING | | | | |
| c. CONTRIBUTING | | | | |

11. TITLE *(Precede with Security Classification Code)* (U) Donor-Specific Bone Marrow and Antithymocyte Preparations for Establishment of Selective Tolerance to Allografted Skin

12. SUBJECT AREAS
0604 Anatomy and Physiology 0605 Medicine and Medical Research

| 13. START DATE | 14. ESTIMATED COMPLETION DATE | 15. FUNDING ORGANIZATION | 16. PERFORMANCE METHOD |
|---|---|---|---|
| 9007 | 9109 | DA | C. In-House |

17. CONTRACT/GRANT — MILITARY RELEVANCY CERTIFIED APPROVED BY

| a. DATE EFFECTIVE | b. CONTRACT/GRANT NUMBER | c. TYPE | d. AMOUNT | e. KIND OF AWARD | f. CUM/TOTAL |
|---|---|---|---|---|---|
| | | | | | |

18. RESOURCES ESTIMATE

| FISCAL YEARS | a. PROFESSIONAL WORKYEARS | b. FUNDS *(In thousands)* |
|---|---|---|
| 90 | 0.1 | 5 |
| 91 | 0.1 | 58 |

19. RESPONSIBLE DOD ORGANIZATION

a. NAME: US Army Institute of Surgical Research
b. ADDRESS *(Include zip code)*: Fort Sam Houston, San Antonio, Texas 78234-5012
c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A
d. TELEPHONE NUMBER *(Include area code)*: 512-221-2720

20. PERFORMING ORGANIZATION

a. NAME: US Army Institute of Surgical Research
b. ADDRESS: Fort Sam Houston, San Antonio, Texas 78234-5012
c. NAME OF PRINCIPAL INVESTIGATOR: RUE, L W
d. TELEPHONE NUMBER *(Include area code)*: 512-221-4440
e. NAME OF ASSOCIATE INVESTIGATOR *(If available)*: CIOFFI, W G
g. NAME OF ASSOCIATE INVESTIGATOR *(If available)*: BECKER, W K

21. GENERAL USE: FINA
MILITARY/CIVILIAN APPLICATION: M

22. KEYWORDS *(Precede EACH with Security Classification Code)* (U) Burns (Injuries); (U) Bone Marrow; (U) Skin Grafts; (U) Immunosuppression; (U) Lymphocytes

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS *(Precede text of each with Security Classification Code)*

23/24. (U) The objectives of this work are to demonstrate selective unresponsiveness to skin allografts using antithymocyte preparations and donor-specific bone marrow, to demonstrate the time course of restoration of the mixed-lymphocyte reaction to third party cells folowing induction of tolerance, and to investigate the need for antithymocyte preparations in induction tolerance in a rat burn model. Groups I and II will be subjected to 30% total body surface area burns. Group I will undergo skin grafting from Brown-Norway rat donors on the second postburn day. Group II will be administered antithymocyte serum on the first postburn day, undergo skin grafting from a Brown-Norway rat donor on the second postburn day, be administered antithymocyte serum on the second postoperative day, and be administered the bone marrow preparation from the Brown-Norway rat donor on the seventh postoperative day. Each of these two groups will undergo mixed-lymphocyte reaction surveillance at the time of burn injury, 1 week postinjury, and monthly thereafter.

25. (U) 8710 - 8809. Not applicable.

(U) 8810 - 8909. Not applicable.

(U) 8910 - 9009. This project was approved by the USAISR Research Council and US Army Institute of Surgical Research Animal Care and Use Committee during the third quarter of fiscal year 1990. Studies will be initiated during fiscal year 1991.

DD FORM 83 MAR 1498 EDITION OF MAR 68 IS OBSOLETE. * USGPO 1988 -491-003/50329

**SUPPLEMENTAL WORK UNIT INFORMATION FOR THE PROJECT ENTITLED "DONOR-SPECIFIC BONE MARROW AND ANTITHYMOCYTE PREPARATIONS FOR ESTABLISHMENT OF SELECTIVE TOLERANCE TO ALLOGRAFTED SKIN"**

**Subrecord/Linking Accession Number:** Not applicable.

**Search Control Data:** W6L46K/W6L47I, 31 May 1990

**Product Identification:** For technical reports, refer to the US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Year 1990.

**Unclassified Special Categories:** Lab Animals: Rats; RA II

# ANNUAL PROGRESS REPORT

**PROJECT NUMBER:** 3M161102BS14-00, RESEARCH

**PROJECT TITLE:** Donor-Specific Bone Marrow and Antithymocyte Preparations for Establishment of Selective Tolerance to Allografted Skin

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

**1 October 1989 - 30 September 1990**

## INVESTIGATORS

Loring W. Rue, III, MD, Major, MC
William G. Cioffi, Jr., MD, Major, MC
William K. Becker, MD, Lieutenant Colonel, MC

## ABSTRACT

**PROJECT NUMBER:** 3M161102BS14-00, RESEARCH

**PROJECT TITLE:** Donor-Specific Bone Marrow and Antithymocyte Preparations for Establishment of Selective Tolerance to Allografted Skin

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

**PERIOD COVERED IN THIS REPORT:** 1 Oct 89 through 30 Sep 90

**INVESTIGATORS:** Loring W. Rue, III, MD, Major, MC
William G. Cioffi, Jr., MD, Major, MC
William K. Becker, MD, Lieutenant Colonel, MC

This study is designed to demonstrate selective unresponsiveness to skin allografts in a rat model of thermal injury using combinations of anti-thymocyte preparations and donor specific bone marrow. Using mixed lymphocyte reactions, the time course of restoration of responsiveness to third party cells following induction of tolerance will be evaluated.

# DONOR-SPECIFIC BONE MARROW AND ANTITHYMOCYTE PREPARATIONS FOR ESTABLISHMENT OF SELECTIVE TOLERANCE TO ALLOGRAFTED SKIN

## INTRODUCTION

Early excision and grafting of full-thickness burns has been shown to diminish the length of hospital stay, the incidence of infectious complications, and the cost of hospitalization. Definitive closure of thermal injuries greater than 40% of the total body surface area is often limited by the availability of skin graft donor sites necessitating multiple operative procedures and prolonging the hypermetabolic and immunosuppressive stresses faced by thermally injured patients.

Alternate approaches to large burn wound coverage have included complete wound excision and coverage with biologic dressings, bilaminar artificial skin substitutes which ultimately necessitate dermal coverage with meshed autografts, cultured keratinocytes requiring prolonged culture times and associated with inconsistent take, and a variety of techniques directed at prolonging cadaver allograft adherence.

The ability of immunosuppression to prolong the take of skin allografts has been demonstrated in rats and humans. Unfortunately, this creates a state of nonspecific immunosuppression, which increases the risk of infectious complications. Wood and Monaco have been successful in inducing long term and even a permanent survival of skin allografts in mice with the use of anti-thymocyte preparations and donor specific bone marrow, essentially inducing a specific state of immune tolerance to allografted skin, but allowing ultimate restoration of the organism's ability to respond to third party allogeneic stimulation (1).

Human applications of these principles has been confined to the renal transplant population. The most recent and comprehensive application of these principles has been undertaken by Barber and colleagues (2). In their series, 20 patients were entered into a donor specific bone marrow protocol, 19 of whom were discharged with functioning renal allografts, and eight of whom were completely off corticosteroids between three to six months following transplantation. The other patients in this protocol were on significantly lower doses of steroids as compared to the 20 patients receiving the contralateral kidney and conventional immunosuppressive regiments. Current allograft survival approached 90% using this protocol, versus a 78% one year graft survival with conventional immunosuppressive regiment, which include cyclosporin.

Extensions of these observations in a burn model may provide a means to affect the early definitive and long-term closure of large thermal injuries, and reduce infectious complications and the length of hospital stay (3). Evaluation of the ability to selectively establish unresponsiveness to skin allografts in the

thermal injury model is of initial interest. Additionally, the time course for restoring immune responsiveness to third party cells following induction of tolerance in the burn model may also have implications with respect to infectious complications. Further, the ability to utilized free/thawed preparations of donor bone marrow and skin for inducing immune tolerance has direct implications with respect to its potential clinical applications and may be investigated in subsequent protocols.

## MATERIALS AND METHODS

The study will involve two groups. Group 1 will consist of 20 Lewis rats, which will be subjected to a 30% total body surface area burn, and will undergo excision of the burn wound to fascia and skin grafting from brown Norway rat donors on the second postburn day. Group 2 will also consist of 20 Lewis rats which will subjected to a 30% total body surface area burn, and be administered anti-thymocyte serum on the first postburn day, undergo skin grafting from a brown Norway rat donor on the second postburn day, administered anti-thymocyte serum on the second postoperative day, and administered the bone marrow preparation from the brown Norway rat donor on the sixth postoperative day. The wounds of both groups of animals will be examined on postoperative day seven, and then daily by tactile and visual inspection for rejection of graft. When less than 10% of the graft remains, it will be considered total rejection. Each of these two groups will undergo mixed lymphocyte reaction surveillance at the time of burn injury, at one week following burn injury, and monthly thereafter to determine the time course of restoration for the mixed lymphocyte reaction.

## RESULTS

Studies will be initiated during fiscal year 1991.

## DISCUSSION

It is expected that the use of a combination of anti-thymocyte preparations and donor-specific bone marrow will promote long-term allograft acceptance in the thermal injury model. Studies will commence in fiscal year 1991 and will be presented in the Annual Report for FY 91.

## PRESENTATIONS/PUBLICATIONS

None.

## REFERENCES

1. Wood ML and Monaco AP: Suppressor cells in specific unresponsiveness to skin allograft in ALS treated, marrow-injected mice. **Transplantation** 29:196-200, 1980.

2. Barber WH, Diethelm AG, Laskow DA, et al: Use of cryopreserved donor bone marrow in cadaver kidney allograft recipients. **Transplantation** 47:66-71, 1989.

3. Clark GT, Moon DJ, Cunningham PRG, et al: Specific unresponsiveness to skin allografts in burns. **J Surg Res** 46:401-404, 1989.

| RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY | 1. AGENCY ACCESSION | 2. DATE OF SUMMARY | REPORT CONTROL SYMBOL |
|---|---|---|---|
| | DA335467 | 01 Oct 90 | DD-DRE(AR) 636 |

| 3. DATE PREV SUM'RY | 4. KIND OF SUMMARY | 5. SUMMARY SCTY | 6. WORK SECURITY | 7. REGRADING | 8. DISP'N INSTR'N | 9. LEVEL OF SUM A. WORK UNIT |
|---|---|---|---|---|---|---|
| 09 Aug 90 | D. Chg | U | U | | CX | |

| 10. NO./CODES: | PROGRAM ELEMENT | PROJECT NUMBER | TASK AREA NUMBER | WORK UNIT NUMBER |
|---|---|---|---|---|
| a. PRIMARY | 61102A | 3M161102BS14 | CA | 331 |
| b. CONTRIBUTING | | | | |
| c. CONTRIBUTING | | | | |

11. TITLE *(Precede with Security Classification Code)* (U) Protein Turnover, Pulmonary Amino Acid Flux, and Nitrogen Balance in Thermally Injured Patients

12. SUBJECT AREAS
0601 Biochemistry 0605 Medicine and Medical Research

| 13. START DATE | 14. ESTIMATED COMPLETION DATE | 15. FUNDING ORGANIZATION | 16. PERFORMANCE METHOD |
|---|---|---|---|
| 9008 | 9209 | DA | C. In-House |

17. CONTRACT/GRANT — MILITARY RELEVANCY CERTIFIED APPROVED BY: [signature]

| a. DATE EFFECTIVE | d. AMOUNT |
|---|---|
| b. CONTRACT/GRANT NUMBER | |
| c. TYPE | |
| e. KIND OF AWARD | f. CUM/TOTAL |

18. RESOURCES ESTIMATE

| a. FISCAL YEARS | a. PROFESSIONAL WORKYEARS | b. FUNDS *(In thousands)* |
|---|---|---|
| 90 | 0.1 | 66 |
| 91 | 0.1 | 69 |

| 19. RESPONSIBLE DOD ORGANIZATION | 20. PERFORMING ORGANIZATION |
|---|---|
| a. NAME: US Army Institute of Surgical Research | a. NAME: US Army Institute of Surgical Research |
| b. ADDRESS *(Include zip code)*: Fort Sam Houston, San Antonio, Texas 78234-5012 | b. ADDRESS: Fort Sam Houston, San Antonio, Texas 78234-5012 |
| c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A | c. NAME OF PRINCIPAL INVESTIGATOR: BECKER, W K |
| d. TELEPHONE NUMBER *(Include area code)*: 512-221-2720 | d. TELEPHONE NUMBER *(Include area code)*: 512-221-5957 |
| 21. GENERAL USE: FINA<br>MILITARY/CIVILIAN APPLICATION: M | e. NAME OF ASSOCIATE INVESTIGATOR *(If available)*: CIOFFI, W G<br>g. NAME OF ASSOCIATE INVESTIGATOR *(If available)*: RUE, L W |

22. KEYWORDS *(Precede EACH with Security Classification Code)* (U) Burns (Injuries); (U) Lung; (U) Protein Metabolism; (U) Hypermetabolism; (U) Amino Acids

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS *(Precede text of each with Security Classification Code)*

23/24. (U) The objective of this work is to measure protein turnover, amino acid flux, and nitrogen balance across the lungs in thermally injured patients. Improvement in the understanding of the metabolic response to thermal injury may improve the outcome of thermally injured soldiers. This is a pilot study to establish techniques and to determine if amino acid flux in the lung can be demonstrated. Ten patients will undergo studies of protein turnover, pulmonary amino acid flux, and nitrogen balance on postburn day 7. Rates of protein turnover, amino acid flux, and nitrogen balance will be correlated with each other and with patient demographics, such as burn size and inhalation injury, using linear regression.

25. (U) 8710 - 8809. Not applicable.

(U) 8810 - 8909. Not applicable.

(U) 8910 - 9009. This project was approved by the USAISR Research Council, the US Army Institute of Surgical Research Human Use Committee, and The Surgeon General's Human Subjects Research Review Board during the fourth quarter of fiscal year 1990. Five patients were enrolled in the study during this reporting period.

DD FORM 83 MAR 1498 — EDITION OF MAR 68 IS OBSOLETE. — * USGPO. 1988 -491-003/50329

**SUPPLEMENTAL WORK UNIT INFORMATION FOR THE PROJECT ENTITLED "PROTEIN TURNOVER, PULMONARY AMINO ACID FLUX, AND NITROGEN BALANCE IN THERMALLY INJURED PATIENTS"**

**Subrecord/Linking Accession Number:** Not applicable.

**Search Control Data:** W6Q13K/W6Q11L, 5 January 1990

**Product Identification:** Not applicable.

**Unclassified Special Categories:** Volunteers: Adults, RA II

**ANNUAL RESEARCH REPORT**

**PROJECT NUMBER:** 3M161102BS14-00, RESEARCH

**PROJECT TITLE:** Protein Turnover, Pulmonary Amino Acid Flux, and Nitrogen Balance in Thermally Injured Patients

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

**1 October 1989 - 30 September 1990**

**INVESTIGATORS**

William K. Becker, MD, Lieutenant Colonel, MC
William G. Cioffi, Jr., MD, Major, MC
David G. Burleson, PhD, Lieutenant Colonel, MS
Elizabeth A. Milner, Captain, SP
Ronald L. Shippee, PhD, Major, MS

## ABSTRACT

**PROJECT NUMBER:** 3M161102BS14-00, RESEARCH

**PROJECT TITLE:** Protein Turnover, Pulmonary Amino Acid Flux, and Nitrogen Balance in Thermally Injured Patients

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

**PERIOD COVERED IN THIS REPORT:** 1 Oct 89 through 30 Sep 90

**INVESTIGATORS:** William K. Becker, MD, Lieutenant Colonel, MC
William G. Cioffi, Jr., MD, Major, MC
David G. Burleson, PhD, Lieutenant Colonel, MS
Elizabeth A. Milner, Captain, SP
Ronald L. Shippee, PhD, Major, MS

Amino acid flux and nitrogen excretion are increased following major injury. The role of the lung in this process is not well understood. To evaluate the role of pulmonary amino acid flux and protein turnover, ten patients will be studied to measure arterial-venous pulmonary amino acid levels, cardiac output, total protein turnover and nitrogen excretion. This protocol was approved and five patients have been entered into the study. No results are available for presentation.

| RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY | | | | 1. AGENCY ACCESSION: DA335423 | 2. DATE OF SUMMARY: 01 Oct 90 | REPORT CONTROL SYMBOL: DD-DR&E(AR) 636 |
|---|---|---|---|---|---|---|
| 3. DATE PREV SUM'RY: 13 Jun 90 | 4. KIND OF SUMMARY: R. Corr | 5. SUMMARY SCTY: U | 6. WORK SECURITY: U | 7. REGRADING: | 8. DISP'N INSTR'N: CX | 9. LEVEL OF SUM / A. WORK UNIT: |

| 10. NO./CODES: | PROGRAM ELEMENT | PROJECT NUMBER | TASK AREA NUMBER | WORK UNIT NUMBER |
|---|---|---|---|---|
| a. PRIMARY | 0601101A | 3A161101A91C | CA | 075 |
| b. CONTRIBUTING | | | | |
| c. CONTRIBUTING | | | | |

11. TITLE (Precede with Security Classification Code)
(U) Development of a Rat Model of Inhalation Injury - A Pilot Study

12. SUBJECT AREAS
0605 Medicine and Medical Research

| 13. START DATE | 14. ESTIMATED COMPLETION DATE | 15. FUNDING ORGANIZATION | 16. PERFORMANCE METHOD |
|---|---|---|---|
| 9006 | 9109 | DA | C. In-House |

17. CONTRACT/GRANT — MILITARY RELEVANCY CERTIFIED BY [illegible]

| a. DATE EFFECTIVE | RESOURCES ESTIMATE: c. FISCAL YEARS | d. PROFESSIONAL WORKYEARS | e. FUNDS (In thousands) |
|---|---|---|---|
| b. CONTRACT/GRANT NUMBER | | | |
| c. TYPE / d. AMOUNT | 90 | 0.1 | 100 |
| e. KIND OF AWARD / f. CUM/TOTAL | 91 | 0.1 | 109 |

| 19. RESPONSIBLE DOD ORGANIZATION | 20. PERFORMING ORGANIZATION |
|---|---|
| a. NAME: US Army Institute of Surgical Research | a. NAME: US Army Institute of Surgical Research |
| b. ADDRESS (Include zip code): Fort Sam Houston, San Antonio, Texas 78234-5012 | b. ADDRESS: Fort Sam Houston, San Antonio, Texas 78234-5012 |
| c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A | c. NAME OF PRINCIPAL INVESTIGATOR: BECKER, W K |
| d. TELEPHONE NUMBER (Include area code): 512-221-2720 | d. TELEPHONE NUMBER (Include area code): 512-221-5703 |
| 21. GENERAL USE: FINA | f. NAME OF ASSOCIATE INVESTIGATOR (If available): CIOFFI, W G |
| MILITARY/CIVILIAN APPLICATION: M | g. NAME OF ASSOCIATE INVESTIGATOR (If available): RUE, L W |

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Burns (Injuries); (U) Inhalation; (U) Lung; (U) Carbon Monoxide; (U) Hydrochloric Acid; (U) Mortality;

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

22. (Continued) (U) Histopathology

23/24. (U) The objective of this work is to develop a reliable rat model of smoke injury and inhalation injury. A smoke delivery system will be developed for the In-Tox small animal exposure device, to include a furnace, an air delivery pump, an in-line carbon monoxide monitor, a mixing and cooling chamber, and a temperature monitor. Initial studies will be performed to ensure uniform smoke and hydrochloric acid exposures. Animal studies will include a time-dose mortality curve and measurement of blood carbon monoxide levels. At various times following exposure, histopathological examination of the lungs will be performed.

25. (U) 8710 - 8809. Not applicable.

(U) 8810 - 8909. Not applicable.

(U) 8910 - 9009. This project was approved by the USAISR Research Council and US Army Institute of Surgical Research Animal Care and Use Committee during the third quarter of fiscal year 1990. Two hundred and twenty-five animals have been studied and results indicate that exposure in the smoke chambers resulted in uniform carbon monoxide levels but caused only minimal smoke injury of the airway. The protocol has been amended to include a second species (guinea pigs) and further studies will be performed in an attempt to increase the severity of pulmonary injury. This corrected

DD FORM 83 MAR 1498 EDITION OF MAR 88 IS OBSOLETE. * U.S.G.P.O.: 1988-491-003/50329

CONTINUATION OF DD FORM 1498 FOR THE PROJECT ENTITLED "DEVELOPMENT OF A RAT MODEL OF INHALATION INJURY - A PILOT STUDY"

DD Form 1498 adjusts the program element, project number, work unit number, and fiscal years 90 and 91 funds.

SUPPLEMENTAL WORK UNIT INFORMATION FOR THE PROJECT ENTITLED "DEVELOPMENT OF A RAT MODEL OF INHALATION INJURY - A PILOT STUDY"

**Subrecord/Linking Accession Number:** Not applicable.

**Search Control Data:** W6R05I/W6R06K, 29 May 1990

**Product Identification:** For technical reports, refer to the US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Year 1990.

**Unclassified Special Categories:** Lab Animals: Rats; Guinea Pigs; RA II

**ANNUAL RESEARCH PROGRESS REPORT**

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

**PROJECT TITLE:** Development of a Rat Model of Inhalation Injury - A Pilot Study

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

**1 October 1989 - 30 September 1990**

**INVESTIGATORS**

William K. Becker, MD, Lieutenant Colonel, MC
William G. Cioffi, Jr., MD, Major, MC
Albert T. McManus, PhD
Loring W. Rue, III, MD, Major, MC

**ABSTRACT**

**PROJECT NUMBER:** 3A16110191C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

**PROJECT TITLE:** Development of a Rat Model of Inhalation Injury - A Pilot Study

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

**PERIOD COVERED IN THIS REPORT:** 1 Oct 89 through 30 Sep 90

**INVESTIGATORS:** William K. Becker, MD, Lieutenant Colonel, MC
William G. Cioffi, Jr., MD, Major, MC
Albert T. McManus, PhD
Loring W. Rue, III, MD, Major, MC

This project was approved by the USAISR Research Council and US Army Institute of Surgical Research Animal Care and Use Committee during the third quarter of fiscal year 1990. Two hundred and twenty-five animals have been studied and results indicate that exposure in the smoke chambers resulted in uniform carbon monoxide levels, but caused only minimal smoke injury of the airway. The protocol has been amended to include a second species (guinea pigs) and further studies will be performed in an attempt to increase the severity of pulmonary injury.

| RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY | | | | 1. AGENCY ACCESSION | 2. DATE OF SUMMARY | REPORT CONTROL SYMBOL |
|---|---|---|---|---|---|---|
| | | | | DA346162 | 01 Oct 90 | DD-DR&E(AR) 636 |
| 3. DATE PREV SUM'RY | 4. KIND OF SUMMARY | 5. SUMMARY SCTY | 6. WORK SECURITY | 7. REGRADING | 8. DISP'N INSTR'N | 9. LEVEL OF SUM A. WORK UNIT |
| 04 May 90 | D. Chg | U | U | | CX | |

| 10. NO./CODES | PROGRAM ELEMENT | PROJECT NUMBER | TASK AREA NUMBER | WORK UNIT NUMBER |
|---|---|---|---|---|
| a. PRIMARY | 63002A | 3M263002D840 | CA | 081 |
| b. CONTRIBUTING | | | | |
| c. CONTRIBUTING | | | | |

11. TITLE (Precede with Security Classification Code) (U) The Effect of High Frequency Oscillatory Ventilation on Smoke Inhalation Injury in Baboons

12. SUBJECT AREAS 0605 Medicine and Medical Research
0612 Medical Facilities, Equipment, and Supplies

| 13. START DATE | 14. ESTIMATED COMPLETION DATE | 15. FUNDING ORGANIZATION | 16. PERFORMANCE METHOD |
|---|---|---|---|
| 9005 | 9109 | DA | C. In-House |

17. CONTRACT/GRANT MILITARY RELEVANCY CERTIFIED APPROVED

| a. DATE EFFECTIVE | b. CONTRACT/GRANT NUMBER | c. TYPE | d. AMOUNT | e. KIND OF AWARD | f. CUM/TOTAL |
|---|---|---|---|---|---|
| | | | | | |

18. RESOURCES ESTIMATE

| FISCAL YEARS | a. PROFESSIONAL WORKYEARS | b. FUNDS (In thousands) |
|---|---|---|
| 90 | 0.1 | 295 |
| 91 | 0.1 | 310 |

| 19. RESPONSIBLE DOD ORGANIZATION | 20. PERFORMING ORGANIZATION |
|---|---|
| a. NAME: US Army Institute of Surgical Research | a. NAME: US Army Institute of Surgical Research |
| b. ADDRESS (Include zip code): Fort Sam Houston, San Antonio, Texas 78234-5012 | b. ADDRESS: Fort Sam Houston, San Antonio, Texas 78234-5012 |
| c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A | c. NAME OF PRINCIPAL INVESTIGATOR: CIOFFI, W G |
| d. TELEPHONE NUMBER (Include area code): 512-221-2720 | d. TELEPHONE NUMBER (Include area code): 512-221-4440 |
| 21. GENERAL USE: FINA<br>MILITARY/CIVILIAN APPLICATION: M | e. NAME OF ASSOCIATE INVESTIGATOR (If available): RUE, L W<br>g. NAME OF ASSOCIATE INVESTIGATOR (If available): JORDAN, B S |

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Burns (Injuries); (U) Inhalation; (U) Pulmonary Edema; (U) Pulmonary Function; (U) Pulmonary Insufficiency;

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

22. (Continued) (U) Respirations; (U) Morbidity

23/24. (U) The objective of this work is to determine the effects of high frequency oscillatory ventilation on pulmonary changes following smoke inhalation injury in a baboon model. Twenty baboons will be randomized to one of four study groups. Group I will serve as the control group. Groups II, III, and IV will be exposed to smoke injury and then be supported with one of three ventilators. Blood gas, blood pressure, airway pressures, and hemodynamic data will be averaged over intervals and plotted at the midpoint of each interval. Physiologic and repetitive biochemical data will be analyzed among groups using repeated measures ANOVA. Data will also be compared at specific time points using ANOVA. Outcome data will be analyzed using Chi square or the Fisher exact test. Nonparametric data will be analyzed using the Kruskall-Wallis test.

25. (U) 8710 - 8809. Not applicable.

(U) 8810 - 8909. Not applicable.

(U) 8910 - 9009. This project was approved by the USAISR Research Council, US Army Institute of Surgical Research Animal Care and Use Committee, and US Army Medical Research and Development Command Animal Use Review Office during the third quarter of fiscal year 1990. Animal studies will be initiated during fiscal year 1991.

DD FORM 83 MAR 1498 EDITION OF MAR 88 IS OBSOLETE. * U.S.G.P.O.: 1988 -491-803/50329

**SUPPLEMENTAL WORK UNIT INFORMATION FOR THE PROJECT ENTITLED "THE EFFECT OF HIGH FREQUENCY OSCILLATORY VENTILATION ON SMOKE INHALATION INJURY IN BABOONS"**

**Subrecord/Linking Accession Number:** Not applicable.

**Search Control Data:** W6L59B/W6M00C, 18 April 1990

**Product Identification:** For technical reports, refer to the US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Year 1990.

**Unclassified Special Categories:** Lab Animals: Baboons; RA II

**ANNUAL RESEARCH PROGRESS REPORT**

**PROJECT NUMBER:** 3M263002D840-00, ADVANCED DEVELOPMENT

**PROJECT TITLE:** The Effect of High Frequency Oscillatory Ventilation on Smoke Inhalation Injury in Baboons

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

**1 October 1989 - 30 September 1990**

**INVESTIGATORS**

William G. Cioffi, Jr., MD, Major, MC
Loring W. Rue, III, MD, Major, MC
Bryan S. Jordan, RN

## ABSTRACT

**PROJECT NUMBER:** 3M263002D840-00, ADVANCED DEVELOPMENT

**PROJECT TITLE:** The Effect of High Frequency Oscillatory Ventilation on Smoke Inhalation Injury in Baboons

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

**PERIOD COVERED IN THIS REPORT:** 1 Oct 89 through 30 Sep 90

**INVESTIGATORS:** William G. Cioffi, Jr., MD, Major, MC
Loring W. Rue, III, MD, Major, MC
Bryan S. Jordan, RN

This project was approved by the USAISR Research Council, US Army Institute of Surgical Research Animal Care and Use Committee, and the US Army Medical Research and Development Command Animal Use Review Office during this period. Animal studies will be initiated during fiscal year 1991.

# THE EFFECT OF HIGH FREQUENCY OSCILLATORY VENTILATION ON SMOKE INHALATION INJURY IN BABOONS

## INTRODUCTION

Smoke inhalation leads to a complex sequence of pulmonary and pathophysiologic events which lead to a high morbidity and mortality when combined with thermal injury. While the volume and composition of the inhaled material clearly play a role in the severity of the pulmonary manifestations of inhalation injury, other data suggest that the mode of ventilatory support may also affect the severity of the disease process.

High frequency ventilation is a ventilatory technique which accomplishes gas exchange and oxygenation of low tidal volumes. It is therefore theoretically possible to maintain a high mean lung volume with peak intraluminal pressures which are lower than those required with conventional tidal ventilation.

High frequency oscillatory ventilation (HFOV) involves the active injection and withdrawal of gas from the lung. The use of active exhalation during high frequency oscillatory ventilation is felt by some investigators to enhance gas egress, thus allowing the use of a higher frequency and lower tidal volume than seen with conventional high frequency ventilations with passive exhalation. The use of active exhalation allows peak and trough volumes and pressures to be held close to mean volumes and pressures, thus approaching a near constant lung volume. Since there is active withdrawal of gas, the oscillator itself results in no net input of gas. A source of fresh gas must be introduced distal to the mechanism which supplies the oscillatory excursions and adjustment of the gas flow and resistance will determine mean airway pressure. The major limiting factor of all types of high frequency ventilation is inadvertent gas trapping. If the tidal volume injected is greater than that which can be eliminated during the expiratory interval, gas trapping and barotrauma will occur. The use of high frequency oscillatory ventilation with active exhalation as well as prolonged lung inspiratory:expiratory times may help to prevent this complication.

The use of high frequency oscillatory ventilation and high mean airway pressures has been shown to markedly alter the progression of both ARDS and hyaline membrane disease in experimental animals. In rabbits with ARDS, use of high frequency oscillatory ventilation prevented edema, hyaline membrane formation and the loss of membrane compliance seen in the surfactant depleted animals treated with conventional ventilation following saline lung lavage (1). Even more dramatic findings have been documented in surfactant deficient premature baboons treated with high frequency oscillatory ventilation. The initiation of HFOV prior to the first breath prevents development of the pathologic, physiologic and morphologic features of hyaline membrane disease when compared to

animals of comparable gestational age treated with conventional positive pressure ventilation and continual positive distending airway pressure (2,3). Of interest is the fact that the development of hyaline membrane disease was associated with increased levels of platelet activating factor-like activity in the lung lavage while no increase in PAF was seen in the HFOV treated animals. These data, coupled with other experimental evidence, have lead to the hypothesis that in the face of surfactant deficiency, conventional tidal ventilation leads to epithelial injury, mediator release and increased parenchymal injury. Various forms of high frequency ventilation have been shown to be efficacious in the management of infants and adults with bronchopleural fistula or respiratory failure unresponsive to conventional respiratory therapy. In some studies, HFOV has been efficacious in the management of infants with diffuse alveolar disease although other studies have shown no dramatic improvement (4). High frequency jet ventilation has not been shown to be of benefit in the management of adults with ARDS. Common to the success of high frequency ventilatory therapy in diffuse alveolar disease has been the use of a high mean airway pressure. Conversely, those studies in infants and adults where high frequency ventilation has not been shown to be effective tended to use lower mean airway pressures than the conventional ventilatory support (5,6). HFOV will allow one to maintain high mean airway pressures while avoiding high peak pressures as compared to conventional ventilation.

Until recently, it has not been possible to use HFOV in large animals or adult humans. This was because the efficiency of available oscillators was insufficient to deliver an adequate tidal volume for adult applications. Development of two new forms of high frequency oscillatory ventilation have made it possible now to study whether the dramatic results obtained in infant models of lung disease can be replicated in adult models. One ventilator is a pure high frequency oscillator. The other is a mixed model HFO ventilator. It allows one to combine HFO with conventional ventilation which may be more advantageous in heterogenous lung disease.

## METHODS

a. Experimental Design: Twenty baboons will be randomized to one of four groups:

(1) Control - no injury, no ventilator, lung lavage only.

(2) Positive pressure conventional ventilation.

(3) Southwest Research high frequency oscillatory ventilation.

(4) Percussionnaire Corporation high frequency ventilation.

At time 0, all animals will be sedated with Ketamine and Valium and a peripheral arterial catheter and Swan-Ganz catheter inserted under local anesthesia. These will be connected to a multi-channel recorder for continual recording of pressures and heart rate. Baseline cardiac output will be determined by thermodilution and arterial and venous gases obtained. Blood will be drawn for CBC, platelet counts and routine chemistries. Pulmonary function tests will be performed. The right lower lobe will then be instrumented using a flexible bronchoscope and lavaged with two 50 cc aliquots of physiologic saline. The recovered lavage fluid will then be combined and an aliquot removed for cell count with the remainder centrifuged and the supernatant decanted. Differential count will be performed on the cell plug. The supernatant will be frozen at -70°C for later assay for protein, surfactant, beta-1 anti-protease, elastase, lipid mediators, and various cytokines. After completion of the baseline studies, the animal will be allowed to recover for one hour. Sedation will be maintained with Ketamine and Valium.

After repeat arterial blood gas, the animal will then be exposed to a six-unit smoke injury using the techniques previously validated at this Institute.

All animals will remain intubated post exposure and treated with 100% oxygen for one hour to reduce the carboxyhemoglobin level. Carbon monoxide levels will be measured immediately prior to smoke exposure, immediately following smoke exposure, q30 minutes for two hours, and q4 hours for 24 hours. Levels will then be obtained daily for the remainder of the experiment.

Intravenous fluids will be administered at a rate of 80 cc/kg/day and will consist of D5 1/2 normal saline with 20 mEq of KCl/L. Arterial lines will be infused with normal saline with 1 unit heparin sodium/cc at 2 cc/hr. Changes in fluid composition and rate of infusion will be based upon hemodynamic data and electrolyte determinations.

Animals will be paralyzed with pancuronium after placement on the ventilator and then sedated with Valium. If Valium sedation is inadequate additional sedation will be accomplished by the use of sodium pentobarbital.

All animals will be intubated with a high-low endotracheal tube allowing proximal airway and distal airway pressure monitoring using a Valine pressure transducer. Blood pressure, pulmonary artery pressure, heart rate, and airway pressures will be recorded continuously.

Arterial and venous blood gases will be obtained hourly until the animal is stable, and then every 4-6 hours. Electrolytes, BUN, creatinine, CBC and platelet counts will be obtained every 12 hours. Chest radiographs will be obtained daily. Pulmonary

function tests will be performed at 0 hours, 24 hours, 72 hours, and 130 hours following injury.

Bronchoalveolar lavage will be performed immediately following pulmonary function testing at each time point. Alveolar lavage fluid will be processed as previously mentioned.

Animals will be turned from side-to-side q4 hours. Tracheal toilet will be performed every 4 hours and as clinically indicated. All animals will receive Gentamicin 2.5 mg/kg q8 hours throughout the experiment.

The animals will be placed on the appropriate ventilator when the $PaO_2$ between 80 and 100 torr. Ventilator adjustments will be made in response to blood gas determination. $PCO_2$ will be maintained between 35 and 45 torr by adjustment of tidal volume and frequency. PEEP will be maintained at 5 cm in water unless the animal requires an $FIO_2$ greater than .6 in order to maintain a $PaO_2$ in the protocol range. In that case, PEEP will be increased to increase mean airway pressures.

High frequency oscillatory ventilation will be accomplished using the two different high frequency oscillatory ventilators. Initial frequency will be set at 10 hertz with oscillatory amplitude sufficient to obtain adequate chest wall motion. Mean airway pressure will be adjusted to maintain the $PaO_2$ between 80 and 100 torr. $FIO_2$ following one hour of 100% will be set at 0.21. Oxygenation will be optimized by adjustment of mean airway pressure and $FIO_2$. Ventilation will be optimized by adjustment of the oscillatory amplitude. If $CO_2$ clearance is inadequate on the max-mode HFO, then conventional tidal breaths will be superimposed upon the high frequency oscillators. If there is a question for either HFO ventilator about the adequacy of the mean airway pressure then the animal will be manually sighed and pre and post sigh arterial blood gases obtained. If the sigh results in an increase of 10 torr or greater in arterial $PO_2$ then the mean airway pressure will be adjusted upwards in increments of 2 cm $H_2O$.

Lung V/Q distribution will be measured prior to injury and then daily, utilizing the multiple inert gas elimination technique previously used at this Institute. Briefly, the animal's IV solution will be switched to a Ringer's lactate solution containing six inert gases (sulphur hexafluoride, euthane, cyclopropane, halothane, ether, and acetone) which will be infused at the rate of 0.1 ml/kg/min. After 30 minutes, arterial and mixed venous blood will be drawn anaerobically into preweighed heparinized syringes simultaneously. Mixed expired gas will be obtained from a temperature-controlled copper coil one minute after obtaining the blood samples. Blood and expired gas samples will be analyzed immediately. Repeat cardiopulmonary parameters will then be measured. MIGET data will be stored and quantified by a software program on the VAX computer system.

All animals will be supported for 130 hours following injury. At the conclusion of the experiment, or sooner if the opinion of the Primary Investigator is that an animal is in irreversible cardiopulmonary failure, all animals will be euthanized with an overdose of barbiturates. Necropsy will be performed in the standard manner on all animals. Sections of all organs will be obtained and fixed for light and possible electron microscopy. The left lower lobe will be inflated to 20 cm of water and fixed by the endotracheal instillation of Carnoy's solution. The trachea will be examined for evidence of gross lesions. A ligature will be placed at the site of the tip of the endotracheal tube prior to fixation. The trachea will be fixed in its entirety, sectioned longitudinally and examined. A section of each of the remaining lobes will be removed and frozen in liquid nitrogen and stored at -80°C.

b. Data Analysis Plan: Blood gas, blood pressure, airway pressures, and hemodynamic data will be averaged over intervals and plotted at the midpoint of each interval. Physiologic and repetitive biochemical data will then be analyzed among groups using analysis of variance for repeated measures. Data will also be compared at specific time points using analysis of variance. Outcome data will be analyzed using Chi square or the Fisher exact test. Nonparametric data will be analyzed using the Kruskall-Wallis statistic.

## RESULTS

The protocol was approved during this fiscal year by the animal use review office of the Office of the Surgeon General. Subsequent to that approval, the Foundation for Biomedical Research Animal Use Committee also approved the protocol. The first animals are to be studied during the first quarter of FY 1991.

## DISCUSSION

None.

## PRESENTATIONS/PUBLICATIONS

None.

## REFERENCES


1. Hamilton PP, Onayemi A, et al: Comparison of conventional and high-frequency ventilation: oxygenation and lung pathology. **J Appl Physiol** 55:131, 1983.

2. deLemos RA, Coalson JJ, et al: Ventilatory management of infant baboons with hyaline membrane disease: the use of high frequency ventilation. **Pediatr Res** 21:594, 1987.

3. Meredith KS, deLemos RA, et al: Role of lung injury in the pathogenesis of hyaline membrane disease in premature baboons. **J Appl Physiol** 66:2150, 1989.

4. HIFI Study Group: High-frequency oscillatory ventilation compared with conventional mechanical ventilation in the treatment of respiratory failure in preterm infants. **N Engl J Med**. 320:88, 1989.

5. Truog WE, Standaert TA, et al: Effects of prolonged high-frequency ventilation in premature primates with hyaline membrane disease. **Amer Rev Respir Dis** 130:76, 1984.

6. Froese AB: Role of lung volume in lung injury: HFO in the atelectasis-prone lung. **Acta Anaesthesiol Scand** 33 (Suppl 90) 126, 1989.

7. Fouke JM, deLemos RA, McFadden ER Jr: Airway response to ultra short term exposure to ozone. **Amer Rev Respir Dis** 137:326, 1988.

8. de los Santos R, Coalson JJ, et al: Hyperoxia exposure in mechanically ventilated primates with and without previous lung injury. **Exp Lung Res** 9:255, 1985.

**UNITED STATES ARMY INSTITUTE OF SURGICAL RESEARCH**
**FORT SAM HOUSTON**
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## PRESENTATIONS

**Duncan DJ**: Burn trauma: initial management and standards of care. Presented at the Baptist Memorial Hospital School of Nursing, San Antonio, Texas, 2 October 1989.

**Duncan DJ**: Initial management of the burn victim. Presented at the Emergency Nursing Conference, Fort Sam Houston, Texas, 4 October 1989.

**Pruitt BA Jr**: Status of the American Trauma Society. Presented at the 49th Annual Meeting of the American Association for the Surgery of Trauma, Chicago, Illinois, 5 October 1989.

**Pruitt BA Jr**: Review of the Ufa, Russia train/fire disaster. Presented at the 49th Annual Meeting of the American Association for the Surgery of Trauma, Chicago, Illinois, 6 October 1989.

**Loresch D**: Prevention of burn injuries. Presented as part of Fire Safety Week, Kelly Air Force Base, San Antonio, Texas, 8 October 1989.

**Molter NC**: Needs of families of critically ill patients. Presented at St. Elizabeth Hospital, Beaumont Texas, 11 October 1989.

**Burleson DG**: Immune function and infection in burned patients. Presented at the Annual Meeting of the Society for Leukocyte Biology, Marco Island, Florida, 12 October 1989.

**Shippee RL**: Primary and secondary humoral response to sheep red blood cells in a rat model. Presented at the Annual Meeting of the Society for Leukocyte Biology, Marco Island, Florida, 12 October 1989.

**Becker WK**: Medical care in the Soviet Union. Presented at the Armed Forces Medical Intelligence Center, Fort Detrick, Frederick, Maryland, 12 October 1989.

**Duncan DJ**: Initial management of the burn victim. Presented at the Wilford Hall USAF Medical Center, Lackland Air Force Base, San Antonio, Texas, 16 October 1989.

**Duncan DJ**: Standards of care for the large burn victim in the resuscitative phase. Presented at the Wilford Hall USAF Medical Center, Lackland Air Force Base, Texas, 16 October 1989.

**Chapman T**: Initial management of burn victims in the theater of operation, Bergstrom Air Force Base, Texas, 24 October 1989.

**Burgess M**: Developing clinical expertise. Presented at the Drusilla Poole Nursing Education and Staff Development Conference, San Antonio, Texas, 25 October 1989.

**Duncan D**: Developing clinical expertise. Presented at the Drusilla Poole Nursing Education and Staff Development Conference, San Antonio, Texas, 25 October 1989.

**Molter N**: Developing clinical expertise. Presented at the Drusilla Poole Nursing Education and Staff Development Conference, San Antonio, Texas, 25 October 1989.

**DePew CL**: Toxic epidermal necrolysis syndrome. Presented at the AACN Spotlights on Critical Care Conference, San Antonio, Texas, 26 October 1989.

**Okerberg CV**: Mammary gland and integument system of laboratory animals. Presented at the International Life Sciences Institute Histopathology Seminary, Baltimore, Maryland, 29 October 1989.

**Kim SH**: Viral infection in severely burned patients: a review of seven years (1981–1987). Presented at the ASCP/CAP Fall Meeting and Exhibit, Washington, DC, 28 October 1989.

**Keenan J**: Initial management of burn victims in the theater of operation. Presented as part of the United States Air Force Battlefield Nursing Course, Brooks Air Force Base, San Antonio, Texas, 31 October 1989.

**Duncan DJ**: Emergency treatment of thermal and chemical burns. Presented at the Pfizer Safety and Health Conference, San Antonio, Texas, 2 November 1989.

**Burgess M**: Initial management of the burn victim. Presented at the New Rochelle, New Rochelle, New York, 2 November 1989.

**Burgess M**: Initial management of the burn victim. Presented at Pace University, Pleasantville, New York, 3 November 1989.

**Pruitt BA Jr**: Fluid resuscitation following injury. Presented to the Department of Surgery, University of South Florida College of Medicine, Tampa, Florida, 3 November 1989.

**Pruitt BA Jr**: Diagnosis and treatment of opportunistic infections in injured man. Presented to the Department of Surgery, University of South Florida College of Medicine, Tampa, Florida, 4 November 1989.

**Kim SH**: Pitfalls in the evaluation of burn wound biopsies. Presented at the 83rd Annual Scientific Assembly of the Southern Medical Association, Washington, DC, 5 November 1989.

**Duncan DJ**: Initial management of the burn victim. Presented at the Wilford Hall USAF Medical Center, Lackland Air Force Base, San Antonio, Texas, 7 November 1989.

**Duncan DJ**: Standards of care for the large burn victim in the resuscitative phase. Presented at the Wilford Hall USAF Medical Center, Lackland Air Force Base, San Antonio, Texas, 7 November 1989.

**Cioffi WG Jr**: High-frequency percussive ventilation in patients with inhalation injury. Presented to the VDR User's Forum at the 21st Annual Dr. Douglas Davis Pulmonary Symposium, Louisville, Kentucky, 9 November 1989.

**McManus WF**: Septic shock. Presented at the Critical Minutes Symposium, Oklahoma City, Oklahoma, 9 November 1989.

**Pruitt BA Jr**: Clinical and research activities of the United States Army Institute of Surgical Research. Presented at the Lab 21 Review, 14 November 1989.

**DePew CL**: Acid base balance. Presented as part of the United States Army Critical Care Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 22 November 1989.

**Becker WK**: Medical experience in the Soviet Union. Presented at the Officers Club, Randolph Air Force Base, San Antonio, Texas, 21 November 1989.

**DePew CL**: Fluid and electrolytes. Presented as part of the United States Army Critical Care Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 22 November 1989.

**Summers TM**: Families and crisis. Presented as part of the United States Army Critical Care Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 30 November 1989.

**Pruitt BA Jr**: Early burn wound excision and closure. Presented at the 14th Annual Meeting of the International Society of Burn Injuries, Denver, Colorado, 1 December 1989.

**Pruitt BA Jr**: Infections in burn patients. Presented at the 14th Annual Meeting of the International Society of Burn Injuries, Denver, Colorado, 1 December 1989.

**Pruitt BA Jr**: Diagnosis and treatment of infection in injured man. Presented at the University of Colorado Health Sciences Center, Denver, Colorado, 2 December 1989.

**Pruitt BA Jr**: Inhalation injury. Presented at the 14th Annual Meeting of the International Society of Burn Injuries, Denver, Colorado, 2 December 1989.

**Pruitt BA Jr**: Unsolved problems in burn care. Presented at the 14th Annual Meeting of the International Society of Burn Injuries, Denver, Colorado, 2 December 1989.

**Summers TM**: Stress management. Presented as part of the United States Army Critical Care Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 6 December 1989.

**McManus WF**: Elderly, high risk burn patients. Presented at the Comprehensive Care of the Burn Patient Conference, Kansas City, Missouri, 8 December 1989.

**DePew CL**: Pacemakers. Presented as part of the United States Army Critical Care Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 8 December 1989.

**Pruitt BA Jr**: Management of the pregnant burn patient. Presented as part of the Ninth Annual Comprehensive Care of the Burn Patient Course, Kansas City, Missouri, 8 December 1989.

**Selzer R**: Initial management of burn victims in the theater of operation. Presented as part of the United States Air Force Battlefield Nursing Course, Brooks Air Force Base, San Antonio, Texas, 12 December 1989.

**Becker WK**: USSR train disaster intervention. Presented at the Seventh Users' Stress Workshop on Training for Psychic Trauma, San Antonio, Texas, 14 December 1989.

**Duncan DJ**: Initial management of burn victims. Presented as part of the Critical Care Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 11 January 1990.

**Pruitt BA Jr**: Progress and problems in burn care. Presented to the San Antonio Surgical Society, San Antonio, Texas, 16 January 1990.

**Pruitt BA Jr**: The pathophysiology of burn injury. Presented to the TSI/DNA Combined Injured Core Group, Orlando, Florida, 17 January 1990.

**Keenan J**: Compassion knows no boundaries: USSR burn mission. Presented to the South Central Emergency Management Association, San Antonio, Texas, 19 January 1990.

**Burleson DG**: Longitudinal changes in T lymphocyte phenotype and function after thermal injury. Presented at the Conference on the Immunocompromised Surgical Patient: Mechanisms and Therapy in Trauma and Burns, Snowbird, Utah, 24 January 1990.

**Driscoll DM**: Compassion knows no boundaries: USSR burn mission. Presented at the Southwest Texas Methodist Hospital, San Antonio, Texas, 25 January 1990.

**Molter NC**: Needs of families of critically ill patients. Presented at the University of Texas Health Science Center, San Antonio, Texas, 30 January 1990.

**Becker WK**: Burn injury pathophysiology. Presented as part of the OT/PT Management of Burns Course, United States Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 5 February 1990.

**Becker WK**: Burn wound management. Presented as part of the OT/PT Management of Burns Course, United States Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 5 February 1990.

**Cioffi WG Jr**: Inhalation injury. Presented as part of the OT/PT Management of Burns, United States Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 5 February 1990.

**Jordan BS**: Review of current research at the United States Army Institute of Surgical Research. Presented as part of the OT/PT Management of Burns, United States Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 5 February 1990.

**Summers TM**: Psychosocial aspects of burn care. Presented as part of the OT/PT Management of Burns, United States Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 6 February 1990.

**Molter NC**: Principles of pain management. Presented as part of the OT/PT Management of Burns, United States Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 6 February 1990.

**Carlson DE**: Nutritional management. Presented as part of the OT/PT Management of Burns, United States Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 6 February 1990.

**Hollan E**: Infection control principles in burn care. Presented as part of the OT/PT Management of Burns, United States Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 7 February 1990.

**Matta CB**: Initial management of burn injuries in the theater of operation. Presented as part of the United States Air Force Battlefield Nursing Course, Brooks Air Force Base, San Antonio, Texas, 13 February 1990.

**Pruitt BA Jr**: Burns complicated by pregnancy and mechanical injury. Presented as part of the OT/PT Management of Burns Course, Fort Sam Houston, San Antonio, Texas, 15 February 1990.

**McManus WF**: Burn mass casualty care in Ufa, USSR. Presented to the International Society of Burns, Maui, Hawaii, 18 February 1990.

**Becker WK**: American military response to the Ufa train disaster. Presented at the Conference on Catastrophic Medicine, Ufa, USSR, 22 February 1990.

**McManus AT**: Microbiologic observations made by the laboratory component of the American burn team sent to aid victims of the Baskarian burn disaster. Presented at the Congress on Catastrophic Medicine, Ufa, Russia, 22 February 1990.

**Cioffi WG Jr**: The use of surfactant replacement in ARDS and smoke inhalation. Presented at the United States Army Institute of Surgical Research, 7 March 1990.

**Duncan DJ**: Initial management of burn victims in the theater of operation. Tri-Service Emergency Medical Conference, San Antonio, Texas, 7 March 1990.

**Chapman T**: Burns/hazardous materials. Presented as part of the Emergency Medical Technician Course, Fort Sam Houston, San Antonio, Texas, 8 March 1990.

**Keenan J**: Compassion knows no boundaries: USSR burn mission. Presented at the FORESCOM Conference, Atlanta, Georgia, 9 March 1990.

**Pruitt BA Jr**: Shock and fluid resuscitation. Presented as part of the Advanced Burn Life Support Provider Course, Los Angeles, California, 14 March 1990.

**Becker WK**: Mass casualty experience in the Soviet Union. Presented to the Committee on Trauma at the 68th Annual Meeting of the American College of Surgeons, Washington, DC, 15 March 1990.

**Burleson DG**: IL2-receptor expression by stimulated lymphocytes from burned patients. Presented at the 14th International Meeting of the Society for Analytical Cytology, Ashville, North Carolina, 18 March 1990.

**Molter NC**: United States Army Institute of Surgical Research. Presented as part of the Nurse Education Tour, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 26 March 1990.

**Pruitt BA Jr**: Shock and Fluid resuscitation. Presented as part of the Advanced Burn Life Support Provider Course, Las Vegas, Nevada, 26 March 1990.

**Pruitt BA Jr**: Epidemiology, pathophysiology, and classification of chemical injury. Presented at the 22nd Annual Meeting of the American Burn Association, Las Vegas, Nevada, 27 March 1990.

**Waymack JP**: Effect of prostaglandin E (PGE) on resistance to sepsis. Presented at the 22nd Annual Meeting of the American Burn Association, Las Vegas, Nevada, 28 March 1990.

**Waymack JP**: Effect of prostaglandin E (PGE) on endotoxin/tumor necrosis factor (TNF) metabolism. Presented at the 22nd Annual Meeting of the American Burn Association, Las Vegas, Nevada, 28 March 1990.

**Cioffi WG Jr**: Advanced burn life support. Presented at the 22nd Annual Meeting of the American Burn Association, Las Vegas, Nevada, 29 March 1990.

**Cioffi WG Jr**: Granulocyte function following thermal injury. Presented at the 22nd Annual Meeting of the American Burn Association, Las Vegas, Nevada, 29 March 1990.

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