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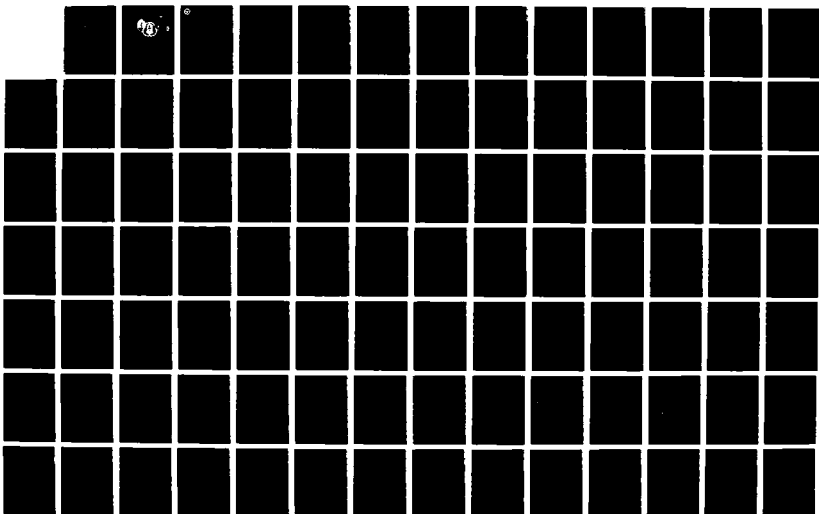
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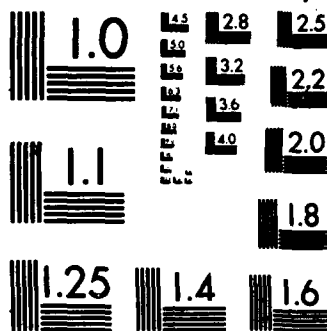
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FOR FISCAL YEAR 1984

1 OCTOBER 1983 - 30 SEPTEMBER 1984

PREPARED BY: US ARMY INSTITUTE OF SURGICAL RESEARCH
FORT SAM HOUSTON
SAN ANTONIO, TEXAS 78234-6200

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1 October 1984

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DEPARTMENT OF THE ARMY

US ARMY INSTITUTE OF SURGICAL RESEARCH
FORT SAM HOUSTON
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1 October 1984

SUBJECT: US Army Institute of Surgical Research Annual Research
Progress Report for Fiscal Year 1984

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Basil A. Pruitt, Jr.

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

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FOREWARD

This and previous annual reports of the US Army Institute of Surgical Research present the outcome of both clinical care and research, i.e., the product of the individual and collaborative scientific activities of the Institute's professionals made possible by the efforts of all members of the organization. The process by which this product is produced is typically disorderly and usually investigator-specific. Advances in clinical care and medical research are so strongly influenced by fortuitous clinical observation, general serendipity, and investigator eccentricities that planning and management techniques useful in nonmedical development projects are irrelevant and counterproductive. The medical research process ultimately depends upon an appropriately trained and experienced professional asking the right question and conducting the right study to obtain a verifiable answer. The professional elitism inherent in medical research has made a world of would-be health care and biomedical research administrators restless and generated a proliferative frenzy in that population.

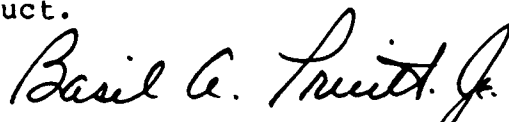
The disproportionate administrative burden in the field of health care is evident in the 25-percent increase in the number of hospital administrators which occurred in the time period 1980 to 1981, a time during which the number of registered nurses increased only 1.2 percent and the number of physicians remained essentially constant (1). A similar burgeoning of biomedical research administrators has occurred in both the Federal and private spheres as indexed by the ever increasing length of managerial rosters and the increase in research overhead. The professional naivete of such administrators limits their ability to influence, let alone assess the outcome of clinical care and biomedical research. That limitation combined with their faith in what are called management principles has caused such administrators to focus their activities on the process of medical research with the resultant blurring of the distinction between the process and the product. Extensive administrative hierarchies have been organized to dictate specifics of patient care and assess the quality of that care, define the scope of physician and other health professional activities, regulate human studies, and restrict the use of animals in research, but to what effect? Benefits of such administrative activity have yet to be identified.

Wohl, S: The Medical Industrial Complex. New York: Harmony Books, 1984, pp 79-80.

In each of the cited areas, professionals or organizations of professionals have traditionally and often by charter discharged, in the course of their other activities, those duties which only confound nonprofessional administrators. The process of clinical care is physician dependent and the quality of such care is related to physician experience and expertise, direct physician involvement in the care process, and the attitude and professionalism of the physician and other health care personnel. The quality of care is best monitored and the excellence of care is best achieved in the course of clinical rounds, staff conferences, and clinical-pathological correlations which can only be conducted by physicians. The certification of professionals has been the province of established peer organizations. There are no data available to suggest that the current certification process requires additional administrative fine tuning. Similarly, medical research involving either human or nonhuman subjects has been guided by commonly appreciated ethical considerations and there is little evidence, judging by daily press reports of business scandal and fraud, that a management background imparts any particular ethical or moral sensitivity.

Rather than achieve research efficiency or economy, greater administrative requirements have simply increased the cost of both patient care and medical research, decreased the velocity of research, promoted an adversarial investigator/administrator relationship, and, by instituting arbitrary species-specific limitations, imperiled the orderly progress of medical research. Moreover, the increased administrative requirements have diverted the efforts of scientists into nonproductive administrative activities to further slow research progress and expended monies to confound the process rather than expedite the product.

The professional product of the physicians and other biomedical scientists at this Institute is best assessed in terms of patient outcome and research results. The productivity evident in this volume and the quality of the clinical care and biomedical research reported attest to the professional excellence and intimate involvement of the Institute's professionals who have no difficulty distinguishing between the process and the product.



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

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 of the Department of Defense.

Partial
TABLE OF CONTENTS;

Page Number

PROJECT NUMBER 3S162772A874-00, APPLIED RESEARCH

CLINICAL OPERATION, CENTER FOR TREATMENT OF BURNED SOLDIERS;

1

COL William F. McManus, MD; COL Anton J. Jirka, MD; Cleon W. Goodwin, MD; LTC George M. Vaughan, MD; LTC Louis H. Aulick, PhD; MAJ Kevin J. Farrell, MD; MAJ Khan Z. Shirani, MD; MAJ Lawrence W. Lehrner, MAJ Roosevelt J. Stallings, MD; MAJ Arthur H. Yancey, II, MD; MAJ Annette R. Aitcheson, RN; CPT Patrick Conarro, MD; CPT James C. McKay, MD; CPT L. Willis Roberts, MD; CPT Kenneth W. Bush, MD; CPT Bonnie E. Hoffman; and CPT Molly S. Maquire, CPT Kathryn R. Robertson, CPT Lise C. Walker, CPT David R. Strome, CPT Nancy K. McLaurin, and COL Basil A. Pruitt, Jr., MD

Anesthesiology;

33

COL Anton J. Jirka, MD, and MAJ Steven I. Schmidt, MD

THE CARDIOPULMONARY RESPONSE TO THERMAL INJURY IN BURNED SOLDIERS;

42

Effect of Plasmapheresis on the Outcome of Critically Injured Burn Patients.....

43

COL William F. McManus and COL Basil A. Pruitt, Jr., MD

Evaluation of Angiotensin II Analogue as a Therapeutic Agent of ARDS.....

49

Tetsuo Yukioka, MD; Noriko Yukioka, MD; LTC Louis H. Aulick, PhD, Cleon W. Goodwin, MD; Arthur D. Mason, Jr., MD; Tsuyoshi Sugimoto, MD; and COL Basil A. Pruitt, Jr., MD

Cont'd

PROJECT NUMBER 3S162772A874-00, APPLIED RESEARCH

An Analysis of the Adaptation of Renal Function
Following Burn Injury..... 71

MAJ Lawrence W. Lehrner, MD; Calvin R.
Kennedy; MAJ James McKay, MD; Arthur D. Mason,
Jr., MD; LTC Khan Z. Shirani, MD; and LTC
George M. Vaughan, MD

Assessment of Non-Nutrient Blood Flow in Criti-
cally Ill Burn Patients..... 79

LTC Kevin J. Farrell, COL William F. McManus,
and COL Basil A. Pruitt, Jr.,

con+
EVALUATION OF BURN WOUND CARE IN TROOPS WITH BURN
INJURY;..... 86

5% Aqueous Sulfamylon Soaks Used in Topical
Treatment of Burned Soldiers..... 87

COL William F. McManus, MD, and COL Basil A.
Pruitt, Jr., MD

Biobrane^R and Porcine - A Comparative Study..... 90

CPT L. Willis Roberts, MD; LTC Khan Z.
Shirani, MD; COL William F. McManus, MD;
Arthur D. Mason, Jr., MD; and Basil A. Pruitt,
Jr., MD

The Use of DuoDERMTM Dressings in the Management
of Skin Graft Donor Sites..... 98

CPT L. Willis Roberts, MD; LTC Khan Z.
Shirani, MD; COL William F. McManus, MD;
Arthur D. Mason, Jr., MD; and Basil A. Pruitt,
Jr., MD

STUDIES OF THE NEUROENDOCRINE ABNORMALITIES IN
BURN INJURY;..... 105

Pineal Function in Burns: Evidence for Parti-
tioned Control of Sympathetic Function..... 106

LTC George M. Vaughan, MD; LTC Thomas J.
Taylor, MD; COL Basil A. Pruitt, Jr., MD, and
Arthur D. Mason, Jr., MD

PROJECT NUMBER 3M161102BS10-00, BASIC RESEARCH

STUDIES OF INFECTION AND MICROBIOLOGIC SURVEILLANCE OF TROOPS WITH THERMAL INJURY.....	127

Albert T. McManus, PhD; Jack R. Henderson, PhD; SSG Timothy E. Lawson; SSG Aldo H. Reyes, SP5 Anne M. Bray; SP5 Charles E. Guymon; and Arthur D. Mason, Jr., MD

A Multicenter Open Study of the Efficacy, Safety, and Tolerance of PRIMAXIN TM (Previously Referred to as MK0787/MK0791 and Thienamycin Formamidine/Potentiator) in the Parenteral Therapy of Infection Caused by Pathogenic Bacteria in Hospitalized Patients.....	174

COL Basil A. Pruitt, Jr., MD; Albert T. McManus, PhD; COL William F. McManus, MD; LTC Khan Z. Shirani, MD; and CPT Alvis T. Perry, MD

<u>Comparison of Virulence of Pseudomonas aeruginosa</u> in Burned Rats and Mice.....	184

CPT George B. Stover, PhD; Albert T. McManus, PhD; LTC Gene B. Hubbard, DVM, MS; and Arthur D. Mason, Jr., MD

Pharmokinetic Study of Amikacin in Burned Patients.....	196

MAJ Lawrence M. Lehrner, MD; MAJ James C. McKay, MD; Albert T. McManus, PhD; and Arthur D. Mason, Jr., MD

Characterization of Biochemical Indicators of Infection in the Thermally Injured.....	200

Kuang-Tzu D. Lin, MD, MAJ David G. Burleson, PhD, and LTC Michael C. Powanda, PhD

THE STUDY OF METABOLISM AND NUTRITIONAL EFFECTS OF BURN INJURY IN SOLDIERS.....	209

Studies of Disturbance of Protein Turnover in Burned Troops: Use of an Animal Model.....	210

Wanda L. Brown, MS; Eleanor G. Bowler, PhM; and Arthur D. Mason, Jr.

PROJECT NUMBER 3M161102BS10-00, BASIC RESEARCH

Role of Lipid Metabolism in Burn Injury.....	217
CPT David R. Strome, PhD; Cleon W. Goodwin, PhD, and Arthur D. Mason, Jr., MD	
An Analysis of the Utility of Amylase Isozyme Differentiation in the Diagnosis of Pancreatitis in Burned Patients.....	224
MAJ Lawrence M. Lehrner, MD; John Dubois, BS; Calvin R. Kennedy; LTC Seung H. Kim, MD; MAJ James C. McKay, MD; and Arthur D. Mason, Jr., MD	
ALTERATION OF HOST RESISTANCE IN BURNED SOLDIERS;	229
Albert T. McManus, PhD; Arthur D. Mason, Jr., MD; COL Basil A. Pruitt, Jr., MD; Camille L. Denton, MA; George T. Daye, Jr., MA; and Virginia C. English, MA	
Therapy with IgG and T ₄ in Burned Patients.....	239
LTC Khan Z. Shirani, MD; LTC George M. Vaughan, MD; Albert T. McManus, PhD; MAJ David G. Burleson, PhD; Richard A. Becker, MD; COL William F. McManus, MD; Arthur D. Mason, Jr., MD; and COL Basil A. Pruitt, Jr., MD	
Double-Blind Study of <u>C. parvum</u> Vaccine in the Prevention of Infection Following Burn Injury...	241
LTC Khan Z. Shirani, MD; Albert T. McManus, PhD; COL William F. McManus, MD; J. Wesley Alexander, MD; Arthur D. Mason, Jr., MD; and COL Basil A. Pruitt, Jr., MD	
ROLES OF THYROID HORMONES IN BURN PATHOPHYSIOLOGY;	246
Central Features of Thyroid Function.....	247
LTC George M. Vaughan, MD, and LTC Khan Z. Shirani, MD	

PROJECT NUMBER 3M161102BS10-00, BASIC RESEARCH

Reduced T_4 and T_3 and Their Altered Serum
Binding after Burn Injury in a Rat Model.....

249

LTC Khan Z. Shirani, MD; LTC George M.
Vaughan, MD; COL Basil A. Pruitt, Jr., MD; and
Arthur D. Mason, Jr., MD

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

Cardiovascular and Endocrine Sequelae of Burn Resuscitation;..... 264

LTC Khan Z. Shirani, MD; Arthur D. Mason, Jr., MD; LTC George M. Vaughan, MD

Mechanisms of Opportunistic Infection in Burned Soldiers;..... 267

Albert T. McManus, PhD; Camille L. Denton, MA; Virginia C. English, MA; George T. Daye, Jr., MA; Arthur D. Mason, Jr., MD

Preliminary Studies of Lymphoid Subpopulations in a Burned Animal Model;..... 276

MAJ David G. Burleson, PhD; LTC George M. Vaughan, MD; Albert T. McManus, PhD; Arthur D. Mason, Jr., MD;

Preliminary Studies on Zinc Homeostatic Control and Immunocompetence in a Burned Animal Model;.... 294

CPT Ronald L. Shippee, PhD

The Study of Metabolism and Temperature Regulation in Animal Models of Human Burn Injury;..... 304

The Effects of Localized and Systemic Infection on Aerobic Metabolism and Core Temperature of the Burned Rat..... 305

LTC Louis H. Aulick, PhD; Albert T. McManus, PhD; Arthur D. Mason, Jr., MD; and COL Basil A. Pruitt, Jr., MD

Topical Antimicrobial Therapy Reduces Postburn Hypermetabolism of the Burned Infected Rat..... 324

LTC Louis H. Aulick, PhD; Albert T. McManus, PhD; Arthur D. Mason, Jr., MD; and COL Basil A. Pruitt, Jr., MD

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

Copy
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Inequality of VA/Q Ratios Following Smoke Inhalation Injury and the Effect of Angiotensin Analogues.....

338

↑
Takeshi Shimazu, MD; Tetsuo Yukioka, MD;
Albert T. McManus, PhD; LTC Gene B. Hubbard,
DVM, MS; Arthur D. Mason, Jr., MD; and COL
Basil A. Pruitt, Jr., MD

	<u>Page Number</u>
PRESENTATIONS.....	346
PUBLICATIONS.....	354
DISTRIBUTION LIST.....	357

FISCAL YEAR 1983		FISCAL YEAR 1984		PAGE NUMBER
<u>Project Number</u>	<u>Work Number</u>	<u>Project Number</u>	<u>Work Number</u>	
3S162772A874				APPLIED RESEARCH
3S162772A874	164	3S162772A874	164	Clinical Operation, Center for Treatment of Burned Soldiers 1
3S162772A874	161	3S162772A874	161	The Cardiopulmonary Response to Thermal Injury in Burned Soldier 42
3S162772A874	162	3S162772A874	162	Evaluation of Burn Wound Care in Troops with Burn Injury 86
3S162772A874	163	3S162772A874	163	Studies of the Neuroendocrine Abnormalities of Burned Soldiers 105
3M161102BS10				BASIC RESEARCH
3M161102BS10	301	3M161102BS10	301	Studies of Infection and Microbiologic Surveillance of Troops with Thermal Injury 127
3M161102BS10	302	3M161102BS10	302	The Study of Metabolism and Nutritional Effects of Burn Injury in Soldiers 209
3M161102BS10	303	3M161102BS10	303	Alteration of Host Resistance in Burned Soldiers 229
3M161102BS10	304	3M161102BS10	304	Roles of Thyroid Hormones in Burn Pathophysiology 246

FISCAL YEAR 1984

FISCAL YEAR 1985

<u>Project Number</u>	<u>Work Number</u>	<u>Project Number</u>	<u>Work Number</u>	<u>PAGE NUMBER</u>
3A161101A91C				
3A161101A91C	075	3A161101A91C	075	264
3A161101A91C	076			267
3A161101A91C	077	3A161102A91C	077	276
×	078	3A161102A91C	078	294
3A161101A91C	083	3A161101A91C	083	304
3A161101A91C	089	3A161102A91C	089	338

IN-HOUSE LABORATORY INDEPENDENT RESEARCH

Cardiovascular and Endocrine Sequelae of Burn Resuscitation

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Preliminary Studies of Lymphoid Subpopulations in a Burned Animal Model

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The Study of Metabolism and Temperature Regulation in Animal Models of Human Burn Injury

Inequality of VA/Q Ratios Following Smoke Inhalation Injury and the Effect of Angiotensin Analogues

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23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code) graft;(U)Ram II						
23. (U) The Clinical Division of the U.S. Army Institute of Surgical Research is the major treatment center for thermally injured military personnel of all services as well as other eligible beneficiaries. The goals of the Clinical Division, in addition to the specialized care of the severely injured patients, include the investigation of diagnostic and therapeutic technics to improve the survival and function of the injured patient as well as promulgation of scientific medical information to health professionals. 24. (U) Thermally injured patients from the Continental United States and throughout the world are transported to the U.S. Army Institute of Surgical Research for intensive, specialized inpatient treatment. Carefully controlled evaluation of new treatment technics is conducted by the professional staff. 25. (U) 8310 - 8409. One hundred eighty eight seriously burned patients were admitted and treated at this Institute in calendar year 1983. Current clinical research activities include host resistance/studies, endocrine changes following injury, development of optimal nutritional support of the burned patient, the use of skin substitutes, and studies in the control of post injury infection.						

ANNUAL PROGRESS REPORT

PROJECT NO. 3S162772A874-00, APPLIED RESEARCH

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

US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234

1 January 1983 - 31 December 1983

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Nancy K. McLaurin, Captain, AMSC
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ABSTRACT

PROJECT No. 3S162772A874-00, APPLIED RESEARCH

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

US Army Institute of Surgical Research, Brooke Army Medical
Center, Fort Sam Houston, Texas 78234

Period covered in this report: 1 January - 31 December 1983

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One hundred and eighty-eight patients were admitted to the Clinical Division of the United States Army Institute of Surgical Research during calendar year 1983. Principle activities of the Clinical Division included care of the severely burned patient, research to improve survival and function of such patients, and education and training of health care professional and para-professional personnel. The areas of research included the cardiopulmonary response to thermal injury in burned soldiers, evaluation of burn wound care in troops with burn injury, studies of neuroendocrine abnormalities in burn injury, study of metabolism and nutritional effects of burn injury in soldiers, utilization of nursing staff for the assessment of dietary intake, assessment of thyroid hormone kinetics in thermally injured patients, and a multicenter open study of the efficacy, safety,

and tolerance of Thienamycin-formamidine/potentiator in the parenteral therapy of infection caused by pathogenic bacteria in hospitalized patients.

Autograft
Allograft
Aeromedical Transfer
Excision
Immunomodulation
Inhalation Injury
Metabolism

Neuroendocrine Abnormalities
Nutrition
Resuscitation
Thermal Injury
Topical Therapy
Zenograft

CLINICAL OPERATION, CENTER FOR TREATMENT OF BURNED SOLDIERS

The Clinical Division, U.S. Army Institute of Surgical Research admitted 188 soldiers and other authorized patients with thermal, chemical or electric injury during the calendar year 1983. Aeromedical teams from this Institute conducted 58 missions to transfer 69 patients (36.7%) of the 188 admitted patients. Fifty-seven missions were within the Continental United States, one mission was OCONUS (23 missions were by rotary wing aircraft [39.6%] and 35 by fixed wing aircraft). One hundred and sixteen (61.7%) of the 188 patients were admitted within 24 hours of injury and 130 (69.1%) were admitted within 48 hours of injury. One hundred and fifty-three of the 188 admitted patients were male and 35 were female. The following statistics are based on 179 patient dispositions during calendar year 1983 and the ages of these 179 patients ranged from two months to 93 years of age with an average age of 30.6 years. Burn size averaged 27.8% of the total body surface with an average full thickness component of 14.7%. Thirty-eight patients were in the pediatric age group (age 15 and under) with an average age of 4.4 years and an average burn size of 20.8% of the body surface. The average hospital stay of all dispositions was 46 days when convalescent leave was included in the calculation and 44.3 days when convalescent leave was subtracted. There were 14 patients with high voltage electric injury and one patient with chemical injury. The source of admission is identified in Table 1 and the cause of burn injury is delineated in Table 2.

MORBIDITY AND MORTALITY

Forty of the 179 patients (22.3%) died during calendar year 1983. Autopsies were performed in 22 (55%) of these hospital deaths. The average burn size of patients who died was 52.0% and the full thickness average was 40.9%. The ages of patients who died ranged from 9 months to 93 years of age. Nineteen of the 40 patients (47.5%) had inhalation injury as a primary or contributing diagnosis as a cause of death. Nine (22.5%) had burn injury exceeding 80% of the total body surface. Three patients died with acute myocardial infarction, one with acute bacterial endocarditis, and one with extensive psoriasis with staphylococcal colonization as a contributing cause of death. Three of the forty deaths were in the pediatric age group (8%) and these three children had an average total body surface burn of 40.3% and an average full thickness burn of 38.3%. The average age of children who died was 17.3 months (9 months through 2 years and 9 months) and two of the three pediatric age group deaths had an autopsy. Infection was once again the most common complication following thermal injury with bacterial pneumonias occurring in

31 patients. The most common organism isolated in patients with bacterial pneumonia was Staphylococcus aureus in nine patients, Klebsiella spp. in six patients, Pseudomonas spp. in five patients, and Escherichia coli in four patients. However, only 23 patients demonstrated septicemia and in only two patients was bacterial invasion of the burn wound identified during this calendar year. Six patients had clinical upper gastrointestinal hemorrhage and all responded to nonoperative therapy.

Only two patients required hemodialysis for acute renal failure. Acute myocardial infarctions were seen in three patients and acute pulmonary emboli in ten patients. Inhalation injury was identified in 53 patients (29.5% of admissions). Eighty-one patients (45%) had some associated injury (includes 53 patients with inhalation injury); fractures or dislocations in 11 patients; lacerations in eight patients; and five patients with head injuries including two patients with subdural hematomas and one with an epidural hematoma necessitating operation.

EDUCATION

The professional staff of the Clinical Division of the U.S. Army Institute of Surgical Research continued to provide education to all professional and paraprofessional levels locally, nationally, and internationally during 1983. A total of 24 resident physicians were attached for periods of one to two months during 1983 including 6 from Brooke Army Medical Center, 3 from Letterman Army Medical Center, 3 from William Beaumont Hospital in Royal Oak, Michigan, 2 from Fitzsimons Army Medical Center, 1 from the Naval Aerospace Medical Institute, 2 from Buffalo General Hospital, 2 from Wilford Hall USAF Medical Center, 2 from Travis AFB Medical Center, and 1 each from Flint, Michigan Osteopathic Hospital, Northwestern University, and Downstate Medical Center of New York. A total of 15 medical students rotated at this Center to include 3 Health Profession Scholarship medical students, 3 from Louisiana State University School of Medicine, 2 from Creighton University School of Medicine and 1 each from the University of Texas at Galveston, George Washington University Medical Center, University of Indiana, University of Kentucky, University of Rochester, and the University of Nebraska. A total of 23 physicians visited this Institute from foreign countries for periods ranging from 1 day to 1 year and included 4 from Yugoslavia, 3 from Pakistan, 2 each from Great Britain, Norway, and Thailand, 1 from Japan, Jordan, France, Australia, Canada, New Zealand, Israel, Ireland, Sweden, and China. The Physical Therapy Branch of this Institute had 21 trainees and the Occupational Therapy Branch had 72 trainees in calendar year 1983. Nineteen scientific publications appeared in refereed medical journals and approximately 163 scientific presentations were conducted for military and civilian audiences. Numerous scientific presentations were made at the Academy of Health

Sciences and various military installations throughout the Continental United States to include support of the Battlefield Medicine Course of the U.S. Air Force and the Combat Casualty Courses of the U.S. Army. In addition, weekly professional staff conferences were conducted for and by Institute personnel.

STATISTICAL RESUME

During calendar year 1983, 188 patients were admitted to the US Army Institute of Surgical Research and there were 179 patient dispositions during the same period. All subsequent data are based on dispositions. There were 153 males and 35 females with an average age of 30.6, ranging from two months to 93 years of age. Thirty-eight patients were less than 15 years old and 40 patients (22.3%) were greater than 45 years of age. The average total burn of the entire population was 27.8% of the total body surface with 14.7% average extent of full-thickness injury. The average hospital stay of all patients excluding convalescent leave for active duty military was 44.3 days. One hundred and thirty patients (69.1%) were admitted within 48 hours of injury.

During 1983, 1,106 operative procedures were performed on 140 patients for an average of 7.9 operative procedures per patient. Two hundred and ninety-one anesthetics were given to 98 patients (2.97 per patient). One hundred and seven patients received a total of 536,145 cc of blood (5,011 cc per patient).

Table 1 identifies the source of admission of patients during the calendar year 1983; Table 2 summarizes burn etiology; Table 3 lists the effect of age and extent of injury on survival; and, Table 4 lists mortality rate associated with increments of 10% total body surface burn for the years 1979 through 1983. Table 5 summarizes the survival of patients with extensive burns from 1958 through 1983 and Table 6 compares mortality before and after the use of topical chemotherapy of the burn wound. Table 7 lists the cause of death for calendar year 1983.

Table 1. Source of Admission, 1983

Area	A	AD	AF	AFD	N	ND	MC	VAB	Other	Total
1st Army	2	1	1	1	0	0	1	1	0	7
3rd Army	4	2	1	0	1	0	0	7	4	19
5th Army	15	15	8	12	2	2	1	11	62	128
6th Army	4	2	3	2	0	0	1	0	0	12
Alaska	1	0	0	0	0	0	0	0	0	1
Korea	2	0	1	0	0	0	0	0	1	4
Germany	3	0	0	0	0	0	0	0	0	3
Hawaii	1	0	0	0	0	0	1	0	0	2
Puerto Rico	0	0	0	0	2	0	0	0	0	2
Mexico	0	0	0	0	0	0	0	0	4	4
Grenada	0	0	0	0	0	0	0	0	1	1
Philippines	0	0	0	0	1	0	0	0	0	1
Lebanon	0	0	0	0	0	0	2	0	0	2
Micronesia	0	0	0	0	0	0	0	0	1	1
Azores	0	0	1	0	0	0	0	0	0	1
	32	20	15	15	6	2	6	19	73	188

A - Army

AF - Air Force

D - Dependent

Other: Civilian Emergency

US Public Health Service Beneficiary

Bureau of Employees Compensation Beneficiary

N - Navy, Marine Corps & US Coast Guard

VAB - Veterans Administration Beneficiary

Table 2. Burn Etiology, 1983 - 179 Dispositions

Causes	Number of Patients	Dispositions	Deaths	Mortality
Gasoline, Diesel & Kerosene	43	24%	8	18%
Structural Fires	21	11%	8	38%
Motor Vehicle Accidents	6	3%	1	16%
Aircraft Accidents	2	1%	1	50%
Open Flames	19	11%	6	32%
Electrical	14	8%	3	21%
Hot Liquids	32	18%	4	13%
Chemical	1	.6%	0	-
Butane, Propane or Natural, Sewer Gas Exp.	11	6%	3	27%
Welding	4	2%	1	25%
Smoking, Clothes, Ignited	5	3%	1	20%
Smoking, Oxygen, Ignited	1	.6%	1	100%
Bomb, Shell, Simulator Grenade, Gunpowder Exp.	9	5%	1	11%
Contact	5	3%	0	-
Others (Suicide, Torched)	6	3%	2	33%
TOTAL	179		40	

Table 3. Age, Body Surface Involvement and Mortality, 1983

Age (Yrs)	Percent Burn										Total Cases	Total Deaths	Mortality %
	0-10	10-20	20-30	30-40	40-50	50-60	60-70	70-80	80-90	90-100			
0-1	1	3	2	1(1)		1(1)					8	2	25%
1-2	2	2	2	3							9		
2-3	3		2	2(1)							7	1	14%
3-4		2									2		
4-5													
5-10	3	1	1	1		1					7		
10-15	1		1		1						3		
15-20	5	2	2	1	1	1					12		
20-30	17(1)	10	8	10(1)	3	3(1)	2(1)	1	2(1)		56	5	9%
30-40	4	5(1)	3	2	1	2(1)	1(1)	2(2)	1(1)		21	6	29%
40-50	5	1	1	2	4		3(3)		1(1)	3(3)	19	7	37%
50-60	4	1	2(1)	3					1(1)	1(1)	12	3	25%
60-70	2	3(1)	5(4)	3(2)	1(1)			1(1)		1(1)	16	10	63%
70-80	1		1(1)	1(1)							3	2	67%
80-90		1(1)		1(1)	1(1)						3	3	100%
90-100					1(1)						1	1	100%
TOTAL	48	31	30	30	13	8	6	4	5	5	179	40	22%
Deaths	1	3	6	7	3	3	5	3	4	5			
% Mortality	2	10	20	23	23	38	83	75	80	100			

Table 4. Percent Body Surface Burn Involvement and Mortality, 1980-1983

% Burn	0-10	10-20	20-30	30-40	40-50	50-60	60-70	70-80	80-90	90-100	TOTAL
(1980)											
No. Burned	34	42	41	37	18	24	16	11	13	6	242
Deaths	0	1	2	3	8	12	12	11	11	6	66
% Mortality	0	2.4	4.9	8.19	44.4	50	75	100	84.6	100	27.3
(1981)											
No. Burned	51	39	24	29	18	26	3	7	3	8	208
Deaths	0	0	3	3	4	15	2	7	1	8	43
% Mortality	0	0	12.5	10.3	22.2	57.7	66.7	100	33.3	100	20.7
(1982)											
No. Burned	42	51	39	29	25	17	12	7	7	2	231
Deaths	0	3	5	6	11	6	8	6	7	2	54
% Mortality	0	5.9	12.8	20.7	44.0	35.3	66.7	85.7	100	100	23.4
(1983)											
No. Burned	47	31	30	30	13	8	6	4	5	5	179
Deaths	1	3	6	7	3	3	5	3	4	5	40
% Mortality	2	10	20	23	23	38	83	75	80	100	22

Table 5. Survival and Death by Year for Patients
With Extensive Burns, 1961-1983

With Extensive Burns, 1961-1983						
Year	Survivors (burns over 30%)			No. Cases	Deaths	
	No.	Average % Burn	Average % Burn			
	Cases	Total	3°		Total	3°
1961	18	44.2	25.0	31	58.0	39.7
1962	18	42.7	21.4	54	59.1	46.2
1963	28	45.8	19.6	57	69.0	41.0
1964	40	41.8	14.8	37	65.0	42.4
1965	47	43.8	21.0	33	66.0	33.4
1966	68	41.5	14.9	59	59.9	31.3
1967	103	42.7	13.3	51	59.9	32.3
1968	143	44.2	12.6	38	54.6	24.6
1969	113	43.2	11.1	70	58.7	26.4
1970	92	39.4	10.7	70	51.9	32.6
1971	63	41.9	14.0	68	60.8	38.0
1972	62	42.0	17.2	103	56.7	35.9
1973	47	43.7	19.6	113	60.3	36.2
1974	55	43.9	12.2	97	60.8	35.9
1975	80	46.1	14.7	94	61.3	32.8
1976	69	45.5	15.0	79	64.2	31.1
1977	66	42.2	14.4	70	56.9	29.0
1978	67	45.7	14.8	69	55.2	33.0
1979	61	45.4	13.4	74	65.0	37.0
1980	62	42.7	15.1	66	64.3	41.8
1981	54	42.7	17.5	43	62.2	39.8
1982	53	43.7	24.8	54	53.9	38.3
1983	37	43.5	17.5	30	62.8	50.7

Table 7. Cause of Death, 1983

Patient	Age	Sex	% Burn Total	30	PEB Death	Cause of Death
1	52	M	97	97	15	*97% total body surface burn, inhalation injury with bronchopneumonia and burn
2	69	M	97	85	0	*97% total body surface burn and inhalation injury
3	45	M	95	95	1	*95% total body surface burn
4	41	M	93	90	8	93% total body surface burn, inhalation injury, pneumonia, Klebsiella species, acute renal failure
5	43	M	92	83	26	92% total body surface burn, acute myocardial infarction, Aspergillus burn wound infection
6	33	M	84	80.5	46	84% total body surface burn, invasive Aspergillus burn wound infection, inhalation injury, bronchopneumonia
7	57	F	83.5	5	11	83.5% total body surface burn, pneumonia with septicemia Staphylococcus aureus
8	25	M	82	39 1/4	70	82% total body surface burn, fungal burn wound infection, pneumonia
9	44	M	80	80	4	*80% total body surface burn, inhalation injury and bronchopneumonia

*Autopsy not performed

Table 7. Cause of Death, 1983 - Continued

Patient	Age	Sex	% Burn Total	% Burn 30	PEB Death	Cause of Death
10	30	M	79.5	79.5	32	*79.5% total body surface burn, inhalation injury, bronchopneumonia, burn wound infection with bacteria and fungi
11	39	F	77	50	51	77% total body surface burn, Staphylococci pneumonia, extensive psoriasis
12	63	M	72	64.5	19	*72% total body surface burn, Pseudomonas burn wound sepsis, Pseudomonas pneumonia
13	34	F	67	65.5	54	67% total body surface burn, inhalation injury, pneumonia and acute bacterial endocarditis
14	49	M	66	10.5	10	66% total body surface burn, aspiration and pulmonary edema, Pseudomonas burn wound infection
15	21	M	65.5	60	5	65.5% total body surface burn, inhalation injury, hypoxic brain death
16	49	M	64.5	62.5	55	64.5% total body surface burn, inhalation injury, bronchopneumonia, cytomegalovirus with renal and pulmonary infection
17	49	M	64	64	1	*64% total body surface burn, inhalation injury

*Autopsy not performed

Table 7. Cause of Death, 1983 - Continued

Patient	Age	Sex	% Burn Total	% Burn 30	PEB Death	Cause of Death
18	38	M	58	53.5	11	*58% total body surface burn, inhalation injury, pneumonia and septicemia
19	9/12	M	54.5	54.5	24	*54.5% total body surface burn, pneumonia Staphylococcus aureus
20	22	M	51	47	1	*51% total body surface burn and inhalation injury
21	64	M	45	45	1	*45% total body surface burn, inhalation injury and acute renal failure
22	93	F	40.5	1.5	22	*40.5% total body surface burn, pneumonia
23	84	F	40.5	18.5	30	*40.5% total body surface burn, inhalation injury and pneumonia and septicemia
24	85	F	38.5	36	0	38.5% total body surface burn, inhalation injury
25	2 9/12	M	34.5	34.5	1	34.5% total body surface burn and inhalation injury
26	22	M	33.5	25.5	38	33.5% total body surface burn, tracheo-esophageal fistula with aspiration
27	68	M	33	10.5	43	33% total body surface burn, cytomegalovirus viremia, fungal burn wound invasion

*Autopsy not performed

Table 7. Cause of Death, 1983 - Continued

Patient	Age	Sex	% Burn Total	30	PEB Death	Cause of Death
28	11 months	F	32	26	16	*32% total body surface burn and pneumonia
29	73	F	32	32	13	*32% total body surface burn, tetanus and wound infection
30	62	M	31	23.5	33	31% total body surface burn, inhalation injury, bronchopneumonia, burn wound infection
31	64	M	29.5	26.5	14	29.5% total body surface burn, Laennec's cirrhosis, with secondary upper gastrointestinal hemorrhage
32	69	M	27.5	7.5	2	27.5% total body surface burn, acute myocardial infarction
33	67	F	26.5	26.5	8	*26.5% total body surface burn, inhalation injury, pneumonia and septicemia
34	71	M	25	4.5	51	25% total body surface burn, massive cerebral vascular accident
35	55	M	21.5	20	106	21.5% total body surface burn, gram negative sepsis from genitourinary tract and burn wounds
36	61	M	21	9.5	55	*21% total body surface burn, pneumonia and hepatic failure secondary to chronic alcoholism

*Autopsy not performed

Table 7. Cause of Death, 1983 - Continued

Patient	Age	Sex	% Burn Total	30	PEB Death	Cause of Death
37	84	F	17	15	16	*17% total body surface burn, acute myocardial infarction
38	61	M	13	0.5	14	13% total body surface burn, inhalation injury, bronchopneumonia, embolic brain death
39	32	M	11	7	1	11% total body surface burn, cervical fracture and multiple injuries
40	29	M	6.5	1	8	6.5% total body surface electric injury, hypoxic encephalopathy
18						

*Autopsy not performed

1983

PRESENTATIONS:

Pruitt B A Jr: Pathophysiology of Thermal Injury,
University of Texas Health Science Center, San Antonio, TX
8 Jan 83

McManus WF: Treatment of Burns. Officers' Basic Course,
Academy of Health Sciences, Fort Sam Houston, TX 14 Jan 83

DeMouy D: Care of the Burn Patient. 91J Course, Academy of
Health Sciences, Fort Sam Houston, TX 17 Jan 83

Robertson KE: Current Management of Thermally Injured
Client, Registered Nurses Club, Fort Sam Houston, TX 18 Jan 83

Pruitt B A Jr: 1) The Burn Patient as a Universal Trauma
Model; 2) Current Methods of Topical Therapy and Burn Wound
Infection Surveillance. USAISR and USAMRDC International Burn
Research Conference, San Antonio, TX 19-21 Jan 83

McManus WF: Treatment of Burn Wound Infection, USAISR and
USAMRDC International Burn Research Conference, San Antonio, TX
19 Jan 83

McManus WF: Management of Burns, Battlefield Medicine
Course, School of Aerospace Medicine, Brooks AFB, TX 20 Jan 83

Maguire M: Body Fat Composition, BAMC Physical Therapists
and Dietitians, Fort Sam Houston, TX 21 Jan 83

Pruitt B A Jr: Epidemiology, Triage, and Pathophysiology of
Thermal Injury. OT/PT Management of Burns in the Theater of
Operations Course, Fort Sam Houston, TX 24 Jan 83

McManus WF: Thermal, Electrical, and Chemical Injuries.
OT/PT Management of Burns in the Theater of Operations Course,
Fort Sam Houston, TX 24 Jan 83

Heeter PA: The Role of Occupational Therapists in the
Management of the Thermally Injured Patient. 91L Students,
Academy of Health Sciences, Fort Sam Houston, TX 24 Jan 83

Robertson KE: Initial Management of Thermal Injuries,
2CF7, Academy of Health Sciences, Fort Sam Houston, TX 24 Jan 83

The following presentations were made at the OT/PT
Management of Burns in a Theater of Operations Course, Academy of
Health Sciences, Fort Sam Houston, TX 24-25 Jan 83:

Robertson KE: 1) Triage and Immediate Care of Thermal Injuries; 2) Initial Management of Thermal Injuries in a Combat Zone

Valdez JS : Infection Control (Thermally Injured)
Schlachta LM : Grafts, Donor Sites, and Dressings
Smith CA : Burn Flight Team

McManus WF: Pathophysiology and Surgical Management of the Burn Wound. OT/PT Management of Burns in Theater of Operations Short Course. Academy of Health Sciences, Fort Sam Houston, TX 31 Jan 83

Heeter PA: Principles of Splinting. OT/PT Management of Burns in Theater of Operations Short Course. Academy of Health Sciences, Fort Sam Houston, TX 31 Jan 83

Hoffman BE: Practical Application of Splinting Principles. OT/PT Management of Burns in Theater of Operations Short Course. Academy of Health Sciences, Fort Sam Houston, TX 31 Jan 83

Warner M: Traction Principles. 365As, 465Bs, 491Ls, US Army Institute of Surgical Research, Fort Sam Houston, TX 1 Feb 83

Cross PJ: Psychosocial Aspects of Burn Injury. OT/PT Management of Burns in a Theater of Operations Short Course, Academy of Health Sciences, Fort Sam Houston, TX 1 Feb 83

Robertson KE: Initial Management of Thermal Injuries. Army Nurse Corps Intensive Care Unit Course, BAMC, Fort Sam Houston, TX 2 Feb 83

Maguire M: The Management of the Thermally Injured Patient in the Theater of Operations. Occupational Therapists and Physical Therapists from CONUS, Academy of Health Sciences, Fort Sam Houston, TX 2 Feb 83

Robertson KE: Complications of Thermal Injuries. Army Nurse Corps Intensive Care Unit Course, BAMC, Fort Sam Houston, TX 3 Feb 83

Pruitt BA Jr: Burn Injury as a Military Problem. Uniformed Services University of the Health Sciences, Bethesda, MD 4 Feb 83

Maguire M: Body Composition Determination. AMSC Educational Technology Course, Academy of Health Sciences, Fort Sam Houston, TX 4 Feb 83

McManus WF: Metabolic Care of the Thermally Injured. University of Tennessee School of Medicine, Knoxville, TN 7 Feb 83

McManus WF: Care of Thermal, Chemical and Electric Injuries. Surgical Society meeting, Knoxville, TN 7 Feb 83

Pruitt B A Jr: Discussion of paper No. 8, Society of University Surgeons' meeting, Oklahoma City, OK, 9-12 Feb 83

Schlachta LM: Wound Care of Thermal Injuries. Army Nurse Corps Intensive Care Unit Course, Fort Sam Houston, TX 10 Feb 83

Robertson KE: 1) Initial Management of Thermal Injuries; 2) Wound Care of Thermal Injuries, 111th Medical Battalion, Texas Army National Guard, Fort Sam Houston, TX 12 Feb 83

Aitcheson AR: Management of Thermal Injuries. To 40-50 Jordanian Army nurses, practical nurses and civilian nurses, Jordan 12-24 Feb 83

Robertson KE: Comprehensive Review of Thermal Injury Care. Baptist Memorial Hospital School of Nursing, San Antonio, TX 15 Feb 83

Cross PJ: Psychosocial Aspects of Burn Injury. Baptist Memorial Hospital School of Nursing, San Antonio, TX 15 Feb 83

Maguire M: Exercise Prescription, BAMC Physical Medicine and Rehabilitation staff, Fort Sam Houston, TX 22 Feb 83

Maguire M: Physical Therapy and the Burn Patient. Intensive Care Unit nurses, Academy of Health Sciences, Fort Sam Houston, TX 23 Feb 83

Heeter PA: OT Management of the Thermally Injured Patient. ICU Nursing Course. BAMC, Fort Sam Houston, TX 25 Feb 83

Robertson KE: Comprehensive Review of Thermal Injury Care. University of Texas at San Antonio Graduate Nursing Students, San Antonio, TX 25 Feb 83

Maguire M: O3C Course. AMSC Planning Conference, Fort Sam Houston, TX 28 Feb 83

Heeter PA: OT Role in the Care of the Thermally Injured Patient. Reserve Component Meeting, San Antonio, TX 4 Mar 83

Maguire M: Thermal Injury Care. USAR personnel, Fort Sam Houston, TX 4 Mar 83

Pruitt BA Jr: Pseudomonas aeruginosa as a Surgical Pathogen; in symposium entitled Pseudomonas aeruginosa Infections. American Society for Microbiology, New Orleans, LA, 6 Mar 83

Pruitt BA Jr: Effect of Resuscitation Fluid Composition on Hemodynamic and Pulmonary Function. Burn Seminar, LSU Medical Center, New Orleans, LA 16 Mar 83

Pruitt BA Jr: 1) Symposium panelist - Bacterial Isolation: Pros and Cons; Invited discussant of 1) Changes in Free and Total Levels of Plasma Cortisol and Thyroxine Following Thermal Injury in Man by Calvano, et al; 2) Non-invasive Detection of Upper Airway Obstruction in Burn Patients by Haponik, et al. American Burn Association, New Orleans, LA 16-19 Mar 83

Schlachta LM: Initial Management of Thermal Injuries. 139th Aeromedical Evacuation Unit of the New York National Guard, Fort Sam Houston, TX 20 Mar 83

Robertson KE: Initial Management of Thermal Injuries. C-22 Army Nurse Course, Academy of Health Sciences, Fort Sam Houston, TX 21 Mar 83

McManus WF: Traumatic Injury. Clinical Pastoral Education In-Service, Academy of Health Sciences, Fort Sam Houston, TX 25 Mar 83

Stallings RJ: Burns. Officers' Basic Course, Academy of Health Sciences, Fort Sam Houston, TX 25 Mar 83

Cross PJ: Communication Skills for Preceptorship. Government Employees Credit Union, Fort Sam Houston, TX 26 Mar 83

Pruitt BA Jr: Acute Care of Minor and Severe Burns. Family Practice Certification Review, University of Texas Health Science Center at San Antonio, TX 8 Apr 83

Robertson KE: Orientation to ISR. New Army Nurse Corps, BAMC, Fort Sam Houston, TX 8 Apr 83

Pruitt BA Jr: Diagnosis and Treatment of Inhalation Injury. Trauma Day, General Surgery Service, BAMC, Fort Sam Houston, TX 9 Apr 83

Pruitt BA Jr: ISR Combat Casualty Care Program Review. Joint Technology Coordinating Group for Combat Casualty Care, Bethesda, MD 11 Apr 83

Heeter PA: Splinting and Positioning of Thermally Injured Patients. Hand Rehabilitation Special Interest Group, San Antonio, TX 12 Apr 83

McManus WF: Treatment of Burns. Battlefield Medicine Course, School of Aerospace Medicine, Brooks AFB, TX 14 Apr 83

Robertson KE: Management of the Burn Patient in the OR.
BAMC OR students, Fort Sam Houston, TX 14 Apr 83

Pruitt BA Jr: Early Diagnosis of Sepsis in Injured Man.
Trauma Symposium, Brown University, Providence, RI 16 Apr 83

Robertson KE: Initial Management of Thermal Injuries and
Definitive Wound Care. East Tennessee State University,
Cookeville, Tennessee, on behalf of the Recruiting Command,
17-19 Apr 83

Pruitt BA Jr: Acute Burn Care. ATLS Provider Course,
University of Texas Health Science Center at San Antonio, TX
24 Apr 83

Robertson KE: 1) Treatment of Thermal Injuries - The ISR Way,
2) Fluid Resuscitation of Thermal Injuries. Emergency Department
Nurses Association Convention, San Antonio, TX 25 Apr 83

Schlachta LM: Management of Thermal Injuries. 13th
Evacuation National Guard, San Antonio, TX 26 Apr 83

Robertson KE: Initial Management of Thermal Injuries, Wound
Care, Psychosocial Concerns. Jamestown College, Jamestown, North
Dakota, on behalf of Recruiting Command 1-3 May 83

Schlachta LM: Initial Management of Thermal Injuries. 2CF7
Aviators, Academy of Health Sciences, Fort Sam Houston, TX
3 May 83

Pruitt BA Jr: Discussant of paper: The Effect of Plasma
Exchange on Postburn Lymphocyte Suppression, Surgical Infection
Society Annual Meeting, Fort Lauderdale, FL 9-11 May 83

Robertson KE: Initial Management and Air Evacuation of
Thermal Injuries. Battlefield Nursing Course, Brooks AFB, San
Antonio, TX 11 May 83

Robertson KE: Introduction to ISR and Burn Nursing. BAMC
NETS Orientees, Fort Sam Houston, TX 13 May 83

Robertson KE: Initial Management and Pulmonary Complications
of Thermal Injury. American Association of Critical Care Nurses,
San Antonio, TX 17 May 83

DeMouy D: Physical Therapy in Burn Care. 91J Course,
Academy of Health Sciences, Fort Sam Houston, TX 17 May 83

McManus WF: Inhalation Injury in Fire Disasters. National
Fire Prevention Association, Kansas City, MO 17 May 83

Pruitt BA Jr: Burn Victim Management. Disaster Planning: Mass Casualties, Southwest Regional Medical Education Center, Long Beach, CA, 18 May 83

The following presentations were made at the 2nd Annual ISR Nursing Service Symposium, San Antonio, TX 18-20 May 83:

Pruitt BA Jr: Burn Wound Sepsis: Diagnosis and Medical Management

Robertson KE: Thermal, Chemical and Electrical Injury

Crawford JL: Care of the Pediatric Burn Patient

Mechanic HF: Infection Control

Smith CA: ISR Flight Team

Robertson KE: Gastrointestinal Complications

Schlachta LM: Wound Care, Grafts, Donors and Dressings

Cross PJ: 1) Psychosocial Aspects: "I Guess Your Mind Gets Burned Too"; 2) Moderator, Panel Discussion: Ex-Burn Patients

Peters GS: DIC and the Burn Patient

Mechanic HF: Current Nursing Research

Miller C : Resocialization and Discharge Planning

Aitcheson AR: A Day in the Life of a Burn Nurse: A Pictorial

Pruitt BA Jr: Briefing on Clinical and Research Activities of USAISR. NATO Surgeons General, Fort Sam Houston, TX 20 May 83

DeMouy D: Early Physical Therapy Intervention and Mobilization of the Thermally Injured Patient. National Association of Rehabilitation Nurses Symposium 19 May 83

Valdez JS: 6F-66E Definitive Wound Closure. Operating Room Nurse Course, BAMC, Fort Sam Houston, TX 8 Jun 83

Pruitt BA Jr: Exposure to Some of the More Common Noxious Gases, and Burn Triage and Care. Mass Casualties from Man Made Disasters, New York Medical College, Valhall, NY, 10 Jun 83

Robertson KE: Initial Management and Transport of Thermal Injuries. 1CF7 Aviators, Academy of Health Sciences, Fort Sam Houston, TX 14 Jun 83

Robertson KE: Initial Management and Transport of Thermal Injuries. Air Force Battlefield Nursing Course, Brooks AFB, San Antonio, TX 29 Jun 83

McManus WF: Traumatic Injury. Clinical Pastoral Education In-Service, Academy of Health Sciences, Fort Sam Houston, TX 28 Jun 83

Robertson KE: Initial Management and Wound Care of Thermal Injuries. Baylor Physical Therapy students, Academy of Health Sciences, Fort Sam Houston, TX 30 Jun 83

Robertson KE: Initial Management of Thermal Injuries, UPSP students, Academy of Health Sciences, Fort Sam Houston, TX 30 Jun 83

Robertson KE: Initial Management of Thermal Injuries. 475th CSH Kentucky Army National Guard, Academy of Health Sciences, 30 Jun 83

DeMouy D: Physical Therapy in Burn Care. 65B Course, Academy of Health Sciences, Fort Sam Houston, TX 30 Jun 83

Robertson KE: Initial Management, Air Evacuation and Wound Care of Thermal Injuries. 34th Aeromedical Evacuation Squadron (Reserve), Kelly AFB, San Antonio, TX 9 Jul 83

Robertson KE: Initial Management and Air Evacuation of Thermal Injuries. 94th General Hospital (Reserve), BAMC, Fort Sam Houston, TX 20 Jul 83

Robertson KE: Initial Management and Air Evacuation of Thermal Injuries. Physicians Assistants, National Guard, Academy of Health Sciences, Fort Sam Houston, TX 21 Jul 83

Robertson KE: Initial Management and Air Evacuation of Thermal Injuries. Combined USAF and University of Texas Trauma Disaster Symposium, University of Texas School of Nursing at Austin, TX 21 Jul 83

Pruitt BA Jr: Opportunistic Infections in Injured Man. Uniformed Services University of the Health Sciences, Bethesda, MD 29 Jul 83

Pruitt BA Jr: Moderator of workshop on Stress Ulcers. National Symposium on the Health Professional's Role in Medical Readiness, Monterey, CA 30-31 Jul 83

Robertson KE: Initial Management of Thermal Injuries. Intensive Care Unit Course, BAMC, Fort Sam Houston, TX 1 Aug 83

Robertson KE: Initial Management and Air Evacuation of Thermal Injuries. Seton Medical Center Austin, TX 2 Aug 83

Robertson KE: Initial Management of Thermal Injuries. Katy Medical Explorer Scouts, ISR, Fort Sam Houston, TX 5 Aug 83

Robertson KE: Initial Management and Air Evacuation of Thermal Injuries. USAF Battlefield Nursing Course, Brooks AFB, TX 9 Aug 83

Robertson KE: Wound Care and Complications of Thermal Injuries. Intensive Care Unit Course, BAMC, Fort Sam Houston, TX 12 Aug 83

Robertson KE: Overview of ISR and Burn Nursing, Prevention of Thermal Injuries. Canyon Lake Lions Club, Canyon Lake, TX 18 Aug 83

Heeter PA: Role of Occupational Therapy in the Treatment of Thermally Injured Patients. ICU Nursing Course, BAMC, Fort Sam Houston, TX 22 Aug 83

Bush K: Physical Therapy in Burn Care. Intensive Care Unit Nursing Course, Academy of Health Sciences, Fort Sam Houston, TX 22 Aug 83

McManus WF: Initial Management of Burns. Wilford Hall Medical Center, Lackland AFB, San Antonio, TX 23 Aug 83

The following presentations were made at the 807th Medical Bde, US Army Reserve, Shreveport, LA 24 Aug 83:

Robertson KE: 1) Initial Management, Early Complications, and Air Evacuation of Thermal Injuries; 2) Cold Injuries

Cross PJ: 1) Psychiatric Care of Thermal; 2) Psychiatric Emergencies; 3) Deployment

Pruitt BA Jr: Behind Armor - Burn and Blast Injuries. Common Problems in the Treatment of the Wounded Soldier Workshop, Munster, West Germany 26-27 Aug 83

Heeter PA: Role of Occupational Therapists in the Management of the Thermally Injured Patient. 91L Students, Academy of Health Sciences, Fort Sam Houston, TX 1 Sep 83

Robertson KE: Initial Management and Air Evacuation of Thermal Injuries. BAMC RN Orientation to ISR, Fort Sam Houston, TX 7 Sep 83

Pruitt BA Jr: The Metabolic Problems of the Burn Patient. Significant Surgical Pathophysiologic Problems in the Severely Traumatized Patient - Symposium honoring Dr. Liljedahl Linkoping, Sweden 9-10 Sept 83

Robertson KE: Initial Management and Air Evacuation of Thermal Injuries. American Association of Critical Care Nurses, Austin, TX 13 Sep 83

Robertson KE: Initial Management and Air Evacuation of Thermal Injuries. 91C School, Fort Sam Houston, TX 19 Sep 83

The following presentations were made to the USAF Reserve, Charleston AFB, at ISR, Fort Sam Houston, TX 20-22 Sep 83:

Robertson KE: Initial Management and Air Evacuation of Thermal Injuries

Robertson KE: Wound Care and Complications of Thermal Injuries

Cross PJ: Psychosocial Concerns of Burn Patients' Families and Staff

Pruitt BA Jr: Opportunistic Infections in Severely Burned Patients. Intravenous Gamma Globulin and the Compromised Host - International Symposium, Dallas, TX 21-23 Sep 83

McManus WF: Where are the Frontiers in the Understanding of Burn Injuries? National Institutes of Health, Bethesda, MD 26-28 Sep 83

McManus WF: Is There a Role for Plasmapheresis/exchange Transfusion in the Treatment of the Burn Patient? Frontiers in Understanding Burn Injury, National Institutes of General Medical Sciences, National Institutes of Health, Bethesda, MD 27 Sep 83

Pruitt BA Jr: Forces and Factors Influencing Trauma Care. Presidential Address, Annual meeting of the American Association for the Surgery of Trauma, Chicago, IL 29 Sep 83

Pruitt BA Jr: The Regional Burn Center and Advances in Burn Care. Burn Center Dedication, Oklahoma City, OK 4 Oct 83

McManus WF: Emergency Care of Thermal, Chemical, and Electric Injuries. Regional EMS Meeting, Sequin, TX 6 Oct 83

Pruitt BA Jr: Care of the Extensively Burned Patient. Department of Surgery, University of Texas Health Science Center, San Antonio, TX, 7 Oct 83

Robertson KE: Emergency Management of Thermal Injuries. University of Texas Undergraduate Nurses, University of Texas at San Antonio, TX 7 Oct 83

Robertson KE: Emergency Management of Thermal Injuries. Visiting Egyptian Military Nurses from Wilford Hall Medical Center, ISR, Fort Sam Houston, TX 11 Oct 83

Pruitt BA Jr: Postburn Impairment of Neutrophil Function. International Surgical Group, Nashville, TN 14 Oct 83

Pruitt BA Jr: Film narration: Burn Wound Management;
Invited discussant: Cine Clinic Session - The Early Surgical
Management of the Major Burn; Discussant of four papers at
Surgical Forum session on "Endocrinology and Tissue Repair".
Clinical Congress of the American College of Surgeons,
Atlanta, GA 16 Oct 83

Robertson KE: Emergency Management of Thermal Injuries.
2CF-7 Class, Aviators, Academy of Health Sciences, Fort Sam
Houston, TX 25 Oct 83

Pruitt BA Jr: Burn Patient Rehabilitation. Department of
Physical Medicine and Rehabilitation, University of Texas Health
Science Center, San Antonio, TX 26 Oct 83

The following presentations were made to the Scientific
Program of the 1983 Annual Meeting of the Association of Life
Insurance Medical Directors of America, San Antonio, TX 2 Nov 83:

Pruitt BA Jr: Panel: 1) Burn Injury Triage and Transportation
of Burn Patients; 2) Burn Prevention

McManus WF: The Hospital Care of Burn Patients

Hoffman BE: Role of Occupational Therapists in the Treatment
of Thermally Injured Patients

Bush K: Physical Therapy in the Treatment of Burns.

Cross PJ: Psychosocial Aspects of Thermal Injuries.
Introduction to Hospital Ministry and Pastoral Care Course, BAMC,
Fort Sam Houston, TX 2 Nov 83

Robertson KE: Initial Management of Thermal Injuries.
Clinical Pastoral Education Group, BAMC, Fort Sam Houston, TX
2 Nov 83

Hoffman BE: Splinting Theory and Techniques. 91L
Advanced Course, BAMC, Fort Sam Houston, TX 28 Oct 83, 1 Nov 83,
and 3 Nov 83

Robertson KE: Initial Management of Thermal Injuries.
Physician's Assistants' Course, Academy of Health Sciences, Fort
Sam Houston, TX 4 Nov 83

Cross PJ: Psychosocial Aspects of Thermal Injuries.
Chaplains assigned to Garrison and Tenant Units, Fort Sam
Houston, TX 10 Nov 83

Pruitt BA Jr: 1) Current Management of the Severely Burned
Patient; 2) Panel - The Hyperdynamic Septic State, 11th Annual
Meeting of the Japanese Association for Acute Medicine, Osaka,
Japan, 9-11 Nov 83

Pruitt BA Jr: Unsolved Problems in Burn Care, Nippon Medical School, Department of Surgery, Tokyo, Japan 9-11 Nov 83

Robertson KE: Initial Management of Thermal Injuries, Inhalation Injury and Transportation of Thermal Injuries, Kelly AFB Emergency and Clinic Staff, San Antonio, TX 18 Nov 83

Robertson KE: Thermal, Chemical and Electrical Injury; Inhalation Injury and Pulmonary Complications; Wound Care; Physical and Occupational Therapy; Aeromedical Evacuation of the Thermally Injured Patient. 57th AES Scott AFB, IL 20-22 Nov 83

Cross PJ: Psychosocial Aspects of Thermal Injuries. Continuing Education Seminar entitled "Care of the Thermally Injured Patient", Scott AFB, IL 21 Nov 83

Hoffman BE: Hand Injuries: Splinting Theory and Principles. 65Bs, 91Js, BAMC, Fort Sam Houston, TX 29 Nov 83

The following presentations were made at the Operating Room Nursing Course, BAMC, Fort Sam Houston, TX 2 Dec 83:

Robertson KE: Initial Management and Air Evacuation of Thermal Injuries

Valdez JS: Operative Interventions in the Thermally Injured

Pruitt BA Jr: Current Management of Severely Burned Patients. Grady Hospital Grand Rounds, Emory University School of Medicine, Atlanta, GA 3 Dec 83

Schlachta LM: Initial Management and Air Evacuation of Thermal Injuries. 2CF7 Course, Academy of Health Sciences, Fort Sam Houston, TX 6 Dec 83

Robertson KE: Initial Management, Inhalation Injury, Air Evacuation, Wound Therapy and Psycho-Social Aspects of Thermal Injuries. University of North Carolina at Charlotte on behalf of Recruiting Command 6 Dec 83

Hoffman BE: Role of Occupational Therapists in the Management of Thermally Injured Patients. 91L Students, Academy of Health Sciences, Fort Sam Houston, Tx 7 Dec 83

Robertson KE: Orientation to ISR and Burn Nursing. BAMC newly assigned RNs, Fort Sam Houston, TX 7 Dec 83

Pruitt BA Jr: Metabolic Responses and Nutritional Support of Burn Patients. International Society for Burn Injuries Annual Burn Seminar, Denver, CO 8-10 Dec 83

Robertson KE: Initial Management, Inhalation Injury and Air
Evacuation of Thermal Injuries, BAMC Short Intensive Care Unit
Cours for RNs, Fort Sam Houston, TX 9 Dec 83

PUBLICATIONS

McLaurin NK, Goodwin CW Jr, Zitzka CA, and Hander EW: Computer-generated graphic evaluation of nutritional status in critically injured patients. JADA 82(1): 49-52, Jan 1983

McManus WF, Goodwin CW Jr, and Pruitt BA Jr: Subeschar antibiotic treatment of burn wound infection. Arch Surg 118: 291-294, Mar 1983

Shirani KZ, Vaughan GM, Robertson GL, Pruitt BA Jr, McManus WF, and Mason AD Jr: Inappropriate vasopressin secretion in burn patients. J Trauma 23(3): 217-224, Mar 1983

Pruitt BA Jr and Goodwin CW Jr: Current Treatment of the Extensively Burned Patient. In Nyhus LM (ed) Surgery Annual 1983. Appleton-Century-Crofts, E Norwalk, CT, Chapter 17, pp 331-364, 1983

Goodwin CW Jr, Dorethy J, Lam V, and Pruitt BA Jr: Randomized trial of efficacy of crystalloid and colloid resuscitation on hemodynamic response and lung water following thermal injury. Ann Surg 197: 520-531, May 1983

Vaughan GM and Taylor TJ: Cushing's Syndrome. In Conn, HF (ed) Current Therapy, WB Saunders Company, Philadelphia, 1983

Goodwin CW Jr and Wilmore DW: Enteral and parenteral nutrition. In Paige, et al (eds) Manual of Clinical Nutrition. Nutrition Publications, Inc., Washington, Chapter 31, pp 1-39, 1983

Aulick LH and Wilmore DW: Thermoregulatory responses and metabolism. In Simmons RL and Howard RJ (eds) Infectious Diseases. Chapman and Hall, New York, 1983

Wilmore DW, Aulick LH, and Becker RA: Hormones and the control of metabolism. In Fisher JE (ed) Surgical Nutrition. Little, Brown and Co, Boston, 1983

Aulick LH and Wilmore DW: Hypermetabolism in trauma. In Girardier L and Stock MJ, eds) Mammalian Thermogenesis. Chapman and Hall, New York, 1983

Strome DR, Newman JJ, Goodwin CW Jr, Mason AD Jr, and Pruitt BA Jr: Mechanism of reduced lipolytic response in rat adipocytes following thermal injury. Surg Forum XXXIV: 102-106, 1983

Becker RA, Vaughan GHM, Goodwin CW Jr, Ziegler MG, Zitzka CA, Mason AD Jr and Pruitt BA Jr: Interactions of thyroid hormones and catecholamines in severely burned patients. Rev Inf Dis 5: S908-S913, Nov-Dec 83

Goodwin CW Jr, Maguire MS, McManus WF, and Pruitt BA Jr: Prospective study of burn wound excision of the hands. J Trauma 23: 510-517, June 1983

McManus WF: Burn mass casualty management: Lessons learned. Disaster Med 1: 2930, 1983

McManus WF: Prevention and treatment of burn wound sepsis. ASEPSIS 5: 21-22, 1983

Pruitt BA Jr, Lindberg RB, McManus WF, and Mason AD Jr: Current approach to prevention and treatment of Pseudomonas aeruginosa infections in burned, traumatized, and surgical patients. Symposium on Pseudomonas Aeruginosa Infections. Rev Infect Dis 5(5):S889-S897, Nov-Dec 1983

Pruitt BA Jr and Goodwin CW Jr: Nutritional Management of the seriously ill burned patient. In Nutritional Support of the Seriously Ill Patient, Chapter 6, pp 63-84, 1983

McManus WF: Subeschar antibiotic infusion in the treatment of burn wound infection. Proceedings of the International Burn Research Conference, pp 58-61, 1983

Newman JJ, Strome DR, Goodwin CW Jr, Mason AD Jr, and Pruitt BA Jr: Neutral proteinase activity in skeletal muscle from thermally injured rats. J Surg Res 35(6) 515-519, Dec 1983

ANNUAL PROGRESS REPORT

PROJECT NO. 3S162772A874-00, APPLIED RESEARCH

REPORT TITLE: CLINICAL OPERATION, CENTER FOR TREATMENT OF
BURNED SOLDIERS--ANESTHESIOLOGY

US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234

1 January 1983 - 31 December 1983

Investigators:

Anton J. Jirka, MD, MPH, Colonel, MC
Steven I. Schmidt, MD, CPT, MC

Reports Control Symbol MEDDH-288(R1)

Unclassified

ABSTRACT

PROJECT NO. 3S162772A874-00, APPLIED RESEARCH

REPORT TITLE: CLINICAL OPERATION, CENTER FOR TREATMENT OF
BURNED SOLDIERS--ANESTHESIOLOGY

US Army Institute of Surgical Research, Brooke Army Medical
Center, Fort Sam Houston, Texas 78234

Period covered in this report: 1 January 1983 - 31 December 1983

Investigators: Anton J. Jirka, MD, MPH, Colonel, MC
Steven I. Schmidt, MD, Captain, MC

Reports Control Symbol MEDDH-288(R1)

In the period covered in this report, 291 anesthetics were administered to 98 patients, an average of 2.97 anesthetics per patient. The most commonly used anesthetic agent was Enflurane (63.23%), followed by ketamine (22.68%), and nitrous oxide (7.56%). Due to the nature and combinations of procedures now performed, regional anesthesia is not used.

Anesthesia

ANESTHESIOLOGY

PREOPERATIVE EVALUATION

Most burn patients are several days postinjury when first seen by the anesthesiologist. In the immediate postburn period, the time is used to gain abundant physiologic data from routine monitoring of various indices: hematologic (hematocrit, electrolytes, liver and renal function tests), pulmonary (arterial blood gases, respiratory rate, daily chest roentgenograms), cardiovascular (blood pressure, central venous pressure, cardiac output measured by use of Swan-Ganz catheters), and renal (urine output, urine chemistry), in addition to the usual preoperative patient interview and physical examination.

All patients, regardless of age, who have electrical injuries have a preoperative electrocardiogram performed to rule out possible myocardial damage.

PREOPERATIVE PREPARATION

All patients are kept NPO after 2400 the day prior to surgery with the exception of children, who may receive clear liquids up to five hours prior to surgery.

Due to extraordinary fluid requirements in most burned patients, an intravenous infusion, if not already in place, is begun the evening prior to surgery.

PREMEDICATION

Glycopyrrolate (Robinul^R) 0.005 mg/kg to a maximum dose of .4 mg, is given intramuscularly as premedication 30 minutes prior to anesthesia. Narcotic premedication is no longer routinely used.

FLUIDS

All fluids except hyperalimentation solutions are changed to D₅RL or RL on arrival in the operating room. Hyperalimentation solutions are continued throughout operative procedures.

TYPES OF ANESTHESIA

The pattern of anesthetic administration has changed from previous years and involves a greater use of enflurane and

ketamine and a lesser use of halothane and regional anesthesia. The reasons for this change will be discussed under individual agent headings.) (Table 1)

TABLE 1. PRIMARY AGENTS

<u>AGENT</u>	<u>1980</u>		<u>1981</u>		<u>1982</u>		<u>1983</u>	
	NUMBER	%	NUMBER	%	NUMBER	%	NUMBER	%
ENFLURANE	252	47.46	252	62.38	335	62.97	184	63.23
KETAMINE	183	34.46	104	25.74	169	31.77	66	22.68
HALOTHANE	10	1.88	16	3.96	1	0.19	0	0
N ₂ O	71	13.37	20	4.95	8	1.50	22	7.56
LOCAL	15	2.82	10	2.48	15	2.81	13	4.47
OTHER	0	0	2	0.49	4	0.75	6	2

1. Enflurane (Ethrane^R)

Enflurane is a halogenated ether which has been commercially available for approximately the past eight years. It provides rapid induction and good muscle relaxation. Biotransformation amounts to less than 2% of an inhaled dose, which perhaps accounts for the few clinical toxic effects observed. Plasma fluoride levels in hypermetabolic burn patients during and after Enflurane administration have been measured and found not to be in the toxic range. Enflurane is presently the most commonly used anesthetic agent at the USAISR.

2. Halothane^R (Fluothane)

The use of halothane is avoided mostly for less than rational reasons related to descriptions of probable hepatotoxicity (incidence 0.7 per 1000) in the literature. Previous studies at the Institute of Surgical Research show its repeated use to be safe in the thermally injured patient, and the National Halothane Study showed halothane to be the anesthetic with the best overall mortality rate. It is a smooth anesthetic, unsurpassed as an agent for pediatric patients. This anesthetic is useful in asthmatics, patients with digitalis toxicity, and children. Its use has decreased as we favor ketamine in the young age group.

3. Nitrous Oxide

This agent is used in concentrations of 50% or 60% with oxygen. It is used mainly in conjunction with other analgesic or anesthetic agents. Succinylcholine has not been used for any purpose in this unit for more than eight years.

4. Ketamine

This agent is used both IM and IV to produce its characteristic dissociative state, with preservation of basal functions and laryngeal reflexes plus stimulation of the cardiovascular system.

Unfortunately, ketamine shares with its parent compound, phenycyclidine, the production of a high incidence of unpleasant hallucinogenic side effects. There seems to have been a "batch" difference in ketamine, and that possessed by ISR in the past had an almost 100% incidence of these effects. New methods of administering the drug, as well as various methods of premedication and patient preparation, appear to have reduced the unpleasant emergence reactions to a level where they are of little consideration in the well selected patient. Laryngospasm, airway obstruction and regurgitation can occur with ketamine. Pronounced blepharospasm prevents its use in eye cases. All ketamine anesthetics, other than in children, are preceded by IV diazepam (0.15-0.2 mg/kg).

5. Subanesthetic Ketamine

Subanesthetic ketamine (single dose 1.5-2 mg/kg IM) has not been used during this reporting period except for dressing changes where it is the anesthetic of choice. Tolerance to ketamine has been noted in several patients after repeated (greater than five) ketamine anesthetics. Ketamine is no longer used for Hubbard tank procedures. Although of limited value, sedation and narcotic analgesia, administered under direction of the surgical staff, have replaced ketamine for this use.

6. Regional Anesthesia

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

MONITORING TECHNIQUES

A. CIRCULATION

1. Precordial and/or esophageal stethoscope
2. Peripheral pulse
3. Blood pressure. Direct arterial lines have been used when necessary. The Dinamap^R blood pressure instrument is routinely used for intraoperative blood pressure monitoring. Since it can be used over dressings and is noninvasive, it is a most practical method of monitoring blood pressure in our patient population. Usually blood pressure is monitored on two sites.
4. CVP
5. Swan Ganz catheter
6. ECG
7. Urine output

B. RESPIRATION

1. Rate
2. Auscultation
3. Arterial blood gases

C. TEMPERATURE

In most cases a temperature monitor is employed. Because of the greatly increased evaporative heat losses in burn patients, hypothermia is a serious problem. Several methods are employed to maintain body temperature during anesthesia:

1. Ambient temperature is maintained at 82-87°F. This is probably the most important method to reduce heat loss.
2. The anesthetic gases may be heated and humidified.
3. A circle system which allows partial rebreathing of warm expired gases may be used to minimize heat loss. A Bain Circuite which achieves the same purpose is used in children.
4. Radiant heat lamps

5. The K-thermia heating blanket can also be used. It is probably used most effectively on children weighing less than 10 kg and for cooling febrile patients.

COMPLICATIONS

There were no intraoperative complications during 1983.

PATIENT DATA AND OPERATIVE PROCEDURES

The following two tables illustrate overall anesthetic patient data for the years 1970 through 1983 (Table 2) and recent trends in operative procedures (Table 3).

TABLE 2. OVERALL PATIENT DATA, USAISR (1970-1983)

Year	No. of Patients	No. Patients Anesthetized (ISR Only)	Percent of Patients Anesthetized	Total Anesthetics Given at ISR	Average Anesthetics Per Patients Anesthetized
1970	321	198	61.7	497	2.51
1971	301	179	59.5	475	2.65
1972	301	183	60.8	575	3.14
1973	273	141	51.6	377	2.67
1974	226	123	54.4	380	3.09
1975	254	142	55.9	490	3.45
1976	277	139	50.2	476	3.43
1977	242	129	53.3	344	2.67
1978	268	151	56.3	435	2.88
1979	267	161	60.3	554	3.44
1980	243	148	60.91	531	3.59
1981	208	127	61.06	404	3.18
1982	231	151	65.37	532	3.52
1983	179	98	54.75	291	2.97

TABLE 3. NATURE OF SURGERY, USALSR

PROCEDURE	1978		1979		1980		1981		1982		1983	
	NUMBER	%	NUMBER	%	NUMBER	%	NUMBER	%	NUMBER	%	NUMBER	%
	PROCEDURES		PROCEDURES		PROCEDURES		PROCEDURES		PROCEDURES		PROCEDURES	
EXCISION	90	19.3	212	30.15	269	37.36	212	36.68	257	33.29	196	42.0
AUTOGRAFT	269	59.9	372	52.91	318	44.17	293	50.69	405	52.46	203	43.6
ORTHOPEDIC	33	7.1	34	4.84	38	5.28	23	3.98	31	4.01	22	4.7
CHONDRECTOMY	4	0.9	1	0.14	4	0.56	3	0.52	0	0	2	0.4
EYE AND LID	6	1.3	21	2.99	17	2.36	3	0.52	14	1.81	8	1.7
INTRA-ABDOMINAL	6	1.3	8	1.13	1	0.14	1	0.17	6	0.78	2	0.4
PLASTIC	6	1.3	3	0.43	5	0.69	3	0.52	15	1.94	2	0.4
OTHER	50	10.8	52	7.39	68	9.44	40	6.92	44	5.70	31	6.7
TOTAL	464	100%	703	100%	720	100%	578	100%	772	100%	466	100%

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1 AGENCY ACCESSION	2 DATE OF SUMMARY	REPORT CONTROL SYMBOL	
				DA OG 6975	84 10 01	DD-DR&BIAR 636	
3. DATE PREV SUMMARY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISB N INSTR N	9. LEVEL OF SUM A WORK UNIT	
83 10 01	D. Change	U	U		CX		
10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER			
a. PRIMARY	62772A	3S162772A874	AF	161			
b. CONTRIBUTING							
c. CONTRIBUTING	STOG 82/83 - 6.2/4						
11. TITLE (Precede with Security Classification Code)							
(U) The Cardiopulmonary Response to Thermal Injury in Burned Soliders							
12. SUBJECT AREAS							
06 05 Clinical Medicine 0615							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING ORGANIZATION		16. PERFORMANCE METHOD	
76 10		CONT		DA		C. In-House	
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE			
a. DATE EFFECTIVE		EXPIRATION		FISCAL YEARS		a. PROFESSIONAL WORK YEARS b. FUNDS (In thousands)	
b. CONTRACT/GRANT NUMBER				84		2.0 130	
c. TYPE		d. AMOUNT		85		2.0 136	
e. KIND OF AWARD				f. CUM/TOTAL			
19. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
a. NAME				a. NAME			
US Army Institute of Surgical Research				US Army Institute of Surgical Research			
b. ADDRESS (include zip code)				b. ADDRESS			
Ft. Sam Houston, Texas 78234-6200				Ft. Sam Houston, Texas 78234-6200			
c. NAME OF RESPONSIBLE INDIVIDUAL				c. NAME OF PRINCIPAL INVESTIGATOR			
Pruitt, BA, Jr				Shirani, KZ			
d. TELEPHONE NUMBER (include area code)				d. TELEPHONE NUMBER (include area code)			
512-221-2720				512-221-4652			
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) Resuscitation Fluids; (U) Burn Injury; (U) Oleic Acid; (U) Cardiac Output; (U) Angiotensin; (U) Volunteers; (U) Lab Animals; (U) Sheep; (U) Goats; (U) Ram II							
23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)							
23. (U) To develop pharmacologic therapy for acute respiratory distress syndrome (ARDS) following combat injury. To define the mechanism of improvement of pulmonary function in ARDS by angiotensin II analogues.							
24. (U) Acute pulmonary edema and ARDS is induced by intravenous infusion of oleic acid. Animals are divided into control and treatment groups. The angiotensin II analogue, [Sar ¹ , Ile ⁸] AII is administered at three dosage regimens: 300 ng/kg/min, 600 ng/kg/min, and 2000 ng/kg/min in three separate groups of animals. Cardiac output, filling pressures, pulmonary mechanics and gas exchange are measured every 30 minutes for five hours.							
25. (U) 8310 - 8409. Improvement in the pulmonary edema, pulmonary function deterioration and systemic hypotension produced by oleic acid infusion occurred in animals given a continuous infusion of [Sar ¹ , Ile ⁸] AII at 2,000 ng/kg/min (515 animals), at 600 ng/kg/min (416 animals) and at 300 ng/kg/min (517 animals). In the drug treated animals, the beneficial effects on the pulmonary function were characterized by a higher Pa O ₂ , lower Qs/Qt and lower pulmonary resistance. Further studies on the efficacy of [Sar ¹ , Ile ⁸] in an experimental model of inhalation injury are currently in progress.							

FINAL REPORT

PROJECT NUMBER: 3S16277A874-00, APPLIED RESEARCH

PROJECT TITLE: THE CARDIOPULMONARY RESPONSE TO THERMAL INJURY IN
BURNED SOLDIERS: Effect of Plasmapheresis on the
Outcome of Critically Injured Burn Patients

US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234-6200

1 October 1983 - 30 September 1984

INVESTIGATORS

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

REPORT CONTROL SYMBOL - MEDDH-288 (R1)

UNCLASSIFIED

ABSTRACT

PROJECT NUMBER: 3S16277A874-00, APPLIED RESEARCH

PROJECT TITLE: THE CARDIOPULMONARY RESPONSE TO THERMAL INJURY IN
BURNED SOLDIERS: Effect of Plasmapheresis on the
Outcome of Critically Injured Burn Patients

US Army Institute of Surgical Research, Brooke Army Medical Center,
Fort Sam Houston, Texas 78234-6200

PERIOD COVERED IN THIS REPORT: 1 Oct 83 through 30 Sep 84

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

REPORT CONTROL SYMBOL: MEDDH-288 (R1)

Plasmapheresis was employed as an adjunct in the resuscitation of severely burned patients who were not responding to standard resuscitation techniques and for burned septic patients in an attempt to improve their hemodynamic response so that definitive treatment of the infection might be accomplished.

Plasmapheresis was no more effective than classic Evans or Brooke formulae utilizing five percent solution in the resuscitation of severely burned patients. Plasmapheresis did not influence the outcome in septic burned patients.

Plasmapheresis
Hemodynamics
Exchange Transfusion
Septicemia
Burn Shock

EFFECT OF PLASMAPHERESIS ON THE OUTCOME OF CRITICALLY INJURED BURN PATIENTS

Two of the major concerns in the care of the burn patient are resuscitation of the extensively burned patient and infection (1). Plasmapheresis has been used in a number of diseases such as systemic lupus erythematosus, severe rheumatoid arthritis, Waldenstrom's macroglobulinemia, and others. Potential complications of plasmapheresis include hepatitis, hypokalemia, transfusion reactions, hemolysis, and air embolism. In this descriptive and preliminary study, two questions were asked. Does plasmapheresis improve the survival of critically burned patients and, secondly, does plasmapheresis reduce the volume of resuscitation fluid required.

The protocol included two groups of patients. The first was the resuscitation group whose age was greater than 18 years, whose burn size exceeded 50 percent of the total body surface, and who, at 10 to 12 hours postinjury, were calculated to require in excess of six cubic centimeters/kilogram body weight/percent burn of resuscitation fluid for adequate resuscitation. The second group were patients generally beyond one week postburn who were undergoing progressive hemodynamic deterioration in the face of some form of invasive infection or sepsis.

The patient is clinically monitored during plasmapheresis by intake and output records, frequent measurement of vital signs, cardiac output, and other hemodynamic observations. In the laboratory, the routine preplasmapheresis and postplasmapheresis exchange included a chest x-ray, routine hematology, coagulation studies, platelet counts, SMAC-20, and repeated arterial blood gas determinations. These were the routine studies in addition to specific studies that may have been done on the effluent.

The first patient in the resuscitation group was a 30-year-old man with a 79.5 percent total body surface burn, all of which was full-thickness. He had a severe inhalation injury and had gross hemochromogens in the urine. At 12 hours postburn, it was found that the calculated volume of replacement exceeded six cubic centimeters/kilograms body weight/percent burn. So, on postburn day zero, plasmapheresis was started. Prior to plasmapheresis, his measured blood pressure was 96/69; following plasmapheresis, the blood pressure was 150/93. The pulmonary capillary wedge pressure rose from six to 14. His cardiac output doubled. His peripheral perfusion and all other clinical signs improved and, during the period of plasmapheresis, the hemochromogens in the urine cleared. On postburn day one, the plasmapheresis was re-

¹Warden GD, Stratta RJ, Saffle JR, Kravitz M, Ninnemann JL: Plasma exchange therapy in patients failing to resuscitate from burn shock. *J Trauma* 23:945-952, 1983.

peated and, at this point, his blood pressure remained stable throughout the procedure; pulmonary capillary wedge pressure, however, increased. Cardiac output remained roughly the same and his clinical condition was unchanged. With the third plasmapheresis on postburn day two, his blood pressure declined slightly. Capillary wedge pressure declined. Cardiac output actually increased and he was stable. This patient died on the thirty-second postburn day of severe bronchopneumonia and systemic sepsis.

The second patient was a 22-year-old man with a 51.25 percent total body surface burn, 47 percent being full-thickness. He had a severe inhalation injury and gross hemochromogens in his urine. His carboxyhemoglobin on admission, approximately a half-hour after the accident, was 40 percent. Prior to plasmapheresis, 12 hours postburn, he was requiring 10 cubic centimeters/kilograms body weight/percent burn for intravenous resuscitation. His carboxyhemoglobin had been reduced by volume ventilation on 100 percent oxygen to six percent. The first plasmapheresis was done on postburn day zero with a resultant increase in blood pressure. The pulmonary capillary wedge pressure rose and his cardiac output was unchanged. The hematocrit fell because of massive hemolysis from deep thermal injury and he required red cell transfusion. He was requiring inotropic support in the form of dopamine and actually required nine cubic centimeters/kilograms body weight/percent for resuscitation in the first 24 hours despite plasmapheresis. His hemochromogens were not cleared from his urine. The procedure was repeated on the second postburn day with resultant improvement in his blood pressure. The pulmonary capillary wedge pressure rose and the hemochromogens in the urine decreased. Following plasmapheresis, he progressed to anuria, required continued support with dopamine and epinephrine, and died of myocardial failure, presumably from severe carbon monoxide intoxication.

During this same period, a 21-year-old man with a 65 percent burn, who was not seen by a physician for over three hours, arrived at this Institute 11 hours postburn. At 12 hours postburn, he was calculated to be receiving in excess of six cubic centimeters/kilograms body weight/percent burn for resuscitation. He was given five percent albumin in physiologic saline. His urine output increased to a total of 1,600 cubic centimeters in the first 24 hours. His blood pressure rose and his pulse rate fell. Albumin was administered until there was a clinical response, which was a total of 150 grams. In retrospective calculation of his resuscitation utilizing the classic Brooke formula, it would have required 91 grams, and for the Evans formula, it would have required 182 grams of five percent albumin solution. He had very satisfactory resuscitation despite the fact that he was in severe trouble on admission.

In summary, in one of two patients, the hemochromogens were cleared; in the other, they were not. Hemolysis is variable, but

is readily replaced with red cell transfusion. Hemodynamic improvement can occur; however, the standard laboratory indices were not changed by plasmapheresis.

In the septic group, the first patient was a 37-year-old man with a 60 percent burn, 46 percent being full-thickness. On the eighth postburn day, he had histologically documented *Pseudomonas* burn wound invasion with positive *Pseudomonas* blood cultures. He was receiving dopamine, intravenous antibiotics, and appropriate topical chemotherapy. Plasmapheresis was begun. He had a modest decrease in his hyperkalemia as well as in his BUN, creatinine, and hematocrit. His arterial pH was minimally improved. His pulmonary capillary wedge pressure fell. His cardiac output blood pressure did not change during the plasmapheresis. The procedure was repeated on the following day and again the arterial pH rose minimally from 7.28 to 7.33. His blood pressure rose to 172/54 and inotropic support was discontinued. On the third plasmapheresis, the BUN fell from 42 to 22. He remained normotensive without inotropic support, his pulmonary capillary wedge pressure rose to 16, and the cardiac output rose to 15 liters. His PTT increased and he received fresh-frozen plasma. The patient appeared, at this point, to be hemodynamically stable. However, he again began to deteriorate hemodynamically on the fourteenth, fifteenth, and sixteenth postburn days and was again treated with three separate plasmapheresis. Hypotension developed prior to the first procedure and dopamine was restarted. No clinical improvement was identified with the second plasmapheresis and hypotension necessitated an increase in dopamine and intravenous fluid volume after the third procedure. His BUN continued to rise and he died of sepsis.

The second patient, a 48-year-old man with a 88 percent burn had a basically similar course. On the nineteenth postburn day, he developed *Pseudomonas* and fungal burn wound invasion with positive blood cultures for *Pseudomonas aeruginosa*. Plasmapheresis was done on three consecutive days. His arterial pH improved. His hematocrit fell and he required red cell transfusion. His platelet count fell and his clinical condition was unchanged following the first plasmapheresis. Following the second procedure, his arterial pH improved. His hematocrit and platelet count again fell, necessitating red cell transfusion. With the third procedure, his arterial pH was again improved; however, his BUN had increased from 25 to 35 milligrams percent. The platelet count again fell. The patient appeared to be hemodynamically stable. However, he again deteriorated on the twenty-fifth postburn day. On the twenty-seventh postburn day, he again underwent plasmapheresis for hemodynamic instability and required inotropic support but did not improve and died of sepsis.

In these two septic patients, there was a modest arterial pH effect and a renal effect which may have been secondary to the volume effect of the administered plasma. Hemolysis was variable

and readily replaced with red cell transfusion. Clotting factor changes were also responsive to replacement. If there is a surgically correctable lesion, for example, an abscess, or infected area that is amenable to excision, an operative approach to the primary problem could be attempted during this period of hemodynamic improvement. However, continued sepsis limits the duration of improvement and, of course, the severity of infection influences the result of the procedures. The repeat plasmapheresis in these patients was ineffective when the infection was not controlled.

After four patients, instead of two questions, there are three. Does plasmapheresis affect the survival of critically burned patients? Our initial and preliminary results indicate that it did not. Does plasmapheresis reduce the volume of resuscitation fluid required? It did not in either patient; however, it did clear hemochromogens in the urine in one. Is plasmapheresis more effective than the classic Brooke or Evans formulae for resuscitation? In our experience, it is not.

PRESENTATIONS/PUBLICATIONS

McManus WF: Is there a role for plasmapheresis/exchange transfusion in the treatment of the septic burn patient? Presented at the National Institutes of Health Conference, Frontiers in Understanding Burn Injury, Bethesda, Maryland, 26-28 September 1983.

McManus WF: Is there a role for plasmapheresis/exchange transfusion in the treatment of the septic burn patient? *J Trauma* 24:S137-S138, 1984.

FINAL REPORT

PROJECT NUMBER: 3S162772A874-00, APPLIED RESEARCH

PROJECT TITLE: THE CARDIOPULMONARY RESPONSE TO THERMAL INJURY
IN BURNED SOLDIERS: Evaluation of Angiotensin
II Analogue as a Therapeutic Agent of ARDS

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1 October 1983 - 30 September 1984

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REPORT CONTROL SYMBOL - MEDDH-288(R1)

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ABSTRACT

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Angiotensin II (1-Sar, 8-Ile) was assessed as a therapeutic agent for acute respiratory distress syndrome using oleic acid pulmonary edema in sheep as an experimental model. Under general anesthesia with controlled mechanical ventilation using 100 percent oxygen, 32 sheep received oleic acid (0.075 ml/kg) intravenously. Following oleic acid infusion, 20 animals were treated with continuous intravenous infusion of the angiotensin II analogue; nine received 300 ng/kg/min, six received 600 ng/kg/min, and five received 2,000 ng/kg/min. Cardiopulmonary measurements were repeated every 30 minutes for 270 minutes. According to time-integrated PaO_2 , six out of 15 animals of the 300 and 600 ng/kg/min groups (43 percent) did not respond to the treatment. All animals responded in the 2,000 ng/kg/min group. Animals in the latter group had lower Q_s/Q_t , $PaCO_2$, and airway resistance than the control animals. Elevation of pulmonary vascular resistance was limited and mean arterial blood pressure was well maintained. These results reveal that angiotensin II is effective in the treatment of oleic acid-induced pulmonary edema.

Acute Respiratory Distress Syndrome
Angiotensin II Analogue
Oleic Acid
Animal Models

EVALUATION OF ANGIOTENSIN II ANALOGUE AS A THERAPEUTIC AGENT OF ARDS

Angiotensin II (AII), an octapeptide, is thought to be the most potent naturally-occurring vasoconstrictor. Some of its analogues antagonize AII pressor activity and have been developed as diagnostic agents for angiotensinogenic hypertension. Clinical trials indicate that these AII analogues have value in the differential diagnosis of hypertension (1-3). Recent studies have shown that two kinds of AII analogues may be effective in the treatment of respiratory disease.

Yukioka et al (4-5) used angiotensin II (1-Sar, 8-Ile) (Figure 1) for the treatment of acute respiratory distress syndrome (ARDS). They found that PaO_2 increased and $PaCO_2$ and Qs/Qt decreased during continuous intravenous infusion of the drug. No changes were detected in systemic or pulmonary circulation. They speculated that the main effect of the AII analogue was on the airway. *Mookherjee et al* (6) have reported the effects of saralasin, angiotensin II, in chronic lung disease. They observed an increase of PaO_2 with no change of $PaCO_2$ or airway resistance and concluded that saralasin had no effect on the airway (6). The mechanism by which these AII analogues increase PaO_2 remains uncertain. The purpose of this study was to evaluate the therapeutic effectiveness of one AII analogue, 1-Sar, 8-Ile, on a form of ARDS.

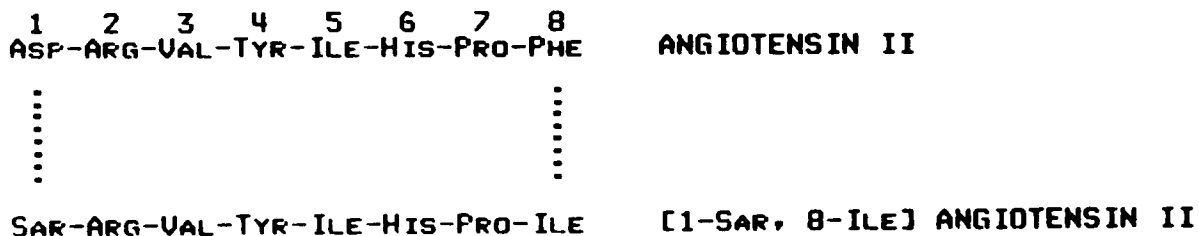


Figure 1. Amino acid sequence of angiotensin II and (1-Sar, 8-Ile) angiotensin II.

¹Ogihara T, Yamamoto T, and Kumahara Y: Clinical applications of synthetic angiotensin II analogue. *Jpn Circ J* 38:997-1003, 1974.

²Ogihara T, Yamamoto T, and Kumahara Y: Angiotensin blockade. *Lancet* 1:219 (Letter), 1974.

³Brunner HR, Gavras H, Laragh JH, et al: Angiotensin-II blockade in man by sar¹-ala⁸-angiotensin II for understanding and treatment of high blood-pressure. *Lancet* 2:1045-1048, 1973.

⁴Yukioka T, Sugimoto H, Yoshioka T, et al: Clinical application of (1-Sar, 8-Ile) angiotensin II for acute respiratory distress syndrome. *Igaku no ayumi* 123:168-170, 1982 (in Japanese).

METHODS AND MATERIALS

Thirty-two healthy adult male sheep (28-40 kg) were studied. All sheep were fasted for 24 hours prior to study. They were anesthetized with intravenous pentobarbital (20 mg/kg IV) and intubated with a cuffed endotracheal tube. The animals were placed in the supine position and ventilated using a volume-limited ventilator (Adult Volume Ventilator, Model 801, Searle Company). Pancuronium bromide (0.065 mg/kg) was initially given to prevent spontaneous breathing, with small supplements added as needed throughout the experiment. Controlled mechanical ventilation, using 100 percent oxygen, was provided during the experiment. Tidal volume was maintained at 12 ml/kg and the respiratory rate was fixed at 15/minute.

A 7F Swan-Ganz catheter (Model 93A-131-7F, Edward Laboratories, Inc.) was passed via the left external jugular vein into the pulmonary artery to monitor pulmonary arterial pressure and pulmonary wedge pressure and also to withdraw mixed venous blood. A polyethylene catheter (ID=0.055", OD=0.075") was inserted into the left femoral artery for blood pressure monitoring and for withdrawal of arterial blood. These pressures were measured with a Statham Model D23P pressure transducer and recorded on a 7754A 4-channel Hewlett-Packard recorder. Another polyethylene catheter (ID=0.055", OD=0.075") was passed into the right atrium via the right external jugular vein. This catheter permitted infusion of lactated Ringer's solution during the infusion at a rate of 3.0 ml/kg/hour.

After a two-hour stabilization period, baseline measurements of the cardiopulmonary variables were collected. Oleic acid (0.075 ml/kg, Sigma Chemical Company) was then infused into the right atrium for a 10-minute period in all animals. The animals were divided into two groups, a control group (n=12) and a treatment group (n=20). The treatment group received an intravenous infusion of (1-Sar, 8-Ile) AII beginning two minutes after termination of the oleic acid infusion and continuing to the end of the experiment. The control group received no drug, while the treatment group received one of three doses. Nine animals received 300 ng/kg/min (300 group), six received 600 ng/kg/min (600 group), and five received 2,000 ng/kg/min (2,000 group). The drug was dissolved in 35 milliliters of normal saline and delivered at a constant rate of 0.12 ml/minute using a Model 660-900 Harvard Infusion Pump. Cardiopulmonary measurements were repeated at 30-minute intervals for the 270 minutes

⁵Yukioka T, Sawada Y, Sugimoto H, et al: Clinical study of (1-Sar, 8-Ile) angiotensin II as a therapeutic agent of ARDS. *Geka chiryo* 46:381, 1982 (in Japanese).

⁶Mookherjee S, Ashutosh K, Smulyan H, et al: Arterial oxygenation and pulmonary function with saralasin in chronic lung disease. *Chest* 83:842-847, 1983.

following oleic acid infusion. At the end of each experiment, the sheep were sacrificed by injection of potassium chloride (25 mEq/animal IV) and a necropsy examination was performed, including light and electron microscopic examination of the lung.

Cardiac output was measured by thermodilution using a Model 9520A Cardiac Output Computer (Edward Laboratories, Inc.). Blood gases were measured with a System 1303 Blood Gas Analyzer (Instrumentation Laboratories) and oxygen content was measured with a CO-Oximeter Model 282 (Instrumentation Laboratories).

The following variables were determined at each measurement: arterial oxygen tension (PaO_2 , Torr), arterial carbon dioxide tension (PaCO_2 , Torr), arterial oxygen content (CaO_2 , ml/100 milliliters blood), mixed venous oxygen content (CvO_2 , ml/100 milliliters blood), mean arterial blood pressure (MBP, Torr), pulse rate (PR, min^{-1}), mean pulmonary arterial pressure (MPAP, Torr), and pulmonary wedge pressure (PWP, Torr). Calculated hemodynamic parameters were: body surface area (BSA, m^2) = $0.084 \times \text{body weight}^{2/3}$ (kg); cardiac index (CI, l/min/m^2) = cardiac output/BSA; left ventricular stroke work index (LVSWI, $\text{g}\cdot\text{m/beat}\cdot\text{m}^2$) = $\text{CI} \times (\text{MBP} - \text{PWP}) / \text{PR} \times 13.6$; pulmonary vascular resistance (PVR, $\text{dyne}\cdot\text{sec/cm}^5$) = $(\text{MPAP} - \text{PWP}) / \text{cardiac output} \times 80$; shunt ratio (Qs/Qt , %) = $(\text{Cc}'\text{O}_2 - \text{CaO}_2 / \text{Cc}'\text{O}_2 - \text{CvO}_2) \times 100$, where $\text{Cc}'\text{O}_2$ is oxygen content of the lung capillary blood calculated assuming that oxygen saturation of hemoglobin was 99 percent.

In addition, to evaluate the drug effect on PaO_2 across time, we used an integrated PaO_2 value (I-PaO_2 , Torr $\cdot\text{min}$), calculated as the sum of the timed average PaO_2 from 30 minutes after oleic acid infusion to the end of the experiment.

Static lung compliance (ml/cm H_2O) and pulmonary resistance (cm $\text{H}_2\text{O/l/sec}$) were measured in 10 sheep (control group, $n=5$; 2,000 group, $n=5$). For this purpose, an esophageal balloon was inserted to obtain intrathoracic pressure (7). Transpulmonary pressure (pressure difference between the airway and esophageal pressure) was monitored by a differential pressure transducer (Model MP45-1, Validyne). Respiratory gas flow was monitored by a pneumotachygraph (Model 17212, Gould, with flow transducer, Model 2, Fleish). Pressure-flow curves were obtained with a Vovetek oscillograph (Model 1901C). The static compliance was calculated as the ratio of the tidal volume to transpulmonary pressure when the breath was held at the end of inspiration. Pulmonary resistance was calculated as ratio of transpulmonary pressure to the peak inspiratory flow rate. The peak flow rate was usually around 0.50 l/sec.

⁷Lemen R, Benson, M, and Jones JG: Absolute pressure measurements with hand-dipped and manufactured esophageal balloons. *J Appl Physiol* 37:600-603, 1974.

Two-way analysis of variance (repeated-measurement design) was used to interpret the data. $P < 0.05$ was defined as statistically significant.

RESULTS

Figure 2 presents the effect of the AII analogue treatment on integrated PaO_2 (I- PaO_2). All treated animals were divided into two groups, responders and nonresponders. We defined a responder as any animal whose I- PaO_2 was at least three standard deviations above the mean of the control animals (mean $\pm$ SD = $15.8 \times 10^3 \pm 3.08 \times 10^3$ Torr·min). Four of nine sheep in the 300 group and two of six in the 600 group were identified as nonresponders. All animals in the 2,000 group were responders. To determine whether responders and nonresponders could be identified prior to treatment, baseline cardiopulmonary variables of responders (n=9) and nonresponders (n=6) of the 300 and 600 groups were compared (Table 1). There were no significant differences between them (Student's t-test).

Since the 300 and 600 group contained some nonresponders, only the data from the 2,000 group were used for detailed analysis. Figures 3 through 11 show changes of the mean and standard error (SE) of each variable of the 2,000 and control groups. PaO_2 decreased following oleic acid infusion in both groups (Figure 3). While the PaO_2 of the control group remained low, that of the 2,000 group significantly increased across time ($p < 0.01$). $\dot{Q}_s/\dot{Q}_t$ of the 2,000 group was lower than that of the control group following oleic acid infusion (Figure 4, $p < 0.05$). The PaCO_2 of the control group increased after oleic acid infusion and remained high during the experiment. In the 2,000 group, PaCO_2 increased transiently following oleic acid infusion and then decreased toward baseline values (Figure 5). Beyond 120 minutes, PaCO_2 was significantly lower in the 2,000 group ($p < 0.05$).

Changes in PVR are shown in Figure 6. Although the mean values of PVR were not different between the two groups, the PVR of the control group continuously increased from 90 minutes after oleic acid infusion to the end of the experiment, while the PVR of the 2,000 group did not change in the same period. The changes of MPAP were essentially the same as PVR. PWP was three to seven mmHg and identical in both groups.

Static compliance was not different between the two groups (Figure 7). Pulmonary resistance increased immediately following oleic acid infusion in both groups. In the control group, pulmonary resistance increased again following a temporary decrease (Figure 8). On the other hand, in the 2,000 group, it continuously decreased and pulmonary resistance of the 2,000

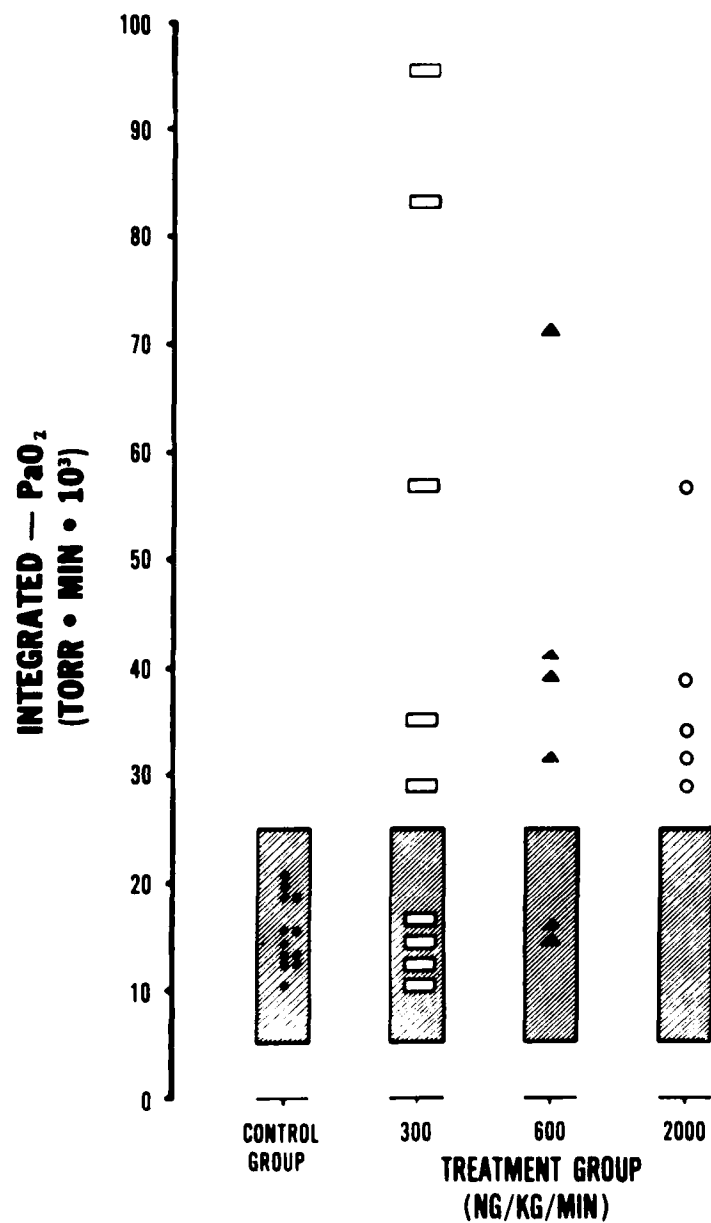


Figure 2. The effect of (1-Sar, 8-Ile) angiotensin II on integrated PaO₂ following oleic acid-induced pulmonary edema. Shaded area represents mean ± 3 SD of the control group. In the 2,000 group (o), all five cases were above this range. In the 600 group (Δ), four out of six, and in the 300 group (□), five out of nine were above the range.

Table 1. Baseline Measurements of Responders and Non-responders

	Responder (n = 9)	Non-responder (n = 6)	Student's <u>t</u>	p
Weight (kg)	34.6 ± 1.9	37.8 ± 2.9	1.90	0.080
PaO ₂ (Torr)	552 ± 19.3	571 ± 9.0	0.75	0.465
PaCO ₂ (Torr)	29.9 ± 1.7	29.8 ± 1.2	0.11	0.918
MBP (Torr)	107 ± 3.7	104 ± 3.8	0.62	0.547
CI (l/min·m ²)	3.06 ± 0.27	3.74 ± 0.16	1.85	0.087
TPR (dyne·sec/cm ⁵)	3342 ± 275	2575 ± 217	2.00	0.067
MPAP (Torr)	8.1 ± 0.71	8.7 ± 0.47	0.65	0.524
PVR (dyne·sec/cm ⁵)	226 ± 26.1	160 ± 20.0	1.84	0.089
LVSWI (g·m/beat·m ²)	33.1 ± 1.9	37.9 ± 1.2	1.90	0.080

MBP = mean blood pressure; CI = cardiac index; TPR = total peripheral resistance; MPAP = mean pulmonary arterial pressure; PVR = pulmonary wedge pressure; LVSWI = left ventricular stroke work index.

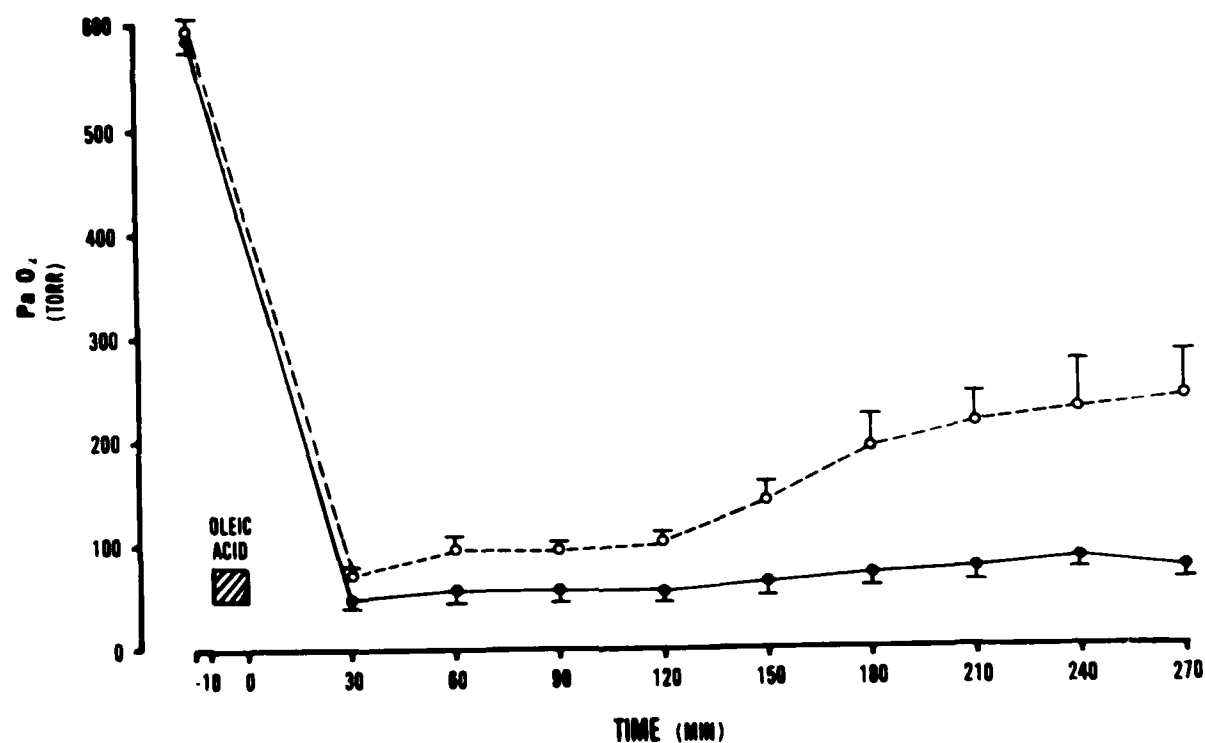


Figure 3. The effects of (1-Sar, 8-Ile) angiotensin II on PaO₂ following oleic acid-induced pulmonary edema. Arterial blood oxygenation was significantly greater in the treated animals (o--o) throughout the period of observation. Mean $\pm$ SE.

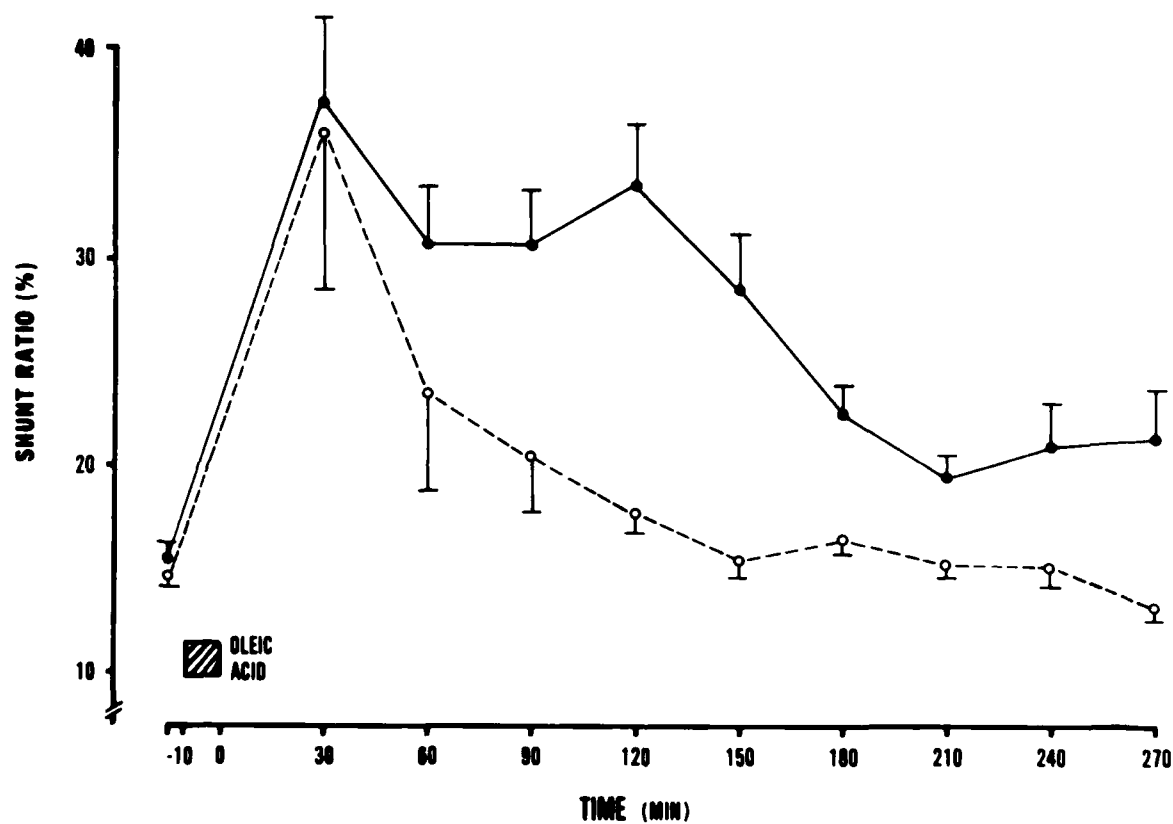


Figure 4. The effect of (1-Sar, 8-Ile) angiotensin II on the shunt ratio following oleic acid-induced pulmonary edema. The mean shunt ratio of the treated animals (o--o) was lower than that of controls (●--●).

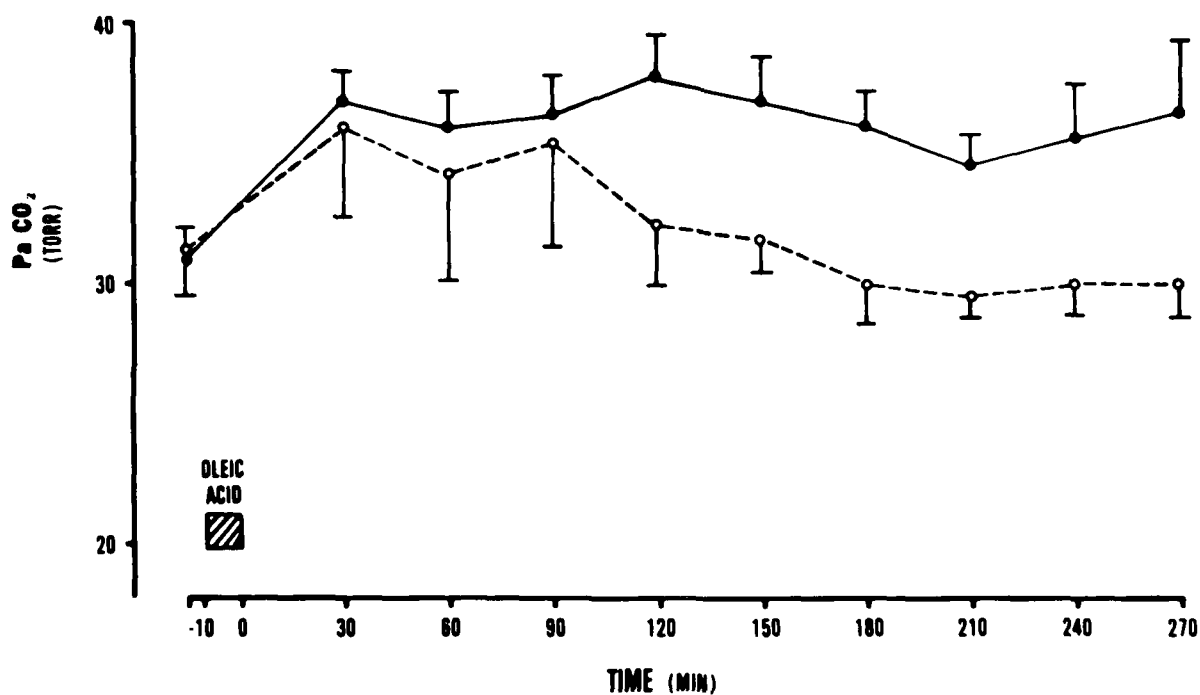


Figure 5. The effects of (1-Sar, 8-Ile) angiotensin II on PaCO₂ following oleic acid-induced pulmonary edema. PaCO₂ of the treated animals (o--o) was lower than controls (●--●) from 120 to 270 minutes post-oleic acid infusion. Mean $\pm$ SE.

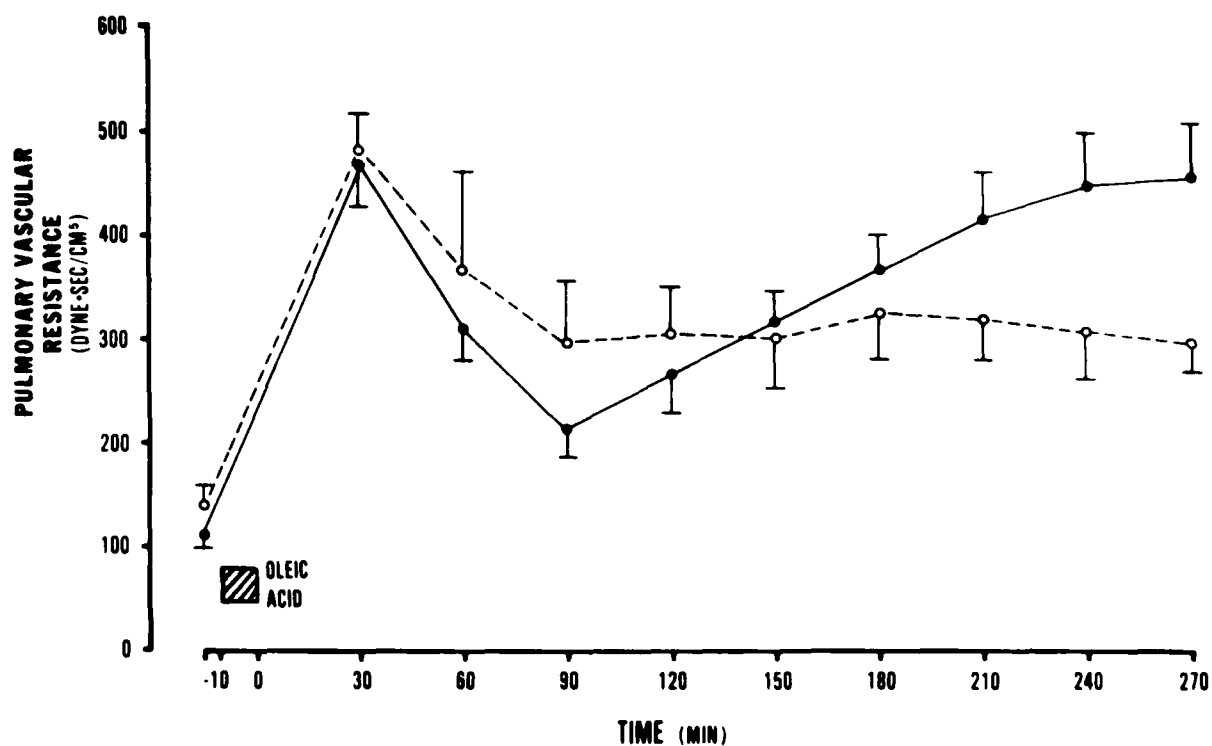


Figure 6. The effect of (1-Sar, 8-Ile) angiotensin II on pulmonary vascular resistance (PVR) following oleic acid-induced pulmonary edema. PVR of the control animals (●--●) continuously increased after 90 minutes post-oleic acid infusion. PVR of the treated animals did not change.

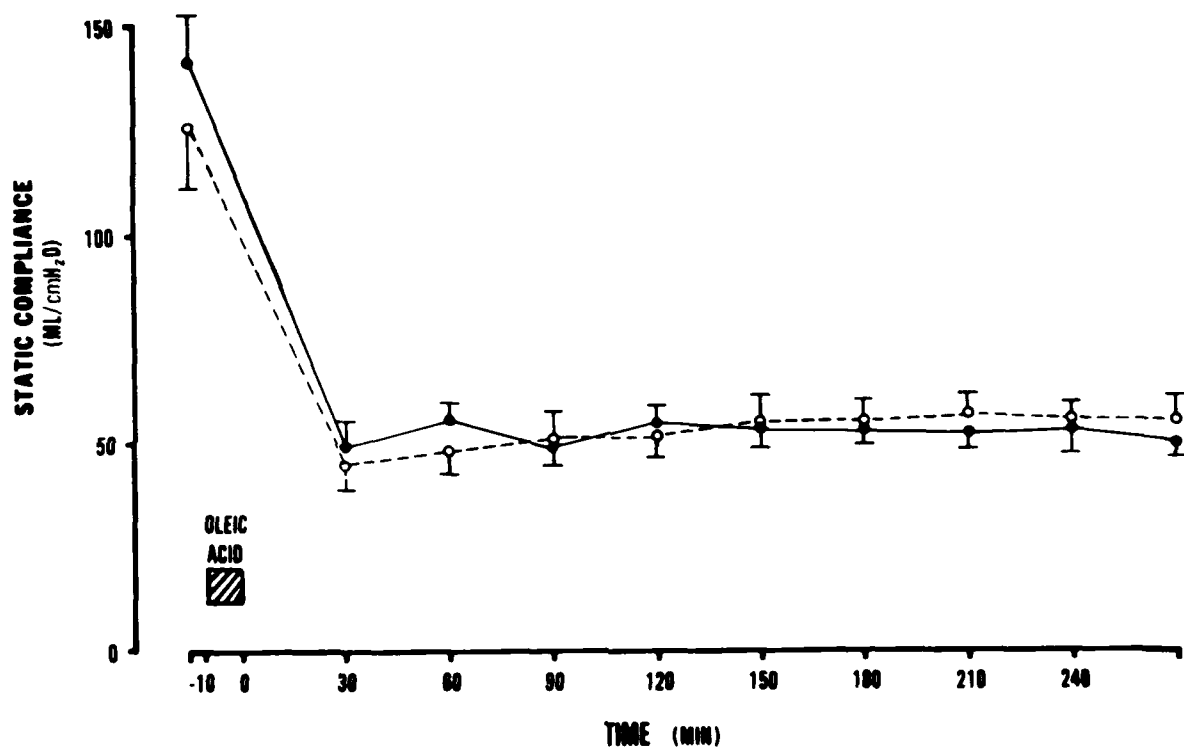


Figure 7. The effect of (1-Sar, 8-Ile) angiotensin II on static compliance. The static compliance was identical in the two groups (o--o, treated animals; ●--●, controls). Mean $\pm$ SE.

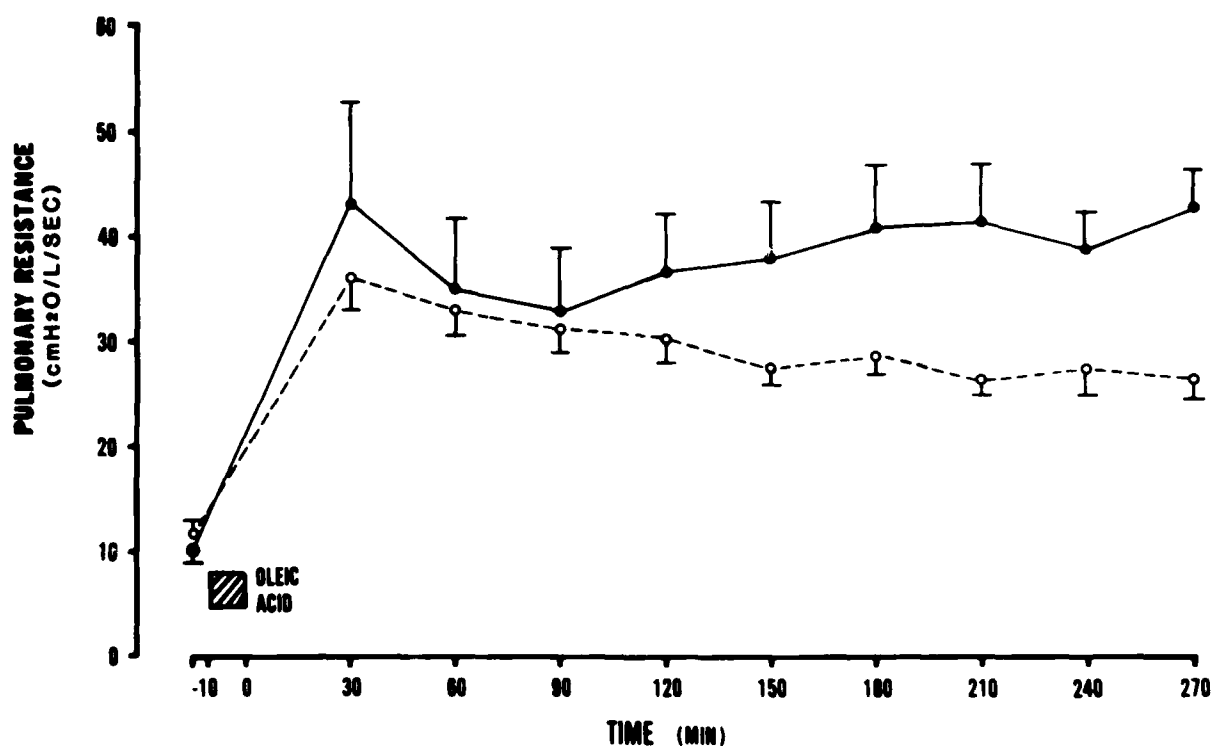


Figure 8. The effect of (1-Sar, 8-Ile) angiotensin II on pulmonary resistance following oleic acid-induced pulmonary edema. Pulmonary resistance of the treated animals (o--o) was lower than controls (●--●) from 180 to 270 minutes post-oleic acid infusion. Mean $\pm$ SE.

group was significantly lower during the last 90 minutes of the experiment ($p < 0.05$).

In the systemic circulation, hypotension and CI depression were observed in both groups following oleic acid infusion. Hypotension was limited in the 2,000 group ($p < 0.05$) where MBP increased across time and returned to baseline value by the end of the experiment (Figure 9). Although the mean values of CI did not differ between the groups, that of the 2,000 group continuously increased in the latter half of the experiment while CI of controls did not change (Figure 10). The pulse rate was 120 to 140/minute in both groups. Figure 11 shows the change of LVSWI. LVSWI was immediately depressed following oleic acid injection in both groups. In the 2,000 group, however, it increased across time and was significantly higher than that of the control group ($p < 0.01$).

Anatomic findings were consistent in all sheep. On gross examination, the lungs were severely congested and edematous, particularly the diaphragmatic lobes. The severity of congestion was identical in the control and treatment groups. Light microscopic examination revealed that there was massive pulmonary edema and congestion in the lung tissue. Alveoli were filled with pink proteinaceous material and parts of the terminal and respiratory bronchioles were flooded with the same material. There was minimal to moderate infiltration of inflammatory cells with a mixture of polymorphonuclear leukocytes and lymphocytes present. The most consistent findings at electron microscopy were edematous changes in the type 1 pneumocyte and increased pinocytotic vesicles in the endothelium.

DISCUSSION

In the present study, we produced severe, histologically confirmed pulmonary edema which was associated with significant deterioration of pulmonary function and systemic hypotension. Continuous intravenous infusion of (1-Sar, 8-Ile) AII improved respiratory function and limited the duration of systemic hypotension following oleic acid infusion.

Oxygen toxicity must be considered in any experiment using 100 percent oxygen. In preliminary studies with the same respiratory management but without either oleic acid or AII analogue, no deterioration of respiratory function could be detected up to seven hours. Since the animals were exposed to 100 percent oxygen less than seven hours in the present study, the effect of oxygen toxicity on the measured variables is assumed to be minimal.

Characteristic differences of pulmonary function in the treated animals included higher PaO_2 , lower $\dot{Q}_s/\dot{Q}_t$, lower $PaCO_2$, and lower pulmonary resistance. Since $\dot{Q}_s/\dot{Q}_t$ represents true in-

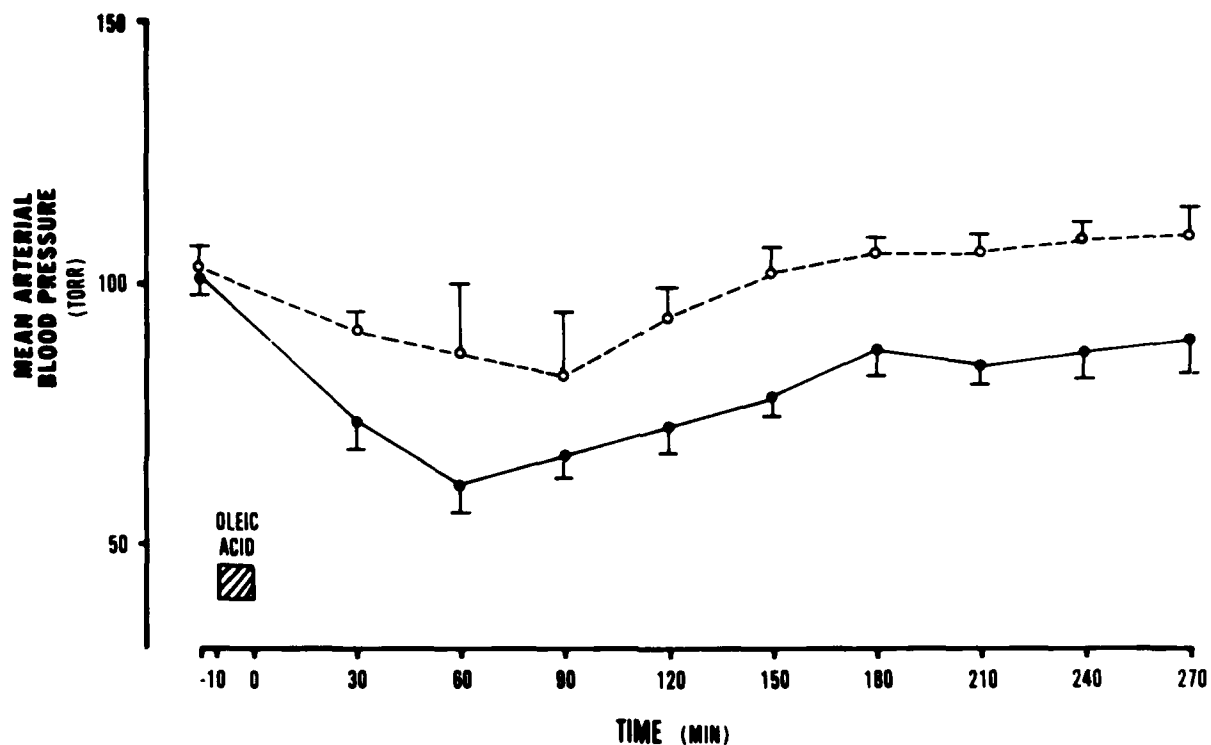


Figure 9. The effect of (1-Sar, 8-Ile) angiotensin II on mean arterial pressure following oleic acid-induced pulmonary edema. Mean arterial blood pressure of the treated animals (o--o) was higher than that of the controls (o--o) throughout the observation period.

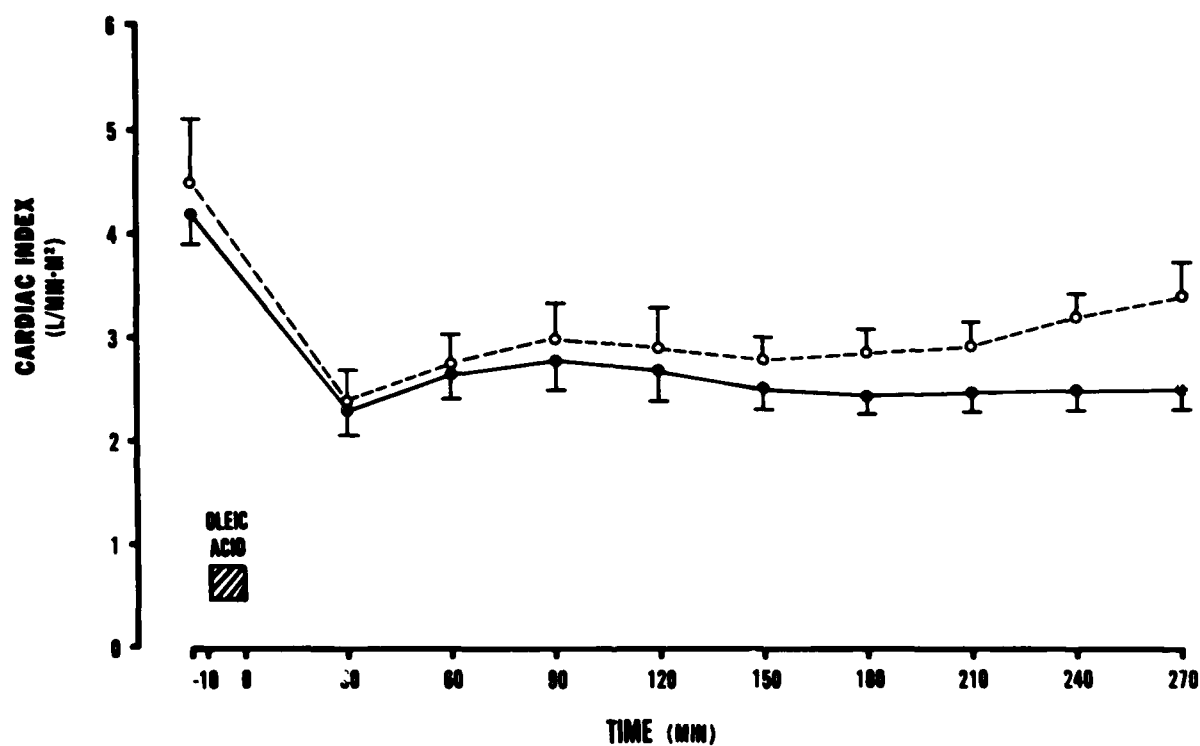


Figure 10. The effect of (1-Sar, 8-Ile) angiotensin II on cardiac index following oleic acid-induced pulmonary edema. Cardiac index of the treated animals (o--o) increased in the latter two hours of the experiment, while that of the controls (o--o) did not change.

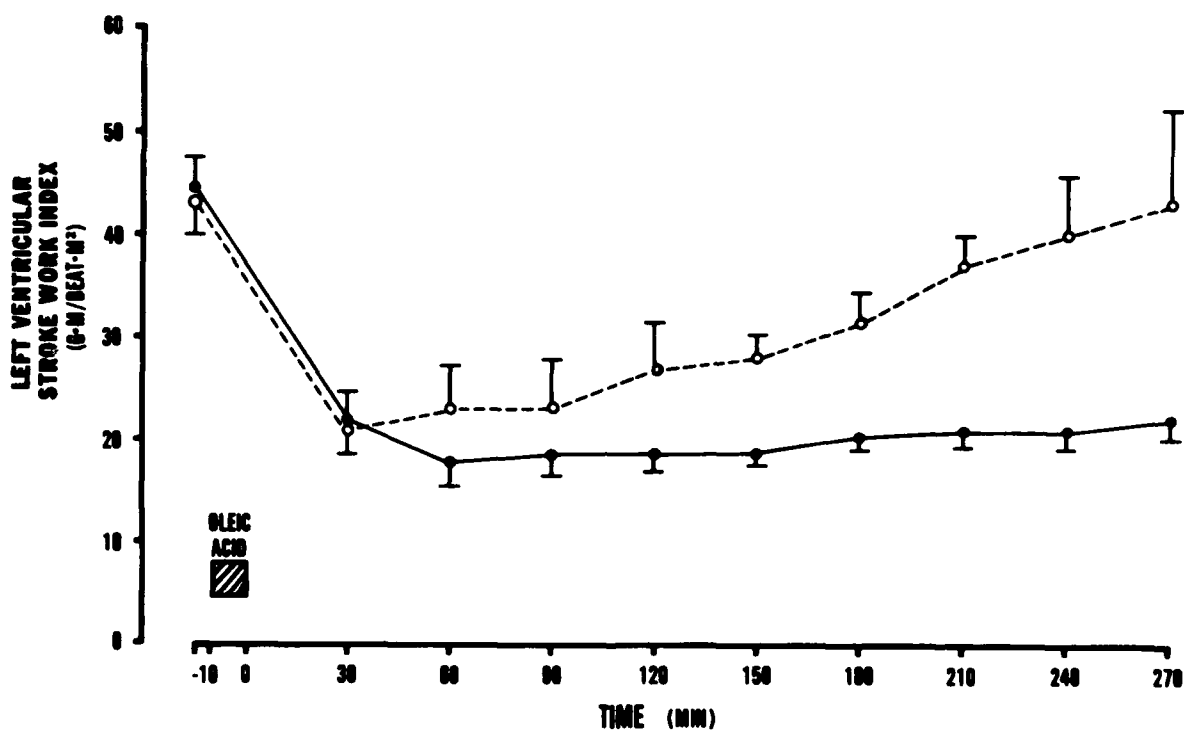


Figure 11. The effect of (1-Sar, 8-Ile) angiotensin II on left ventricular stroke work index. Left ventricular stroke work index of the treated animals (o--o) was greater than controls (o--o) throughout the observation period.

trapulmonary shunt under 100 percent oxygen, AII analogue appears to ameliorate true intrapulmonary shunt following oleic acid administration. Since minute ventilation did not change throughout the experiment, the decreased PaCO_2 in the treated animals suggests increased effective alveolar ventilation.

To analyze the effect of this drug on pulmonary function, it is necessary to consider not only ventilatory status but also circulatory changes. If the drug redistributes some shunt flow to alveoli previously ventilated but without blood flow, the lower $\dot{Q}_s/\dot{Q}_t$ and PaCO_2 and higher PaO_2 could be the result of alteration of circulatory status. No drug effect on pulmonary circulation was clearly identified in the present study, but since the PVR of the treated animals did not increase in the latter half of the experiment, some drug effect should not be ruled out (Figure 6). We do not have data concerning intrapulmonary blood distribution and further study is necessary for more detailed analysis of an effect on pulmonary circulation.

The pulmonary resistance of the treated animals was lower than that of the control group during the later stages of the experiment, despite the fact that both groups had identical static compliance (Figures 7 and 8). Pulmonary resistance is the sum of airway resistance and tissue resistance (8). Since compliance has a close relation with tissue resistance (9), both groups should have identical tissue resistance. If this is so, the lower pulmonary resistance of the treated animals can best be explained by lower airway resistance. These results suggest a drug effect on ventilatory status and are compatible with higher PaO_2 , lower $\dot{Q}_s/\dot{Q}_t$, and lower PaCO_2 .

Airway resistance is related to the tone of bronchial smooth muscle as well as edema of bronchial tissue and mucosal congestion. Since histologic examination revealed an equal severity of lung injury in all sheep, the lower airway resistance of the treated animals may result from a decrease in smooth muscle tone. Thus, the relationship between AII and bronchial smooth muscle is important in analyzing the effect of AII analogue on respiratory function. *Türker et al* (10) have reported that AII relaxed bronchial smooth muscle in the cat and that the action of AII was

⁸Comroe JH: Mechanical factors in breathing. In *Physiology of Respiration*, 2nd edition, Chicago: Year Book Medical Publishers, pp 94-141, c1974.

⁹Bachofen H: Lung tissue resistance and pulmonary hysteresis. *J Appl Physiol* 24:296-301, 1968.

¹⁰Türker RK and Ercan ZS: The effects of angiotensin I and angiotensin II on the isolated tracheal muscle of the cat. *Pharm Pharmacol* 28:298-301, 1976.

blocked by aspirin. Conversely, Lung (11) reported that AII constricted bronchial smooth muscle of both the guinea pig and the rat. Such constriction was blocked by (1-Sar, 8-Ala) AII (saralasin), but was not blocked by aspirin. It thus appears that the effect of AII on bronchial smooth muscle varies according to species. In our study, however, the changes in PaCO_2 and airway resistance strongly suggest that (1-Sar, 8-Ile) AII causes airway dilatation.

In the 300 and 600 groups, according to our criterion of integrated PaO_2 (I- PaO_2), six out of 15 animals (40 percent) did not respond to the drug (Figure 2). These are the usual dosages employed in clinical studies (12,13). Since we could not detect any difference in the baseline data between responders and non-responders (Table 1), it was impossible to predict which subjects would respond to the AII analogue.

We cannot explain why some animals responded and others did not, but altered drug metabolism could be responsible for part of the interanimal variation. Angiotensinase inactivates not only AII but also AII analogues (14). This enzyme is ordinarily found in lung tissue, especially in the lysosomal fraction (15). Although AII is not metabolized in the pulmonary circulation under normal conditions when the lung becomes edematous, AII is inactivated while passing through the lung (16). Since oleic acid pulmonary edema is a typical permeability type edema (17), (1-Sar, 8-Ile) AII may also be degraded in the pulmonary circulation and its concentration maintained at levels too low to be effective. This speculation is consistent with the observed dose response.

¹¹Lung MA and Souhrada JF: Response of airway smooth muscle to angiotensin II. *Fed Proc* 38:1374 (Abstract), 1979.

¹²Yamamoto T, Doi K, Ogihara T, et al: Changes of blood pressure, plasma renin activity, and plasma aldosterone concentration following the infusion of Sar¹-Ile⁸-angiotensin II in hypertensive, fluid, and electrolyte disorders. *Prog Biochem Pharmacol* 12:174-189, 1976.

¹³Ogihara T, Hata T, Mikami H, et al: Sodium depletion and blood pressure response to 1-sarcosine, 8-isoleucine angiotensin II in hypertension. *Clin Pharmacol Ther* 23:566-572, 1978.

¹⁴Ody CE, Marinkovic DV, Hammon KJ, et al: Purification and properties of prolylcarboxypeptidase (angiotensinase C) from human kidney. *J Biol Chem* 253:5927-5931, 1978.

¹⁵Kumamoto K, Stewart TA, Johnson AR, et al: Prolylcarboxypeptidase (angiotensinase C) in human lung and cultured cells. *J Clin Invest* 67:210-215, 1981.

¹⁶Bakhle YS, Reynard AM, and Vane JR: Metabolism of the angiotensins in isolated perfused tissues. *Nature* 222:956-959, 1969.

¹⁷Staub NC: Pulmonary edema due to increased microvascular permeability to fluid and protein. *Circ Res* 43:143-151, 1978.

In the systemic circulation, hypotension was limited in the treated animals despite an initial decrease in CI comparable to the untreated animals. This suggests greater vasoconstriction in the treatment group at this stage of the experiment (Figures 9 and 10). (1-Sar, 8-Ile) AII acts not only to antagonize the AII pressor effect but can also act agonistically to increase blood pressure (18). Administration of AII analogue has also been reported to release catecholamines (19) and this action of the drug might be responsible for limitation of hypotension in the treatment group.

In the latter half of the experiment, the increased CI of the treated animals plays some part in increasing blood pressure. This improvement of the CI was associated with increased cardiac performance as measured by a significant increase of LVSWI (Figure 10). Some factors in the treatment group, such as improvement of blood oxygenation and limited elevation of PVR which limits the increase in right heart afterload, might indirectly improve cardiac performance during the latter half of the experiment. The direct effect of the drug on cardiac function is uncertain. These results suggest that (1-Sar, 8-Ile) AII may have some desirable effects on systemic circulation when used as a therapeutic agent for ARDS.

Information concerning the pulmonary effects of AII analogues is limited (4-6). The only consistent observation is an increase in PaO_2 following AII analogue infusion. Yukioka *et al* (4,5) used 300 ng/kg/min of (1-Sar, 8-Ile) AII in the treatment of patients with ARDS. They found that PaO_2 increased while PaCO_2 decreased. No changes occurred in the pulmonary or systemic circulation. The patients in that study, however, had relatively stable circulation with normal blood pressure. In such patients, (1-Sar, 8-Ile) AII at that dose could increase PaO_2 without pronounced effects on the circulatory system.

Mookherjee *et al* (6) used saralasin in the treatment of chronic lung disease. They reported no change of PaCO_2 or airway resistance. The differences between that study and ours include not only the differences between acute and chronic lung disease and species, but also the AII analogue used. The pharmacologic effect of (1-Sar, 8-Ile) AII on hypertensive patients is different from that of saralasin. The depressor effect of saralasin is usually accompanied by reduction of cardiac output with or without a

¹⁸Hata T, Ogiwara T, Mikami H, *et al*: Effect of two angiotensin II analogues on blood pressure, plasma aldosterone concentration, plasma renin activity, and creatinine clearance in normal subjects on different sodium intakes. *Eur J Clin Pharmacol* 18:295-299, 1980.

¹⁹Sen S, Smeby RR, and Bumpus FM: Angiotensin antagonist and possible release of catecholamine. *Proc Soc Exp Biol Med* 147:847-849, 1974.

decrease of peripheral resistance (20,21). (1-Sar, 8-Ile) AII has less of a depressive effect on cardiac function and reduces blood pressure and peripheral resistance without changing cardiac output (22). In the treatment of chronic lung disease with saralasin, PaO_2 increase correlated with cardiac output decrease (6). In the present study, we found no depressive effect of (1-Sar, 8-Ile) AII on cardiac function or systemic circulation. Since patients with severe ARDS may also exhibit circulatory instability, (1-Sar, 8-Ile) AII might be preferred to saralasin in the treatment of such patients.

Other drugs have been studied as therapeutic agents for ARDS. Nitroprusside decreases PVR but also decreases PaO_2 . Glucagon improves oxygenation but increases PVR (23). Prostacyclin has been reported to have some useful effects in the treatment of pulmonary embolism (24). Prostacyclin, however, depresses the systemic circulation and this may limit its clinical application. Compared to these drugs, (1-Sar, 8-Ile) AII improved not only blood gas data but also the pulmonary circulation while maintaining or even improving systemic circulation. These effects suggest that this AII analogue may have some promise as a therapeutic agent for the clinical treatment of ARDS.

PRESENTATIONS/PUBLICATIONS

Yukioka T: The effects of an angiotensin II analogue on ARDS. Accepted for presentation at the 70th Annual Clinical Congress of the American College of Surgeons, San Francisco, California, 21-26 October 1984.

Yukioka T, Yukioka N, Aulick LH, Goodwin CW, Mason AD Jr, Sugimoto T, and Pruitt BA Jr: Evaluation of (1-Sar, 8-Ile) angiotensin II as a therapeutic agent for oleic acid-induced pulmonary edema. In preparation.

²⁰de Carvalho JGR, Dunn FG, Kem DC, et al: Hemodynamic correlates of saralasin-induced arterial pressure changes. *Circulation* 57:373-378, 1978.

²¹Mookherjee S, Obeid A, Warner R, et al: Systemic and pulmonary hemodynamic effects of saralasin infusion in hypertension: predictability of plasma renin status from hemodynamic changes. *Am J Cardiol* 42:987-992, 1978.

²²Morimoto S, Yamamoto I, Uchida K, et al: Hemodynamic effects of (Sar¹, Ile⁸) AII, an angiotensin II analog, in the renin subgroups of essential hypertension. *Cardiology* 67:219-229, 1981.

²³Weigelt JA, Gewertz BL, Aurbakken CM, et al: Pharmacologic alterations in pulmonary artery pressure in the adult respiratory distress syndrome. *J Surg Res* 32:243-248, 1982.

²⁴Krausz MM, Utsunomiya T, Feuerstein G, et al: Prostacyclin reversal of lethal endotoxemia in dogs. *J Clin Invest* 67:1118-1125, 1981.

ANNUAL PROGRESS REPORT

PROJECT NUMBER: 3S162772A874-00, APPLIED RESEARCH

PROJECT TITLE: THE CARDIOPULMONARY RESPONSE TO THERMAL INJURY
IN BURNED SOLDIERS: An Analysis of the Adapta-
tion of Renal Function Following Burn Injury

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REPORT CONTROL SYMBOL - MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NUMBER: 3S162772A874-00, APPLIED RESEARCH

PROJECT TITLE: THE CARDIOPULMONARY RESPONSE TO THERMAL INJURY
IN BURNED SOLDIERS: An Analysis of the Adaptation of Renal Function Following Burn Injury

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REPORT CONTROL SYMBOL: MEDDH-288(R1)

This report describes a proposed protocol to study renal function adaptation following burn injury. Thermal injuries place great demands on the homeostatic mechanisms responsible for maintenance of fluid, electrolyte, and acid-base status and excretion of both metabolic and drug waste products. Kidney function is critical to all these processes. The study is designed to describe the response of the kidney to thermal injury and burn treatment procedures and define mechanisms of renal adaptations to trauma. The protocol has received all required approvals and contracts to perform assays not available within the Institute have been signed. Patients will be enrolled during FY 85.

Human
Volunteer
Renal
Kidney
Glomerular Filtration Rate
Proteinuria
Sodium
Potassium
Beta-2-Microglobulin
Burns
Thermal Injury

AN ANALYSIS OF THE ADAPTATION OF RENAL FUNCTION FOLLOWING BURN INJURY

Introduction

Burn injury and its therapy place great demands on the homeostatic mechanisms responsible for maintenance of fluid, electrolyte, and acid-base status and excretion of both metabolic and drug waste products. The function of the kidneys is critical to all these processes. Therefore, it is suprising that renal function in the postburn period has received scant attention (1-13).

¹Loirat P, Rohan J, Baillet A, Beaufils F, David R, and Chapman A: Increased glomerular filtration rate in patients with major burns and its effect on the pharmacokinetics of tobramycin. *N Eng J Med* 299:915-919, 1978.

²O'Neill JA Jr, Pruitt BA Jr, and Moncrief JA: Studies of renal function during the early postburn period. In *Research in Burns*. Matter P, Barclay TL, and Konicková Z (eds). Bern: Hans Huber Publishers, c1971, pp 95-99.

³Haynes BW, DeBakey ME, and Denman FR: Renal function studies of severely burned patients: a preliminary report. *Ann Surg* 134:617-625, 1951.

⁴Sevitt S: Distal tubular necrosis with little or no oliguria. *J Clin Path* 9:12-30, 1956.

⁵Graber IG and Sevitt S: Renal function in burned patients and its relationship to morphological changes. *J Clin Path* 12:25-44, 1959.

⁶Eklund J, Granberg PO, Liljedahl SO: Studies on renal function in burns. I. Renal osmolal regulation, glomerular filtration rate, and plasma solute composition related to age, burned surface area, and mortality probability. *Acta Chir Scand* 136:627-640, 1970.

⁷Eklund J: Studies on renal function in burns. II. Early signs of impaired renal function in lethal burns. *Acta Chir Scand* 136:735-740, 1970.

⁸Eklund J: Studies on renal function in burns. III. Hyperosmolal states in burned patients related to renal osmolal regulation. *Acta Chir Scand* 136:741-751, 1970.

⁹Vertel RM and Knochel JP: Nonoliguric acute renal failure. *JAMA* 200:598-602, 1967.

¹⁰Davies DM, Pusey CD, Rainford DJ, Brown JM, and Bennett JP: Acute renal failure in burns. *Scand J Plast Reconstr Surg* 13:189-192, 1979.

¹¹Planas M, Wachtel T, Frank H, and Henderson LW: Characterization of acute renal failure in the burned patient. *Arch Intern Med* 142:2087-2091, 1982.

The studies which have been conducted had various limitations: patients studied only once at random times postburn (1), studied only during the first week postburn (2), small number of patients (3), prior renal function not defined (4-8), no comment on the development of nonrenal complications which can affect renal function (4-8), study limited to patients who developed renal failure (9-11), and summary article without original data (12-13). There have been no studies of proximal tubular renal function.

The following study was proposed to define the normal physiologic adaptation of renal function to burn injury. The objectives of the study are to measure glomerular and tubular functions in patients suffering at least 30 percent total body surface area burns who had normal renal, hepatic, and cardiovascular function prior to burn injury. The patients will be followed sequentially from admission until death or discharge. Specific goals of the study include:

1. Determining glomerular filtration rate (GFR) and determining whether endogenous creatinine clearance is an accurate measure of GFR. In the literature, it is stated that GFR postburn is decreased, normal, or increased. Some of the differences probably relate to the time postburn when the measurements were made and some of the measurements may have been made in patients with prior abnormal renal function or in patients with complications (sepsis, hypotension, myocardial infarction, aminoglycoside antibiotic therapy, etc.) which depress renal function. From a literature review, it appears that less than 15 to 20 (1-3, 5) patients have had creatinine clearances validated by simultaneous inulin clearance measurements and all but two (5) of these measurements were performed in patients with normal renal function. Only five to 10 (1) patients have had creatinine clearance verified by the glomerular filtration technique and all these patients had normal renal function. The importance of verifying the endogenous creatinine clearance by another technique is that the creatinine assay is nonspecific and interference can be produced by hyperalbuminemia, lipemia, hemolysis, bilirubinemia >50 mg/dl, elevated acetoacetate, ascorbic acid, or cephalospor-

¹²Sevitt S: Renal function after burning. *J Clin Path* 18:572-578, 1965.

¹³Gellman DD: The renal complications of burns. *Canad Med Ass J* 97:440-444, 1967.

in antibiotics (14). An accurate GFR is of critical importance clinically since dosages of many drugs, including aminoglycosides, are based upon the GFR.

2. Defining the pattern of proteinuria and specifically the fractional excretion of b2-microglobulin. In the literature, there are few reports of proteinuria postburn (15-17). One states that all patients suffering a "large" burn will have a transient proteinuria of 0.55 grams/24 hours from postburn day five through seven (15). b2-microglobulin is freely filtered at the glomerulus and normally 98 percent is reabsorbed in the proximal tubules. To our knowledge, there has been no prior study of b2-microglobulin excretion in the burned patient. By defining the excretion of b2-microglobulin, an index of proximal tubular function can be established. It will then be possible to study the problem of low sodium fractional excretion (FeNa) renal failure in burned patients. In patients with low FeNa, this would imply that the proximal tubule is damaged and the low FeNa is secondary to increased distal reabsorption. If the b2-microglobulin is low, it would imply that the proximal tubule is functioning normally and that the lesion is either pre-renal or glomerular.
3. Defining the excretion of phosphorous and calcium. To our knowledge, this has never been done, and recently, we have observed several patients with low serum concentrations of both calcium and phosphorous. Since calcium (ionized) and phosphorous are required for proper function of many organ systems, it is important to determine if there is excessive excretion or merely decreased intake so that proper replacement therapy can be given.

¹⁴IL TEST Creatinine, Category Number 35164, Instrumentation Laboratory, Inc., 1980.

¹⁵Shakespeare PG, Coombes EJ, Hambleton J, and Furness D: Proteinuria after burn injury. *Ann Clin Biochem* 18:353-360, 1981.

¹⁶Coombes EJ, Shakespeare PG, and Batstone GF: Urine proteins after burn injury. *Clin Chim Acta* 95:201-209, 1979.

¹⁷Eades CH Jr, Pollack RL, and Hardy JD: Thermal burns in man. IX. Urinary amino acid patterns. *J Clin Invest* 34:1756-1759, 1955.

4. Defining the serum concentration of parathyroid hormone (PTH). PTH secretion is controlled by the serum concentration of ionized calcium and requires a permissive concentration of magnesium. PTH affects many organ systems, especially bone. If hypocalcemia is indeed a common occurrence postburn, then elevated PTH levels should result. Bedrest tends to cause excessive bone resorption and elevated PTH levels would accelerate the resorption.
5. Determining if the excessive renal potassium excretion is secondary to increased aldosterone. This will require determination of sodium and potassium balance, renin, and aldosterone. Two studies have shown elevated levels of aldosterone in burned patients. If our balance studies show a correlation between the levels of aldosterone and potassium excretion, then further studies will be required to separate a primary from a secondary effect. The studies would use two drugs - one to block sodium entry into the tubules to determine the sodium dependence of the potassium excretion and the second drug would be an aldosterone inhibitor.

METHODS AND MATERIALS

A maximum of 25 patients age 18 or older admitted to this Institute who are admitted within 48 hours of injury, have at least a 30 percent burned body surface area, have no prior history of renal dysfunction, have no prior history of myocardial dysfunction (a history of mild treated hypertension is allowed), have a serum creatinine less than 1.4 mg/dl after initial resuscitation, and have no evidence of ascites will be entered in the study if they or their substitute provide written informed consent. Patients will be selected to include a spectrum of burn size and age.

While patients are in the intensive care unit at this Institute, all intake and output will be recorded, with particular attention to sodium and potassium, calcium, and phosphorous. During each 24-hour period, all urine will be collected and saved for chemical analyses. At the time of routine daily blood sampling, an additional maximum of 30 milliliters of blood will be obtained for research analyses.

As soon as the ward physician determines that the patient has been stabilized, the GFR will be determined by endogenous creatinine and compared against the inulin and glofil (125I-

iothalamate 20 μ Ci) methods (18). The inulin GFR may be performed as often as once a week while the patient remains in the intensive care unit. The glofil GFR will only be repeated if the patient's serum creatinine increase one mg/dl. The patient's urine output must be greater than 30 ml/hr for the inulin or glofil GFR to be performed.

After the patient is transferred to the intermediate care ward, a 24-hour urine and a maximum of 30 milliliters of blood will be collected only once per week. A final inulin and endogenous creatinine GFR will be determined immediately prior to discharge.

Urine assays will include sodium potassium, chloride, UUN, creatinine, OSM, phosphorous, calcium, total protein, albumin, IgG, and b2-microglobulin. In addition, selected samples will be analyzed by electrophoresis and isoelectrofocusing.

Serum assays will include sodium, potassium, chloride, BUN, creatinine, OSM, total protein, albumin, phosphorous, calcium (total and ionized), b2-microglobulin, renin, angiotensin II, aldosterone, PTH, and ADH. Selected samples will be analyzed by electrophoresis and isoelectrofocusing.

Clinical parameters which will be monitored include weight, blood pressure, medications, percentage body surface area covered and type covering, and complications, such as cardiac, pulmonary, sepsis, survival/death.

It is expected that the patients will be divided into at least uncomplicated/survived, complicated/survived, and complicated/died. If enough patients are available, the complications may be analyzed separately, cardiac, pulmonary, sepsis, etc. The analysis of data will be by multiple correlation-regression techniques.

RESULTS

This protocol was approved by the Office of The Surgeon General on 13 October 1983 with the requirement that the use of 125I-iodothalamate be approved by the Brooke Army Medical Center Radioisotope Committee. That committee's approval has been obtained. Additionally, a contract has been signed with Nichols Institute to measure hormones and ionized calcium, tests which are not available at this Institute. The first patient was enrolled in this study during November 1984. Final results will be provided with the next annual report.

¹⁸Israelit AH, Long DL, White M, and Hull A: Measurement of glomerular filtration rate utilizing a single subcutaneous injection of 125I-iothalamate. *KI* 4:346-349, 1973.

PRESENTATIONS/PUBLICATIONS

None.

ANNUAL PROGRESS REPORT

PROJECT NUMBER: 3S162772A874-00, APPLIED RESEARCH

PROJECT TITLE: Assessment of Non-Nutrient Blood Flow In Critically
Ill Burn Patients

US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
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1 October 1983 - 30 September 1984

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REPORT CONTROL SYMBOL - MEDDH-288(R1)

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ABSTRACT

ASSESSMENT OF NON-NUTRIENT BLOOD FLOW IN CRITICALLY ILL BURN PATIENTS

(A Preliminary Report)

Seventy-three determinations of non-nutrient flow (N-NBF) were made in 18 critically ill patients. Included in these measurements were 12 sets of oxygenation hemodynamic profiles, in five septic (blood culture positive) patients, (average percent total body surface burn was $57\frac{1}{2} \pm 17.7\%$) where the N-NBF ranged from +25% to +70%. In these patients a significant inverse relationship ($p < .01$) between cardiac index (range 4.2 - 6.8 L/min/m²) and hemoglobin concentration (Hb) (range 8.4 - 14.1 grams/100 ml) was noted. The data suggest that hemoglobin and by implication oxygen demand, remains a determinant of cardiac output in these patients where oxygen delivery is increased out of proportion to oxygen consumption.

ASSESSMENT OF NON-NUTRIENT BLOOD FLOW IN CRITICALLY ILL BURN PATIENTS (A Preliminary Report)

Some data (1,2) indicate that cardiac output (CO) is increased out of proportion to the increase in oxygen consumption ($\dot{V}O_2$) during the hypermetabolic phase in patients with extensive thermal injuries. Such a physiologic setting is consistent with "peripheral shunting" of oxygenated blood or in other words the presence of non-nutrient blood (N-NBF). To what extent does N-NBF occur in patients with major thermal injury? What is its relationship to oxygen demand? What is the effect of sepsis on N-NBF in the burn patient? These are but a few of the unanswered questions regarding this complex clinical pathophysiologic problem. In order to begin to answer these questions it will be necessary to measure N-NBF.

N-NBF, that fraction of the CO that does not participate in oxygen exchange at the blood-tissue interface, has heretofore not been able to be determined by a clinically applicable method. A mathematical model for N-NBF that interrelates two sets of values for oxygen delivery (DO_2) and VO_2 has recently been proposed by

1. Gump, FE, Price, JB, and Kinney, JM: Blood flow and oxygen consumption in patients with severe burns. Surg Gynecol Obstet, 130:23, 1970

2. Wilmore, DW, Aulick, LH, Mason, AD, Jr, and Pruitt, BA, Jr: Influence of the burn wound on local and systemic responses to injury. Ann Surg, 186:444, 1977

3. Farrell, K, Bowen, R, and Beatty, J: Non-nutrient blood flow in critically ill patients. Surg Forum 33:75, 1982

by the author of this report. It is as follows: (revised from
(3))

For "normal" DO_2 and decreased VO_2

$$\% \text{ N-NBF} = [\dot{\text{VO}}_2]_a / (\dot{\text{VO}}_2)_b - 1] \times 100$$

For increased DO_2 and "normal" VO_2

$$\% \text{ N-NBF} = [(\text{DO}_2)_b / (\text{DO}_2)_a - 1] \times 100$$

For decreased DO_2 and "normal" VO_2

$$\% \text{ N-NBF} = -[(\text{DO}_2)_a / (\text{DO}_2)_b - 1] \times 100$$

For "normal" DO_2 and increased VO_2

$$\% \text{ N-NBF} = -[(\dot{\text{VO}}_2)_b / (\dot{\text{VO}}_2)_a - 1] \times 100$$

For increased DO_2 and decreased VO_2

$$\% \text{ N-NBF} = [(\text{DO}_2)_b / (\text{DO}_2)_a - 1] + [(\dot{\text{VO}}_2)_a / (\dot{\text{VO}}_2)_b - 1] \times 100$$

For decreased DO_2 and decreased VO_2

$$\% \text{ N-NBF} = [(\dot{\text{VO}}_2)_a / (\dot{\text{VO}}_2)_b - 1] - [(\text{DO}_2)_a / (\text{DO}_2)_b - 1] \times 100$$

For increased DO_2 and increased VO_2

$$\% \text{ N-NBF} = [(\text{DO}_2)_b / (\text{DO}_2)_a - 1] - [(\dot{\text{VO}}_2)_b / (\dot{\text{VO}}_2)_a - 1] \times 100$$

For decreased DO_2 and increased VO_2

$$\% \text{ N-NBF} = - [(\text{DO}_2)_a / (\text{DO}_2)_b - 1] + [(\dot{\text{VO}}_2)_b / (\dot{\text{VO}}_2)_a - 1] \times 100$$

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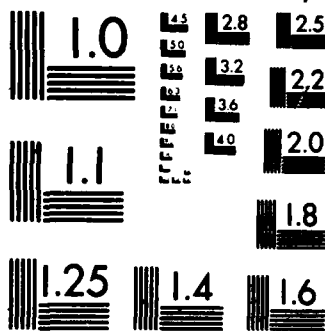
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The subscript (a) refers to "normal" values for DO_2 and VO_2 . These are based on known basal metabolic rates for age and sex and the assumption of a normal respiratory quotient and a normal oxygen utilization coefficient. The subscript (b) values are those observed in the patients. Negative values for N-NBF indicate the utilization of reserve oxygen transport capacity. This has been the subject of separate report. (4)

METHODS

All data were obtained in critically ill burn patients who previously had had a pulmonary artery catheter placed for clinical indications. N-NBF was calculated, using the above described mathematical model, from hemodynamic-oxygenation profiles that were obtained in these patients in order to guide clinical management.

RESULTS AND DISCUSSION

Seventy-three determinations of N-NBF were obtained in 18 patients. The range of values was -92.5% to +91.1%. The distribution was:

	>	+40%	18
+16%	to	+39%	12
-15%	to	+15%	27 ("normal" range for N-NBF)
-16%	to	-39%	9
	<	-40%	7

4. Farrell, K, Bowen, R, and Beatty, J: Quantification of utilization of reserve oxygen transport capacity - initial studies in critically ill patient. Adv Exp Med Biol 169:775, 1984.

An example of sequential data obtained in a 25 year old male with an 82% total body surface burn and candida sepsis is shown below.

PvO ₂ (torr)	46.8	54.5	51	58	55	52
% N-NBF	31.2	38.8	29.2	65.4	62.3	69.9
CI (L/min/m ²)	6.8	5.63	6.36	5.22	5.17	5.89
Hb (grams/100 ml)	8.4	10.7	10.3	12.2	12.1	11.1
DO ₂ (ml/min/m ²)	816	856.3	915.2	908.7	883.7	931.2
VO ₂ (ml/min/m ²)	163.2	163.4	190.7	177.6	139.5	141.5
Date	11/9/83	11/12/83	11/13/83	11/14/83	11/15/83	11/16/83

Twelve profiles where N-NBF ranged from +25% to +70% were obtained in 5 septic burn patients. They were all clinically septic and had positive blood cultures for candida species and/or gram negative bacilli. The average percent total body surface burn was $57 \pm 17.7\%$. The interrelationship of cardiac index (CI) (range 4.2 - 6.8 L/min/m²) and hemoglobin concentration (Hb) (range 8.4 - 14.1 grams/100 ml) was evaluated by linear regression analysis. From this the following equation can be written:

$$CI = -.41 (Hb) + 10.37 \quad (r = -.868 \text{ and } n = 12)$$

Changes in body temperature between 37.5°C and 39.1°C did not correlate with changes in CI. There is a significant inverse relationship between CI and Hb ($p < 0.01$) in this subset of hyperdynamic septic burn patients. The implication from these data is that despite oxygen delivery being increase out of proportion to

any increase in oxygen demand, Hb and by implication oxygen demand remains a determinant of cardiac output.

Preliminary analysis of additional data suggests that there is a separate inverse relationship between CI and Hb in those patients who do not have increased N-NBF. Further work will involve the correlation of N-NBF with the clinical course of the patients and an analysis of the interrelationships of N-NBF with PvO_2 , DO_2 , VO_2 , CI, Hb, P_{50} , A-V difference and derived cardiovascular parameters (i.e., stroke volume index and stroke work indices).

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
				DA OG 6971	84 10 01	DD-DR&RIAR) 636
3. DATE PREV SUMMARY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISB'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
83 10 01	D. Change	U	U		CX	
10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER		
a. PRIMARY	62772A	3S162772A874	AF	162		
b. CONTRIBUTING						
c. CONTRIBUTING	STOG 82/83 - 6.2/4					
11. TITLE (Precede with Security Classification Code)						
(U) Evaluation of Burn Wound Care in Troops with Burn Injury						
12. SUBJECT AREAS						
06 05 Clinical Medicine 0615						
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING ORGANIZATION		16. PERFORMANCE METHOD
76 10		CONT		DA		C. In-House
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE		
a. DATE EFFECTIVE		EXPIRATION		FISCAL YEARS	a. PROFESSIONAL WORK YEARS	b. FUNDS (In thousands)
b. CONTRACT/GRANT NUMBER				84	1.5	80
c. TYPE		d. AMOUNT		85	1.5	84
e. KIND OF AWARD		f. CUM/TOTAL				
19. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION		
a. NAME US Army Institute of Surgical Research				a. NAME US Army Institute of Surgical Research Clinical Division		
b. ADDRESS (include zip code) Ft. Sam Houston, Texas 78234-6200				b. ADDRESS Ft. Sam Houston, Texas 78234-6200		
c. NAME OF RESPONSIBLE INDIVIDUAL Pruitt, BA, Jr				c. NAME OF PRINCIPAL INVESTIGATOR McManus, WF		
d. TELEPHONE NUMBER (include area code) 512-221-2720				d. TELEPHONE NUMBER (include area code) 512-221-3301		
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) Burn Injury; (U) Topical Therapy; (U) Sulfamylon; (U) 5% Sulfamylon Acetate Solution; (U) Volunteers; (U) Autografts; (U) Ram II						
23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)						
23. (U) The cause of infection in the wounds of burn patients has continued to be a major area of study in order to improve the survival of the severely burned patient. Such studies have included the use of 5% aqueous Sulfamylon soaks, the effects of burn wound excision on survival and function, and the effectiveness of skin substitutes. 24. (U) Patients admitted to the U.S. Army Institute of Surgical Research for care following thermal, chemical or electric injury may be, depending on the specific injury, included in studies of these newer modalities of care. 25. (U) 8310 - 8409. One hundred twenty eight patients were treated with 5% aqueous Sulfamylon soaks during the period of this report. Fifteen of these 128 patients exhibited mild cutaneous atopy. This low incidence of mild side effects of 5% aqueous Sulfamylon and its continued clinical effectiveness speak for the continued use of this valuable therapeutic agent.						

ANNUAL PROGRESS REPORT

PROJECT NO. 3S162772A874-00, APPLIED RESEARCH

REPORT TITLE: EVALUATION OF BURN WOUND CARE IN TROOPS WITH BURN
INJURY: 5% AQUEOUS SULFAMYLON SOAKS USED IN TOPICAL
TREATMENT OF BURNED SOLDIERS

US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234

1 October 1983 - 30 September 1984

Investigators:

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

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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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 twenty-eight 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 11.7% 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.

Burn injury
Topical therapy
5% Sulfamylon acetate solution
Humans

EVALUATION OF BURN WOUND CARE IN TROOPS WITH BURN INJURY: 5%
AQUEOUS SULFAMYLON SOAKS USED IN TOPICAL TREATMENT OF BURNED
SOLDIERS

During this reporting period of 1 October 1983 through 30 September 1984, the evaluation of 5% Sulfamylon acetate solution for topical treatment of the burn wound has continued at this Institute and was used in 128 patients (64%) of the 199 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 elements in preparation for split thickness autograft procedures or as continuous wet dressings to protect freshly excised wounds that are not autografted. In addition, when meshed cutaneous autografts are applied, dressings are soaked with 5% Sulfamylon acetate to decrease the rate of bacterial growth and to prevent desiccation of tissue exposed in the interstices of such grafts.

Fifteen patients (11.7%) demonstrated allergic reactions (atopy) with the use of 5% aqueous Sulfamylon solution and these fifteen patients demonstrated rapid resolution of the atopic reaction following administration of an antihistamine and/or discontinuation of the 5% aqueous Sulfamylon soaked dressings. Saline or other aqueous topical antimicrobial agents were substituted once 5% aqueous Sulfamylon dressings were discontinued 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 premature 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 speaks for continued use of this solution.

ANNUAL PROGRESS REPORT

PROJECT NO. 3S162772A874-00, APPLIED RESEARCH

REPORT TITLE: EVALUATION OF BURN WOUND CARE IN TROOPS WITH BURN INJURY:
BIOBRANE^R AND PORCINE - A COMPARATIVE STUDY

US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234

1 October 1983 - 30 September 1984

Investigators:

L. Willis Roberts, MD, Captain, MC
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Reports Control Symbol MEDDH-288(R1)

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ABSTRACT

PROJECT NO. 3S162772A874-00, APPLIED RESEARCH

REPORT TITLE: EVALUATION OF BURN WOUND CARE IN TROOPS WITH BURN
INJURY: BIOBRANE^R AND PORCINE - A COMPARATIVE
STUDY

US Army Institute of Surgical Research, Brooke Army Medical
Center, Fort Sam Houston, Texas 78234

Period covered in this report: 1 October 1983 - 30 September 1984

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Basil A. Pruitt, Jr., MD, Colonel, MC

Reports Control Symbol MEDDH-288(R1)

Biobrane^R has been compared with porcine cutaneous xenograft for initial coverage of excised burn wounds awaiting autografting in 12 adult patients.

The patients had burn sizes ranging from 29-92% total body surface burn (TBSB). They had both Biobrane^R and porcine cutaneous xenograft applied either to opposite extremities or adjacent to each other on the excised surface(s). This was done either intra-operatively or within 24 hours postexcision. Neither dressing was applied to a wound with visible evidence of suppuration. Each dressing was examined daily. Any spotty areas of nonadherence due to residual nonviable tissue were exposed and treated topically. Wounds were evaluated for two factors, granulation tissue formation and suppuration. The dressings were evaluated for adherence, conformation, and pliability. Each of these five factors was ranked on a scale of 1-5, 1 equalling the least and 5 equalling the most or best of each factor. Success or failure was also judged by percent autograft "take" on those wounds carried to grafting.

In every case, "take" of subsequently applied autografts was comparable on wounds treated with either material. Friedman's two-way analysis of variance by rank evaluation of the five factors revealed no statistically significant difference between the two dressings.

When percent viable cutaneous autograph "take" and these five factors were compared, this synthetic dressing was as efficacious as porcine cutaneous xenograft for initial coverage of the excised burn wound.

Synthetic membrane
Temporary wound closure

BIOBRANE^R AND PORCINE - A COMPARATIVE STUDY

Biologic dressings aid in the production of fibrovascular tissue, act as a barrier to exogenous microorganisms, control water vapor loss, and protect a freshly excised burn wound from desiccation. The prolonged adherence of a biologic dressing also implies successful "take" of subsequently applied autograft. Ideally, biologic dressings should also be without antigenicity or local and systemic toxicity (1). They should be pliable for conformation to the wound surface and they should be resterilizable with a long shelf life and low relative cost. Cadaver allograft and porcine cutaneous xenograft are used as biologic dressings at the US Army Institute of Surgical Research. Porcine cutaneous xenograft is used most frequently as it is more readily available. Numerous synthetic dressings have been designed to provide the same protective coverage as biologic dressings for excised burn wounds awaiting autografting. The purpose of this study^R has been to determine quantitatively the efficacy of Biobrane^R, a synthetic bilaminate membrane, when used as a burn wound dressing. Comparison with porcine cutaneous xenograft^R is being made to determine if the characteristics of Biobrane^R offer any clinical advantages over porcine cutaneous xenograft, a currently accepted device.

MATERIALS AND METHODS

Biobrane^R is a bilaminar composite membrane of a 6u thick silastic membrane bonded to a 360u thick nylon mesh. The outer layer of silastic is designed to have the water vapor permeability of human skin (2). The inner layer of nylon mesh is designed to provide adherence due to fibrous tissue ingrowth after the first 72 hours. The bilayer is biochemically bonded with purified porcine collagen to provide a hydrophilic surface for granulation tissue and to aid in initial adherence to the wound bed from binding with fibrin.

Patients with uncomplicated postresuscitation hospital courses have been studied. They were debrided prior to the availability of viable cutaneous autograft or their debrided wounds were judged clinically unacceptable for autografting. No more than 10% total body surface was tested by the application of Biobrane^R at a single procedure.

1. Pruitt BA Jr and Levine NS: Characteristics and uses of biologic dressings and skin substitutes. Arch Surg 119: 312-322, 1984

2. Wise DL: Biobrane^R, a biosynthetic skin prosthesis. In Burn Wound Coverings. Vol. II, CRC Press, Inc., Boca Raton, FLA, Chap. I, pp. 1-26

Twelve patients had burn sizes ranging from 29-92% TBSB. The patients had both Biobrane^R and porcine cutaneous xenograft applied either to opposite extremities or adjacent to each other on the excised surfaces(s) in random order. This was done either intraoperatively or within 24 hours postexcision. When application was not performed intraoperatively, it was to allow treatment of the wound bed with 5% sulfamylon solution soaked coarse mesh gauze for 24 hours. This provided further mechanical debridement of the wound bed in an antibacterial environment.

Intraoperative hemostasis was achieved with thrombin soaked telfa pads. The Biobrane^R was then placed with the dull side in contact with the wound bed, stretched (sometimes up to twice its original length in one dimension), and secured in place with staples. A dressing was then applied over the Biobrane^R in such a fashion as to assure contact with the wound surface. The dressing was removed after 24-48 hours when the Biobrane^R was adherent. The porcine cutaneous xenograft was also applied after hemostasis, generally in a transverse or circumferential orientation to the torso or limb. Each piece was placed in contact with the adjacent porcine cutaneous xenograft or normal epidermis. The porcine cutaneous xenograft was also held in place with a dressing or surgical netting for at least 24-48 hours.

Neither dressing was applied to a wound with visible evidence of suppuration. Each dressing was examined daily. Any spotty areas of nonadherence due to residual nonviable tissue were exposed and treated topically, e.g., application of 5% sulfamylon soaked laparotomy pads twice daily for debridement.

Wounds were evaluated for two factors, granulation tissue formation and suppuration. The dressings were evaluated for pliability and adherence and conformation to the wound surface. Each of these five factors was ranked on a scale of 1-5. The higher the score, the better the factor that is being evaluated.

Definition of Grading Scales

A. Formation of Granulation Tissue

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

B. Presence of Suppuration

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

C. Adherence of Test Dressing

1. None - Slides off test site spontaneously
2. Slight - Easily dislodged
3. Moderate - Easily removed with forceps
4. Good - Strips off with resistance
5. Excellent - Strips off with resistance and brisk bleeding

D. Conformation to Wound Surface

1. None - Buckled, wrinkled, poor wound contact
2. Slight - Conforms only to flat wound surface
3. Moderate - Torsion and tension must be applied to gain maximal wound surface contact with minimal wrinkling present
4. Good - As #3 but with no wrinkling of dressing
5. Excellent - Mimics skin

E. Pliability of Test Dressing

1. None - Stiff prior to wound application
2. Slight - Loses pliability unrelated to recipient test site condition
3. Moderate - Loses pliability related to absorption of wound exudate
4. Good - Maintains most of its pliability despite absorption of wound exudate
5. Excellent - Mimics skin

Success or failure was also judged by percent autograft "take" on those wounds carried to grafting.

RESULTS

The porcine cutaneous xenograft required changing every 3-5 days. Biobrane^R did not require changing unless there were large areas of nonadherence due to inadequately excised burn wound. Porcine cutaneous xenograft was noted to shift position initially when held in place using our standard technique as described. The method of attaching Biobrane^R precludes an appreciable amount of shift of the Biobrane^R. Two patients had wounds treated with porcine cutaneous xenograft and Biobrane^R for periods of time greater than three weeks which led to fibrotic granulation tissue formation in the excised wound beds treated with porcine xenograft but not the Biobrane^R treated wounds. General anesthesia was required for removal of adherent Biobrane^R if attempted after 72-96 hours. Biobrane^R removal early in the postoperative course was associated with large areas of nonadherence and, therefore, was amenable to pain prevention with parenteral analgesics. Removal of porcine xenograft did not require general anesthesia.

Friedman two-way analysis of variance by rank evaluation was performed on the appearance of the test sites. The mean ranks for granulation tissue formation were 3.38 for the porcine cutaneous xenograft and 3.5 for Biobrane^R treated wounds. A p value of 0.72 was not statistically significant. The mean ranks for the presence of suppuration were 3.55 and 3.89 for the porcine cutaneous xenograft and Biobrane^R respectively. A p value of 0.51 revealed no statistical significance.

Evaluation of the test dressings revealed no statistically significant difference between the xenograft and Biobrane^R with respect to adherence, conformation, or pliability. The mean ranks for adherence were 3.22 for the porcine cutaneous xenograft and 3.55 for the Biobrane^R. The p value equalled 0.74. The mean ranks were 4.4 for porcine cutaneous xenograft and 4.2 for Biobrane^R in the evaluation of conformation to the wound surface. The p value equalled 0.75. The mean ranks for pliability were 4.55 and 4.66 for the porcine cutaneous xenograft and Biobrane^R respectively. This had a p value of 0.74.

In every case, "take" of subsequently applied autografts has been comparable on wounds treated with either material.

CONCLUSIONS

Biobrane^R requires fewer dressing changes than porcine cutaneous xenograft. Biobrane^R does not shift initially due to the method of fixing it to the wound surface. Removal of adherent Biobrane^R requires general anesthesia. Removal of porcine cutaneous xenograft does not. Biobrane^R is readily available and resterilizable.

Both Biobrane^R and porcine cutaneous xenograft allow moderate to good granulation tissue formation. Suppuration under either dressing is rare. Porcine cutaneous xenograft and Biobrane both had moderate to good adherence to the wound bed and good to excellent conformation and pliability. When percent autograft "take" is compared, Biobrane^R is as efficacious as porcine cutaneous xenograft for initial coverage of the excised burn wound.

PRESENTATIONS

Roberts LW: Biobrane^R and porcine - a comparative study.
Seventeenth Annual Meeting of the American Burn Association,
Orlando, Florida, 28-30 March 1985

PUBLICATIONS

None

FINAL REPORT

PROJECT NUMBER: 3S162772A874-00, APPLIED RESEARCH

PROJECT TITLE: EVALUATION OF BURN WOUND CARE IN TROOPS WITH
BURN INJURY: The Use of DuoDermTM Dressings
in the Management of Skin Graft Donor Sites

US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234-6200

1 October 1983 - 30 September 1984

INVESTIGATORS

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REPORT CONTROL SYMBOL - MEDDH-288 (R1)

UNCLASSIFIED

ABSTRACT

PROJECT NUMBER: 3S162772A874-00, APPLIED RESEARCH

PROJECT TITLE: EVALUATION OF BURN WOUND CARE IN TROOPS WITH
BURN INJURY: The Use of DuoDermTM Dressings
in the Management of Skin Graft Donor Sites

US Army Institute of Surgical Research, Brooke Army Medical Center, Fort Sam Houston, Texas 78234-6200

PERIOD COVERED IN THIS REPORT: 1 Oct 83 through 30 Sep 84

INVESTIGATORS: L. Willis Roberts, MD, Captain, MC
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REPORT CONTROL SYMBOL: MEDDH-288 (R1)

DuoDermTM has been compared with fine mesh gauze for the management of skin graft donor sites in 20 patients. These patients had burn sizes ranging from 13 to 59 percent of the total body surface area. DuoDermTM and fine mesh gauze (FMG) were applied either to mirror image donor sites or to adjacent donor sites with eight centimeters of intervening healthy skin. The FMG was removed from the donor site as soon as re-epithelization was complete. The DuoDermTM was changed whenever "leakage" occurred. The patient evaluated donor site pain on a scale of 0 to 10 before each change of DuoDermTM (0 = no pain, 10 = intolerable pain). Any pain during dressing changes was also noted. Gram stain and cultures were done if there were clinical signs of infection. The DuoDermTM donor site wound was left open on the sixth to seventh post-operative day if it was re-epithelized.

Re-epithelization occurred after an average of 7.35 days for DuoDermTM and 12.47 days for FMG donor sites. Student's t-test revealed a p value of <0.001. Reharvest time was an average of 10.72 and 14.16 days for the DuoDermTM and FMG sites, respectively (p = <0.001). The average time between re-epithelization and reharvest was 3.37 days for DuoDermTM and 1.69 days for FMG sites. Student's t-test revealed a p value of 0.0069. Of the 116 tests for pain, 72 percent had a value of 1 or 0. FMG was more painful in 50, DuoDermTM in 6. Sixty tests were neutral. FMG was more painful in one or more tests of 15 patients, DuoDermTM in four of the same patients. Five patients never identified a difference. Student's t-test of the average difference in

DONOR SITE DRESSING
HYDROCOLLOID

pain revealed that $p = 0.045$. Seventy-six percent of the DuoDERM™ dressing changes were painful to the patient. One patient had not pain at any DuoDERM™ dressing changes.

DuoDerm™ permits donor site re-epithelization five days earlier than FMG. The time between re-epithelization and reharvesting is longer for the DuoDerm™ site, but reharvesting time may still be shortened by three days. The pain felt by patients with either dressing is minimal. DuoDerm™ causes less pain between dressing changes, but pain was noted at dressing change 76 percent of the time.

THE USE OF DuoDermTM DRESSINGS IN THE MANAGEMENT OF SKIN GRAFT DONOR SITES

In patients requiring multiple viable cutaneous autograft procedures, donor site healing time can impact the length of hospital stay and survival. Fine mesh gauze (FMG) is sufficient for re-epithelization of donor sites without infection in the vast majority of patients. However, it has been shown that a moist environment, free of infection, permits more rapid re-epithelization of partial thickness wounds (1). DuoDermTM was compared with FMG for the management of skin graft donor sites in an attempt to determine whether an occlusive dressing provides faster re-epithelization and subsequent earlier reharvesting of the donor site and whether this dressing reduced donor site pain.

MATERIALS AND METHODS

DuoDermTM is a bilaminate structure with an outer layer of polyurethane foam which is oxygen and water vapor impermeable. Its inner layer is hydrophilic and consists of a hydrocolloid polymer complex. The inner layer is adherent to normal skin but not to open wounds (2).

Patients included were awake, alert, and responsive; required mirror image of adjacent donor sites; and had burns less than 60 percent of the total body surface area. Patients who had only one of the donor sites harvested previously were excluded from the protocol.

Twenty consecutive patients who met the above criteria were studied. They had burn sizes ranging from 13 to 59 percent of their total body surface area. In an attempt to maintain consistency in depth of donor sites, the same physician harvested the donor skin with the same blade and dermatome setting when possible. They had DuoDermTM and FMG applied earlier to the mirror image donor sites or to the adjacent donor sites with eight centimeters of intervening healthy skin.

The FMG was placed over the fresh donor sites in complete contact with the wound. Hot saline-soaked laparotomy pads were then placed on the FMG. Post-operatively, the FMG donor sites were treated according to the standard protocol using heat lamps and were generally dry within 24 hours. No soaking of the FMG donor site occurred until at least the eighth post-operative

¹Winter GD: Formation of the scab and the rate of epithelization of superficial wounds in the skin of the young domestic pig. *Nature* 193:293-294, 1962.

²DuoDermTM Hydroactive Dressings (Technical Bulletin). ConvaTec, PO Box 4000, Princeton, New York 08540, pp 4-5.

day. The FMG was separated from the wound bed as soon as re-epithelization was complete.

The DuoDerm™ was applied to the donor site(s) after hemostasis was obtained using thrombin-soaked telfa pads. The wound exudate was absorbed by the hydrophilic layer forming a pocket under the outer DuoDerm™ layer that made the DuoDerm™ analogous to a "blister." When the "blister" became large enough, the fluid dissected between the DuoDerm™ and the normal epidermis and a leak at the edge of the DuoDerm™ resulted. It was at this time that the DuoDerm™ was changed. The DuoDerm™ sites were managed utilizing sterile technique and the wound was irrigated with saline. The surrounding epidermis was then carefully dried and the DuoDerm™ reapplied. The dressing was placed so that at least 1.5 centimeters extended beyond the wound margins to allow for adequate adherence. The edges of the dressing were not taped. The DuoDerm™ donor site was inspected on the sixth to seventh post-operative day and left open if re-epithelization had occurred.

The patient was asked to evaluate the pain at each donor site on a scale of zero to 10 before each change of DuoDerm™ with zero equalling no pain and 10 equalling intolerable pain. Pain during dressing changes was also noted. Patients were maintained on routine pain control regimens. Gram stain and cultures were done if there was clinical signs of infection. Signs of infection were considered to be erythema around the wound margins, fluctuance with gross purulence, an increase in pain or tenderness, and an increase in wound edema.

RESULTS

The DuoDerm™ dressings frequently had to be changed twice per day for the first two to three post-operative days. The frequency of dressing changes then decreased due to the decrease in leakage from under the DuoDerm™. Posterior donor sites forced an increase in frequency of DuoDerm™ dressing changes as the weight of the patient did not allow accumulation of exudate under the dressing. Posterior dressing changes entailed moving the patient, thus increasing chances of graft disruption. One patient dislodged his posterior DuoDerm™ dressing repeatedly, thus contaminating his donor sites. Maintenance of sterile technique was difficult in patients with large donor sites and more frequent dressing changes were needed. There were not enough large donor sites to substantiate this clinical impression.

The fluid under the DuoDerm™ had a unique odor that was noted at each dressing change and was not associated with any signs of infection. The appearance of this fluid is a thick, creamy fluid exactly like purulence. This appearance can be distressing to those unfamiliar with the nature of this fluid.

The wound bed itself was clean and pink after saline irrigation with small areas of soft but adherent hydrocolloid, which was not removed but was covered by the next DuoDermTM dressing. Punctate skin buds were frequently visible on the wound bed on the second or third day following harvesting of the autograft. This was noted to be the time when exudate and frequency of dressing changes decreased. The DuoDermTM donor sites were smoother in texture than the FMG donor sites after re-epithelization (3). Although the DuoDermTM-covered donor sites re-epithelized earlier, the new epidermis initially appeared clinically to be monolayer. Blanching with pressure was prominent as were angiomatous vascular formations under the "monolayer." Therefore, the donor sites were not deemed reharvestable immediately after this early re-epithelization.

Re-epithelization occurred after an average of 7.35 days for DuoDermTM and 12.47 days for FMG donor sites. Student's t-test revealed a p value of <0.001 . Reharvest time was an average of 10.72 and 14.16 days for the DuoDermTM and FMG sites, respectively. The average time between re-epithelization and reharvest was 3.37 days for DuoDermTM and 1.69 days for FMG sites. Of the 116 tests for pain, 72 percent had revealed a value of 1 or 0. FMG was more painful in 50, DuoDermTM in 6. Sixty tests were neutral. FMG was more painful in one or more tests of 15 patients, DuoDermTM in four of the same patients. Five patients never identified a difference. Student's t-test of the average difference in pain revealed that $p = 0.049$. Seventy-six percent of the DuoDermTM dressing changes were painful to the patient. One patient had no pain at any DuoDermTM donor site when the "blister" was large or had already leaked just prior to the dressing change.

No patients had donor site infections on either the DuoDermTM or FMG donor sites. Six patients had a total of 18 cultures of the "blister" fluid, most of which were done at the beginning of the study because of the appearance of this fluid. One patient had *staphylococcus epidermidis* cultured from the donor site. One additional patient had *staphylococcus aureus* cultured when one area of his donor site was not healing at the same rate as the rest. His donor site was healed two days later at the time the culture results were returned.

DISCUSSION

Re-epithelization of the donor site leads to a decrease in wound exudate, which continues to decrease until re-epithelization is complete. The healed DuoDermTM donor site is smoother in appearance than the FMG donor site. This may mean there is

³Miller TA and White WL: Healing of second degree burns. Comparison of effects of early application of homograft and coverage with tape. *Plast Reconstr Surg* 49:552-557, 1972.

no loss of dermis due to desiccation as in the wound that is allowed to re-epithelize in a dry environment.

It is not easy to care for large DuoDerm™ donor sites. The ambient temperature causes partial melting of the DuoDerm™, which then shifts off the wound bed due to its own weight and the weight of the "blister" fluid. DuoDerm™ does not seem to be suitable for posterior donor sites because the weight of the patient does not allow accumulation of "blister" fluid. This results in frequent dressing changes or possibly a missed leak if dressing inspection is not thorough.

DuoDerm™ permits donor site re-epithelization five days earlier than FMG. The time between re-epithelization and reharvest is longer for the DuoDerm™ site, but reharvesting time may still be shortened by three days. Thus, the use of DuoDerm™ may shorten a hospital stay. No increase in donor site infection occurs when sterile technique is used.

The pain felt by patients with either dressing is minimal. DuoDerm™ causes less pain between dressing changes, but causes pain at dressing changes 76 percent of the time. Dressing changes are frequent, generally twice a day for two to three days post-operatively. The pain at the DuoDerm™ donor site prior to dressing changes may be due to fluid shifting under the DuoDerm™ and irritating the wound bed. Pain at dressing change or the frequency of dressing changes needs to be decreased for DuoDerm™ to make a significant decrease in patient pain.

PUBLICATIONS/PRESENTATIONS

None.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1 AGENCY ACCESSION	2 DATE OF SUMMARY	REPORT CONTROL SYMBOL
				DA 06 6970	84 10 01	DD-DR&BIAR) 636
3. DATE PREV SUMMARY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISB'N INSTR'N	9. LEVEL OF SUM A WORK UNIT
83 10 01	D. Change	U	U		CX	
10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER		
a. PRIMARY	62772A	3S162772A874	AF	163		
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11. TITLE (Precede with Security Classification Code)						
(U) Studies of the Neuroendocrine Abnormalities in Burn Injury						
12. SUBJECT AREAS						
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13. START DATE	14. ESTIMATED COMPLETION DATE		15. FUNDING ORGANIZATION		16. PERFORMANCE METHOD	
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17. CONTRACT/GRANT				18. RESOURCES ESTIMATE		
a. DATE EFFECTIVE		EXPIRATION		FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
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a. NAME US Army Institute of Surgical Research				a. NAME US Army Institute of Surgical Research Internal Medicine Branch		
b. ADDRESS (include zip code) Ft. Sam Houston, Texas 78234-6200				b. ADDRESS Ft. Sam Houston, Texas 78234-6200		
c. NAME OF RESPONSIBLE INDIVIDUAL Pruitt, BA, Jr				c. NAME OF PRINCIPAL INVESTIGATOR Vaughan, GM		
d. TELEPHONE NUMBER (include area code) 512-221-2720				d. TELEPHONE NUMBER (include area code) 512-221-5416		
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23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)						
23. (U) To determine the hormonal abnormalities in burned soldiers.						
24. (U) To characterize pineal activity as an index of the extent and nature of the altered sympathetic function in burned soldiers.						
25. (U) 8310 - 8409. The pineal gland of human and Syrian hamsters is controlled by the sympathetic nervous system: the nocturnal surge of melatonin secretion depends on intact pineal sympathetic innervation. Despite the known tonic sympathetic hyperactivity in burns and its expression in the cardiovascular system, we could find no evidence for excess melatonin secretion. In fact, burned soldiers had a diminished, though persisting, nocturnal melatonin surge and hamsters had reduced daytime pineal melatonin content. Sympathetic activity controlling melatonin secretion is regulated independently from that controlling other systems. The inhibitory responses in melatonin indicate pineal involvement in the overall endocrine response to injury and suggest another avenue of endocrine modulation, in that the pineal is known to be a neuroendocrine transducer which integrates other endocrine systems, biorhythms, and environmental influences. A SWAG milestone, perhaps achievable in two or three years, will be the determination of which metabolic alterations are adaptive and which require therapy. The "end product" will be the ability to alter one or more metabolic variables in such a way as to prevent mortality and reduce morbidity in injured soldiers.						

ANNUAL RESEARCH PROGRESS REPORT

PROJECT NO.: 3S162772A874, APPLIED RESEARCH

REPORT TITLE: STUDIES OF THE NEUROENDOCRINE
ABNORMALITIES IN BURN INJURY:
PINEAL FUNCTION IN BURNS: EVIDENCE FOR
PARTITIONED CONTROL OF SYMPATHETIC
FUNCTION

US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
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1 October 1983 - 30 September 1984

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ABSTRACT

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Burn injury in humans or rats is a model of marked elevation of general sympathetic activity lasting for weeks, manifested in part by increased heart rate, metabolic rate, core temperature, and elevated plasma and urinary catecholamines. Plasma melatonin was sampled at 2-h intervals for 24 h in 9 control subjects and 11 patients with severe burn injury. Daytime melatonin was not different between the groups but nighttime values were significantly lower at night in the burn patients. A significant nocturnal surge still occurred in the patients. Resting heart rate and rectal temperature were elevated in the burn patients. In male Sprague-Dawley rats, pineal melatonin content did not differ between controls and those with an experimental burn at 4 h into the light phase nor during the nocturnal surge. Male Syrian hamsters with burns had lower daytime pineal melatonin content than did controls, but the nocturnal surge in pineal melatonin was not significantly different between groups, nor was daytime morning serum melatonin. Sympathetic activity appears partitioned, with that controlling melatonin (nocturnal surge) regulated independently. In agreement with our previous findings in other models, melatonin is not a marker for general sympathetic activity, even following severe burn injury.

Melatonin
burns
sympathetic

pineal
human
nervous system

INTRODUCTION

Extensive information in the literature indicates that the nocturnal rise of pineal melatonin synthesis in rats, hamsters, and humans is determined by the pineal's sympathetic innervation (1,2,3). Furthermore, in the rat, this sympathetic control of melatonin includes norepinephrine as the post-ganglionic neurotransmitter in the pineal (4). Burn injury in humans (5,6) and rats (7,8) is a model of marked persistent elevation of general sympathetic activity, manifested in part by marked elevations of plasma and urinary catecholamine content, heart rate, and resting heat production. We have investigated endogenous melatonin in this setting of sympathetic hyperactivity, in order to determine if melatonin is a marker for general sympathetic activity.

1. Reiter, R.J., M.K. Vaughan, G.M. Vaughan, S. Sorrentino, Jr., and R.J. Donofrio (1975) The pineal as an organ of internal secretion. In: *Frontiers of Pineal Physiology*. M.D. Altschule, ed. MIT Press, Cambridge, pp. 54-174.

2. Reiter, R.J. (1980) The pineal and its hormones in the control of reproduction in mammals. *Endocr. Rev.*, 1:109-131.

3. Vaughan, G.M. (1984) Melatonin in humans. In: *Pineal Research Reviews*. R.J. Reiter, ed. Alan R. Liss, Inc., New York, Vol. 2, pp. 141-201.

4. Klein, D.C., ed. (1982) *Melatonin Rhythm Generating System*. Karger, Basel.

5. Vaughan, G.M., and R.A. Becker (1984) Thyroid hormones and catecholamines in burn patients: A hypermetabolic low T3 syndrome. In: *Norepinephrine*. M.G. Ziegler and C.R. Lake, eds. Williams and Wilkins Company, Baltimore, Chap. 30, pp. 450-470.

6. Wilmore, D.W., J.M. Long, A.D. Mason, Jr., R.W. Skreen, and B.A. Pruitt, Jr. (1974) Catecholamines: mediator of the hypermetabolic response to thermal injury. *Ann. Surg.*, 180:653-669.

7. Aprille, J.R., N. Aikawa, T.C. Bell, H.H. Bode, and D.F. Malamud (1979) Adenylate cyclase after burn injury: resistance to desensitization by catecholamines. *J. Trauma*, 19:812-818.

8. Herndon, D.N., D.W. Wilmore, A.D. Mason, Jr., and B.A. Pruitt, Jr. (1977) Humoral mediators of nontemperature-dependent hypermetabolism in 50% burned adult rats. *Surg. Forum*, 28:37-39.

MATERIALS AND METHODS

We sampled blood through an indwelling venous catheter into heparinized tubes at 2-h intervals over 24 h in 11 accidentally burned patients on postburn day (mean $\pm$ SE) 15 ± 3 with total burn size $41 \pm 6\%$ of body surface area, age 36 ± 4 years, and weight 88 ± 8 kg. Nine uninjured control subjects (normal, except that four had alopecia areata) were similarly sampled on a hospital ward, at age 36 ± 4 years and weight 89 ± 4 kg. All individuals were sampled in the supine position. The burn patients slept between 23 h and 07 h when the ward lights were dimmed. The controls slept between 23 h and 07 h when the lights were turned out. Although light intensity measurements were not taken and the ambient lighting at night may have been a little greater for the burn patients than for the controls, nocturnal samples were taken usually while the burns and controls remained asleep with their eyes closed. Morning (08-11 h) resting recumbent pulse rate and rectal temperature was recorded.

One non-burned man with residual hypothalamic destruction from a suprasellar extension of a pituitary tumor was sampled hourly around the clock, sleeping in darkness 23-07 h. Melatonin measurements on other samples from this patient taken on a previous occasion have been reported (9).

Adult male Syrian hamsters housed in L/D 14/10h received a standard full-thickness scald burn of 23% of body surface under general anesthesia (burn), while others (sham) received only fur clipping (10, 11). Hamsters were sacrificed 4 h into the light phase as separate groups (6-10/group) on various postburn days or during the dark phase on postburn day 7 or 14. Pineals were immediately frozen (-70°C) and in one case serum was saved for later analysis.

9. Vaughan, G.M., S.D. McDonald, R.M. Jordan, J.P. Allen, R. Bell, and E.A. Stevens (1979) Melatonin, pituitary function and stress in humans. *Psychoneuroendocrinol.*, 4:351-362.

10. Walker, H.L., and Mason, A.D., Jr. (1968) A standard animal burn. *J. Trauma* 8:1049-1051.

11. Vaughan, G.M. (1982) Studies of neuroendocrine abnormalities in burn injury: II. Thyroidal, reproductive and pineal function in a hamster burn model. In: U.S. Army Institute of surgical Research Annual Research Progress Report, FY 82. U.S. Army Medical Research and Development Command, Frederick, Maryland, pp. 81-105.

Adult male Sprague-Dawley rats (L/D 14/10h) received a standard full-thickness burn of 60% of body surface (burn), or only fur clipping (sham) under anesthesia. Others were left untreated (control). Rats were sacrificed 4 h into the light phase on postburn day 8 or 14 (9-14 per treatment group at each postburn day) or members of a group were sacrificed at times during the dark phase on postburn day 22. Pineals were saved for analysis.

Melatonin was radioimmunoassayed with the Rollag antibody and ¹²⁵I-melatonin analogue tracer as previously described (12), with further modification. Plasma, or pineal sonicated and diluted in pH 7 phosphate-buffered saline containing 0.1% gelatin (Sigma, No. G2625) (0.5 ml), was extracted in 12 X 75 mm borosilicate glass culture tubes (Fisher) with 2 ml of chloroform (Burdick-Jackson, No. 048), which was washed with 0.5 ml 0.1 N HCl then 0.5 ml 0.1 N NaOH, then evaporated in a vacuum centrifuge and reconstituted in 0.5 ml gelatin-phosphate-buffered saline overnight at 4C. These samples were washed twice with 2 ml of petroleum ether (Burdick-Jackson, No. 317), which was aspirated, and the samples were placed at 4C for at least 30 min. Duplicate 0.2 ml aliquots were placed in separate 12 X 75 mm glass tubes in an ice bath. Extraction and all washes were by vortexing for 7 sec, and aspiration followed centrifugation at 1700G for 10 min. Antibody (0.1 ml of 1:64,000 dilution, in 1:400 normal rabbit gammaglobulin from Cappel, in pH 7 phosphate-buffered saline with EDTA) and ¹²⁵I-analogue tracer (0.05 ml, initially 8000 cpm, usable for up to two months stored at 4C in gelatin-phosphate-buffered saline, tracer obtained from Meloy Laboratories) were added and incubated together in the sample for 24 h at 4C. Bound tracer was isolated by addition of 3 ml 95% ethanol at 4C, incubation at 4C for 10 min, centrifugation at 1700G for 45 min at 4C, and discarding of the supernatant. Standards in gelatin-phosphate-buffered saline were carried through all the above procedures. The bound counts (corrected for nonspecific binding) were analyzed in standard logit-log

12. Vaughan G.M., J.P. Allen, W. Tullis, T.M. Siler-Khodr, A. de la Pena, and J.W. Sackman (1978) Overnight plasma profiles of melatonin and certain adenohipophyseal hormones in men. *J. Clin. Endocrinol. Metab.* 47:566-571.

fashion. Assay recovery of 50 pg/ml melatonin added to serum from 9 individuals averaged 97.4% (not significantly different from 100%). Bound counts in the zero-standard tubes were 40% and the half-inhibiting standard concentration was 47 pg/ml. The least detectable concentration (at mean B/Bo minus 2 SD for zero-standard tubes) was 4 pg/ml. The within-assay coefficient of variation was 8% at 15-20 pg/ml, 10% at 31-35 pg/ml, and 9% at 57 pg/ml. The between-assay coefficient of variation was 11% at 57 pg/ml. Dilution of nighttime human and hamster serum pools showed parallelism, and addition of 50 or 100 pg/ml to daytime pools was associated with quantitative recovery (Fig. 1). Fractionation of pooled extracts by high-pressure liquid chromatography (13) yielded a single peak of immunoactivity for human and hamster serum at the retention time for authentic melatonin. Assay of serum and heparinized plasma gave the same results.

Melatonin values for each human subject were fitted to a periodic function (14) with clock time converted to angular units both on a 24 h scale (0-360 degrees for 0-24 h) and on a 12 h scale (0-360 degrees for 0-12 h and for 12-24 h) and expressed as the cos and the sin of the angle with respect to each scale. This allowed the time of a sample to be expressed as four separate terms, two for the 24-h scale and two for the 12-h scale, which were the independent variables in a regression in which the dependent variable was melatonin concentration. Nighttime melatonin was evaluated by reading the peak value and the time of its occurrence from the best-fit curve. This analysis was done for each subject and on data pooled within the burn or control group. Subject-specific results were analyzed with a t-test for independent means.

For the rat daytime data, a t test for several means was used, employing the Bonferroni correction to protect against bias toward significance resulting from multiplicity of comparisons (15). The rat nighttime data

13. Tamarkin, L., P. Abastillas, H.-A. Chen, A. McNemar, and J.B. Sidbury (1982) The daily profile of plasma melatonin in obese and Prader-Willi syndrome in children. *J. Clin. Endocrinol. Metab.*, 55:491-501.

14. Batschelet, E. (1974) Statistical rhythm evaluation. In: *Biorhythms and Human Reproduction*. M. Ferin, F. Halberg, R.M. Richart, and R.L. Vande Wiele, eds. John Wiley & Sons, New York, Chap. 2, pp. 25-35.

15. Brown, M.B., L. Engelman, J.W. Frane, M.A. Hill, R.I. Jennrich, J.D. Toporek (1983) *BMDP Statistical Software*. W.J. Dixon, ed. University of California Press, Berkeley.

were subjected to stepwise multiple regression analysis with group (control and sham together versus burn) and linear and quadratic functions of time as independent variables. This allowed testing of whether the nocturnal pineal melatonin surge was different between the burn group and the other two groups combined. All analyses were done on a DEC VAX 11/780 computer with the BMDP statistical package (15).

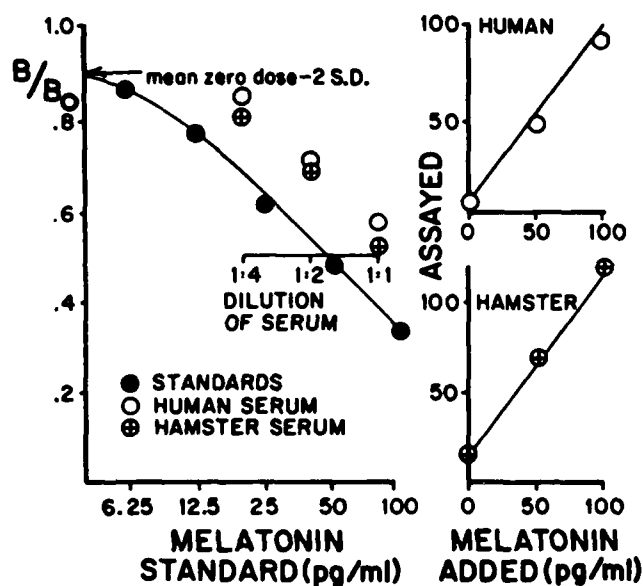


Figure 1. Parallelism and quantitative recovery in the melatonin assay.

RESULTS

Figure 2 indicates that resting pulse and temperature were elevated in burns compared to controls, but plasma melatonin was lower in burns, with differences which were statistically significant at night. Table 1 gives the sex, month of sampling, and the values for melatonin and the time at the best-fit curve peak for each subject. Not indicated in the table is that the rhythm was statistically significant in each subject of both groups. Table 2 gives reanalyses of the curve peak melatonin values, subdividing the groups in several ways. The results indicate that the lower values in burns were not influenced by inclusion of patients with alopecia areata in the control group, nor by month of sampling.

The one patient with hypothalamic destruction (Fig. 2) did not have a statistically significant melatonin rhythm, as reported earlier in this patient (9) and indicates the necessity for an intact neural pathway (which courses through the hypothalamus and finally through the sympathetics) for occurrence of the nocturnal melatonin surge (3).

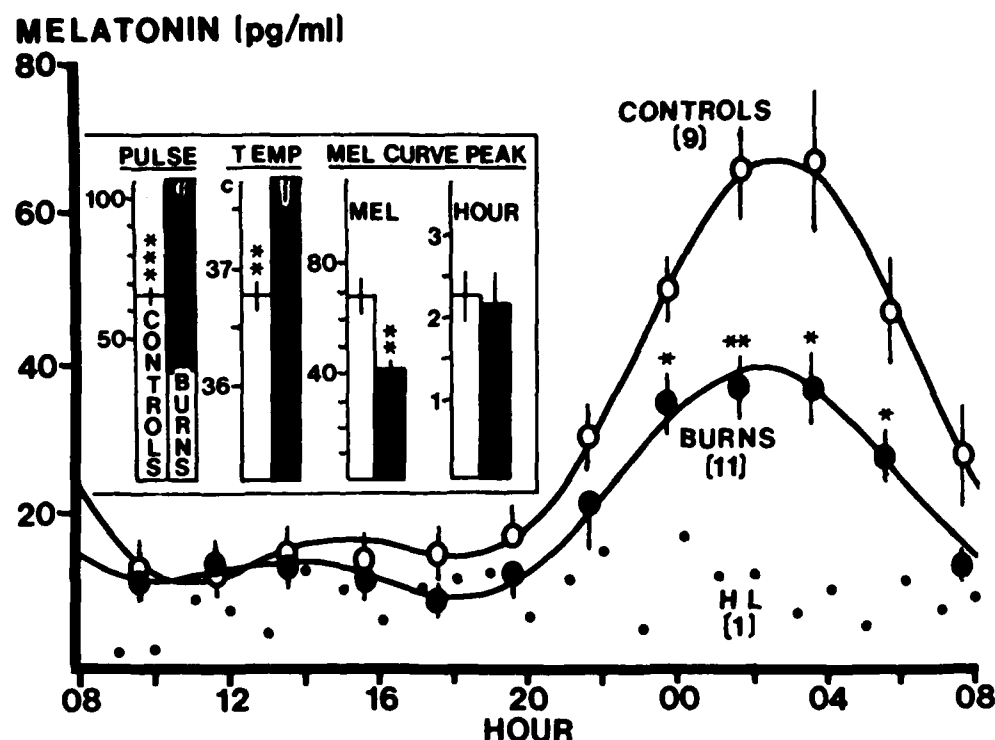


Figure 2. Circulating melatonin (mean $\pm$ SE) in human subjects. Numbers in parentheses indicate the number of subjects. Pulse and rectal temperature (TEMP) representing resting recumbent morning values (08-11 h), the melatonin value (MEL) from the peak of the individual best-fit periodic function curves, and the corresponding peak time (hour) are shown in the inset. The group curves are based on pooled data. "HL" represents one unburned patient with a hypothalamic lesion whose values are shown by unconnected dots.

* $p < 0.05$, and ** $p < 0.01$, burns versus controls.

Table 1.

	Subject	Sex	Month	Melatonin Peak	
				Mel (pg/ml)	Time (h)
Controls	1	M	May	100	3.5
	2	M	May	46	0.4
	3	M	Apr	44	2.5
	4*	M	May	95	3.6
	5*	M	May	75	2.7
	6*	M	Mar	73	2.3
	7*	M	Mar	42	2.0
	8	M	Aug	76	1.8
	9	M	Sep	68	1.6
Burns	10	M	May	60	3.4
	11	F	May	57	23.7
	12	M	May	40	2.2
	13	M	May	27	2.4
	14	M	May	26	2.1
	15	F	Sep	39	0.5
	16	M	Sep	44	1.5
	17	M	Sep	45	2.1
	18	M	Sep	30	4.0
	19	M	Aug	54	2.8
	20	M	Sep	34	3.2

* Alopecia areata

Table 2.

CURVE PEAK MELATONIN [pg/ml $\pm$ SE (n)]

Controls	A. All (7 in Mar-May, 2 in Aug-Sep)	68.8 $\pm$ 7.1 (9)
	B. Without AA (3 in Apr-May, 2 in Aug-Sep)	66.8 $\pm$ 10.3 (5)
	C. Only AA (2 in Mar, 2 in May)	71.3 $\pm$ 10.9 (4)
	D. Mar-May	67.9 $\pm$ 9.2 (7)
	E. Aug-Sep	76, 68 (2)
Burns	F. All (5 in May, 6 in Aug-Sep)	41.5 $\pm$ 3.6 (11) A, B
	G. Only men (4 in May, 5 in Aug-Sep)	40.0 $\pm$ 4.0 (9) A, B
	H. May	42.0 $\pm$ 7.2 (5) ()D
	I. Aug-Sep	41.0 $\pm$ 3.5 (6) All below each in E.

AA, alopecia areata.

(*) $p = 0.05$; * $p < 0.05$, ** $p < 0.01$, versus controls specified by letter.

Figure 3 shows that daytime (4 h into light) pineal melatonin in burned hamsters was significantly less than that in shams at the end of the second week post burn. Values at various times during the night on postburn day 7 and at 04 h on postburn day 14 were not significantly different between burn and sham, but at 20 h on postburn day 7 (end of the light phase) the burn group had significantly lower pineal melatonin. Figure 4 indicates that on postburn day 14, burned hamsters had significantly suppressed pineal melatonin at 4 h into the light phase, though serum melatonin was unchanged.

PINEAL MELATONIN

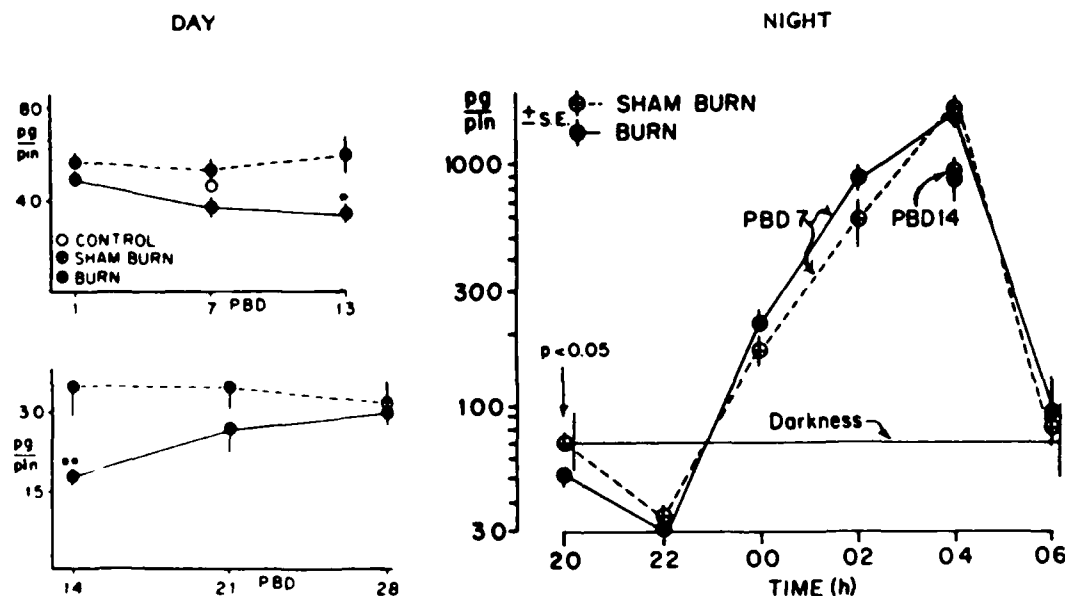


Figure 3. Syrian hamster pineal melatonin, mean $\pm$ SE. PBD, postburn day. * $p < 0.05$, and ** $p < 0.01$, burn versus sham burn.

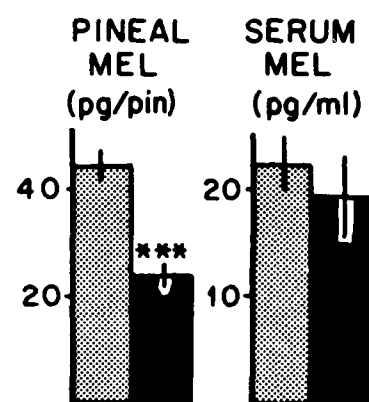


Figure 4. Hamster pineal and serum melatonin (mean $\pm$ SE) during the day on postburn day 14. Shaded bars, sham burns. Solid bars, burns. ***p < 0.001.

Figure 5 shows that daytime (4 h into light) pineal melatonin did not change in burned rats, and the nocturnal surge was the same among controls, shams, and burns. Stepwise regression analysis of the night data indicated a highly significant relationship of melatonin with time. After accounting for this source of variation in melatonin, there was no significant difference in melatonin between burn and other groups.

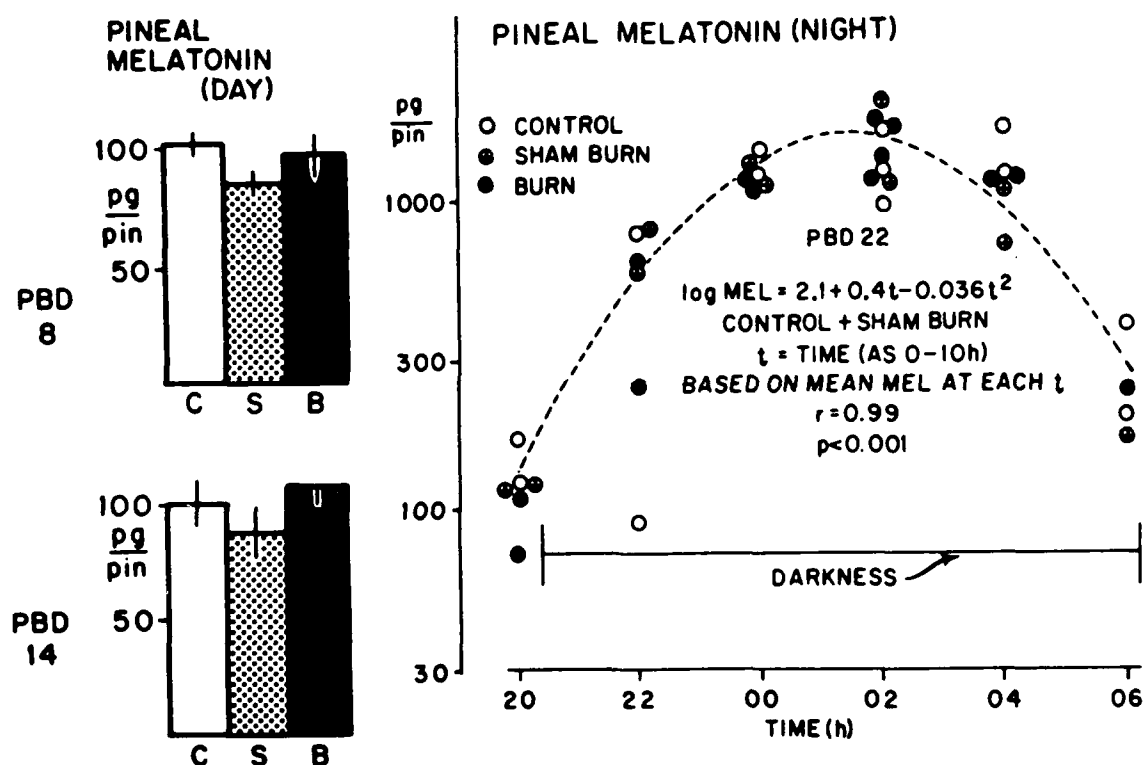


Figure 5. Rat pineal melatonin (mean $\pm$ SE). C, control; S, sham burn; B, burn; PBD, postburn day. The dashed line represents the best fit quadratic regression for combined control and sham burn groups.

DISCUSSION

The principal results of this study are that in three species, burn injury was not associated with elevated melatonin values during the day or night and a significant nocturnal surge of melatonin occurs whether or not burn injury is present. These results are interesting in light of two other observations in the literature. First, the sympathetic innervation of the pineal in all three species controls melatonin production, in that bilateral lesions that interrupt the central or peripheral sympathetic pathway to the pineal prevent the nocturnal rise of pineal melatonin synthesis or of circulating or excreted melatonin (1-3, 9, 16-21).

Second, burn injury is a model of increased tonic sympathetic activity associated with tachycardia, increased blood flow to the wound and to splanchnic organs with relative vasoconstriction (normal blood flow) in uninjured skin, increased glucose production and lypolysis with maintenance of normal or elevated insulin secretion, increased muscle protein breakdown and synthesis with net weight loss, increased metabolic rate (heat production) in proportion to elevated circulating and excreted catecholamines, and increased core temperature in

16. Eichler, V.B., and R.Y. Moore (1971) Pineal hydroxyindole-O-methyltransferase and gonadal responses to blinding or continuous darkness blocked by pineal denervation in the male hamster. *Neuroendocrinol.*, 8:81-85.

17. Klein, D.C., J.L. Weller, and R.Y. Moore (1971) Melatonin metabolism: Neural regulation of pineal serotonin:acetyl coenzyme A N-acetyltransferase activity. *Proc. Nat. Acad. Sci. U.S.A.*, 68:3107-3110.

18. Kneisley, L.W., M.A. Moskowitz, and H.J. Lynch (1978) Cervical spinal cord lesions disrupt the rhythm in human melatonin excretion. *J. Neural. Transm.*, [Suppl.] (13):311-323.

19. Moore, R.Y., and D.C. Klein (1974) Visual pathways and the central neural control of a circadian rhythm in pineal serotonin N-acetyltransferase activity. *Brain Res.*, 71:17-33.

20. Panke, E.S., M.D. Rollag, and R.J. Reiter (1979) Pineal melatonin concentrations in the Syrian hamster. *Endocrinol.*, 104:194-197.

21. Tetsuo, M., R.J. Polinsky, S.P. Markey, and I.J. Kopin (1981) Urinary 6-hydroxymelatonin excretion in patients with orthostatic hypotension. *J. Clin. Endocrinol. Metab.*, 53:607-610.

proportion to elevated metabolic rate (5, 6, 8, 22-29). This pattern includes a strong component of beta-adrenergic response, further evidenced by blunting of the hypermetabolism after propranolol administration (6) and of weight loss with other beta-blocking drugs (30). Patients with a burn size of 41% studied at 2 weeks after injury (corresponding to the mean values in the present study) have resting morning plasma values of norepinephrine and epinephrine elevated about three-fold above the normal mean (5).

22. Aulick, L.H., and D.W. Wilmore (1983) Hypermetabolism in trauma. In: Mammalian Thermogenesis. L. Girardier and M.J. Stock, eds. Chapman and Hall, New York, Chap. 9, pp. 259-304.

23. Danielsson, U., G. Arturson, and L. Wennberg (1976) The elimination of hypermetabolism in burned patients: a method suitable for clinical use. Burns, 2:110-114.

24. Harrison, T.S., J.F. Seaton, and I. Feller (1967) Relationship of increased oxygen consumption to catecholamine excretion in thermal burns. Ann. Surg., 165:169-172.

25. Vaughan, G.M., R.A. Becker, J.P. Allen, C.W. Goodwin, Jr., B.A. Pruitt, Jr., and A.D. Mason, Jr. (1982) Cortisol and corticotrophin in burned patients. J. Trauma 22:263-273.

26. Wilmore, D.W. (1976) Hormonal responses and their effect on metabolism. Surg. Clin. North Am., 56:999-1018.

27. Wilmore, D.W., J.M. Long, A.D. Mason, Jr., and B.A. Pruitt, Jr. (1976) Stress in surgical patients as a neurophysiologic reflex response. Surg. Gynecol. Obstet., 142:257-269.

28. Wilmore, D.W., and L.H. Aulick (1978) Metabolic changes in burned patients. Surg. Clin. North Am., 58:1173-1187.

29. Wolfe, R.R., and M.J. Durkot (1982) Evaluation of the role of the sympathetic nervous system in the response of substrate kinetics and oxidation to burn injury. Circulatory Shock, 9:395-406.

30. Szabo, K. (1979) Clinical experiences with beta adrenergic blocking therapy on burned patients. Scand. J. Plast. Reconstr. Surg., 13:211-215.

Undoubtedly, even greater elevations occur during the day when the patients are disturbed and receive wound care, etc. It is presumed that sympathetic tone is continuously elevated (tachycardia does not resolve at night) in burn injury to provide increased substrate flow for the benefit of the wound until it is healed, when the hypermetabolism resolves. Rats with a burn size of 60% have total 24-h catecholamine excretion elevated to six-fold normal on postburn day 9 (31,32). In rats with burns of 20% of body surface, epinephrine excretion was elevated two-fold above normal at least from postburn days 3-11 (7).

Although the post-ganglionic sympathetic neurotransmitter for stimulation of melatonin synthesis in the pineal is not yet well established for humans and Syrian hamsters, it is norepinephrine acting through a beta-receptor and adenylyl cyclase stimulation in rats (4). Injection of beta-agonists apparently does not stimulate melatonin synthesis in humans or Syrian hamsters (3, 33-34), though it does in rats (35,36). Acute adverse stimuli do not alter human plasma melatonin (3), though

31. McManus, A.T. (1983) Examination of neutrophil function in a rat model of decreased host resistance following burn trauma. *Rev. Infec. Dis.*, 5(Suppl 5):S898-S907.

32. Herndon, D.N., D.W. Wilmore, and A.D. Mason, Jr. (1978) Development and analysis of a small animal model simulating the human postburn hypermetabolic response. *J. Surg. Res.*, 25:394-403.

33. Lipton, J.S., L.J. Petterborg, S. Steinlechner, and R.J. Reiter (1982) In vivo responses of the pineal gland of the syrian hamster to isoproterenol or norepinephrine. In: *The Pineal and its Hormones*. Alan R. Liss, Inc., New York, pp. 107-115.

34. Steinlechner, S., T.S. King, T.H. Champney, K. Spaniel-Borowski, and R.J. Reiter (1984) Comparison of the effects of B-adrenergic agents on pineal serotonin N-acetyltransferase activity and melatonin content in two species of hamsters. *J. Pineal Res.*, 1:23-30.

35. Deguchi, T., and J. Axelrod (1973) Supersensitivity and subsensitivity of the B-adrenergic receptor in pineal gland regulated by catecholamine transmitter. *Proc. Nat. Acad. Sci.*, 70:2411-2414.

36. Romero, J.A. (1976) Influence of diurnal cycles on biochemical parameters of drug sensitivity: the pineal gland as a model. *Fed. Proc.*, 35:1157-1161.

they may elevate rat pineal melatonin synthesis (37-39). Thus, differences among species for acute beta-adrenergic responsiveness of pineal melatonin may further complicate interpretation of lack of melatonin responsiveness to the more chronic elevation of sympathetic activity of burn injury. For this, there are perhaps three potential explanations that involve adrenergic activity at the level of the pineal, and they focus on the rat, in which species norepinephrine has become accepted as an intrapineal neurotransmitter.

In normal rats, injection of beta-agonists down-regulates the response in pineal melatonin synthesis (35,36) and activation of fat cell adenylyl cyclase (7) upon subsequent application of the agent. Against this explanation is the reported resistance to desensitization of the fat cell adenylyl cyclase response (normally induced by short-term injections of isoproterenol) in burned rats and in other rat models of chronic elevation of catecholamines, in which adenylyl cyclase remained responsive to isoproterenol (7). This and the continued long-term hypermetabolism (O₂ consumption) in the burned rats of that study suggests that in burn injury, tissue beta-responsiveness is not eliminated by down regulation. The pineals of our burned rats also retained their responsiveness, because their nocturnal surge of melatonin was normal. Secondly, normal rat sympathetic endings take up circulating endogenous catecholamines (re-uptake phenomenon) and thus may protect pinealocytes against activation of melatonin synthesis by adverse stimuli (40).

37. Allen, J.P., J.W. Sackman, W. Tullis, M.K. Vaughan, R.A. Becker, and G.M. Vaughan (1981) Nyctohemeral rhythms in rat pineal: Epinephrine uptake and N-acetyltransferase response to ether stress. In: Pineal Function. C.D. Matthews and R.F. Seamark, eds. Elsevier/North-Holland Biomedical Press, Amsterdam, pp. 211-216.

38. Lynch, H.J., J.P. Eng, and R.J. Wurtman (1973) Control of pineal indole biosynthesis by changes in sympathetic tone caused by factors other than environmental lighting. Proc. Nat. Acad. Sci. U.S.A., 70:1704-1707.

39. Lynch, H.J., M. Ho, and R.J. Wurtman (1977) The adrenal medulla may mediate the increase in pineal melatonin synthesis induced by stress but not that caused by exposure to darkness. J. Neural Transm., 40:87-97.

40. Parfitt, A.G., and D.C. Klein (1976) Sympathetic nerve endings in the pineal gland protect against acute stress-induced increase in N-acetyltransferase (E.C.2.3.1.5.) activity. Endocrinol., 99:840-851.

This explanation assumes that the increased sympathetic activity in the burned rats did not include elevated neuronal transmission in the pineal, but rather that pinealocytes did not respond to burn injury because they were protected from the humoral component of the sympathetic hyperactivity. This explanation, like the first, is unlikely because it would mean that the pineal is different from other tissues since the hyperdynamic metabolic status continues in burns until healing. But the normally innervated rat pineal reportedly does respond to humoral sympathetic activation apparently from the adrenal medullae during acute physical immobilization (39). A third explanation, presynaptic inhibition of pineal nerve endings (41) by the alpha component of activity of the elevated norepinephrine exposure in burns, does not explain lack of response to humoral catecholamines in burns and is further unlikely in that the nocturnal melatonin surge was normal in the burned rats.

The reduction only of nocturnal plasma melatonin in burned humans and the reduced daytime pineal melatonin in burned hamsters remains unexplained. The unavoidable administration of small doses of narcotic analgesic (usually 5 mg oxycodone orally once or twice) during study of the burn patients appears not to explain our results, because it was administered only during the day in the patients and was not given to the hamsters or rats. Morphine administration to rats was associated with a slight reduction of pineal N-acetyltransferase (42) but a reported elevation of plasma melatonin (43), and in vitro incubation of rat or hamster pineals with morphine or naloxone produced no effect on the secretion of melatonin (Vaughan, 1984, unpublished results). Whether altered clearance of melatonin in burns could have contributed to the results of measurements of circulating melatonin is not known, but such an effect appears not to have influenced the overall results, because pineal melatonin

41. Pelayo, F., M.L. Dubocovich, and S.Z. Langer (1977) Regulation of noradrenaline release in the rat pineal through a negative feedback mechanism mediated by presynaptic alpha-adrenoceptors. *Europ. J. Pharmacol.*, 45:317-318.

42. Zatz, M., and M.J. Brownstein (1979) Central depressants rapidly reduce nocturnal serotonin N-acetyltransferase activity in the rat pineal gland. *Brain Res.*, 160:381-385.

43. Esposito, D., G. Esposito, and F. Fraschini (1984) Effects of morphine on plasma melatonin in the rat. Third Colloquium of the European Pineal Study Group, *EPSG Newsletter, Suppl. 5, Aug., 1984, p. 57 (abstract).*

content was not elevated in burned animals. The possibility of greater dim light exposure at night may have had some role in the lower nocturnal plasma melatonin in the burn patients. We have presented the melatonin rhythm as it occurs in the actual circumstance of their care, and neither day nor night values were above normal despite their hyperadrenergic state. We think that possible differences in nocturnal light exposure in these patients was not a factor because suppression of nocturnal plasma melatonin in normal humans requires light intensity even much greater than the usual indoor daytime intensity (44).

The extent to which local neuronal transmission in peripheral tissues contributes to the overall sympathetic hyperactivity of burn injury, which at least includes the humoral element of markedly elevated circulating and excreted catecholamines, remains unknown. Whether or not neuronal transmission provides a major contribution, the present results are best explained by the concept that measurements of circulating or pineal melatonin do not provide a positive index of general sympathetic activity. This agrees with the lack of a melatonin response in humans also seen after acute sympathetic activation (3). Control of sympathetic activity appears partitioned, with pineal melatonin synthesis regulated independently of cardiovascular and metabolic functions. Whether other hormones (such as adrenocorticoids and glucagon) that usually surge in conjunction with general sympathetic activation or the fall in thyroid hormones that accompanies burn injury (5), can have a role in preventing pineal activation at central control sites or at the pineal is not known. Whether chronic changes in these additional hormones might prevent a pineal response to humoral but not locally released catecholamine in burned rats perhaps should be investigated.

44. Lewy, A.J., T.A. Wehr, F.K. Goodwin, D.A. Newsome, and S.P. Markey (1980) Light suppresses melatonin secretion in humans. *Science*, 210:1267-1269.

PRESENTATIONS/PUBLICATIONS

Presented to the Symposium on Immune Consequences of Thermal and Traumatic Injury, Snowbird, Utah, January 23, 1984.

Presented to the '84 Pineal Satellite Symposium of the VIIth International Congress of Endocrinology, Digby, Nova Scotia, Canada, July 7-10, 1984.

Presented to the Third Colloquium of the European Pineal Study Group, Pecs, Hungary, August 13-17, 1984.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
				DA OG 6972	84 10 01	DD-DR&E(IAR) 636
3. DATE PREV SUMMARY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISB'N INSTR'N	9. LEVEL OF SUMMARY WORK UNIT
83 10 01	D. Change	U	U		CX	
10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER		
a. PRIMARY	61102A	3M161102BS10	BB	301		
b. CONTRIBUTING						
c. CONTRIBUTING	STOG 82/83 - 6.2/4					
11. TITLE (Precede with Security Classification Code)						
(U) Studies of Infection and Microbiologic Surveillance of Troops with Thermal Injury						
12. SUBJECT AREAS						
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13. START DATE	14. ESTIMATED COMPLETION DATE		15. FUNDING ORGANIZATION		16. PERFORMANCE METHOD	
76 10	CONT		DA		C. In-House	
17. CONTRACT/GRANT			18. RESOURCES ESTIMATE			
a. DATE EFFECTIVE	EXPIRATION		FISCAL YEARS	a. PROFESSIONAL WORK YEARS	b. FUNDS (In thousands)	
b. CONTRACT/GRANT NUMBER			84	2.5	260	
c. TYPE	d. AMOUNT		85	0.5	272	
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19. RESPONSIBLE DOD ORGANIZATION			20. PERFORMING ORGANIZATION			
a. NAME			a. NAME			
US Army Institute of Surgical Research			US Army Institute of Surgical Research			
b. ADDRESS (include zip code)			b. ADDRESS			
Ft. Sam Houston, Texas 78234-6200			Ft. Sam Houston, Texas 78234-6200			
c. NAME OF RESPONSIBLE INDIVIDUAL			c. NAME OF PRINCIPAL INVESTIGATOR			
Pruitt, BA, Jr			McManus, AT			
d. TELEPHONE NUMBER (include area code)			d. TELEPHONE NUMBER (include area code)			
512-221-2720			512-221-3411			
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) Pseudomonas; (U) Klebsiella; (U) Staphylococcus; (U) Wound Infection; (U) Antibiotic Resistance; (U) Sepsis; (U) Topical Chemotherapy;						
23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code) (U) Volunteers; (U) Ram II						
23. (U) Burns constitute a large component of military injuries sustained in combat. Military relevance of this research lies in the fact that infection and ensuing sepsis are major problems among burned soldiers. Control of surface infection is a major objective, and species of organisms causing sepsis, epidemiology, response of significant species to topical chemotherapy modalities, and relation of antibiotics to sepsis control are major study areas. 24. (U) Cultures of human wounds, tissues and body fluids are carried out with precise strain speciation and differentiation being employed. Virulence is assessed in burn wound models which are also used to assess effectiveness of experimental drugs, both topical and systemic. 25. (U) 8310 - 8409. In FY84, 227 burned patients were cultured. Streptococcus viridans was the most common isolate (1,819 strains). This finding documents a dramatic decrease in the rate of upper respiratory colonization with Gram (-) flora. The next nine most common isolates (all sources) were: S. aureus (1,456), N-hemolytic Non-GRD streptococci (1,062), P. aeruginosa (545), P. mirabilis (500), K. pneumoniae (448), S. epidermidis (445), E. coli (441), C. albicans (351), and E. cloacae (286). Blood cultures yielded 239 organisms from a total of 1,616 cultures. S. aureus was the most common blood stream pathogen.						

ANNUAL PROGRESS REPORT

PROJECT NO. 3M161102BS10-00, BASIC RESEARCH

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

US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234

1 October 1983 - 30 September 1984

Investigators:

Albert T. McManus, Ph.D.
Jack R. Henderson, Ph.D.
Timothy E. Lawson, SSG
Aldo H. Reyes, SSG
Anne M. Bray, SP5
Charles H. Guymon, SP5

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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REPORT TITLE: STUDIES OF INFECTION AND MICROBIOLOGIC SURVEILLANCE OF
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US Army Institute of Surgical Research, Brooke Army Medical Center,
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Reports Control Symbol MEDDH-288(R1)

Cultures were received from 195 burned patients admitted during FY 84. Blood cultures were positive for 45 patients. Staphylococcus aureus was the most common isolate and was found in 20 patients. The next eight most common blood isolates (number of patients indicated in parentheses) were: Staphylococcus epidermidis (13), Candida albicans (8), Candida rugosa (8), Klebsiella pneumoniae (7), Pseudomonas aeruginosa (5), Serratia marcescens (5), Escherichia coli (4) and Enterobacter cloacae (3).

Burn microbiology
Pseudomonas
Klebsiella
Staphylococcus
Antibiotic resistance
Blood culture
Biopsy
Humans

STUDIES OF INFECTION AND MICROBIOLOGIC SURVEILLANCE OF TROOPS WITH THERMAL INJURY

AUTOMATED MICROBIOLOGY DATA BASE

This is the second reporting period in which microbiology results have been stored and retrieved from a computer data base. The microbiology data base was added to a new patient data base on 1 October 1983. This data base was created by Major Lawrence Lehrner and Dr. David Strome and allows simultaneous displays of all available laboratory data (microbiology, chemistry, pathology, etc.) directly to the burn ward. Microbiology data are displayed retrospectively on a per patient basis from the most recent results to 2 weeks before the current date. Upon specific request, patient data can be reviewed for any period in the patient's records.

Epidemiological data are updated daily from the patient data base. The epidemiological data base is used as originally described (1).

ANTIBIOTIC SENSITIVITY DETERMINATION

The FY 84 antibiotic testing panels are presented in Table 1. Bacterial organisms were tested by agar overlay disc diffusion. Broth dilution minimal inhibitory concentrations (MIC) and minimal bactericidal concentration (MBC) were available upon specific request. Organisms selected for in vitro testing were selected by the following criteria: isolation from blood culture, predominant organisms in biopsy cultures, predominant organism in urine cultures with $\geq 10^5$ CFU/ml, predominant gram-negative isolates from respiratory sources, Staphylococcus aureus isolates, Pseudomonas aeruginosa isolates and any other organisms requested.

MICROBIAL SURVEILLANCE

The microbial surveillance protocol established in FY 83 was continued through this reporting period (2). Patients were cultured from wound, sputum, urine and rectum on admission. While in intensive care (Ward 14A) or for one month after admission, sputum and urines were cultured three times per week and stools and wound surfaces twice per week. Patients transferred to Ward 14B and hospitalized more than 30 days were cultured once per week. Gentamicin resistant gram-negative organisms

1. McManus AT, Henderson JR, Alderson TC, Brownell LA, Lawson TE, Reyes AH: Studies of infection and microbiologic surveillance of troops with thermal injury. USAISR Annual Report FY 1982, Ft Sam Houston, Texas, pp 153-221.

2. McManus AT, Henderson JR, Lawson TE, Alderson TC, Brownell LA, Reyes AH: Studies of infection and microbiologic surveillance of troops with thermal injury. USAISR Annual Report FY 1983, Ft Sam Houston, Texas, pp 183-226.

Table 1. In vitro Sensitivity Panels (FY 84)

ENTERIC ORGANISMS

1. AMIKACIN ^{a,c}	13. CHLORAMPHENICOL
2. GENTAMICIN ^{a,c}	14. TETRACYCLINE
3. TOBRAMYCIN ^{a,c}	15. CEFOXITIN ^a
4. TICARCILLIN ^a	16. CEFAMANDOLE ^a
5. MEZLOCILLIN ^{a,c}	17. AMPICILLIN ^a
6. PIPERACILLIN ^{a,c}	18. NEOMYCIN
7. MOXALACTAM ^{a,c}	19. TRIMETHOPRIM
8. CEFOTAXIME ^a	20. TRIMETH & SULFA
9. CEFOPERAZONE	21. NALIDIXIC ACID
10. SULFADIAZINE	22. MK0787 ^{b,c}
11. NETILMICIN ^a	23. STREPTOMYCIN
12. KANAMYCIN	

NON-ENTERIC GRAM (-) ORGANISMS

1. AMIKACIN ^{a,c}	10. CEFSULODIN ^a
2. GENTAMICIN ^{a,c}	11. COLISTIN
3. TOBRAMYCIN ^{a,c}	12. SULFADIAZINE ^a
4. TICARCILLIN ^a	13. NETILMICIN
5. MEZLOCILLIN ^{a,c}	14. KANAMYCIN
6. PIPERACILLIN ^{a,c}	15. CHLORAMPHENICOL
7. MOXALACTAM ^{a,c}	16. TETRACYCLINE
8. CEFOTAXIME ^a	17. MK0787 ^{b,c}
9. CEFOPERAZONE ^a	18. AZLOCILLIN ^a

GRAM (+) ORGANISMS

1. AMIKACIN ^{a,c}	13. CEPHALOTHIN ^a
2. GENTAMICIN ^{a,c}	14. VANCOMYCIN ^{a,c}
3. TOBRAMYCIN ^{a,c}	15. KANAMYCIN
4. TICARCILLIN	16. CHLORAMPHENICOL ^a
5. MEZLOCILLIN ^{a,c}	17. TETRACYCLINE
6. PIPERACILLIN ^{a,c}	18. AMPICILLIN
7. MOXALACTAM ^c	19. MK0787 ^{b,c}
8. CEFOTAXIME	20. CLINDAMYCIN ^a
9. CEFOPERAZONE	21. PENICILLIN ^a
10. CEFSULODIN	22. ERYTHROMYCIN ^a
11. SULFADIAZINE	23. STREPTOMYCIN
12. METHICILLIN ^a	

^a - Reported daily on daily clinical microbiology report (hard copy).

^b - Experimental drug.

^c - Reported on computer screen from patient data base.

from sputum or stool specimens were also screened by plating on MacConkey agar containing gentamicin sulfate (20 µg/ml).

MICROBIOLOGIC FINDINGS IN BURN PATIENTS (1 October 1983-30 September 1984)

A total of 195 patients admitted in FY 84 were cultured. Species isolated and number of patients yielding each species are presented in Table 2. A summary of the 10 most common isolates is presented in Table 3. The table contains 77.7% of all isolates identified. The relative frequencies of sites of isolation are presented in Figure 1. The relative frequencies of sites of isolation for gram-negative organisms, gram-positive organisms and yeasts are shown in Figure 2.

FLORA RECOVERED FROM RESPIRATORY SYSTEM

A total of 6468 organisms were recovered from respiratory system specimens. The majority of these were sputum cultures collected in the surveillance system. The 10 most frequent species are presented in Table 4. Of particular note is the absence of P. aeruginosa from the top 10 list. Pseudomonas aeruginosa was recovered from 30 of the 185 patients cultured. This is not a significant change from FY 83. The principal changes compared to FY 83 were increases in patients colonized with Enterobacter cloacae and beta-hemolytic (not Group A, B or D) streptococci.

FLORA RECOVERED FROM BURN WOUND

A total of 830 contact plates were examined and 471 yielded growth (56.7%), a significant increase from FY 82 ($P < .05$). The relative frequency of species isolated from surface cultures is presented in Figure 3. Subsurface flora as measured in biopsy specimens was examined in 352 specimens taken from 41 patients. The 10 most frequent organism types are presented in Table 5. For the second reporting period, filamentous fungi, principally Aspergillus sp., were the most common finding. The incidence of fungi was not different from FY 83.

FLORA RECOVERED FROM URINARY TRACT OF BURNED PATIENTS

Urine specimens from 182 patients were cultured. A total of 1033 organisms were identified. The 10 most common isolates are presented in Table 6. Significantly increased incidences in colonization compared to FY 83 were noted in eight of the 10 top species. The top 10 organisms found in urine cultures with more than 10^5 CFU/ml are presented in Table 7. Significant increase in frequency of patients colonized was found in seven of the top 10 organisms.

FLORA RECOVERED FROM BLOOD CULTURES

Blood cultures (1480) were collected from 103 patients. Positive cultures were found in 45 patients. This incidence is not different from FY 83. Three patients had single isolations of obligate anaerobic organisms (Propionibacterium acnes). The principal organisms isolated

Table 2. Distribution by Organism

Organism	No. Isolates	No. Patients Colonized	Organism	No. Isolates	No. Patients Colonized
<i>Acinetobacter anitratus</i>	34	5	<i>Providencia stuartii</i>	31	4
<i>Acinetobacter lwoffii</i>	4	2	<i>Pseudomonas aeruginosa</i>	569	58
<i>Actinomyces flavus</i>	1	1	<i>Pseudomonas cepacia</i>	2	1
<i>Alcaligenes faecalis</i>	1	1	<i>Pseudomonas fluorescens</i>	3	3
<i>Bacillus</i>	37	29	<i>Pseudomonas maltophilia</i>	3	3
<i>Branhamella catarrhalis</i>	2	2	<i>Pseudomonas putida</i>	4	4
<i>Candida albicans</i>	289	51	<i>Serratia liquefaciens</i>	1	1
<i>Candida parapsilosis</i>	1	1	<i>Serratia marcescens</i>	257	26
<i>Candida pseudotropicalis</i>	1	1	<i>Staphylococcus aureus</i>	1416	134
<i>Candida rugosa</i>	135	33	<i>Staphylococcus epidermidis</i>	425	108
<i>Candida tropicalis</i>	51	19	<i>Staphylococcus saprophyticus</i>	49	24
<i>Citrobacter freundii</i>	18	15	<i>Alpha Streptococcus</i>	12	10
<i>Citrobacter diversus</i>	64	29	<i>Beta Streptococcus</i>		
<i>Enterobacter aerogenes</i>	235	44	not Group A, B, D	97	45
<i>Enterobacter agglomerans</i>	10	10	Group A Streptococcus	4	3
<i>Enterobacter cloacae</i>	356	56	Group B Streptococcus	1	1
<i>Escherichia coli</i>	331	74	Group D Streptococcus		
<i>Haemophilus influenzae</i>	7	2	not Enterococcus	139	64
<i>Haemophilus parahaemolyticus</i>	1	1	Group D Enterococcus	81	44
<i>Haemophilus parainfluenzae</i>	31	5	Non-hemolytic Streptococcus	14	12
<i>Klebsiella oxytoca</i>	75	23	Non-hemolytic Streptococcus		
<i>Klebsiella ozaenae</i>	3	3	not Group D	1021	169
<i>Klebsiella pneumoniae</i>	393	85	Streptococcus pneumoniae	23	16
<i>Morganella morganii</i>	30	17	Streptococcus viridans	1788	179
<i>Mucor</i> sp.	1	1	<i>Torulopsis candida</i>	1	1
<i>Neisseria</i> sp.	21	12	True fungi sp.	154	43
<i>Neisseria mucosa</i>	80	29	Yeast sp.	36	27
<i>Propionibacterium acnes</i>	3	3			
<i>Proteus mirabilis</i>	468	60			
<i>Proteus rettgeri</i>	1	1			
<i>Proteus vulgaris</i>	68	19			

Total isolates = 8906; total patients = 195

Table 3. Ten Most Frequent Isolates (FY 84)

Organism	Patients Colonized	% Patients	Number Isolates	% Total Isolates
<i>Streptococcus viridans</i>	179	91.8	1788	20.1
Non-hemolytic <i>Streptococcus</i> not Group D	169	86.7	1021	11.5
<i>Staphylococcus aureus</i>	134	68.7	1416	15.9
<i>Staphylococcus epidermidis</i>	108	55.4	425	4.8
<i>Klebsiella pneumoniae</i>	85	43.6	393	4.4
<i>Escherichia coli</i>	75	38.5	331	3.7
Group D <i>Streptococcus</i> not <i>Enterococcus</i>	64	32.8	139	1.6
<i>Proteus mirabilis</i>	60	30.8	468	5.3
<i>Pseudomonas aeruginosa</i>	58	29.7	569	6.4
<i>Enterobacter cloacae</i>	56	28.7	356	4.0
			6906	77.7
Total patients cultured = 195				
Total isolates = 8906				

Table 4. Ten Most Frequent Isolates from Respiratory Sources (FY 84)

Organism	Patients Colonized	% Patients	Number Isolates	% Total Isolates
<i>Streptococcus viridans</i>	176	95.1	1727	26.7
Non-hemolytic <i>Streptococcus</i> not Group D	166	89.7	923	14.3
<i>Staphylococcus aureus</i>	115	62.2	1172	18.1
<i>Staphylococcus epidermidis</i>	75	40.5	254	3.9
<i>Klebsiella pneumoniae</i>	47	25.4	168	2.6
Beta-hemolytic <i>Streptococcus</i> not Group A, B, or D	42	22.7	93	1.4
Group D <i>Streptococcus</i> not <i>Enterococcus</i>	41	22.2	131	2.0
<i>Enterobacter cloacae</i>	37	20.0	179	2.8
<i>Escherichia coli</i>	34	18.4	152	2.4
<i>Enterobacter aerogenes</i>	33	17.8	190	2.9
Total patients cultured = 185			4989	77.1
Total isolates = 6468				

Table 5. Principal Organisms Recovered in Biopsy Specimens (FY 84)

Organism	Patients Colonized	% Patients	Number Isolates	% Isolates
Filamentous fungi	18	43.9	69	38.5
Escherichia coli	8	19.5	18	10.1
Enterobacter cloacae	7	17.1	15	8.4
Candida rugosa	7	17.1	12	6.7
Klebsiella pneumoniae	5	12.2	12	6.7
Candida albicans	4	9.8	8	4.5
Pseudomonas aeruginosa	4	9.8	6	3.4
Proteus mirabilis	3	7.3	5	2.8
Serratia marcescens	3	7.3	5	2.8
Group D Enterococcus	3	7.3	4	2.2
Total patients = 41			154	86.1
Total isolates = 179				

Table 6. Ten Most Common Organisms from Urinary Specimens (FY 84)

Organism	Patients Colonized	% Patients	Number Isolates	% Isolates
<i>Klebsiella pneumoniae</i>	54	29.7*	144	13.9
<i>Escherichia coli</i>	45	24.7	92	8.9
<i>Proteus mirabilis</i>	44	24.2*	179	17.3
Non-hemolytic <i>Streptococcus</i> not Group D	35	19.2*	59	5.7
<i>Enterobacter cloacae</i>	31	17.0*	83	8.0
<i>Pseudomonas aeruginosa</i>	31	17.0*	70	6.8
<i>Candida albicans</i>	28	15.4*	124	12.0
<i>Staphylococcus epidermidis</i>	27	14.8*	39	3.8
<i>Staphylococcus aureus</i>	17	9.3	20	1.9
<i>Enterobacter aerogenes</i>	16	8.8*	26	2.5
Total patients = 182			836	80.8
Total isolates = 1039				

* $P < .05$ greater incidence FY 84 vs FY 83.

Table 7. Ten Most Frequent Organisms from Urinary Specimens with
> 10⁵ CFU (FY 84)

Organism	Number Patients	% Patients	Number Isolates	% Isolates
<i>Proteus mirabilis</i>	37	20.3*	128	19.0
<i>Klebsiella pneumoniae</i>	34	18.7*	92	13.7
<i>Escherichia coli</i>	27	14.8*	51	7.6
<i>Pseudomonas aeruginosa</i>	25	13.7*	47	7.9
<i>Enterobacter cloacae</i>	22	12.1*	62	9.2
Non-hemolytic <i>Streptococcus</i> not Group D	21	11.5*	34	5.1
<i>Candida albicans</i>	19	10.4	84	12.5
<i>Staphylococcus epidermidis</i>	15	8.2	21	3.1
<i>Enterobacter aerogenes</i>	11	6.0*	15	2.2
<i>Candida rugosa</i>	10	5.5	23	3.4
			557	82.8
Total patients = 182				
Total isolates = 673				

* P < .01 greater incidence FY 84 vs FY 83.

FREQUENCY OF POSITIVE SOURCES

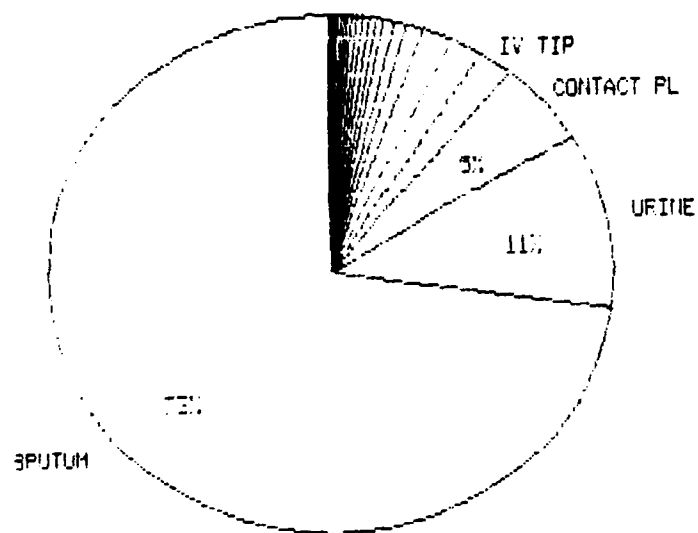
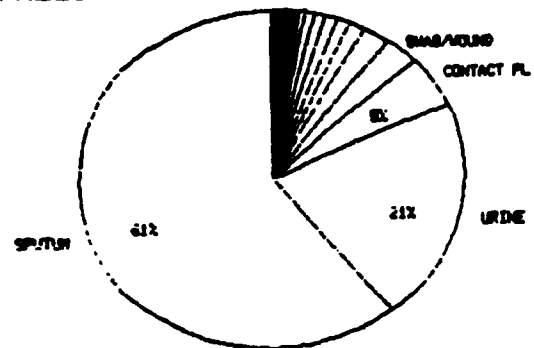
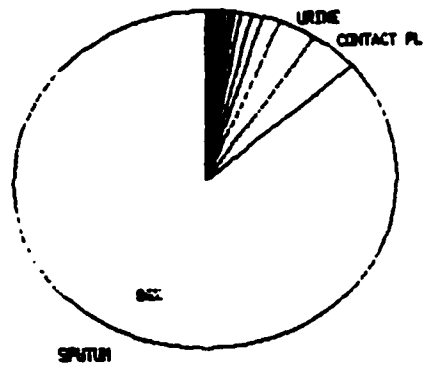


Figure 1. Display of the relative frequency of specimen sources yielding isolates in FY 84.

FREQUENCY OF GRAM(-) SOURCES



FREQUENCY OF GRAM(+) SOURCES



FREQUENCY OF YEAST SOURCES

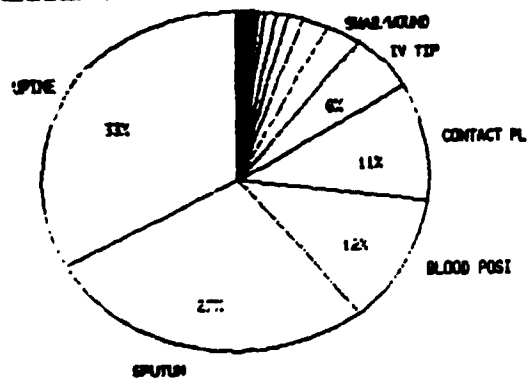


Figure 2. Display of the relative frequency of specimen sources yielding gram-negative, gram-positive or yeast-like organisms.

FREQUENCY OF SURFACE RECOVERY

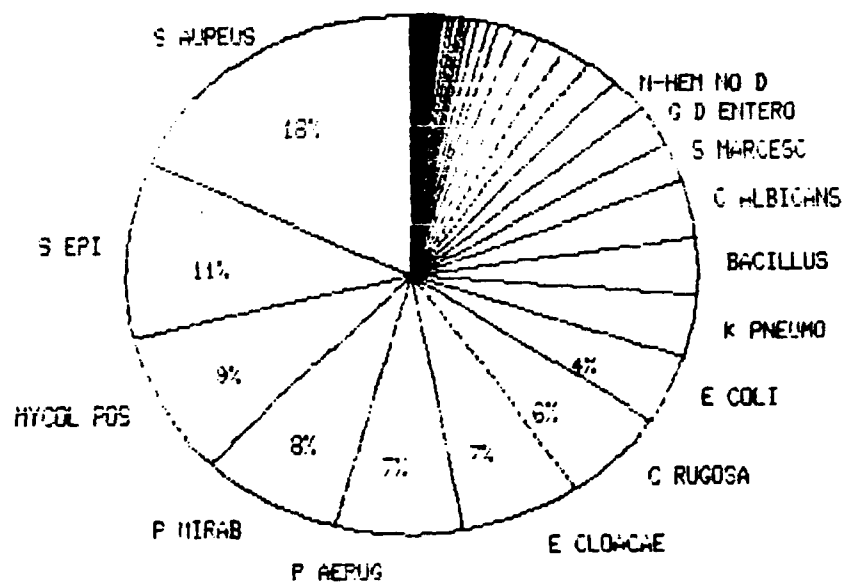


Figure 3. Display of the relative frequency of organism types isolated from surface wound cultures.

from blood cultures are presented in Table 8. A case of bacteremia was described as the isolation of an organism from blood culture. If the same organism was again cultured within 30 days of the first it was considered one case. A total of 170 isolates representing 92 cases were observed. The ratio of cases to positive patients was 2.04. This ratio was 1.68 in FY 83. The average number of positive cultures for patients with at least one positive blood culture was 3.77 in FY 84 as to compare with 3.8 in FY 83. A comparison of blood culture results between FY 84 and FY 83 is presented in Table 9. The principal difference between FY 84 and FY 83 was an increased frequency of S. aureus and Serratia marcescens isolations.

SUMMARY OF ANTIBIOTIC TESTING

A total of 3060 bacterial isolates were tested for in vitro sensitivity to antibiotics. A comparison of sources of tested strains is presented in Figure 4. The frequency of organisms tested is displayed in Figure 5. The proportions of tested organisms were similar to those tested in FY 83.

Gentamicin resistance was again used as a plasmid surveillance marker. Testing was done on 3053 isolates. Figure 6 displays the relative frequency of tested organisms. Figure 7 displays the frequency of species resistant to gentamicin. As can be seen, a major difference from FY 83 was the establishment in FY 84 of E. cloacae as an endemic gentamicin resistant organism. The epidemiology of this event will be presented in another section of this annual report. The overall incidence of gentamicin resistance was not different from FY 83.

Antibiotic sensitivity patterns for the principal bacterial organisms isolated from blood cultures will be presented separately.

STAPHYLOCOCCUS AUREUS

The sources of S. aureus strains tested for in vitro activity are presented in Figure 8. The results of in vitro testing are presented in Table 10. An overall increase in antibiotic resistance occurred during FY 84. The number of methicillin resistant isolates was about 5% (8.63) for the first time in six reporting periods, although these strains were not clinically evident. Histogram displays of select antibiotics are presented in Figure 9a-m.

PSEUDOMONAS AERUGINOSA

The frequency of sources of P. aeruginosa strains tested in vitro is presented in Figure 10. The results of testing are presented in Table 11. A comparison of frequency of resistances to selected antibiotics is presented in Table 12. Sensitivity to aminoglycoside antibiotics has continued to improve. The relative frequency of gentamicin resistance for the last four reporting periods is presented in Figure 11. Significant increases in the frequency of resistance were noted for mezlocillin and azlocillin, while piperacillin was not changed from FY 83. Sulfadiazine sensitivity was at its highest frequency since the drug has been tested at this Institute. The relative frequency of sulfonamide resistance for the last four reporting periods is presented in Figure 12. Histogram displays

Table 8. Principal Organisms Found in Blood Cultures from Burned Patients
(FY 84)

Organism	Number Patients	% Patients	Number Cases	% Cases	Number Isolates	% Isolates
<i>Staphylococcus aureus</i>	20	19.4	20	21.7	37	21.8
<i>Staphylococcus epidermidis</i>	13	12.6	13	14.1	14	8.2
<i>Candida albicans</i>	8	7.8	8	8.7	29	17.1
<i>Candida rugosa</i>	8	7.8	8	8.7	28	16.5
<i>Klebsiella pneumoniae</i>	7	6.8	7	7.6	8	4.7
<i>Pseudomonas aeruginosa</i>	5	4.9	5	5.4	9	5.3
<i>Serratia marcescens</i>	5	4.9	5	5.4	6	3.5
<i>Escherichia coli</i>	4	3.9	4	4.3	10	5.9
<i>Enterobacter cloacae</i>	3	2.9	3	3.3	5	2.9
				79.2		85.9

Total isolates = 170 Total cases = 92
 Total positive cultures = 170
 Total cultures = 1480
 Total patients cultured = 103
 Total patients positive = 45

Table 9. Patients with Positive Blood Cultures

Organism	FY 83	FY 84	FY 83 <u>vs</u> FY 84
Staphylococcus aureus	8	20	P < .01 ↑
Staphylococcus epidermidis	14	13	N.S.
Candida albicans	7	8	N.S.
Candida rugosa	14	8	N.S.
Klebsiella pneumoniae	5	7	N.S.
Pseudomonas aeruginosa	8	5	N.S.
Serratia marcescens	0	5	P < .01
Escherichia coli	4	4	N.S.
Enterobacter cloacae	3	3	N.S.
Patients sampled	145	103	

Table 10

ANTIBIOTIC SENSITIVITY DATA

DATES : 83-10-01 TO 84-09-30
ORGANISM : S AUREUS

ANTIBIOTIC	RESISTANT Percent Number	INTERMEDIATE Percent Number	SENSITIVE Percent Number	Total Number
AMIKACIN	0.30%	2.81%	96.89%	965
GENTAMICIN	14.14%	0.20%	85.66%	854
TOBRAMYCIN	13.76%	0.90%	85.34%	850
TICARCILLIN	1.91%	7.34%	90.75%	903
MEZLOCILLIN	93.58%	1.00%	5.42%	54
PIPERACILLIN	93.49%	0.60%	5.91%	59
MOXALACTAM	10.23%	22.47%	67.30%	671
CEFOTAXIME	4.41%	7.72%	87.86%	876
CEFOPERAZONE	3.21%	13.96%	82.83%	825
CEFSULODIN	0.94%	1.25%	97.81%	939
SULFADIAZINE	17.47%	16.67%	65.86%	656
METHICILLIN	8.63%	4.41%	86.96%	867
CEPHALOTHIN	1.40%	0.90%	97.70%	975
VANCOMYCIN	0.00%	0.00%	100.00%	998
KANAMYCIN	20.68%	0.40%	78.92%	786
CHLORAMPHENICOL	3.61%	1.91%	94.48%	941
TETRACYCLINE	11.35%	0.10%	88.55%	882
AMPICILLIN	13.70%	9.16%	77.14%	766
MK0787	0.20%	0.41%	99.39%	976
CLINDAMYCIN	2.71%	0.00%	97.29%	970
PENICILLIN	84.95%	8.73%	6.32%	63
ERYTHROMYCIN	13.04%	1.20%	85.76%	855
STREPTOMYCIN	14.99%	0.40%	84.61%	841

Table 11

ANTIBIOTIC SENSITIVITY DATA

DATES : 83-10-01 TO 84-09-30

ORGANISM : P AERUG

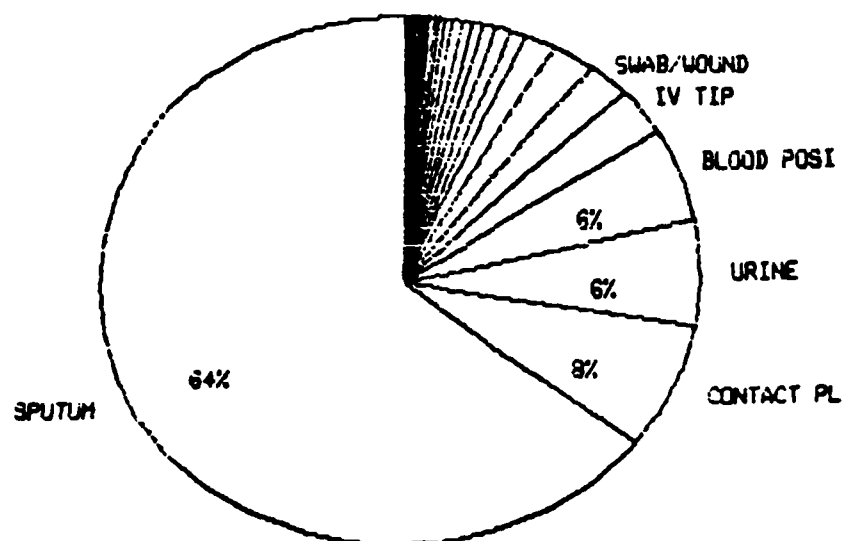
ANTIBIOTIC	RESISTANT Percent Number	INTERMEDIATE Percent Number	SENSITIVE Percent Number	Total Number
AMIKACIN	0.64%	4.88%	94.48%	471
GENTAMICIN	4.03%	8.92%	87.05%	471
TOBRAMYCIN	1.27%	0.85%	97.88%	471
TICARCILLIN	8.07%	12.10%	79.83%	471
MEZLOCILLIN	29.94%	9.98%	60.08%	471
PIPERACILLIN	11.68%	9.34%	78.98%	471
MOXALACTAM	29.72%	49.26%	21.02%	471
CEFOTAXIME	32.91%	58.60%	8.49%	471
CEFOPERAZONE	14.01%	12.74%	73.25%	471
CEFSULODIN	7.78%	0.86%	91.36%	463
COLISTIN	0.00%	0.64%	99.36%	471
SULFADIAZINE	17.83%	12.95%	69.21%	471
NETILMICIN	1.27%	0.85%	97.88%	471
KANAMYCIN	99.36%	0.21%	0.42%	471
CHLORAMPHENICOL	82.59%	16.56%	0.85%	471
TETRACYCLINE	83.86%	15.07%	1.06%	471
MK0787	16.38%	0.00%	83.62%	470
AZLOCILLIN	27.39%	3.18%	69.43%	471

Table 12. Comparison of Resistance in Pseudomonas aeruginosa Isolates
between FY 83 and FY 84

Antibiotic	FY 83		FY 84		$\chi^2(1)$	P
	Resistant/Sensitive	% Res.	Resistant/Sensitive	% Res.		
Amikacin	12/352	3.4	3/468	0.64	8.6	< .01
Gentamicin	69/295	18.9	19/452	4.2	48.5	< .01
Tobramycin	165/199	45.3	6/465	1.3	24.4	< .01
Ticarcillin	59/305	16.2	38/433	8.8	13.3	< .01
Mezlocillin	49/315	13.5	141/330	42.7	31.7	< .01
Azlocillin	46/315	12.7	129/342	27.4	26.4	< .01
Piperacillin	32/332	8.8	55/416	13.2	1.83	N.S.*
Moxalactam	70/291	19.4	140/331	42.3	11.56	< .01
Cefotaxime	59/305	16.2	155/316	49.1	30.04	< .01
Cefsulodin	49/306	13.8	36/427	8.4	7.83	< .01
Sulfadiazine	218/145	60.1	84/387	21.7	15.8	< .01

* Not significant.

FREQUENCY OF FY83 SOURCES



FREQUENCY OF FY84 SOURCES

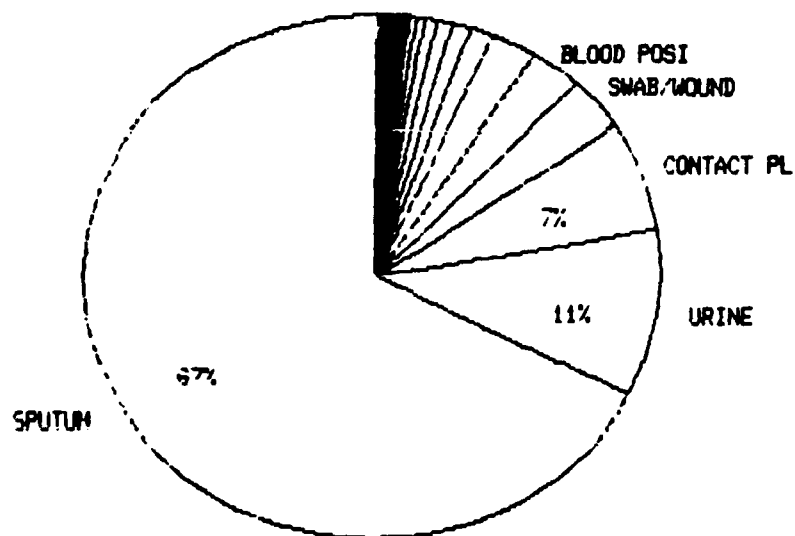
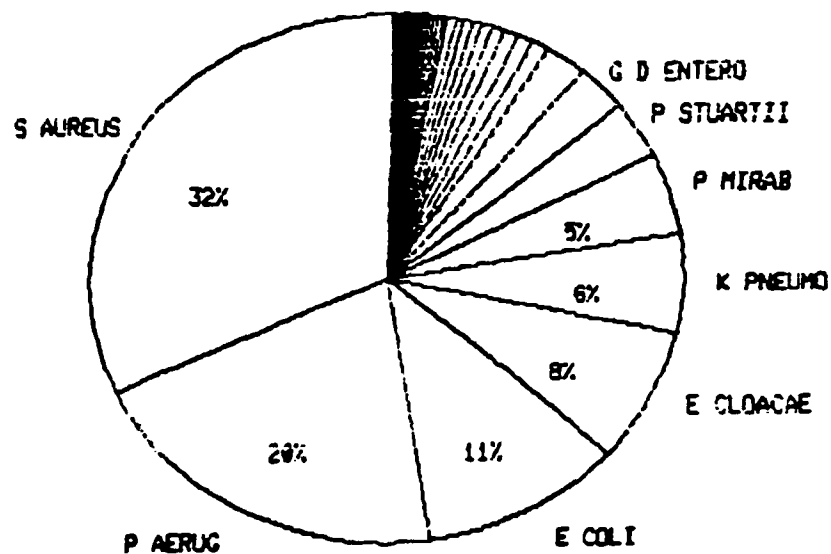


Figure 4. Display of the relative frequencies of sources yielding organisms tested for in vitro sensitivity to antibiotics in FY 83 and FY 84.

FREQUENCY OF FY83 TEST



FREQUENCY OF FY84 TEST

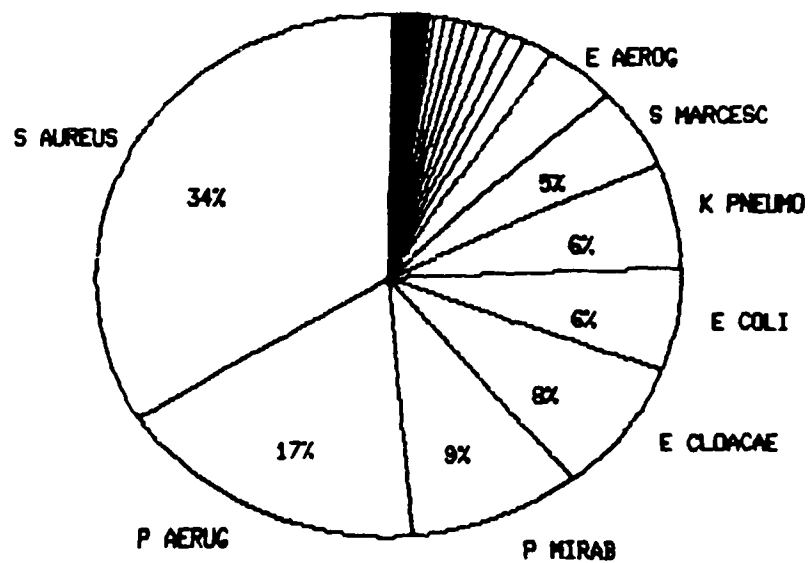
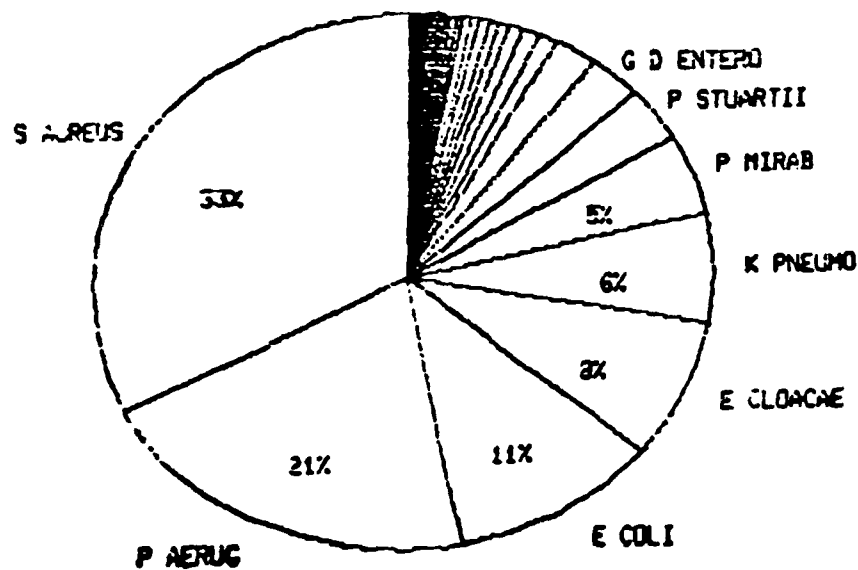


Figure 5. Display of the relative frequencies of organisms tested for in vitro sensitivity to antibiotics in FY 83 and FY 84.

FREQUENCY OF FY83 GM TEST



FREQUENCY OF FY84 GM TEST

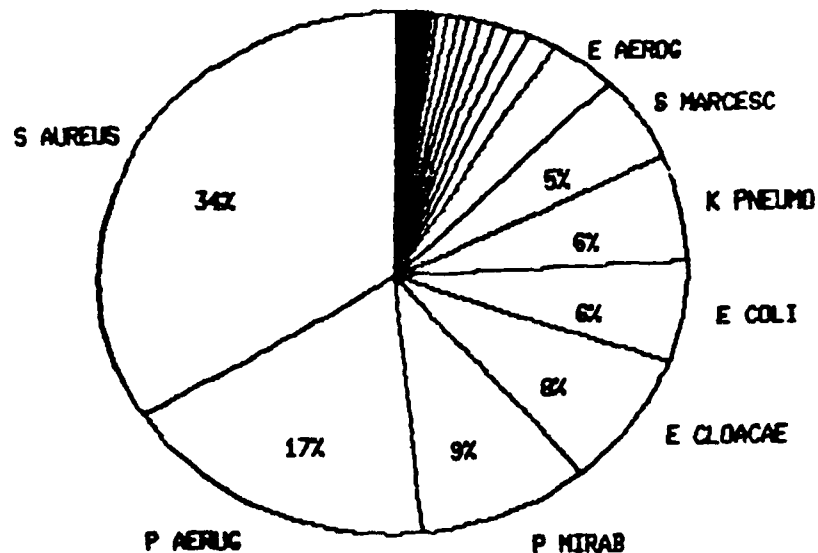
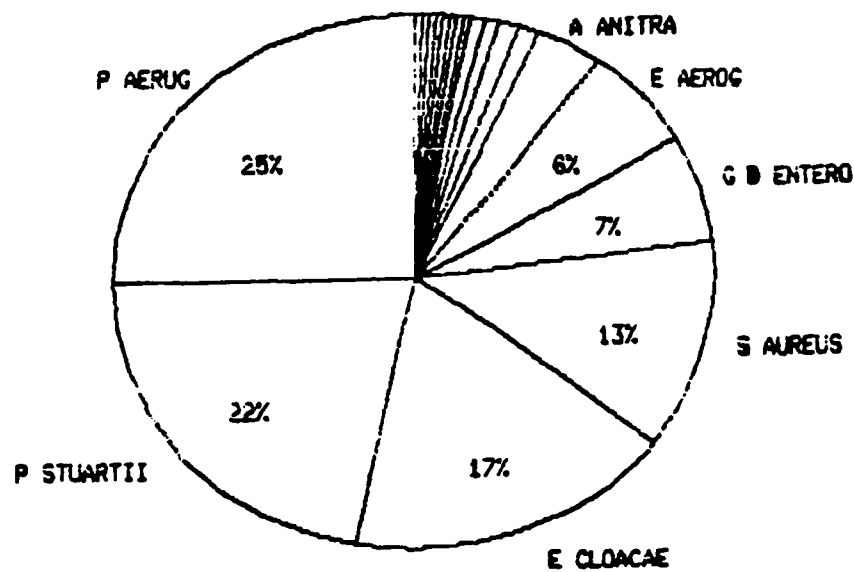


Figure 6. Display of the relative frequency of organisms tested for in vitro sensitivity to gentamicin (GM) in FY 83 and FY 84.

FREQUENCY OF GM RES FY83



FREQUENCY OF GM RES FY84

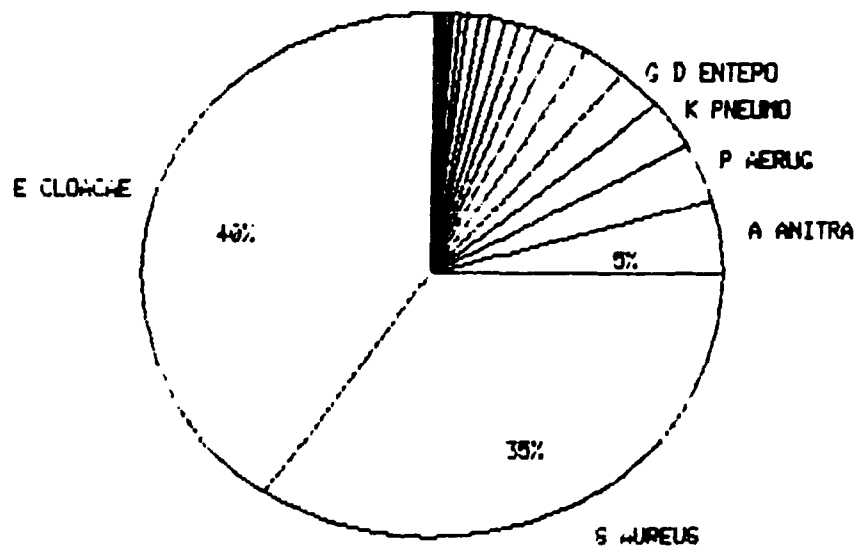


Figure 7. Display of the relative frequency of gentamicin resistant organisms isolated in FY 83 and FY 84.

FREQUENCY OF FY84 S.AUREUS TESTING

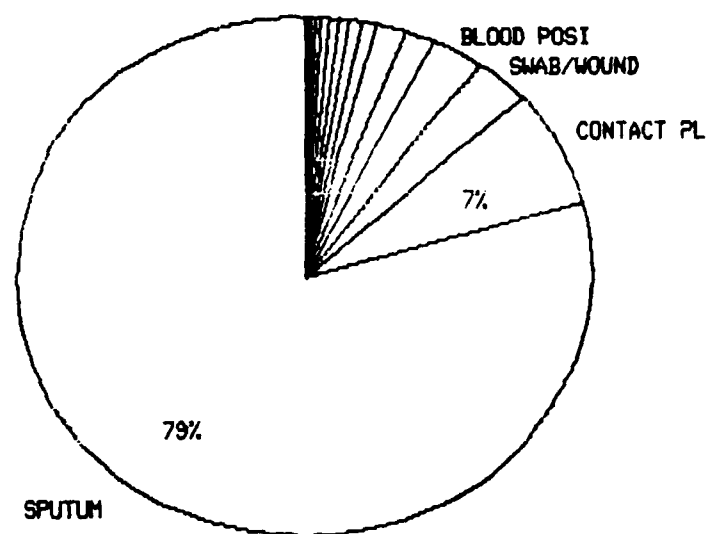


Figure 8. Display of the relative frequency of sources yielding S. aureus tested for in vitro sensitivity to antibiotics in FY 84.

ANTIBIOTIC SENSITIVITY DATA
 DATES : 83-10-01 TO 84-09-30
 ORGANISM : S. AUREUS

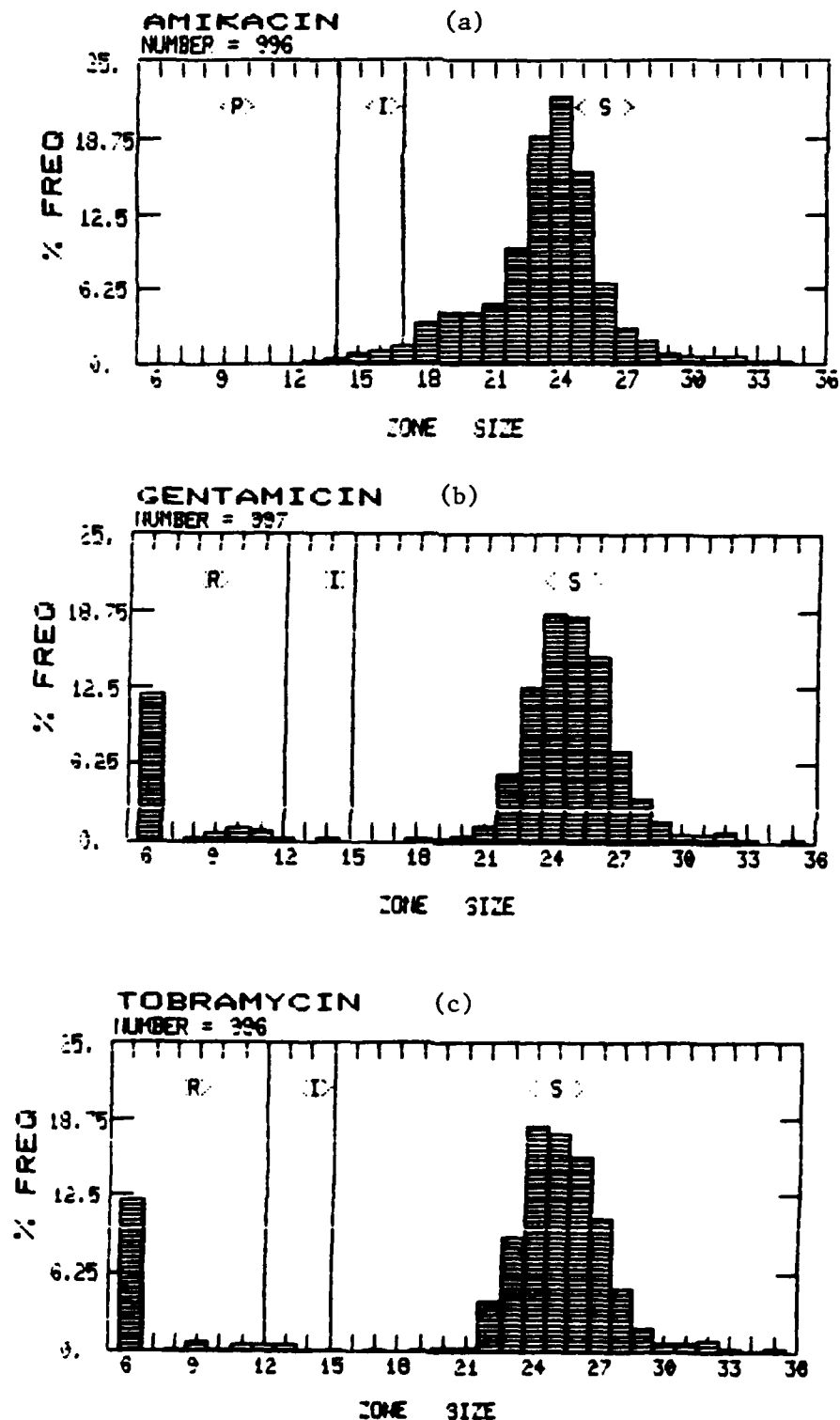


Figure 9. Histogram display of the distribution of zones of inhibition of growth of *Staphylococcus aureus*. Antibiotics tested: (a) amikacin (30 mcg), (b) gentamicin (10 mcg), (c) tobramycin (10 mcg).

INTERPRETED SENSITIVITY DATA
 DATES : 83-10-01 TO 84-09-30
 ORGANISM : S AUREUS

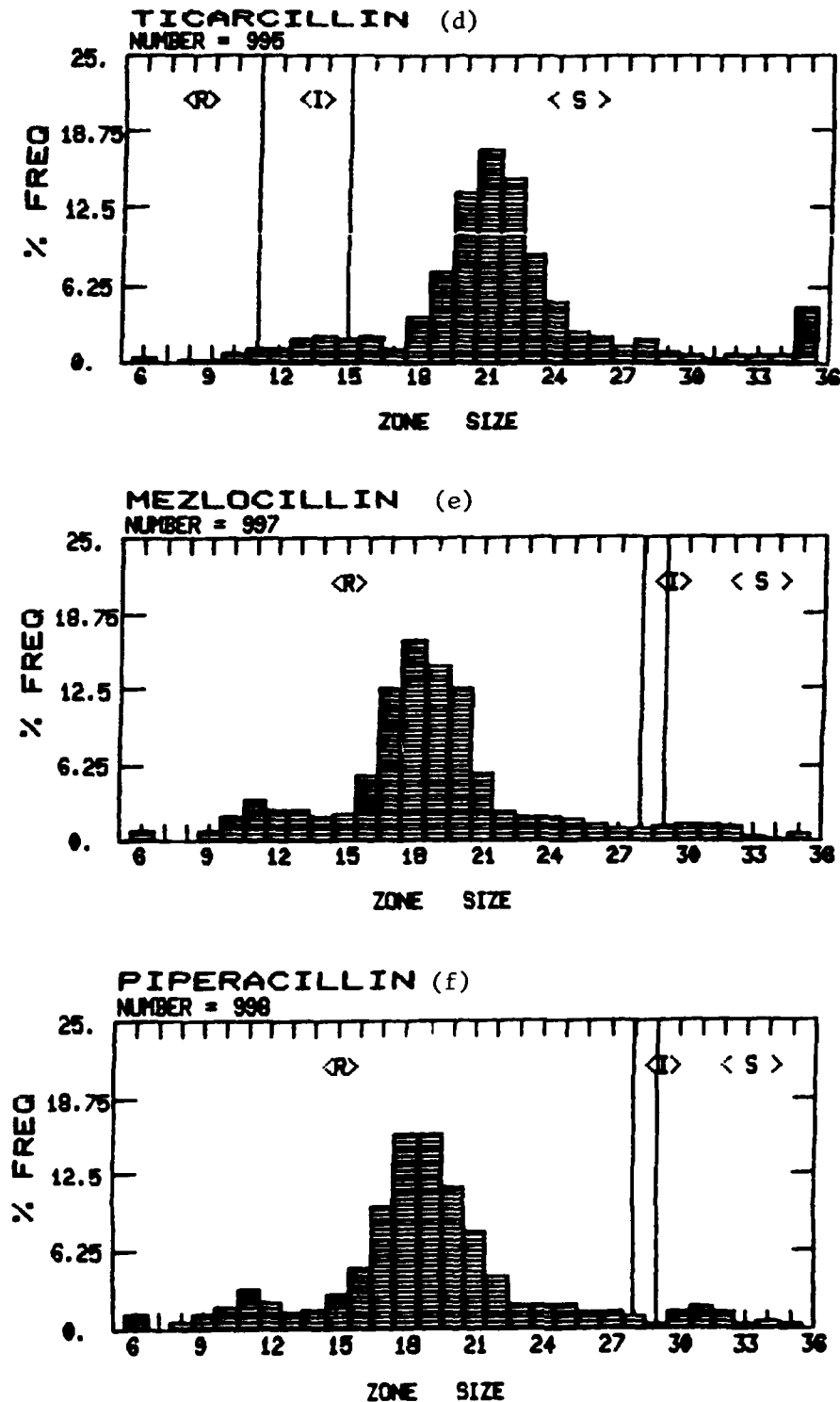


Figure 9. Histogram display of the distribution of zones of inhibition of growth of *Staphylococcus aureus*. Antibiotics tested: (d) ticarcillin (75 mcg), (e) mezlocillin (75 mcg), (f) piperacillin (100 mcg).

ANTIBIOTIC SENSITIVITY DATA
 DATES : 83-10-01 TO 84-09-30
 ORGANISM : S. AUREUS

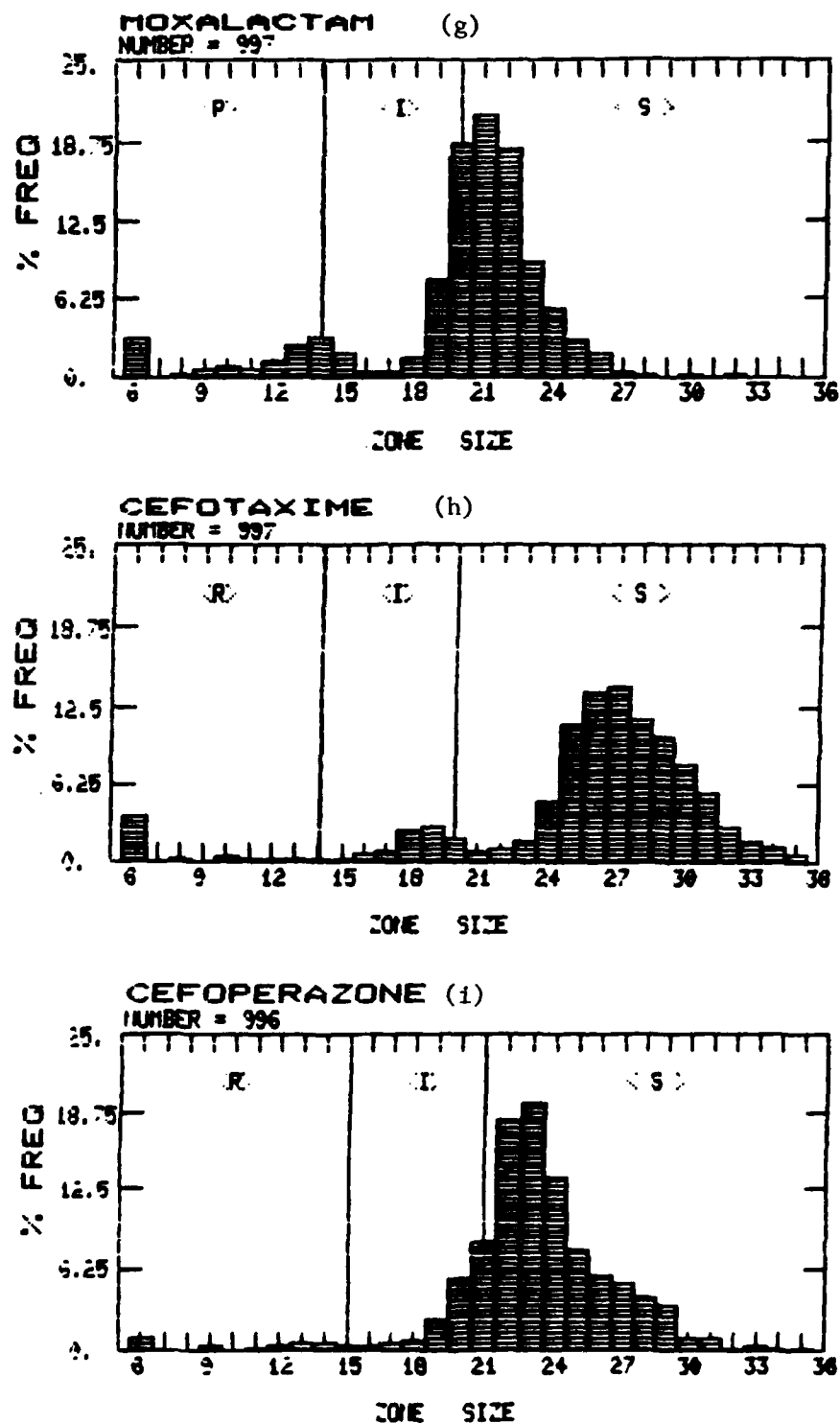


Figure 9. Histogram display of the distribution of zones of inhibition of growth of *Staphylococcus aureus*. Antibiotics tested: (g) moxalactam (30 mcg), (h) cefotaxime (30 mcg), (i) cefoperazone (30 mcg).

ANTIBIOTIC SENSITIVITY DATA
 DATES : 83-10-01 TO 84-09-30
 ORGANISM : S AUREUS

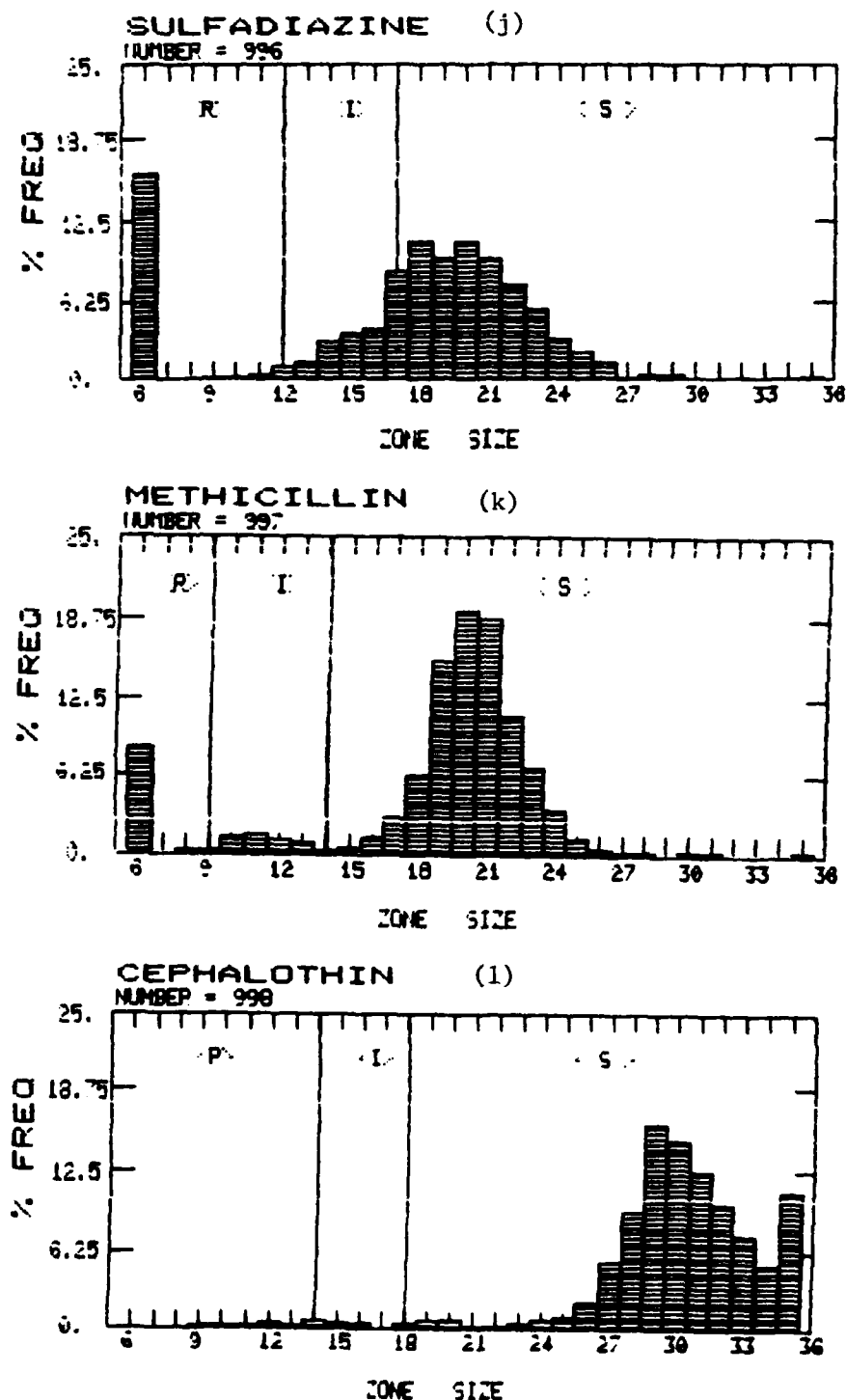


Figure 9. Histogram display of the distribution of zones of inhibition of growth of *Staphylococcus aureus*. Antibiotics tested: (j) sulfadiazine (250 mcg), (k) methicillin (5 mcg), (l) cephalothin (30 mcg).

ANTIBIOTIC SENSITIVITY DATA
 DATES : 83-10-01 TO 84-09-30
 ORGANISM : S AUREUS

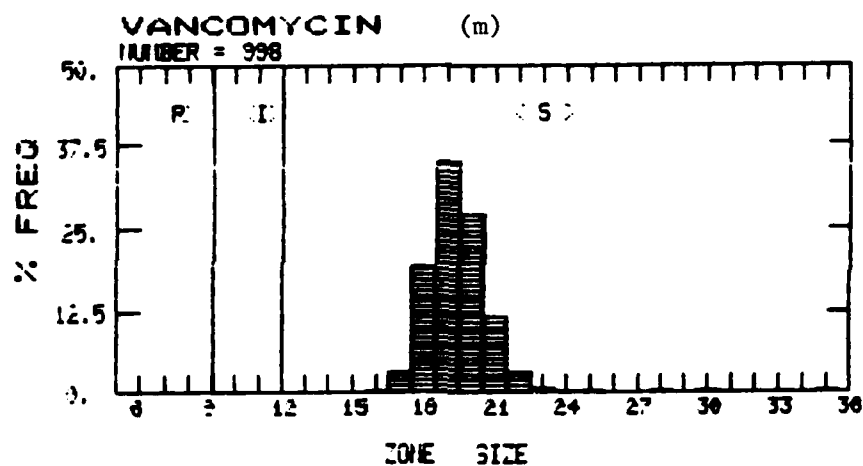


Figure 9. Histogram display of the distribution of zones of inhibition of growth of Staphylococcus aureus. Antibiotics tested: (m) vancomycin (30 mcg).

FREQUENCY OF FY84 P.A. TESTING

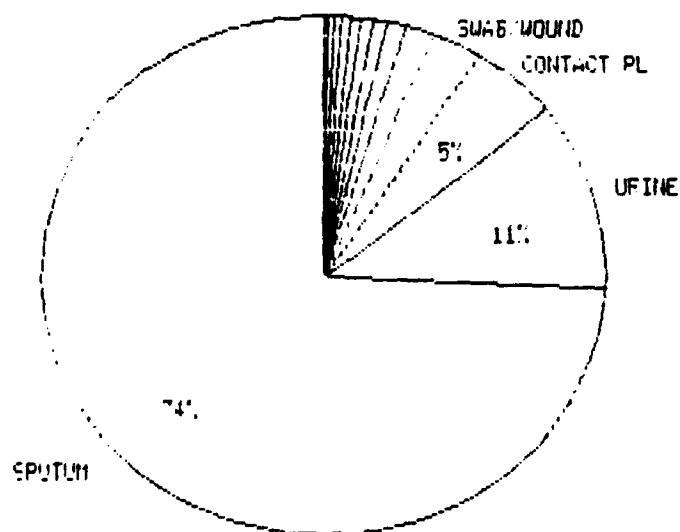


Figure 10. Display of the relative frequency of sources yielding *P. aeruginosa* (P.A.) tested for in vitro sensitivity to antibiotics in FY 84.

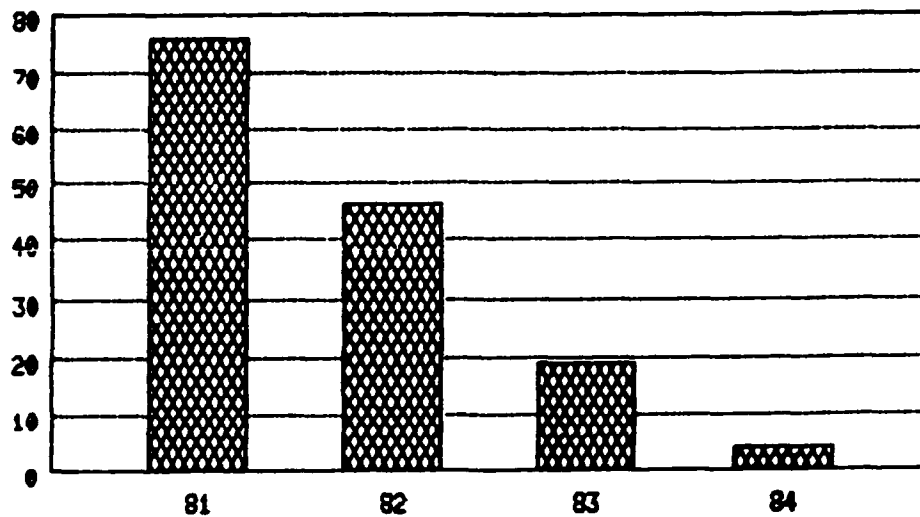


Figure 11. Relative frequency of *P. aeruginosa* resistance to gentamicin (%), FY 81 through FY 84.

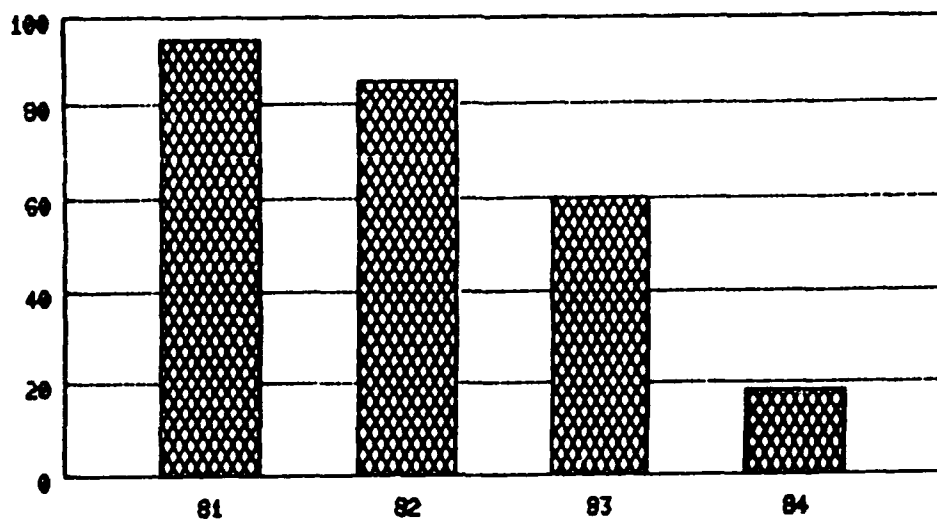


Figure 12. Relative frequency of *P. aeruginosa* resistance to sulfonamides (%), FY 81 through FY 84.

of the distributions of zone sizes for selected antibiotics are presented in Figure 13a-k. A display of the association of zones of inhibition of selected anti-Pseudomonas penicillins is presented in Figure 14. The relative resistances and mean zones of inhibition are presented in Table 13. The reason for the selective loss of mezlocillin and azlocillin activity in this reporting period when compared to FY 83 is unknown.

KLEBSIELLA PNEUMONIAE

A total of 288 isolates were tested for in vitro sensitivities to antibiotics. The sources of isolation for tested strains are presented in Figure 15. The sensitivity results are presented in Table 14.

SERRATIA MARCESCENS

Serratia marcescens occurred at its highest incidence in several years. The organism was isolated from 26 patients in FY 84 while only from six patients in FY 83 ($P < .01$). The sources of isolation are presented in Figure 16. The results of in vitro antibiotic testing are presented in Table 15. The epidemiology of the outbreak will be presented in a separate section of this annual report.

ENTEROBACTER CLOACAE

Gentamicin and sulfonamide resistant E. cloacae strains appeared in May 1984. This was after a 9-month separation from our previously reported endemic (AR 1983). The in vitro sensitivity results are presented in Table 16. Antibiotic histograms are presented in Figure 17a-i.

PRESENTATIONS

McManus AT: Microbial colonization in a new intensive care burn unit. Fourth Annual Meeting of the Surgical Infection Society, Montreal, Canada, 1 May 1984.

PUBLICATIONS

McManus AT, McManus WF, Mason AD Jr, Aitcheson AR, Pruitt BA Jr: Microbial colonization in a new intensive care burn unit. Arch Surg, accepted for publication.

Table 13. Relative in vitro Sensitivity of 471 Burn Patient Isolates of Pseudomonas aeruginosa to Ticarcillin, Mezlocillin, Azlocillin and Piperacillin

Antibiotic	Number Resistant Strains	Percent Resistant	$\bar{X}$ Zone
Ticarcillin	38	8.1	20.6
Mezlocillin	141	29.9	16.9
Azlocillin	129	27.4	21.3
Piperacillin	55	11.7	24.4

Table 14

ANTIBIOTIC SENSITIVITY DATA

DATES : 83-10-01 TO 84-09-30
ORGANISM : K PNEUMO

ANTIBIOTIC	RESISTANT		INTERMEDIATE		SENSITIVE		Total Number
	Percent	Number	Percent	Number	Percent	Number	
AMIKACIN	0.00%	0	2.18%	5	97.82%	224	229
GENTAMICIN	5.70%	13	0.44%	1	93.86%	214	228
TOBRAMYCIN	6.11%	14	0.00%	0	93.89%	215	229
TICARCILLIN	46.05%	105	32.89%	75	21.05%	48	228
MEZLOCILLIN	6.99%	16	7.42%	17	85.59%	196	229
PIPERACILLIN	6.55%	15	3.93%	9	89.52%	205	229
MOXALACTAM	0.44%	1	0.44%	1	99.13%	227	229
CEFOTAXIME	0.44%	1	1.75%	4	97.82%	224	229
CEFOPERAZONE	1.31%	3	5.68%	13	93.01%	213	229
SULFADIAZINE	67.25%	154	2.18%	5	30.57%	70	229
NETILMICIN	2.62%	6	0.87%	2	96.51%	221	229
KANAMYCIN	9.61%	22	0.00%	0	90.39%	207	229
CHLORAMPHENICOL	9.61%	22	0.00%	0	90.39%	207	229
TETRACYCLINE	15.28%	35	8.30%	19	76.42%	175	229
CEFOXITIN	1.76%	4	1.76%	4	96.48%	219	227
CEFAMANDOLE	3.51%	8	3.95%	9	92.54%	211	228
AMPICILLIN	88.60%	202	7.46%	17	3.95%	9	228
NEOMYCIN	4.39%	10	0.88%	2	94.74%	216	228
TRIMETHOPRIM	2.67%	6	0.44%	1	96.89%	218	225
TRIMETH & SULFA	2.63%	6	9.65%	22	87.72%	200	228
NALIDIXIC ACID	0.88%	2	2.64%	6	96.48%	219	227
MK0787	0.00%	0	0.00%	0	100.00%	227	227
STREPTOMYCIN	10.53%	24	31.14%	71	58.33%	133	228

Table 15

ANTIBIOTIC SENSITIVITY DATA

DATES : 83-10-01 TO 84-09-30
ORGANISM : S MARCESC

ANTIBIOTIC	RESISTANT Percent Number	INTERMEDIATE Percent Number	SENSITIVE Percent Number	Total Number
AMIKACIN	3.13%	7.50%	89.38%	160
GENTAMICIN	0.00%	0.00%	100.00%	160
TOBRAMYCIN	5.63%	10.00%	84.38%	160
TICARCILLIN	0.00%	1.88%	98.13%	160
MEZLOCILLIN	6.88%	18.75%	74.38%	160
PIPERACILLIN	1.88%	5.00%	93.13%	160
MOXALACTAM	0.00%	0.00%	100.00%	160
CEFOTAXIME	0.00%	11.25%	88.75%	160
CEFOPERAZONE	1.25%	22.50%	76.25%	160
SULFADIAZINE	4.38%	11.88%	83.75%	160
NETILMICIN	0.63%	7.50%	91.88%	160
KANAMYCIN	1.25%	15.63%	83.13%	160
CHLORAMPHENICOL	0.00%	9.38%	90.63%	160
TETRACYCLINE	85.63%	13.75%	0.63%	160
CEFOXITIN	1.25%	41.25%	57.50%	160
CEFAMANDOLE	91.88%	8.13%	0.00%	160
AMPICILLIN	100.00%	0.00%	0.00%	160
NEOMYCIN	0.00%	1.25%	98.75%	160
TRIMETHOPRIM	0.00%	5.00%	95.00%	160
TRIMETH & SULFA	0.00%	0.00%	100.00%	160
NALIDIXIC ACID	0.00%	0.00%	100.00%	160
MK0787	0.00%	0.00%	100.00%	160
STREPTOMYCIN	0.00%	3.13%	96.88%	155

Table 16

ANTIBIOTIC SENSITIVITY DATA

DATES : 83-10-01 TO 84-09-30
ORGANISM : E CLOACAE

ANTIBIOTIC	RESISTANT Percent Number	INTERMEDIATE Percent Number	SENSITIVE Percent Number	Total Number
AMIKACIN	2.51%	58.29%	39.20%	199
GENTAMICIN	75.38%	0.50%	24.12%	199
TOBRAMYCIN	75.88%	0.50%	23.62%	199
TICARCILLIN	75.88%	7.34%	24.12%	199
MEZLOCILLIN	100.00%	1.00%	5.42%	199
PIPERACILLIN	97.49%	1.51%	1.01%	199
MOXALACTAM	0.50%	8.04%	91.46%	199
CEFOTAXIME	8.54%	7.72%	91.46%	199
CEFOPERAZONE	12.56%	63.32%	24.12%	199
SULFADIAZINE	82.91%	0.50%	16.58%	199
NETILMICIN	69.35%	5.53%	25.13%	199
KANAMYCIN	76.26%	0.51%	23.23%	198
CHLORAMPHENICOL	76.88%	3.52%	19.60%	199
TETRACYCLINE	6.53%	35.68%	57.79%	199
CEFOXITIN	93.97%	0.50%	5.53%	199
CEFAMANDOLE	70.85%	5.53%	23.62%	199
AMPICILLIN	94.97%	1.01%	4.02%	199
NEOMYCIN	0.50%	1.51%	97.99%	199
TRIMETHOPRIM	1.01%	0.00%	98.99%	199
TRIMETH & SULFA	1.51%	3.02%	95.48%	199
NALIDIXIC ACID	0.00%	3.02%	96.98%	199
MK0787	0.20%	0.41%	100.00%	199
STREPTOMYCIN	9.05%	3.52%	87.44%	199

INTERATED SENSITIVITY NoTo
 DATES : 83-10-01 TO 84-09-30
 ORGANISM : P. AERUG

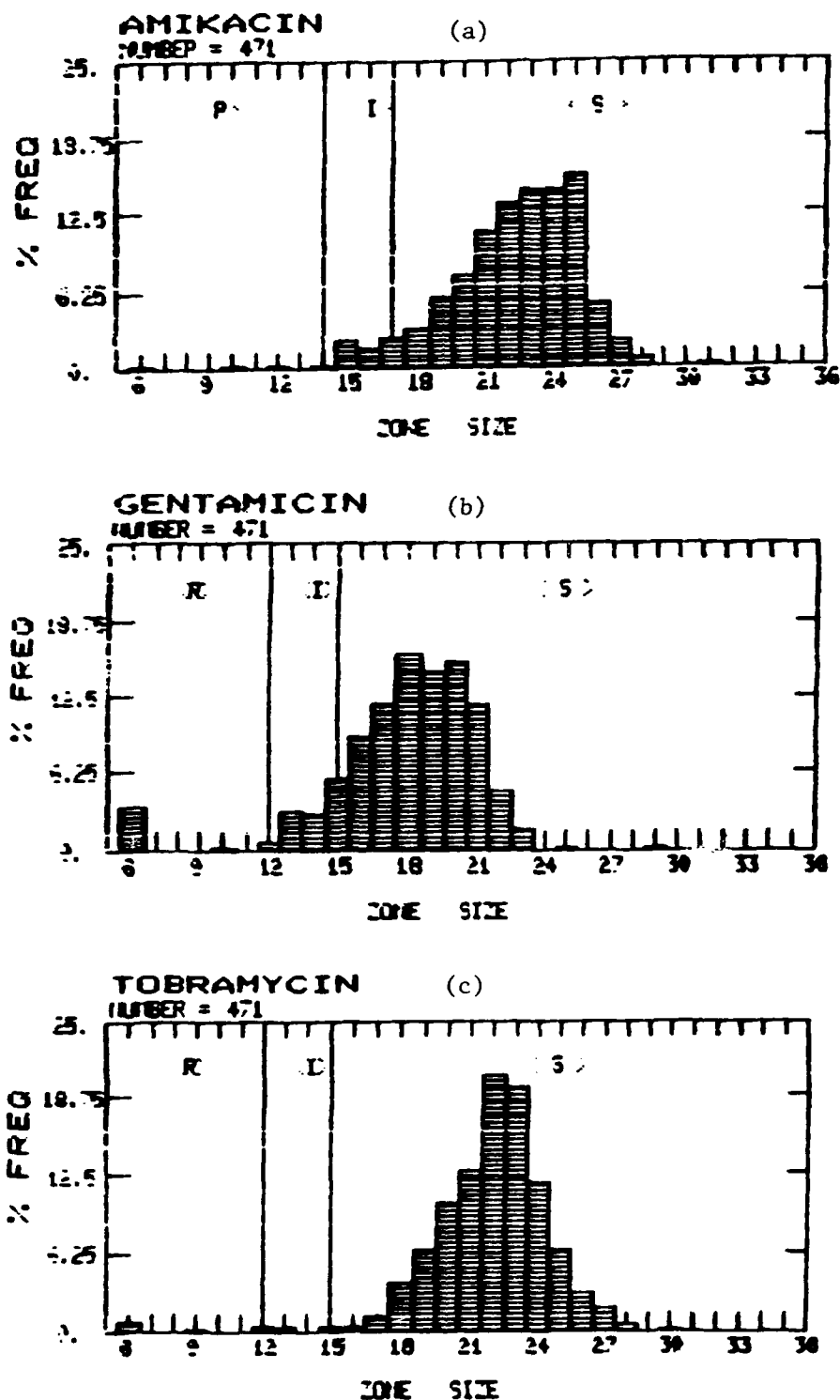


Figure 13. Histogram display of the distribution of zones of inhibition of growth of *Pseudomonas aeruginosa*. Antibiotics tested: (a) amikacin (30 mcg), (b) gentamicin (10 mcg), (c) tobramycin (10 mcg).

ANTIBIOTIC SENSITIVITY DATA
 DATES : 83-10-01 TO 84-09-30
 ORGANISM : P AERUG

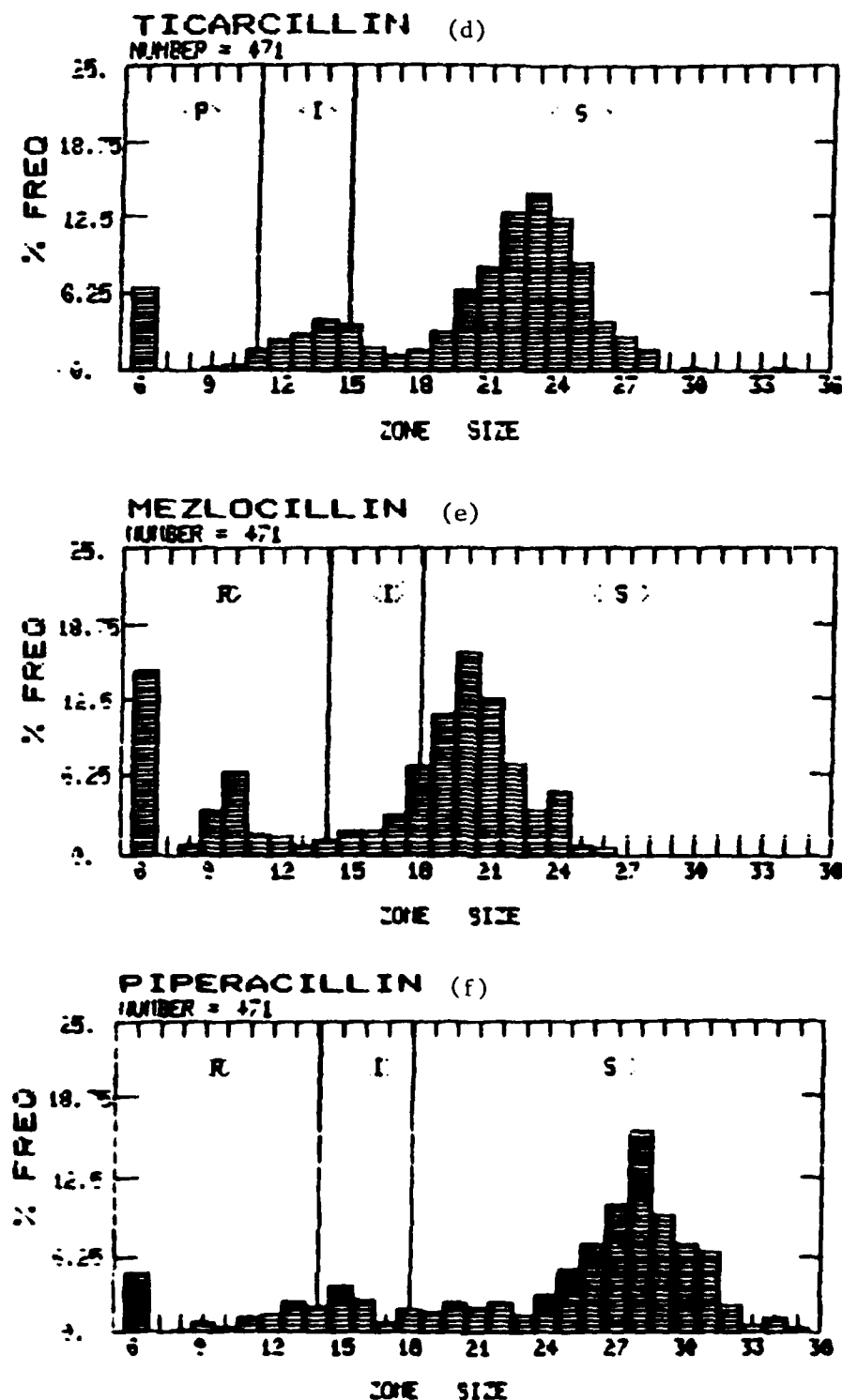


Figure 13. Histogram display of the distribution of zones of inhibition of growth of *Pseudomonas aeruginosa*. Antibiotics tested: (d) ticarcillin (75 mcg), (e) mezlocillin (75 mcg), (f) piperacillin (100 mcg).

ANTIBIOTIC SENSITIVITY DATA
 DATES : 83-10-01 TO 84-09-30
 ORGANISM : P. AERUG

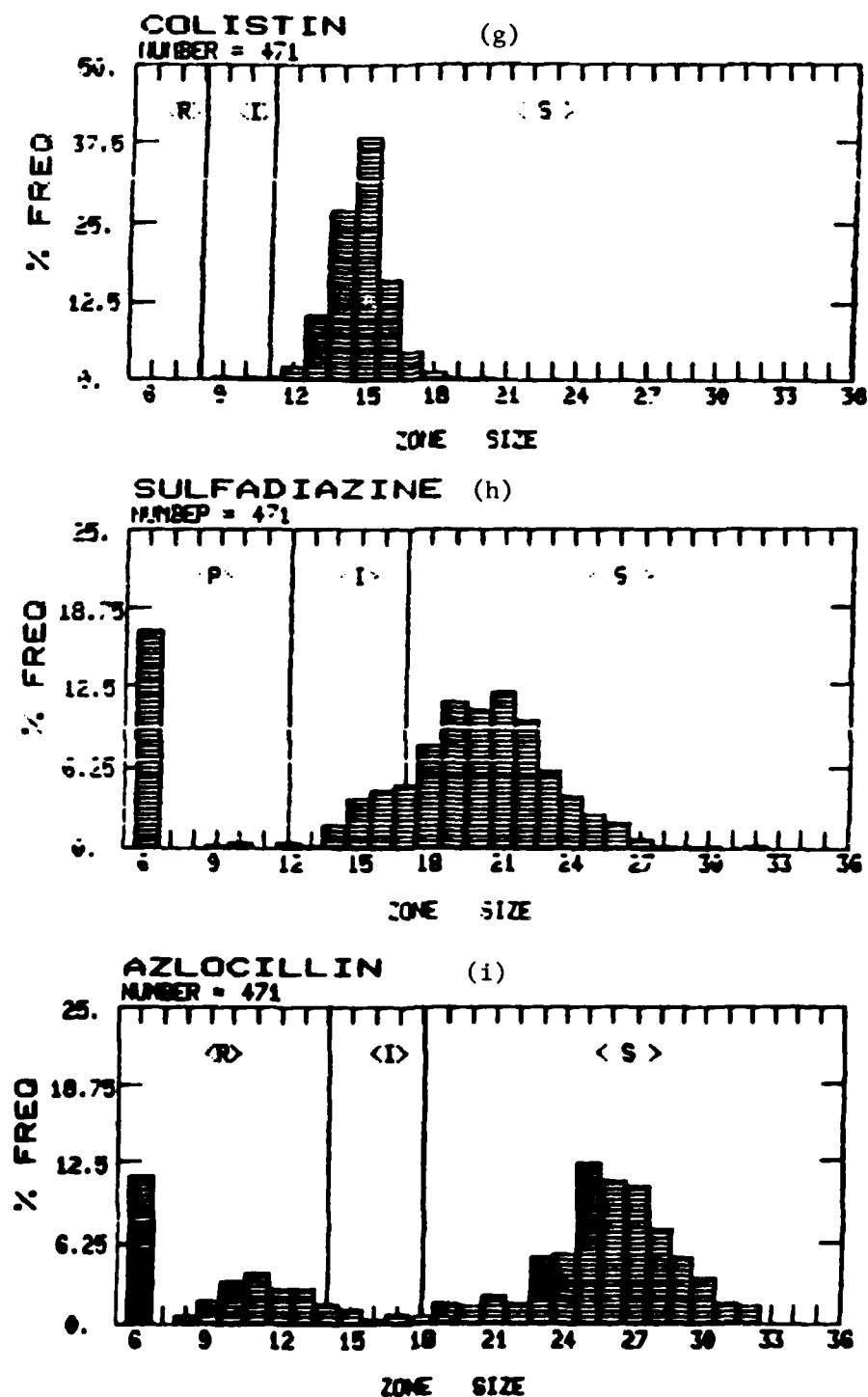


Figure 13. Histogram display of the distribution of zones of inhibition of growth of *Pseudomonas aeruginosa*. Antibiotics tested: (g) colistin (10 mcg), (h) sulfadiazine (250 mcg), (i) azlocillin (75 mcg).

ANTIBIOTIC SENSITIVITY DATA
 DATES : 83-10-01 TO 84-09-30
 ORGANISM : P AEPUG

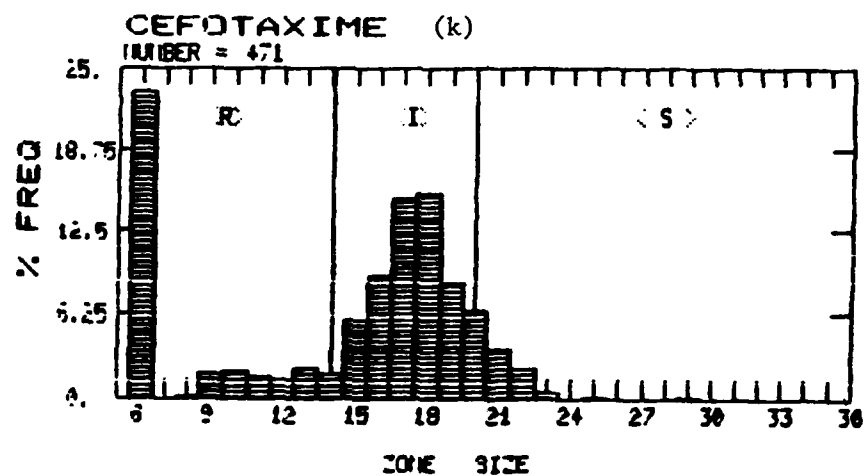
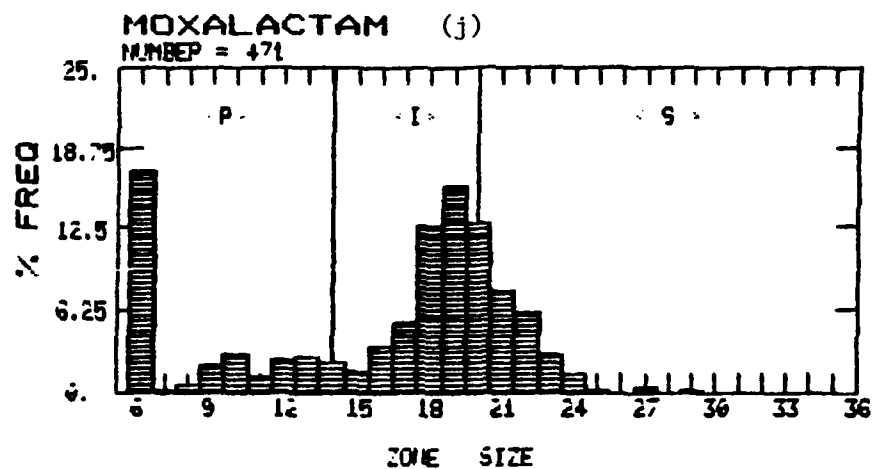


Figure 13. Histogram display of the distribution of zones of inhibition of growth of *Pseudomonas aeruginosa*. Antibiotics tested: (j) moxalactam (30 mcg), (k) cefotaxime (30 mcg).

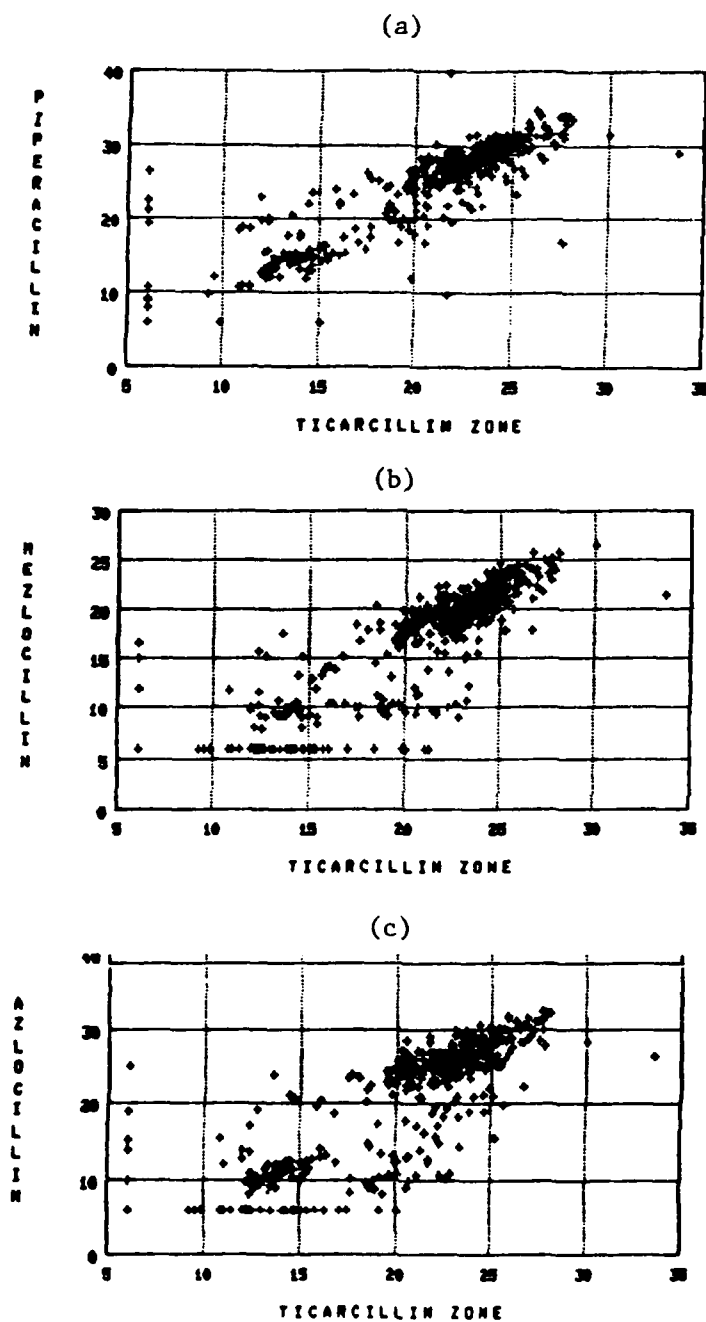


Figure 14. Scatter plot display of the distribution of zones of inhibition of tested *P. aeruginosa*.
 (a) ticarcillin (75 mcg) vs piperacillin (100 mcg),
 (b) ticarcillin (75 mcg) vs mezlocillin (75 mcg),
 and (c) ticarcillin (75 mcg) vs azlocillin (75 mcg).

FREQUENCY OF FY84 K.PNEUMO. TESTING

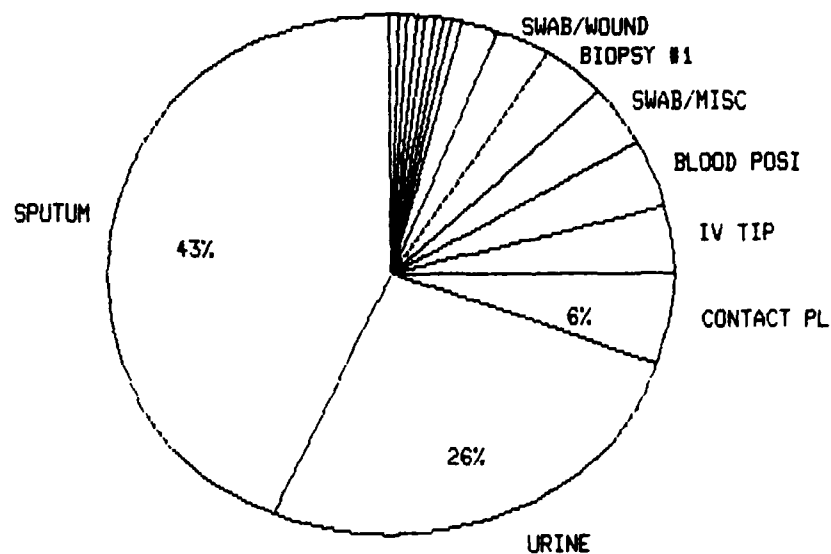


Figure 15. Display of the relative frequency of sources yielding K. pneumoniae (K. Pneumo.) tested for in vitro sensitivity to antibiotics in FY 84.

FREQUENCY OF FY84 S.MARCES. TESTING

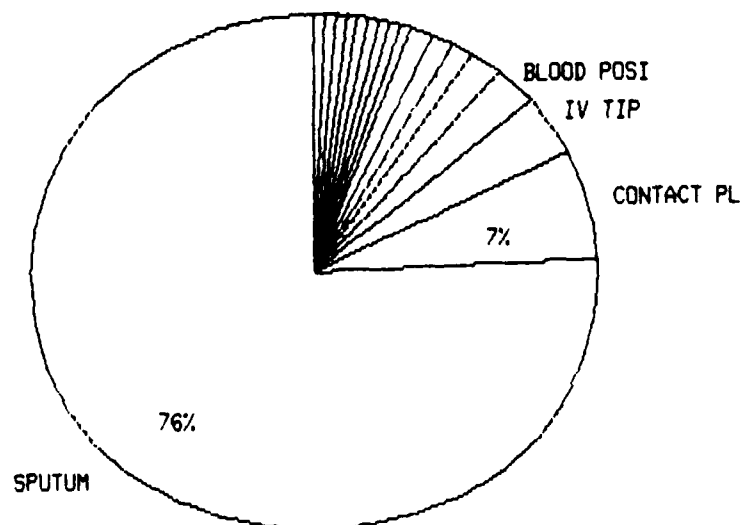


Figure 16. Display of the relative frequency of sources yielding S. marcescens (S. Marces.) tested for in vitro sensitivity to antibiotics in FY 84.

ANTIBIOTIC SENSITIVITY DATA
 DATES : 83-10-01 TO 84-09-30
 ORGANISM : E. CLOACAE

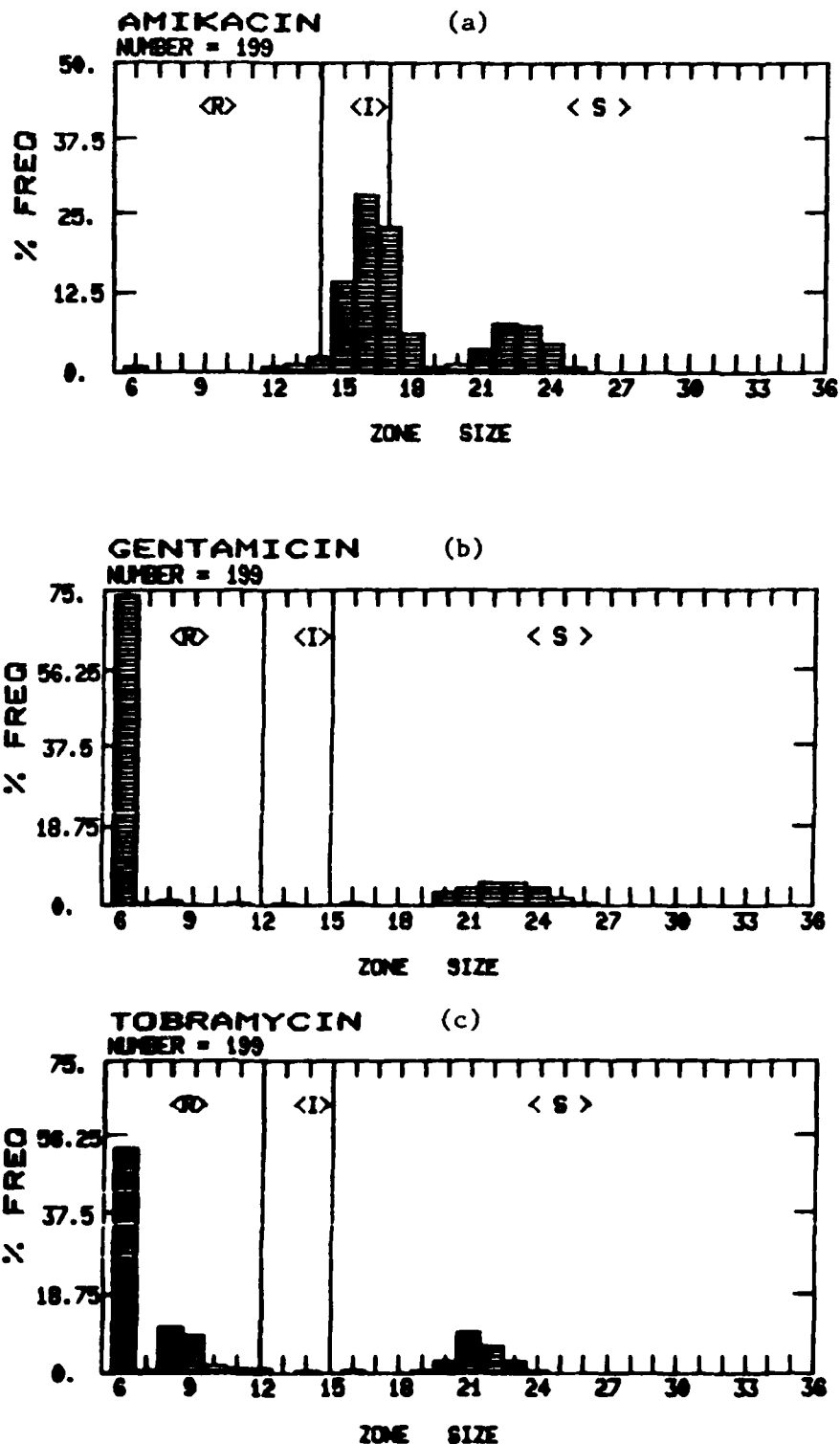


Figure 17. Histogram display of the distribution of zones of inhibition of growth of Enterobacter cloacae. Antibiotics tested: (a) amikacin (30 mcg), (b) gentamicin (10 mcg), (c) tobramycin (10 mcg).

ANTIBIOTIC SENSITIVITY DATA
 DATES : 83-10-01 TO 84-09-30
 ORGANISM : E CLOACAE

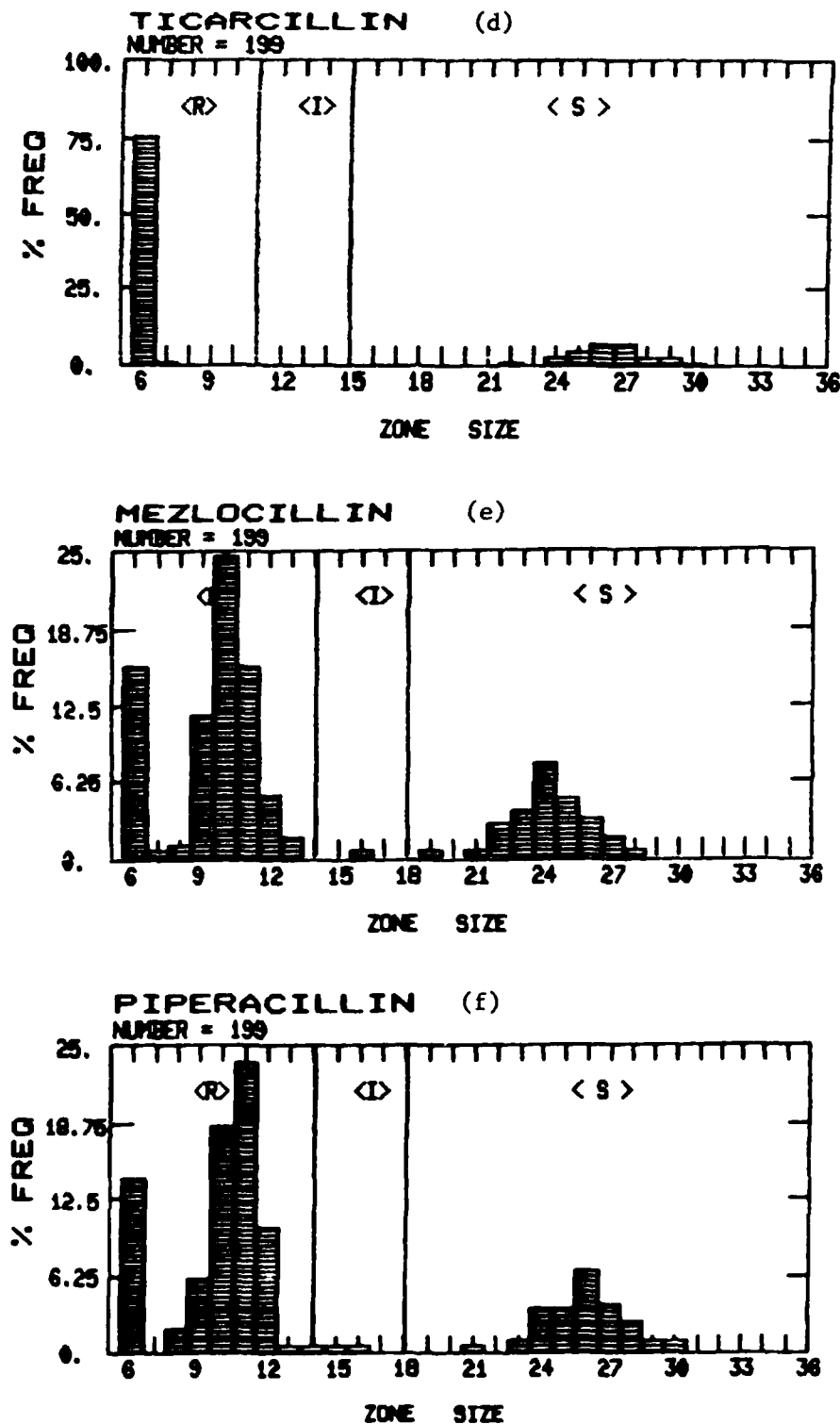


Figure 17. Histogram display of the distribution of zones of inhibition of growth of *Enterobacter cloacae*. Antibiotics tested: (d) ticarcillin (75 mcg), (e) mezlocillin (75 mcg), (f) piperacillin (100 mcg).

ANTIBIOTIC SENSITIVITY DATA
 DATES : 83-10-01 TO 84-09-30
 ORGANISM : E CLOACAE

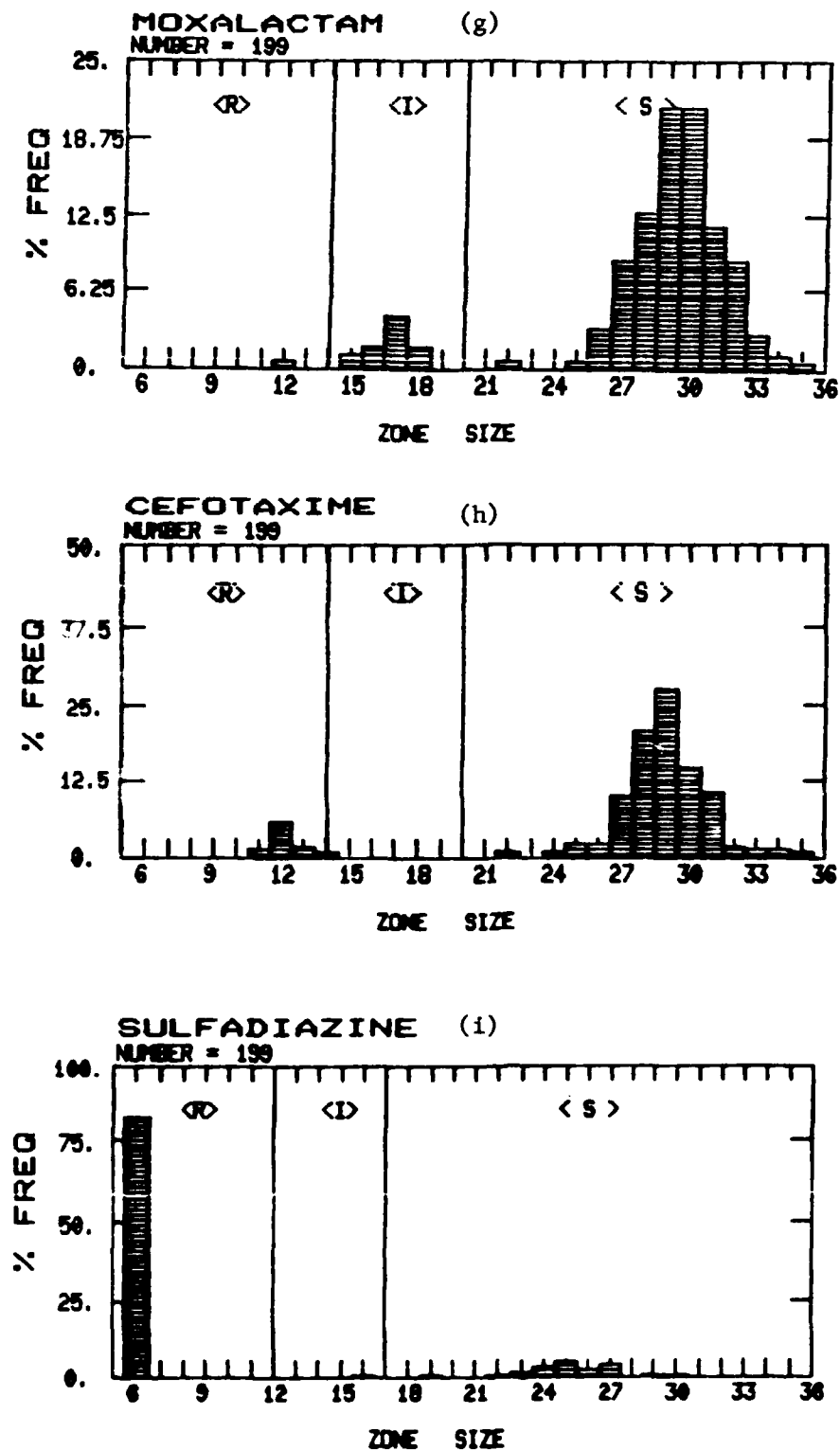


Figure 17. Histogram display of the distribution of zones of inhibition of growth of *Enterobacter cloacae*. Antibiotics tested: (g) moxalactam (30 mcg), (h) cefotaxime (30 mcg), (i) sulfadiazine (250 mcg).

ANNUAL PROGRESS REPORT

PROJECT NO. 3M161102BS10, BASIC MECHANISMS IN BURN INJURY

REPORT TITLE: A MULTICENTER OPEN STUDY OF THE EFFICACY, SAFETY
AND TOLERANCE OF THIENAMYCIN FORMAMIDINE/POTENTIATOR
IN THE PARENTERAL THERAPY OF INFECTION CAUSED BY
PATHOGENIC BACTERIA IN HOSPITALIZED PATIENTS

US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234

1 October 1983 - 30 September 1984

Investigators:

Basil A. Pruitt, Jr., M.D., Colonel, MC
Albert T. McManus, Ph.D.
William F. McManus, M.D., Colonel, MC
Khan Z. Shirani, M.D., LTC, MC
Alvis T. Perry, M.D., Captain, MC

Report Control Symbol MEDDH-288(R1)
UNCLASSIFIED

ABSTRACT

PROJECT NO. 3M161102BS10, BASIC MECHANISMS IN BURN INJURY

REPORT TITLE: A MULTICENTER OPEN STUDY OF THE EFFICACY, SAFETY AND TOLERANCE OF THIENAMYCIN FORMAMIDINE/POTENTIATOR IN THE PARENTERAL THERAPY OF INFECTION CAUSED BY PATHOGENIC BACTERIA IN HOSPITALIZED PATIENTS

US Army Institute of Surgical Research, Brooke Army Medical Center, Fort Sam Houston, Texas

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Alvis T. Perry, M.D., Captain, MC

Reports Control Symbol MEDDH-288(R1)

The efficacy, safety, and tolerance of Imipenem® (a combination of a novel derivative of Thienamycin and an inhibitor of renal dihydropeptidase-I) have been evaluated in the treatment of infection caused by pathogenic bacteria in 13 severely burned patients. In 7 patients with a mean extent of burn of 47% of the total body surface, the infection cleared and survival was achieved. In 6 patients with a mean extent of burn of 67% of the total body surface who died, treatment effect was variable - no improvement was evident in 2, improvement was evident in 2, and the treatment effect was considered indeterminate in 2. Duration of treatment averaged 8.3 days (range 3 to 15 days) with no toxicity or significant side effects noted. Development of microbial resistance was noted in 5 patients, three of whom survived and were discharged from the hospital. The broad antimicrobial spectrum of Imipenem®, its minimal toxicity, and low incidence of side effects justify its use in the treatment of infections caused by organisms resistant to other available antibiotics.

Infection
Antibiotic effects
Gram-negative bacteria
Humans

A MULTICENTER OPEN STUDY OF THE EFFICACY, SAFETY,
AND TOLERANCE OF THIENAMYCIN FORMAMIDINE/POTENTIATER
IN THE PARENTERAL THERAPY OF INFECTION CAUSED BY PATHOGENIC
BACTERIA IN HOSPITALIZED PATIENTS

Post-injury immunosuppression, the imperfect protection afforded by topical therapy, and the emergence of antibiotic resistance during therapy contribute to the persistence of infection as the most common cause of morbidity and mortality in severely burned patients. The organisms causing infections in a burn center change across time and such infections can be viewed as a series of miniepidemics caused by a broad range of opportunistic organisms. Similarly, the antibiotic sensitivity of the causative organisms may change with time and, in general, resistance to a given antibiotic increases in parallel with its use. These factors, which all contribute to the frequency of infection caused by organisms resistant to available antibiotics, mandate continued evaluation of the effectiveness and safety of newly developed antibiotics in the treatment of septic complications in severely injured and burned patients.

MATERIALS AND METHODS

Imipenem® (MK0797/MK0791) is a new combination of the N-formimidoyl monohydrate derivative of Thienamycin (a novel beta lactam antibiotic produced by *Streptomyces cattleya*) and a sodium salt of a derivatized heptenoic acid which inhibits renal dehydropeptidase-I. The Thienamycin derivative has a greater potency than has been reported for any other natural or semi-synthetic beta lactam antibiotic with 92% to 94% of cephalosporin resistant *Pseudomonas* organisms sensitive to between 4 and 8 mcg/ml. Imipenem® shows good antimicrobial activity against *Pseudomonas aeruginosa*, *Serratia*, *Enterobacter*, *Enterococcus*, the gram-negative anaerobes, *Streptococcus pyogenes*, and *Staphylococcus aureus*. Cilastatin, the inhibitor of renal dihydropeptidase-I, increases renal clearance of Imipenem® above the glomerular filtration rate acting in a competitive and freely reversible manner to decrease renal beta lactam ring hydrolysis, reduce nephrotoxicity, and increase urinary tract bioavailability.

Burn patients greater than 12 years in age with acute bacterial infections caused by organisms presumed or proved to be susceptible to Imipenem® have been entered in this study. Each patient is treated with an intravenous course of therapy for 5 to 42 days. Imipenem® is administered at a dosage ranging from 1 to 3 gm per day, or in cases with severe life threatening infection, a dosage of 4 gm per day. The clinical and bacteriologic course of each patient has been followed and documented, with evaluation made of bacteriologic and clinical efficacy as well as the safety and tolerance of the antibiotic regimen. Laboratory data have been obtained before, during, and after therapy to identify hematologic, renal, and hepatic dysfunction.

RESULTS

To date, 13 burn patients have been entered in this study. (Table I) The study patients consist of 12 males and 1 female ranging in age from 13 to 70 years, with an average age of 35.5 years. Seven patients with a mean extent of burn of 47% of the total body surface (range 25% to 71%) survived. Six patients with a mean extent of burn of 67% of the total body surface (range 32% to 84%) expired. The duration of Imipenem® treatment ranged from 3 to 15 days with an average duration of treatment of 8.3 days. Eleven patients had monomicrobial infection caused by *E. coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Serratia marcescens*, *Hemophilus parainfluenzae*, and *Streptococcus viridans*. Two patients had polymicrobial infections caused by *Staphylococcus* plus a gram-negative organism. Four patients with polymicrobial infections expired and both of the patients with polymicrobial infections expired. (Table II)

Clinical assessment revealed no response or no improvement in four patients of whom two expired. An indeterminant response occurred in two patients both of whom expired and improvement or cure occurred in seven patients of whom only two expired. (Table III) In terms of microbiologic effect, the infecting organisms were reduced in number in 10 patients from five of whom resistant organisms were recovered. In three patients in none of whom were resistant organisms recovered, the infecting organism was eradicated. (Table IV)

DISCUSSION

Outcome in the 13 patients studied to date has appeared to be most closely related to extent of burn injury and disease process. (Table V) All three of the patients treated for tracheobronchitis have survived, while only four of the 10 patients treated for bronchopneumonia have survived. The overriding effect of extent of burn is evident in the latter group, particularly in the six patients who expired. In four of the patients who expired clinical and/or roentgenologic improvement of the pulmonary infection was recorded but the patients subsequently expired with burn wound infection, other soft tissue infection, or septicemia caused by a different organism (bacterial or fungal) either during treatment with Imipenem® (one patient) or three to 94 days following cessation of Imipenem® therapy.

Toxicity and side effects were minimal with a transient skin rash noted at the infusion site in one patient and evanescent premature ventricular contractions of one day's duration occurring in another patient, both of whom survived. Of interest was the recovery of resistant organisms from five of the treated patients in whom the duration of treatment ranged from three to

nine days. Three of the patients from whom resistant organisms were recovered survived, but only one of those patients showed clinical improvement while receiving Imipenem®. Imipenem® appears to be an antimicrobial agent of low toxicity with minimal side effects which is useful in the treatment of burn patients with pulmonary infections caused by a wide variety of opportunistic organisms. Injury severity related infection susceptibility influences outcome as indicated by the strong correlation between burn size and subsequent life threatening infections in other sites.

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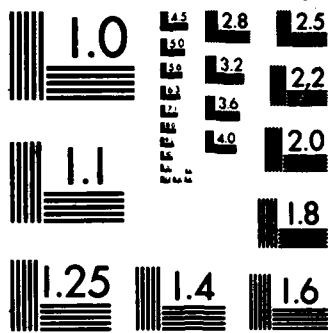
PROGRESS REPORT FOR FISCAL YEAR 1984(07 ARMY INS
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The figure consists of a 10x10 grid of 100 small square images. Each image shows a different pattern of light and dark spots, representing various stages of a process. The patterns are arranged in a grid, with the first row showing a single bright spot in the center, and subsequent rows showing more complex patterns of spots and lines. The patterns are arranged in a grid, with the first row showing a single bright spot in the center, and subsequent rows showing more complex patterns of spots and lines. The patterns are arranged in a grid, with the first row showing a single bright spot in the center, and subsequent rows showing more complex patterns of spots and lines.



MICROCOPY RESOLUTION TEST CHART
NATIONAL BUREAU OF STANDARDS-1963-A

TABLE I

OPEN CLINICAL TRIAL OF IMIPENEM®
IN BURN PATIENTS

TOTAL PATIENTS TREATED: AVG AGE 35.5 (13-70)	13
SURVIVED (MEAN EXTENT OF BURN 47% TBS)	7
DIED (MEAN EXTENT OF BURN 67% TBS)	6
DURATION OF TREATMENT - AVG 8.3 DAYS (3-15)	

TABLE II

CAUSATIVE ORGANISMS - IMIPENEM® CLINICAL TRIAL

	TOTAL	SURVIVED	DIED
MICROBIAL INFECTIONS:			
E. COLI	2	0	2
PSEUDOMONAS AERUGINOSA	3	2	1
STAPHYLOCOCCUS AUREUS	2	1	1
SERRATIA MARCESCENS	1	1	
HEMOPHILUS PARAINF	2	2	
STREPTOCOCCUS VIRIDANS	1	1	
POLYMICROBIAL INFECTIONS:			
STAPHYLOCOCCUS AUREUS PLUS GRAM-NEGATIVE ORGANISM	2	0	2

TABLE III

RESPONSE TO TREATMENT
IMIPENEM® CLINICAL TRIAL

	TOTAL NUMBER OF PATIENTS	DEATHS
NO RESPONSE OR NO IMPROVEMENT	4	2
INDETERMINATE RESPONSE	2	2
IMPROVED OR CURED	7	2

TABLE IV

MICROBIOLOGIC EFFECT OF TREATMENT
IMIPENEM® CLINICAL TRIAL

	TOTAL NUMBER OF PATIENTS	DEVELOPMENT OF RESISTANCE
INFECTING ORGANISM ERADICATED	3	0
INFECTING ORGANISMS REDUCED IN NUMBER	10	5

TABLE V

INFECTIONS TREATED
IMIPENEM® CLINICAL TRIAL

BRONCHOPNEUMONIA	10
SURVIVED - 4	
DIED - 6	
TRACHEOBRONCHITIS	3
SURVIVED - 3	

ANNUAL PROGRESS REPORT

PROJECT NUMBER: 3M161102BS10-00, BASIC RESEARCH

PROJECT TITLE: STUDIES OF INFECTION AND MICROBIOLOGIC SURVEILLANCE OF TROOPS WITH THERMAL INJURY: Comparison of Virulence of *Pseudomonas aeruginosa* in Burned Rats and Mice

US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234-6200

1 October 1983 - 30 September 1984

INVESTIGATORS

George B. Stover, Captain, MS
Albert T. McManus, PhD

CO-INVESTIGATORS

Gene B. Hubbard, DVM, Lieutenant Colonel, VC
Arthur D. Mason, Jr., MD

REPORT CONTROL SYMBOL - MEDDH-288 (R1)

UNCLASSIFIED

ABSTRACT

PROJECT NUMBER: 3M161102BS10-00, BASIC RESEARCH

PROJECT TITLE: STUDIES OF INFECTION AND MICROBIOLOGIC SURVEILLANCE OF TROOPS WITH THERMAL INJURY: Comparison of Virulence of *Pseudomonas aeruginosa* in Burned Rats and Mice

US Army Institute of Surgical Research, Brooke Army Medical Center, Fort Sam Houston, Texas 78234-6200

PERIOD COVERED IN THIS REPORT: 1 Oct 83 through 30 Sep 84

INVESTIGATORS: George B. Stover, Captain, MS
Albert T. McManus, PhD

CO-INVESTIGATORS: Gene B. Hubbard, DVM, Lieutenant Colonel, VC
Arthur D. Mason, Jr., MD

REPORT CONTROL SYMBOL: MEDDH-288 (R1)

Gram-negative sepsis resulting from invasion of burn wounds is classically associated with *Pseudomonas aeruginosa*. Previous reports from this Institute have documented the Walker-Mason scalded rat model as a means of investigating burn wound sepsis and its treatment. We have examined the mouse as an experimental model for this process. Anesthetized CE-1 mice weighing 26 grams were subjected to a 12 square centimeter full-thickness dorsal burn using a watertight template. A seven-second exposure to 80°C water resulted in a third-degree burn. Susceptibility to topically-applied organisms (strain 59-1244) was examined. Dose response experiments showed the LD₅₀ to be less than 10⁴ CFU, with most deaths occurring within four days. Pathogenesis was examined by quantitative bacteriologic and histologic evaluation of serially sacrificed animals. The inoculum was 6 x 10⁵ CFU. A progressive increase in wound counts preceded bacteremia. Cultures of the liver, spleen, and lungs were positive by 36 hours postburn. Liver and spleen counts were more than 10⁷ CFU/gram by 60 hours postburn. Histologic examination of moribund mice showed little morphologic correlation with the observed tissue counts. The most significant morphologic finding was multifocal hepatic necrosis with minimal inflammatory cell infiltration. The lack of host tissue response suggests that the scalded mouse may be exceedingly susceptible to *Pseudomonas* infection.

Mouse Model
Infection
Pseudomonas
Sepsis

COMPARISON OF VIRULENCE OF *Pseudomonas aeruginosa* IN BURNED RATS AND MICE

Pseudomonas aeruginosa is an important pathogen for the immunocompromised patient (1-3). The invasive nature of this pathogen and its frequent resistance to antimicrobial agents has made it a particularly difficult problem for patients with large burns (4). An animal model of *Pseudomonas* burn invasion was defined for the scalded rat in 1964 (5). This model showed the bacteriologic and histopathologic changes characteristic of human burn invasion (6). For this reason, it has been widely used for testing topical antimicrobial agents and other therapeutic burn modalities. The space and cost requirements of the rat model are a limiting feature. We have investigated the outbred mouse as a possible alternative model of invasive *Pseudomonas* burn wound sepsis. The establishment of a valid model in the mouse would offer savings in space and cost as well as opportunities to investigate a wider variety of genetic variables. Although mouse models of increased susceptibility to *Pseudomonas aeruginosa* have been previously reported (7,8), bacteriologic and histologic evidence of invasive wound sepsis has not been established.

MATERIALS AND METHODS

Bacteria. *Pseudomonas aeruginosa* strains ISR 59-1244 (9) and a streptomycin-resistant mutant (MIC greater than one mg/ml) of 1244 were used throughout this study.

¹Pruitt BA Jr and McManus AT: Opportunistic infections in severely burned patients. *Am J Med* 76:146-154, 1984.

²Young LS: The clinical challenge of infections due to *Pseudomonas aeruginosa*. *Rev Infect Dis* 6:S603-S607, 1984.

³Pollack M: The virulence of *Pseudomonas aeruginosa*. *Rev Infect Dis* 6:S617-S626, 1984.

⁴Morrison AJ Jr and Wenzel RP: Epidemiology of infections due to *Pseudomonas aeruginosa*. *Rev Infect Dis* 6:S627-S642, 1984.

⁵Walker HL, Mason AD Jr, and Raulston GL: Surface infection with *Pseudomonas aeruginosa*. *Ann Surg* 160:297-305, 1964.

⁶Teplitz C, Davis D, Mason AD Jr, and Moncrief JA: *Pseudomonas* burn wound sepsis. I. Pathogenesis of experimental *Pseudomonas* burn wound sepsis. *J Surg Res* 4:200-216, 1964.

⁷Stieritz DD, Bondi A, McDermott D, and Michaels EB: A burned mouse model to evaluate anti-*Pseudomonas* activity of topical agents. *J Antimicrob Chemother* 9:133-140, 1962.

⁸Stieritz DD and Holder IA: Experimental studies of the pathogenesis of infections due to *Pseudomonas aeruginosa*: description of a burned mouse model. *J Infect Dis* 131:688-691, 1975.

⁹Walker HL, McLeod CG, Leppla SH, and Mason AD Jr: Evaluation of *Pseudomonas aeruginosa* toxin A in experimental rat burn wound sepsis. *Infect Immun* 25:828-830, 1979.

Quantitation of Bacteria. Bacteria were grown in brain heart infusion broth for 16 hours at 37°C on a gyratory shaker. Cells were harvested by centrifugation (4,000 x g for 15 minutes), washed, and diluted in a standard diluent (25 mM potassium phosphate, pH 7.0, 0.9% NaCl, 0.01% gelatin, 0.2 mM MgSO₄·7H₂O). Viable cell counts were determined by serial dilution plate counts done in duplicate on trypticase soy agar plates.

Laboratory Animals. Male CD-1 mice (Charles River Breeding Laboratories, Inc.) weighing approximately 26 grams were used in all experiments. Prior to experimental use, the animals were maintained in cages at 30 mice per cage with food and water available *ad libitum*.

Experimental Burn Procedure. Experimental and control animals were anesthetized with intraperitoneal injection of sodium pentobarbital (0.5 milliliters of a 1:20 dilution). The backs of the mice were then shaved with an Oster small animal clipper (blade size 40). Each mouse was placed in a fixed area (12 square centimeters) shield and the dorsum was immersed in 80°C water for seven seconds. This resulted in a third-degree burn of approximately 15 percent of the total body surface area. Following the scald, all mice were injected intraperitoneally with 0.5 milliliters of sterile physiological saline. Animals were challenged immediately postburn by topical application of 0.3 milliliters of a bacterial suspension. Control mice were burned but not inoculated.

Bacterial Quantitation of Animal Tissue and Blood: At various times postburn, animals were sacrificed by decapitation and the liver, spleen, lungs, and burned skin were aseptically removed. The burned skin was divided into two parts, one of which was immersed in 70 percent isopropanol, ignited, and alcohol flamed for six seconds. The alcohol immersion-flaming was repeated twice to insure destruction of bacteria colonizing the surface of the burned skin (6). The skin and organs were weighed and disrupted in plastic bags containing five milliliters of sterile diluent. The samples were homogenized for two minutes using a Stomacher lab-blender 80 (Seward Laboratory, London). Bacterial content of the homogenates and blood was determined by spiral plating (Spiral Systems, Bethesda, Maryland) on cetrimide agar plates. Plates were counted after 24 hours' incubation at 37°C. Bacterial counts were expressed as CFU per gram weight of tissue. Numbers of bacteria in the blood were similarly determined from samples collected after decapitation. Blood, collected immediately after death, was diluted 1:10 in standard diluent to prevent clotting and the bacterial counts were expressed as CFU per 0.1 milliliter. Results were expressed as the average counts from three mice.

Histologic Examination. The depth of the burn wound was determined in mice sacrificed after scalding for five, six, seven,

or eight seconds. Infected mice were housed one per conventional wire bottomed, stainless steel cage. Mice that died spontaneously or were sacrificed were necropsied. Complete sets of tissue were fixed in 10 percent neutral buffered formalin and processed by standard methods.

RESULTS

A seven-second exposure to 80°C water provided the most consistent full-thickness burn without excessive damage to subdermal tissue (Figure 1). Diffuse uniform thermal necrosis of all tissue within the template was observed. The necrosis extended to the subdermal skeletal muscle. Microscopic examination of the burn wounds revealed colonization of the necrotic tissue by a uniform population of Gram-negative bacteria morphologically consistent with *Pseudomonas* (Figures 2 and 3). Inflammatory cell infiltrates associated with the burn foci were comprised primarily of neutrophils and macrophages. Bacteria were also seen in viable tissue subjacent to the burn focus. The most significant morphologic change in the mice was acute multifocal hepatic necrosis (Figure 4).

Increased susceptibility of mice to *Pseudomonas aeruginosa* following burning has been previously reported (8). Strain 59-1244 is the standard challenge strain used in the Walker-Mason scalded rat model. The virulence of this strain was tested in scalded mice. As seen in Table 1, the strain is also virulent for the scalded mouse. The LD₅₀ following surface inoculation was less than 10⁴ CFU. The streptomycin mutant of strain 59-1244 also had an LD₅₀ of less than 10⁴ CFU (Table 2).

The bacteriologic course of the model is shown in Figure 5. When 10⁵ CFU were topically applied to burned mice, there was rapid multiplication of bacteria in the burned skin. Counts in unflamed skin reached 10⁹ CFU within 36 hours. Intraeschar colonization (flamed skin) was detected by 12 hours postburn and reached levels of 10⁷ CFU by 36 hours postburn. Bacteremia was first detected at 12 hours postburn and reached levels of 10²-10⁴ CFU/0.1 milliliter. Bacterial invasion of the liver, spleen, and lungs occurred within 48 hours. Within 60 hours, 10⁷-10⁸ CFU/gram were detected in the liver and spleen while 10⁵ CFU/gram were present in the lungs. All control mice survived. Histological examination of control burns showed no evidence of bacterial infection.

DISCUSSION

Application of 10⁵ CFU of *Pseudomonas aeruginosa* strain 59-1244 clearly resulted in a progressive invasive infection as demonstrated in the bacteriologic and histologic results. Burn wound invasion was the first manifestation of *Pseudomonas* infection. Intraeschar growth was confirmed in alcohol flamed burned skin, where bacteria on the surface were destroyed by alcohol-

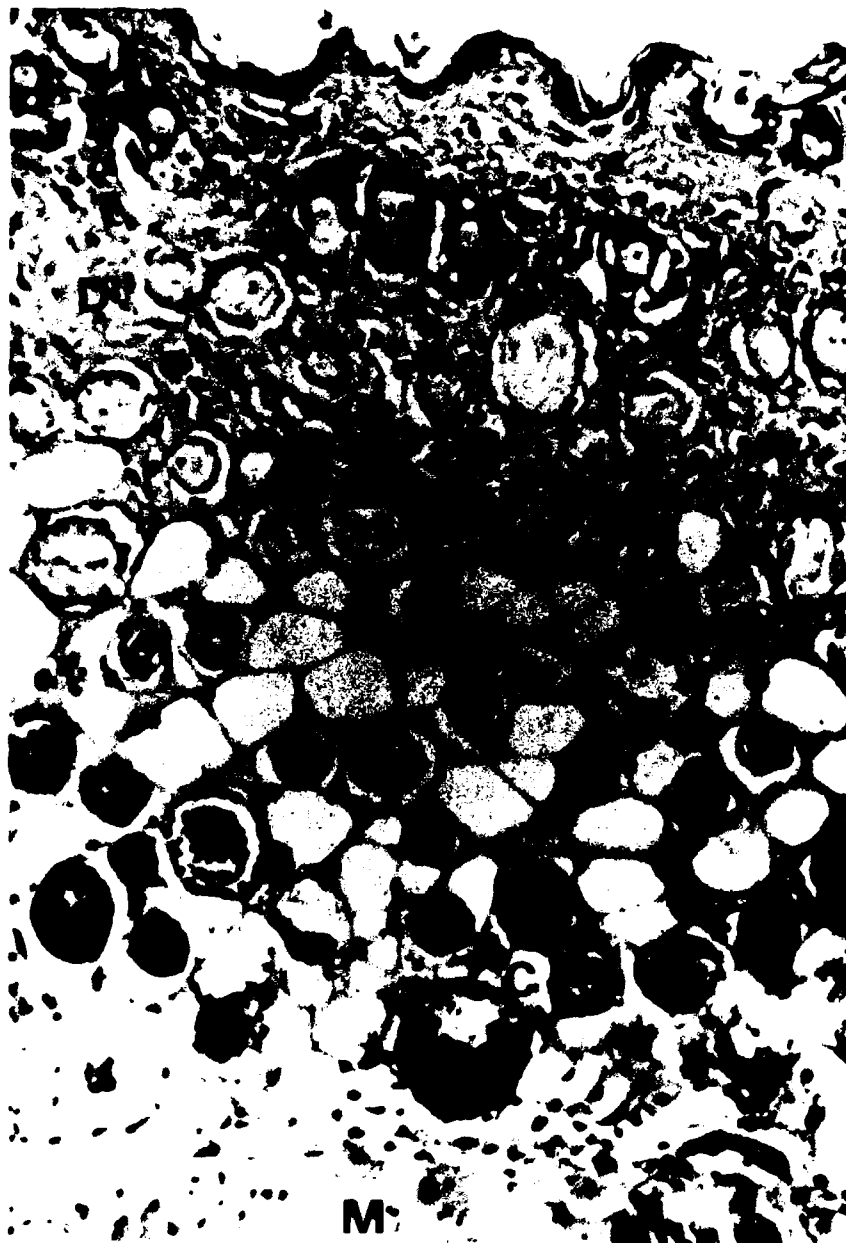


Figure 1. This photograph illustrates the histologic changes associated with a full-thickness burn caused by a seven-second exposure of skin to 80°C water. Mouse was sacrificed immediately postburn. Necrosis of the epidermis, adnexial structures (A), dermal connective tissue (D), capillaries (C), and skeletal muscle (M) are visual proof of the burn exposure. Note the nuclear pyknosis, granular coagulation of cytoplasm, hemogenization of dermal collagen, and fragmentation of skeletal muscle. (H AND E STAIN)



Figure 2. Bacterial colonization of the burn wound is evidenced by colonies of Gram-negative pseudomonads (P). These bacteria are especially numerous in the epithelium and dermis. Approximately 52 hours postburn. (GRAM STAIN)



Figure 3. Bacterial colonization of the subdermal skeletal muscle (M) by pseudomonads (P). Approximately 52 hours postburn. (H AND E STAIN)

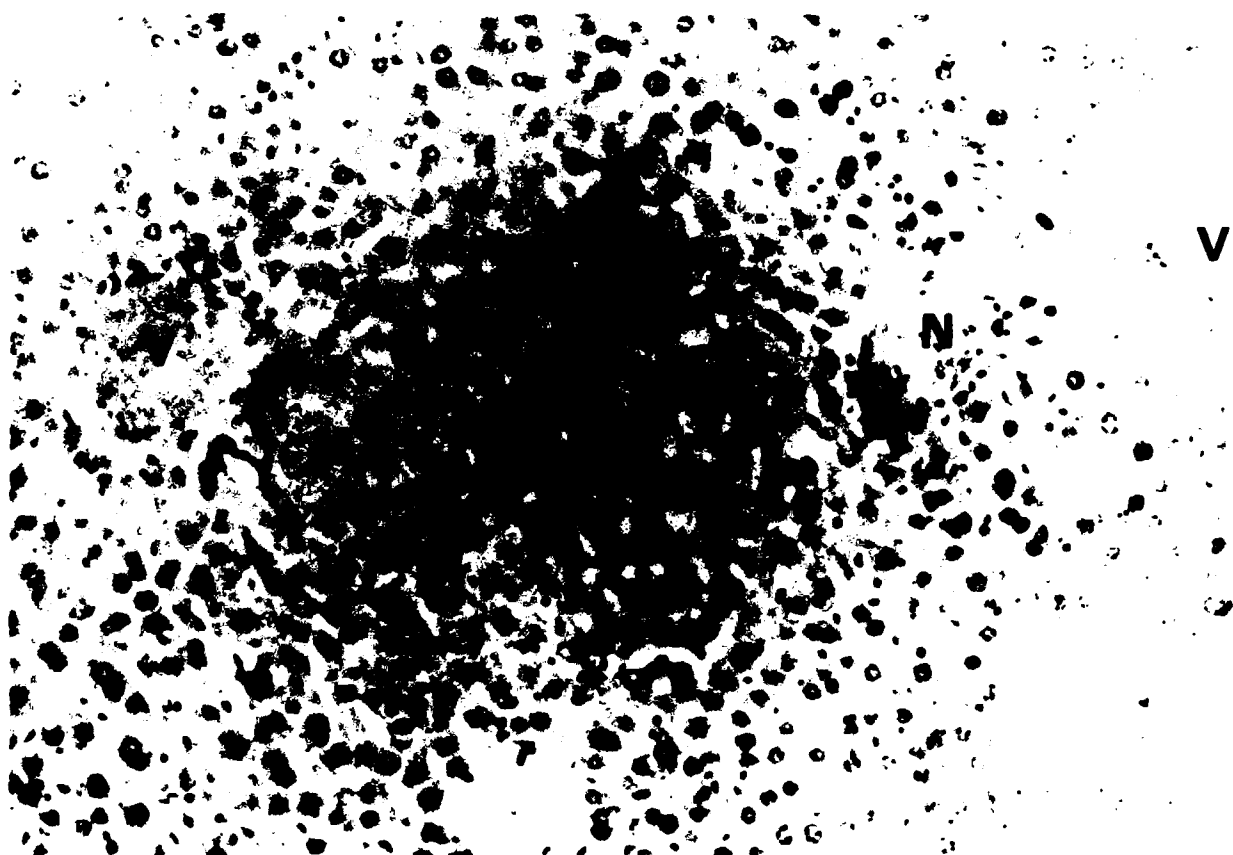


Figure 4. Multifocal hepatic necrosis (N) with minimal inflammatory cell infiltration. Note the close proximity to portal veins (V). These changes support the hemogenous spread of the pseudomonads. Approximately 52 hours postburn. (H AND E STAIN)

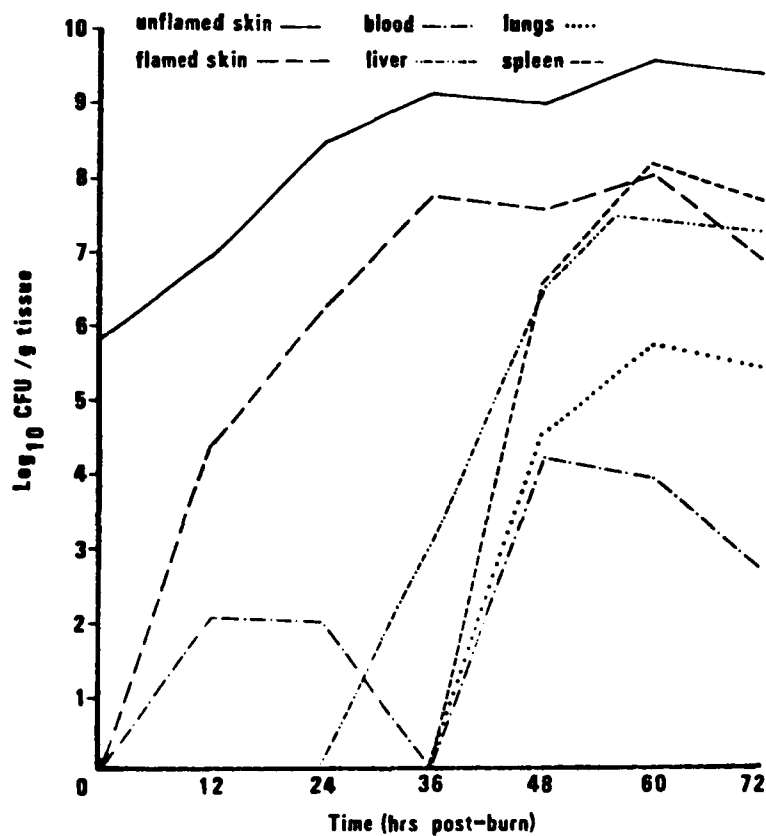


Figure 5. Quantitative bacteriology of tissues and blood after topical inoculation of burned skin with 6×10^5 CFU immediately postburn.

TABLE 1. MORTALITY IN BURNED, 1244 INFECTED MICE

Challenge Dose	Mortality/Day ^a									
	1	2	3	4	5	6	7	8	9	10 ^b
Control	1/10 [†]	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10
1.11x10 ⁸	1/10 [†]	5/10	8/10	9/10	10/10	—	—	—	—	—
1.11x10 ⁷	0/10	5/10	9/10	10/10	—	—	—	—	—	—
1.11x10 ⁶	0/10	2/10	6/10	7/10	7/10	9/10	9/10	9/10	9/10	9/10
1.11x10 ⁵	0/10	2/10	9/10	10/10	—	—	—	—	—	—
1.11x10 ⁴	0/10	1/10	4/10	5/10	5/10	6/10	8/10	8/10	9/10	9/10
1.11x10 ³	0/10	2/10	5/10	6/10	6/10	6/10	7/10	7/10	7/10	8/10

† died ≤ 2 hrs postburn

a # dead/# in group

b no further deaths for 21 days postburn

TABLE 2. MORTALITY IN BURNED, 1244 Sm^R INFECTED MICE

Challenge Dose	Mortality/Day ^a											
	1	2	3	4	5	6	7	8	9	10	11	12 ^b
Control	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
1.22 x 10 ⁸	0/10	6/10	8/10	10/10	—	—	—	—	—	—	—	—
1.22 x 10 ⁷	1/10	9/10	9/10	10/10	—	—	—	—	—	—	—	—
1.22 x 10 ⁶	0/10	9/10	10/10	—	—	—	—	—	—	—	—	—
1.22 x 10 ⁵	0/10	1/10	2/10	5/10	5/10	6/10	6/10	6/10	6/10	6/10	6/10	7/10
1.22 x 10 ⁴	0/10	1/10	1/10	2/10	3/10	3/10	5/10	5/10	5/10	5/10	5/10	5/10

a # dead/# in group

b no further deaths for 21 days postburn

immersion flaming. Histologic evidence of invasion correlated with tissue counts of 10^5 or greater (6,10). Subsequent bacteremia and invasion of deep body tissues were also demonstrated. Invasion of the liver occurred after intraeschar colonization had reached levels of 10^6 CFU/gram tissue. Despite the high bacterial counts in organs, however, little tissue reactivity was observed. This result suggests that the scalded mouse may be a useful model for the study of microbial pathogenesis in a severely immunodepressed host.

PRESENTATIONS/PUBLICATIONS

None.

¹⁰Nathan P, Holder IA, and MacMillan BG: Burn wounds: microbiology, local host defenses, and current therapy. *CDC Crit Rev Clin Lab Sci* 4:61-100, 1973.

ANNUAL PROGRESS REPORT

PROJECT NUMBER: 3M161102BS10-00, BASIC RESEARCH

PROJECT TITLE: STUDIES OF INFECTION AND MICROBIOLOGIC SURVEILLANCE OF TROOPS WITH THERMAL INJURY: Pharmacokinetic Study of Amikacin in Burned Patients

US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234-6200

1 October 1983 - 30 September 1984

INVESTIGATORS:

Lawrence M. Lehrner, MD, Major, MC
James McKay, MD, Major, MC
Albert T. McManus, PhD
Arthur D. Mason, Jr., MD

REPORT CONTROL SYMBOL - MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NUMBER: 3M161102BS10-00, BASIC RESEARCH

PROJECT TITLE: STUDIES OF INFECTION AND MICROBIOLOGIC SURVEILLANCE OF TROOPS WITH THERMAL INJURY: Pharmacokinetic Study of Amikacin in Burned Patients

US Army Institute of Surgical Research, Brooke Army Medical Center, Fort Sam Houston, Texas 78234-6200

PERIOD COVERED IN THIS REPORT: 1 Oct 83 through 30 Sep 84

INVESTIGATORS: Lawrence M. Lehrner, MD, Major, MC
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REPORT CONTROL SYMBOL: MEDDH-288(R1)

A major complication of thermal injury is infection, frequently with Gram-negative organisms. Amikacin is often the drug of choice. Amikacin is excreted by the kidneys and is nephrotoxic. The study was designed to determine the proper dosage of amikacin in thermally-injured patients. During the past year, the protocol was approved by the appropriate human subject review committees. The US Army Institute of Surgical Research Biochemistry Branch acquired a machine which measures amikacin concentrations in serum. Computer programs to calculate the pharmacokinetics were written. We have performed the kinetic modeling phase of the experiment (drawing five blood samples after infusion of the first dose of amikacin) in one patient.

Human
Volunteer
Drug
Amikacin
Glomerular Filtration Rate
Pharmacokinetics
Burns
Thermal Injury

PHARMOKINETIC STUDY OF AMIKACIN IN BURNED PATIENTS

INTRODUCTION

Amikacin is frequently used in the treatment of burned patients, either to treat infections or perioperatively. During fiscal year 1982, final disposition was made on 231 patients. Of these, 131 received at least one dose of amikacin.

Several studies, including one of the 10 burned patients treated with amikacin, have demonstrated that the recommended dosages of aminoglycosides frequently yield serum concentrations which are lower than the therapeutic range (1-5). However, the studies do not define the mechanism responsible for the low serum levels. Therefore, the following study is proposed.

METHODS AND MATERIALS

A maximum of 25 patients will be studied. All patients age 16 or older will be eligible for entry into the study. When the ward physician determines that a patient age 16 or older needs to be started on amikacin, one of the investigators will explain this protocol to the patient or appropriate substitute for the purpose of obtaining informed consent prior to the institution of drug treatment.

After obtaining informed consent, all patients will have the pharmacokinetics of amikacin determined by a standard technique (1). Briefly, amikacin (3-5 mg/kg) will be infused intravenously for 60 minutes. Serum samples will be obtained at 15 and 30 minutes, one to two hours, and four to five hours after infusion. Amikacin serum concentrations will then be measured using an Abbott TDx drug analyzer. After the kinetic infusion,

¹Zaske DE, Irvine P, Strand LM, Strate RG, Cipolle RJ, and Rotschafer J: Wide interpatient variations in gentamicin dose requirements for geriatric patients. *JAMA* 248:3122-3126, 1982.

²Zaske DE, Sawchuk RJ, Gerding DN, and Strate RG: Increased dosage requirements of gentamicin in burn patients. *J Trauma* 16:824-828, 1976.

³Zaske DE, Cipolle RJ, and Strate RG: Gentamicin dosage requirements: wide interpatient variations in 242 surgery patients with normal renal function. *Surgery* 87:164-169, 1980.

⁴Zaske DE, Sawchuk RJ, and Strate RG: The necessity of increased doses of amikacin in burn patients. *Surgery* 84:603-608, 1978.

⁵Zaske DE, Bootman JL, Solem LB, and Strate RG: Increased burn patient survival with individualized dosages of gentamicin. *Surgery* 91:142-149, 1982.

amikacin will be continued at a dosage determined by the patient's ward physician.

Subsequently, peak (30 minutes after an intravenous dose) and trough (immediately prior to a dose) levels of amikacin will be determined once daily while the patient is receiving the drug. In addition, a 24 hour urine will be collected daily for determination of drug and creatinine clearance. If toxic levels of the drug are found, the ward physician will be immediately notified so that appropriate adjustments of the dosage can be made.

This protocol places no restrictions on any other therapy the patient may receive. Any decision to discontinue amikacin will be made by the patient's ward physician.

The only special invasive monitoring (requiring only a venous blood sample) is the determination of peak and trough serum levels of the aminoglycoside since the standard procedure at this Institute is to obtain serum electrolytes and creatinine daily.

RESULTS

During FY 84, an Abbott TDx drug analyzer was acquired by this Institute. Amikacin levels can now be determined in less than five minutes. Computer programs to calculate the pharmacokinetics have been written. We have performed the kinetic modeling phase of the experiment (drawing five blood samples after infusion of the first dose of amikacin) in one patient and used the computer programs to calculate the pharmacokinetics. We expect to complete this protocol during FY 85.

DISCUSSION

The relationship of the pharmacokinetics and renal clearance of amikacin to patient age, sex, renal function, percent initial burn, percent total body surface area not covered at the time of drug administration, weight, and percent change from pre-burn weight will be determined by multiple correlation and regression techniques.

PRESENTATIONS/PUBLICATIONS

None.

ANNUAL PROGRESS REPORT

PROJECT NUMBER: 3M161102BS10-00, BASIC RESEARCH

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

US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234-6200

1 October 1983 - 30 September 1984

INVESTIGATORS

Kuang-Tzu D. Lin, MD, PhD
David G. Burleson, PhD, Major, MS
Michael C. Powanda, PhD, Lieutenant Colonel, MS

REPORT CONTROL SYMBOL - MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NUMBER: 3M161102BS10-00, BASIC RESEARCH

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

US Army Institute of Surgical Research, Brooke Army Medical Cen-
ter, Fort Sam Houston, Texas 78234-6200

PERIOD COVERED IN THIS REPORT: 1 Oct 83 through 30 Sep 84

INVESTIGATORS: Kuang-Tzu D. Lin, MD, PhD
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REPORT CONTROL SYMBOL: MEDDH-288(R1)

Several components of plasma and whole blood were evaluated in a burned rat model and in burned human patients to assess their usefulness in discriminating burn infection from burn injury only. The fluorescent factor F 355/420 appears to be a good indicator for infection in both rats and humans. However, this factor is not elevated in human burn patients until the terminal stage of burn sepsis. The spectral similarity between F 355/420 and conjugated diene (lipid peroxidation product) as well as the increased malonaldehyde concentration in PCA filtrates of plasma from burned and burned-infected rats suggests that F 355/420 may be generated by lipid peroxidation. Among all organs measured, lipid peroxidation was highest in the rat kidney.

Burns
Infection
Indicators of Infection
Lipid Peroxidation
Plasma
Whole Blood
Kidney
Malonaldehyde

CHARACTERIZATION OF BIOCHEMICAL INDICATORS OF INFECTION IN THE THERMALLY INJURED

INTRODUCTION

Burns are among the most severe injuries of man. Depending on the depth of the burn, this injury affects the dermal, vascular, and muscular tissues as well as tendon and bone. When the extent of burn exceeds 30 percent total body, the injury affects all organ systems in the body. Among the severe consequences is lowered resistance to bacterial infection. The devitalized skin harbors bacteria and/or fungi which can then invade the deeper, normal underlying tissues. Compounding this, the immune system is compromised and severely burned patients are more susceptible to infection. The metabolic and immunological changes induced by severe burn injury hamper timely detection of sepsis because of altered febrile and leukocyte responses.

Several metabolic changes in the burned patient have been noted. Resting metabolic rate and oxygen consumption increase in proportion to the proportion of surface burned and at 70 percent reach 100 percent over control level (1). An increased flow of glucose from the liver to the periphery also parallels the hypermetabolic phase of injury. The close relationship of heat production to protein synthesis indicates that the hypermetabolic response to thermal injury is linked with repair and protein synthesis requirements. The mediator of this hypermetabolic response is catecholamines (2). Urinary catecholamine is markedly elevated during burn infection and may be inappropriately high for the metabolic response measured. Plasma acute phase proteins have been found to be elevated in burn injury and in burn injury complicated with infection (3). The ratio of these acute phase proteins can be used to differentiate burns alone from burns with infection or burns with infection and other complications. The levels of two plasma fluorescent factors, Ex 280/Em 340 and Ex 355/Em 420, are increased in burned

¹Wilmore DW: Metabolic changes in burns. In Artz CP, Moncrief JA, and Pruitt BA Jr eds, *Burns: A Team Approach*. WB Saunders Company, Philadelphia, PA, 1979:120.

²Wilmore DW, Long JM, Mason AD Jr, et al: Catecholamines: mediator of the hypermetabolic response to thermal injury. *Ann Surg* 180:653-669, 1974.

³Powanda MC and Moyer ED: Plasma protein alterations during infection: potential significance of these changes to host defense and repair systems. In Powanda MC and Canonico PG eds, *Infection: The Physiologic and Metabolic Responses of the Host*. Elsevier/North-Holland Publishing Company, Amsterdam, 1981:271-296.

rat models and are further increased when bacterial infection is present (4).

The present study is intended to characterize the nature of these two factors in the plasma after burn injury, to correlate their presence with the clinical course of treatment in human patients, and to determine their origin in the tissues of a burned animal model.

MATERIALS AND METHODS

Male albino rats (350 to 450 grams) purchased from Holtzman Company, Madison, Wisconsin, were anesthetized with 2.5 milligrams sodium pentobarbital per 100 grams body weight and shaved on both the dorsal and ventral surfaces. A 30 percent total body surface dorsal full-thickness burn was achieved by immersing anesthetized rats (placed in a mold to define the extent of injury) in a 97° to 98°C water bath for 10 seconds (5). A 60 percent burn was achieved by immersing the ventral surface for another two seconds. The rats with 60 percent burns were resuscitated with 20 milliliters of normal saline injected intraperitoneally.

To induce a *Pseudomonas aeruginosa* infection, one milliliter of a 16-hour broth culture containing about 10^8 organisms (ISR 59-1244) was applied with a cotton-tipped swab to the dorsum of each rat. At 24, 48, 72, 96, and 168 hours after thermal injury, rats from each study group were sacrificed under sodium pentobarbital anesthesia. Blood for culture and biochemical studies was obtained from the abdominal aorta using aseptic technique. Plasma was separated from heparinized whole blood (10 to 20 units heparin per milliliter of blood) by centrifugation.

One milliliter of heparinized plasma was mixed with four milliliters of chilled perchloric acid (0.8 M). After 10 minutes, the mixture was centrifuged at 4°C for 10 minutes at 3,000 g. The supernatant was then centrifuged at 20,000 g for 30 minutes. Fluorescence was measured using an Aminco-Bowman spectrofluorometer at excitation 355 nm/emission 420 nm. The fluorometer was standardized using a standard quinidine sulfate solution and fluorescence reported as relative fluorescence units (rfu). For tissue assay, one gram of liver, kidney, heart, lung, or burned skin (and subcutaneous tissue) was homogenized separately in four milliliters of 0.8 M chilled perchloric acid (PCA) and processed as above. After the initial centrifugation

⁴Powanda MC, Dubois J, Villarreal Y, et al: Detection of potential biochemical indicators of infection in the burned rat. *J Lab Clin Med* 97:672-679, 1981.

⁵Walker HL and Mason AD Jr: A standard animal burn. *J Trauma* 8:1049-1051, 1968.

(3,000 g) and recentrifugation (20,000 g), the ultraviolet absorbance and fluorescent measurement was carried out on the supernatant fraction. The thiobarbituric acid (TBA) assay of malonaldehyde in the PCA supernatant was performed immediately after the fluorescence determination (6).

For the Sulfamylon treatment study, four grams of Sulfamylon cream was applied to the burn wound evenly with a cotton-tipped swab once a day except on the day of sacrifice. The blood and tissues were obtained for biochemical study as in control and burned groups. Plasma Sulfamylon levels were quantitated on a C-18 reverse phase column eluted with potassium phosphate buffer containing methanol (7).

RESULTS AND DISCUSSION

In initial studies, the F 355/420 was found in high levels compared to other tissues (676.1 ± 142.6 rfu/.2 grams, $n = 46$) in kidney PCA filtrates in all three groups of rats (control, burned, and burned-infected). The level of this fluorescent factor from liver extract was less than 20 percent (145.7 ± 35.2 rfu/.2 grams, $n = 30$) of that in kidney extracts and that from heart and lung extracts was less than 10 percent (93.3 ± 40.4 rfu/.2 grams, $n = 43$). Plasma levels averaged 22.5 rfu/.1 milliliter ($n = 24$). Thus, blood contamination of organs appears to contribute little, if any, to tissue F 355/420 levels.

The F 355/420 and TBA assay results from plasma PCA supernatant and kidney PCA supernatant are shown at Table 1. The plasma F 355/420 in burned-infected rats increased two-fold over control and burned rats on days two and three. All rats in the burned-infected group died after day three. In human patients who died of terminal burn infection, the plasma and whole blood F 355/420 were found to exceed 100 units during the last four to six days before death (data not shown). An increase in the level of F 355/420 in kidney PCA supernatant was observed after burn and burn-infection; the proportional increase was relatively small since measurements on kidney tissue from control animals had a high background level of F 355/420.

The lipid peroxidation product (measured as malonaldehyde extracted from the kidney) increased three to four-fold over control after 60 percent burn-infection. The lipid peroxidation product from the liver, heart, and lungs of the three groups of rats showed little difference (data not shown). In 60 percent

⁶Högberg J, Örrenius S, and Larsen RD: Lipid peroxidation in isolated hepatocytes. *Eur J Biochem* 50:595-602, 1975.

⁷Lin KD, Burleson DG, and Johnson A: Determination of Sulfamylon and p-carboxy derivatives in plasma and urine by reverse phase high pressure liquid chromatography (to be submitted to the *J Chromatogr*).

TABLE 1

RELATIVE LEVELS OF LIPID PEROXIDATIVE PRODUCT
AND FLUORESCENT INDICATORS FROM KIDNEY AND PLASMA
FROM CONTROL, 60 PERCENT BURNED, AND BURNED-INFECTED RATS

	K 355/420 rfu/.2 gm tissue	K TBA OD 530	K%BW	P 355/420 rfu/.1 ml plasma
<u>CONTROL</u>				
Day 1 (5)	908 ± 103.0	.087 ± .015	.666 ± .051	25.8 ± 5.4
Day 2 (5)	752 ± 47.6	.095 ± .015	.692 ± .056	13.8 ± 4.2
Day 3 (5)	816 ± 79.2	.093 ± .013	.636 ± .039	11.0 ± 2.5
<u>BURNED</u>				
Day 1 (4)	922 ± 143	.294 ± .063	.609 ± .053	20.6 ± 7.1
Day 2 (5)	1092 ± 72	.264 ± .040	.654 ± .064	15.0 ± 3.2
Day 3 (5)	1128 ± 77	.244 ± .039	.740 ± .110	17.6 ± 6.0
Day 4 (3)	1157 ± 72	.139 ± .020	.828 ± .043	22.7 ± 8.3
<u>BURNED-INFECTED</u>				
Day 1 (4)	1132 ± 31	.230 ± .022	.568 ± .044	19.6 ± 2.3
Day 2 (3)	1184 ± 92	.291 ± .038	.685 ± .059	43.4 ± 24.0
Day 3 (3)	1140 ± 342	.303 ± .001	.716 ± .114	44.3 ± 24.8

TABLE 1. Relative levels of lipid peroxidative product and fluorescent indicators from kidney and plasma from control, 60 percent burned, and burned-infected rats. K%BW indicates the kidney weight expressed as percent of body weight. Results expressed as mean ± SD from kidney (K) or plasma (P). The number in parentheses indicates the number of determinations for that group. rfu = relative fluorescence units.

burned-only rats, the lipid peroxidation product in the kidney was found to be increased to three-fold over controls at postburn day one. The level increased to 1.5-fold at postburn day four. In contrast, in 60 percent burned-infected rats, the level was 2.5-fold at postburn day one and increased to four-fold at postburn day three.

The kidney weight expressed as percent of body weight gradually increased from postburn day one to postburn day four in both burned and burned-infected groups. By postburn day four, the average percent of body weight in the burned group increased 36 percent, while in the burned-infected group, a 26 percent increase was observed by postburn day three.

Table 2 shows the effect of a topical antibacterial agent, Sulfamylon, on the level of F 355/420 and lipid peroxidation product from the plasma and kidney. The lipid peroxidation, as measured with the TBA assay, increased by 50 percent over control in plasma PCA filtrate from burned rats by postburn day two. In burned rats treated with Sulfamylon, lipid peroxidation increased to 92 percent above control on day two, as measured with the TBA assay, increased by 50 percent over control in plasma PCA filtrate from burned rats by postburn day two. In burned rats treated with Sulfamylon, lipid peroxidation increased to 92 percent above control on postburn day two. Lipid peroxidation product from kidney homogenate was increased to an even greater degree. The level from burned rats increased to 100 percent above control on postburn day two, while in burned-Sulfamylon treated rats, the level increased to 156 percent above control. The level of F 355/420 increased slightly in plasma filtrate on postburn day two, but showed no significant change in the kidney for all three study groups.

There is no readily apparent explanation for the increased peroxidation seen after topical application of Sulfamylon. Direct interference with the TBA assay by Sulfamylon or its major para-carboxy metabolite seems unlikely but has not been ruled out. Many sulfonamides can participate in redox reactions, so direct participation of Sulfamylon in the synthesis of the peroxide may be a possibility.

These results suggest that there is an increase in lipid peroxidation in kidney tissue after severe burn injury and, under the conditions of this study, burns complicated by infection increases the amount of lipid peroxidation seen after postburn day two over that in animals subjected to burns alone.

The high levels of F 355/420 and malonaldehyde detected in the kidney and the additional increase in these substances in the kidney and blood after burn injury with infection make it tempting to propose the kidney as the site of synthesis for plasma F 355/420. Whether the increase in lipid peroxidation is related to the increase in F 355/420 appearing in the blood after infection will be subjected to further study.

PUBLICATIONS/PRESENTATIONS

Burleson D: Indicators of infection in burned patients. Presented to the American Burn Association Annual Meeting, San Francisco, CA, 10-12 April 1984.

Lin KD: Plasma alpha-2 acute phase globulin and zinc as indicators of burn infection in rats. Presented to the Federa-

	PLASMA		KIDNEY			
	355/420	TBA	355/420	TBA		
	rfu/.1 ml plasma	OD 530	rfu/.2 gm tissue	OD 530	%BW	
<u>CONTROL</u>						
Day 2 (4)	23.0 ± 1.8	.018 ± .003	1075 ± 117	.142 ± .018	.767 ± .084	
Day 4 (4)	18.8 ± 3.8	ND	1034 ± 99	.118 ± .004	.712 ± .037	
<u>BURNED</u>						
Day 2 (4)	26.0 ± 1.7	.027 ± .004	1073 ± 178	.283 ± .033	.749 ± .037	
Day 4 (4)	19.0 ± 2.4	ND	1163 ± 138	.158 ± .013	.777 ± .066	
<u>BURNED-SULFAMYLON TREATED</u>						
Day 2 (4)	29.0 ± 2.1	.033 ± .006	1050 ± 71	.364 ± .078	.682 ± .037	
Day 4 (4)	19.0 ± 2.2	ND	944 ± 31	.195 ± .015	.801 ± .043	

TABLE 2. Effect of topical Sulfamylon treatment on the lipid peroxidative product and fluorescent indicators from the plasma and kidney of burned rats compared to untreated control and burned animals. Results from 60 percent burned rats are expressed as mean ± SD in relative fluorescence units (rfu). %BW indicates the kidney weight expressed as percent body weight. The number in parentheses indicates the number of determinations for that group. ND = not determined; TBA = thiobarbituric acid assay.

tion of American Societies for Experimental Biology Annual Meeting, St. Louis, MO, 1-6 April 1984.

Lin KD: Plasma indicators of infection in burn injury. Presented at the University of Tennessee College of Medicine, Knoxville, TN, 11 April 1984.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
				DA OG 6969	84 10 01	DD-DR&B(R) 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
83 10 01	D. Change	U	U		CX	
10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER		
a. PRIMARY	61102A	3M161102RS10	BB	302		
b. CONTRIBUTING						
c. CONTRIBUTING	STOG 82/83 - 6.2/4					
11. TITLE (Precede with Security Classification Code)						
(U) The Study of Metabolism and Nutritional Effects of Burn Injury in Soldiers						
12. SUBJECT AREAS						
06 05 Clinical Medicine 0615						
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING ORGANIZATION		16. PERFORMANCE METHOD
76 10		CONT		DA		C. In-House
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE		
a. DATE EFFECTIVE		EXPIRATION		FISCAL YEARS	a. PROFESSIONAL WORK YEARS	b. FUNDS (In thousands)
b. CONTRACT/GRANT NUMBER				84	5.5	343
c. TYPE		d. AMOUNT		85	5.5	359
e. KIND OF AWARD		f. CUM/TOTAL				
19. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION		
a. NAME				a. NAME		
US Army Institute of Surgical Research				US Army Institute of Surgical Research		
b. ADDRESS (include zip code)				b. ADDRESS		
Ft. Sam Houston, Texas 78234-6200				Ft. Sam Houston, Texas 78234-6200		
c. NAME OF RESPONSIBLE INDIVIDUAL				c. NAME OF PRINCIPAL INVESTIGATOR		
Pruitt, BA, Jr				Shirani, KZ		
d. TELEPHONE NUMBER (include area code)				d. TELEPHONE NUMBER (include area code)		
512-221-2720				512-221-4652		
21. GENERAL USE				f. NAME OF ASSOCIATE INVESTIGATOR (if available)		
FINA				g. NAME OF ASSOCIATE INVESTIGATOR (if available)		
MILITARY/CIVILIAN APPLICATION: M						
22. KEYWORDS (Precede EACH with Security Classification Code) (U)Nitrogen Balance;(U)Burn Injury;(U)Computer Surveillance;(U)Mitochondria;(U)Volunteers;(U)Lab Animals;(U)Rats;(U) Beta Oxidation;(U)Bam II						
23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)						
23. (U) To identify the cellular mechanism of postburn hypermetobлизм and altered thermo-regulation in burned soldiers and establish nutritional requirements following thermal injury. 24. (U) An animal model of injury has been developed to characterize the time course of postburn hypermetabolism and associated change in substrate delivery following trauma. Lipolysis of both stimulated and unstimulated adipocytes has been measured and the effect of adenosine inhibition assessed as an index of receptor activity. The kinetics of protein and water transfer in rats with 20% burns have been determined by radioactive tracer techniques. 25. (U) 8310 - 8409. Glycerol production following epinephrine stimulation of adipocytes from burned animals was significantly less than that from adipocytes from unburned animals. Dose response studies documented decreased lipolytic responses at all concentrations of epinephrine. Right shifting of the dose for half maximal stimulation in the burned adipocytes is compatible with diminution in receptor sensitivity. Adenosine removal was associated with further augmentation of glycerol production from adipocytes of both normal and burned animals. The significantly greater response to adenosine inhibition in cells from the burned rats is compatible with altered receptor sensitivity following thermal injury. Plasma volume decreases rapidly after burning but returns to normal within 24 hours. Wound water and albumin content are maximal at 24 hours after injury. Kinetic measurements using 125 I-labelled albumin indicate that the wound albumin pool is in dynamic equilibrium with plasma albumin. Hypoalbuminemia is a consequence of alteration of albumin distribution rather than a failure of protein synthesis.						

209

ANNUAL PROGRESS REPORT

PROJECT NO. 3M161102BS10-00, BASIC RESEARCH

PROJECT TITLE: THE STUDY OF METABOLISM AND NUTRITIONAL EFFECTS OF
BURN INJURY IN SOLDIERS - STUDIES OF DISTURBANCE OF
PROTEIN TURNOVER IN BURNED TROOPS: USE OF AN ANIMAL
MODEL

US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234

1 October 1983 - 30 September 1984

Investigators:

Wanda L. Brown, M.S.
Eleanor G. Bowler, Ph.M.
Arthur D. Mason, Jr., M.D.

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3M161102BS10-00, BASIC RESEARCH

PROJECT TITLE: THE STUDY OF METABOLISM AND NUTRITIONAL EFFECTS OF
BURN INJURY IN SOLDIERS - STUDIES OF DISTURBANCE OF
PROTEIN TURNOVER IN BURNED TROOPS: USE OF AN ANIMAL
MODEL

US Army Institute of Surgical Research, Brooke Army Medical Center,
Fort Sam Houston, Texas 78234

Period covered in this report: 1 October 1983 - 30 September 1984

Investigators: Wanda L. Brown, M.S.
Eleanor G. Bowler, Ph.M.
Arthur D. Mason, Jr., M.D.

Reports Control Symbol MEDDH-288(R1)

Studies of 1-hour transfer rates of ^{125}I -labeled albumin between plasma and burn wound showed that albumin continues to enter the burn wound at an elevated rate for at least 3 days postburn. Treatment of the burn wound with subcutaneous injection of hyaluronidase accelerated the rate of flow through the burn wound and reduced the total quantity of edema in the treated burn wound. This, plus previously completed 6-day labeled albumin disappearance studies and measurements of total albumin by radioimmunoassay, show that the burn wound albumin pool is not static, as has been proposed by others, but continues to exchange with the plasma albumin pool.

Albumin
Labeled albumin
Rat
Burn
Edema
Hyaluronidase

STUDIES OF DISTURBANCE OF PROTEIN TURNOVER IN BURNED TROOPS: USE OF AN ANIMAL MODEL

In previous experiments in this study, it was found that when ^{125}I -labeled albumin was injected into rats just before burn or sham burn, the half-time of labeled albumin disappearance from the burn wound was longer than that from the sham wound. In order to determine if this indicated that the burn wound albumin pool was not exchangeable, as has been proposed by others (1-3), we have measured the 1-hour transfer rates of labeled albumin injected at later times postburn (PB).

The procedures used in this set of experiments have been previously described in detail (4). Briefly, rats subjected to a 20% body surface scald burn (100° C, 10 seconds) or sham burn were untreated (SU and BU) or treated by subcutaneous injection of hyaluronidase (SHY and BHY) into the wound at 1-hour PB. At various times PB, ^{125}I -labeled rat albumin (0.4 μCi in 0.5 ml) was injected into the tail vein 1 hour before the selected time of sacrifice. Five minutes before the time of sacrifice, ^{131}I -labeled human albumin (0.4 μCi in 0.5 ml) was injected into the tail vein and blood samples for the determination of plasma volume and ^{125}I -labeled albumin content were drawn within 4 to 6 minutes after the ^{131}I -labeled albumin injection. Wound samples were rapidly excised, homogenized, and the supernate was used to determine total albumin content and the protein-bound activity of ^{125}I and ^{131}I . Total albumin was determined independently by radioimmunoassay. Plasma samples were assayed in the same manner. The results are expressed as gram plasma equivalents (Gm Pl Eq) transferred per hour from plasma and into wound. One Gm Pl Eq is equal to the mg albumin in 1 gm plasma (5).

RESULTS AND DISCUSSION

The plasma albumin concentrations (Gm Pl Eq) of all four groups of rats were lower than normal during the first 4 hours PB (Table 1). After 24 hours PB the plasma albumin concentrations of Groups SU and SHY were

1. Jackson, T.M. and W.H. Lee, Jr. The role of plasma protein denaturation on the thermal burn syndrome. *Am. Surg.* 30: 26-34, 1964.
2. Lynch, J.B., J.P. Bray, S.R. Lewis, and T.G. Blocker. Studies with radioisotope labeled albumin in experimental burn wounds. *J. Surg. Res.* 4: 226-232, 1964.
3. Moritz, A.R. Studies of thermal injury. III. The pathology and pathogenesis of cutaneous burns: An experimental study. *Am. J. Path.* 23: 915-941, 1947.
4. Brown, W.L., E.G. Bowler, A.D. Mason, Jr. and B.A. Pruitt, Jr. Protein metabolism in burned rats. *Am. J. Physiol.* 231: 476-482, 1976.
5. Studer, R. and J. Potchen. The radioisotopic assessment of regional microvascular permeability to macromolecules. *Microvasc. Res.* 3: 35-48, 1971.

TABLE 1. Plasma Albumin^a

Hour Postburn	Gram Plasma Equivalent ^b			
	SU	SHY	BU	BHY
1	22.1 ± 0.8 (3)	- ^c	21.5 ± 0.5 (3)	- ^c
3	24.9 ± 2.4 (3)	27.2 ± 1.7 (3)	21.6 ± 0.4 (3)	26.2 ± 0.7 (3)
4	24.8 ± 1.6 (3)	25.2 ± 1.8 (3)	21.4 ± 0.8 (3)	24.1 ± 0.8 (3)
24	36.0 ± 1.2 (4)	28.7 ± 1.8 (3)	27.0 ± 1.0 (5)	23.0 ± 0.5 (4)
48	36.4 ± 0.7 (3)	29.7 ± 1.1 (3)	24.6 ± 0.4 (4)	22.0 ± 0.8 (3)
72	36.4 ± 1.0 (3)	30.8 ± 1.2 (3)	26.0 ± 1.2 (3)	23.9 ± 0.6 (3)

^a Values are means ± SEM for rats in 1-hour labeled albumin transfer study. () = number rats in group.

^b Gram plasma equivalent = mg albumin per g plasma.

^c Hyaluronidase was injected subcutaneously into the wound at 1 hour PB.

within normal range, but those of Groups BU and BHY remained lower than normal through 72 hours PB.

The rate (Gm Pl Eq/hour) of labeled albumin disappearance from plasma and the rate of entry of labeled albumin into wounds of burned rats (Table 2) was greatest during the first hour PB. The disappearance rate from plasma of Group BHY was 1.5 times that of Group BU at 4 and 24 hours PB ($P < 0.05$) but was lower than that of Group BU at 48 hours PB ($P < 0.05$). At 72 hours PB the disappearance rates from plasma of Groups BU and BHY were not significantly different. Labeled albumin disappearance rates from plasma of Groups SU and SHY were similar except at 48 hours PB when Group SHY rate was greater ($P < 0.05$). The hourly rates of disappearance of labeled albumin from plasma of burned rats (BU + BHY) were significantly lower than those of sham rats (SU + SHY) from 3 to 24 hours PB ($P < 0.01$) and at 48 hours PB ($P < 0.05$) but were not significantly different at 72 hours PB. It should be noted that the significance of the difference at 24 hours PB was due solely to the marked decrease in the rate of disappearance from plasma of Group BU; the rate for Group BHY was similar to that of Groups SU and SHY at that time.

The rate (Gm Pl Eq/hour) of entry of labeled albumin into wounds of Group BHY was 1.3 to 1.8 times greater than that into Group BU wounds from 3 to 24 hours PB ($P < 0.01$), then decreased to 60% of the rate of Group BU at 48 hours PB ($P < 0.01$). At 72 hours PB Group BU and BHY rates were not significantly different. The rates of entry of labeled albumin into wounds of Groups SU and SHY were not significantly different at any time. The rates of entry of labeled albumin into wounds of burned rats (BU + BHY) were significantly higher than those into wounds of sham rats (SU + SHY) at each time measured ($P < 0.001$).

The rate of entry of labeled albumin into the remainder of the tissues (other than wound) in Group BU rats was equal to that of Group SU rats during the first hour PB, but then decreased. The rate of entry of labeled albumin into other tissues of rats in Group BHY was 1.5 times that in Group BU at 4 and 24 hours PB ($P < 0.05$) but lower than Group BU at 48 hours PB. The rates were not significantly different at 3 and 72 hours PB. The rates of entry of labeled albumin into other tissues of Groups SU and SHY were not significantly different at any time. The mean rate of entry of labeled albumin into other tissues of Groups (SU + SHY) was significantly greater than in Groups (BU + BHY) from 3 to 48 hours PB ($P < 0.05$). As noted above for plasma disappearance rates, the significant difference at 24 hours PB was due to the marked decrease in the rate of entry into other tissues of rats in Group BU and the rate for Group BHY was similar to that of the sham groups at that time. There were no significant differences among the rates at 72 hours PB.

The measurements of the hourly transfer rates of labeled albumin into wounds of sham rats agreed closely with those reported for skin of

TABLE 2. One-hour Transfer Rates of ^{125}I -labeled Rat Albumin

Gm Pl Eq ^a Albumin Transferred Per Hour													
Hour	From Plasma				Into Wound				Into Other Tissue ^c				
	Postburn ^b	SU	SHY ^d	BU	BHY ^d	SU	SHY	BU	BHY	SU	SHY	BU	BHY
1	2.761	-	6.317	-	0.092	-	3.691	-	2.669	-	2.626	-	-
3	2.473	2.656	1.739	1.862	0.055	0.062	0.517	0.686	2.418	2.594	1.222	1.176	1.176
4	2.065	2.212	1.280	1.932	0.065	0.062	0.223	0.332	2.000	2.150	1.047	1.600	1.600
24	2.566	2.556	1.891	2.737	0.071	0.075	0.122	0.220	2.495	2.481	1.769	2.517	2.517
48	2.561	3.044	2.622	2.128	0.070	0.060	0.208	0.124	2.491	2.984	2.414	2.004	2.004
72	2.672	2.524	2.500	2.714	0.079	0.087	0.223	0.193	2.593	2.437	2.277	2.521	2.521

^a One gram plasma equivalent (Gm Pl Eq) = mg albumin per gm plasma.

^b Time of sacrifice; labeled albumin injected intravenously 1 hour earlier.

^c Determined by difference of plasma and wound values.

^d Hyaluronidase was injected subcutaneously into the wound at 1 hour postburn.

normal rats by Studer et al. (5). The lower than normal plasma albumin concentrations and the lower rates of entry of labeled albumin into wounds of sham rats from 1 to 4 hours PB may have been due to reduced physical activity or to an effect of the anesthetic agent (6). Increased interstitial pressure created by the rapid influx of fluid into the burn wound (7), associated with increased lymphatic return of labeled albumin to plasma during the first hour PB, makes it likely that the hourly transfer rates for burned rats (BU + BHY) during the first hour PB, and to a lesser extent at 3 and 4 hours PB, represent net exchange rates. From 24 to 72 hours PB the rates for burned rats were probably unidirectional rates, as they were at all times for sham rats. The continued elevation of the rate of entry of labeled albumin into the burn wound was at the apparent expense of transfer into other tissues of rats in Group BU from 3 to 24 hours PB and in Group BHY at 3 and 4 hours PB. The greater rate of entry of labeled albumin into wounds of Group BHY from 3 to 24 hours PB was probably due to decreased interstitial resistance of the tissue in which the hyaluronate concentration had been lowered by the hyaluronidase.

The fact that the wound albumin pool size did not change from 24 to 72 hours PB, during which times the hourly rates of entry of labeled albumin into the burn wound continued to be two to three times greater than normal, indicates that the rate of return of albumin from wound to plasma was equal to the entry rate. Thus, the burn wound albumin pool is not static but continues to exchange with the plasma albumin pool.

6. Polderman, H., J.D. McCarrell, and H.K. Beecher. Effect of anesthesia on lymph flow (local procaine, ether, and pentobarbital sodium). *J. Pharmacol. Exp. Ther.* 78: 400-406, 1943.

7. Taylor, A.E., W.H. Gibson, H.J. Granger, and A.C. Guyton. The interaction between intracapillary and tissue forces in the overall regulation of tissue volume. *Lymphology* 6: 192-208, 1973.

PRESENTATIONS/PUBLICATIONS - None.

TERMINATION REPORT

PROJECT NUMBER: 3M161102BS10-00, BASIC RESEARCH

PROJECT TITLE: THE STUDY OF METABOLISM AND NUTRITIONAL EFFECTS
OF BURN INJURY IN SOLDIERS: Role of Lipid Me-
tabolism in Burn Injury

US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234-6200

1 October 1983 - 30 September 1984

INVESTIGATORS

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REPORT CONTROL SYMBOL - MEDDH-288 (R1)

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ABSTRACT

PROJECT NUMBER: 3M161102BS10-00, BASIC RESEARCH

PROJECT TITLE: THE STUDY OF METABOLISM AND NUTRITIONAL EFFECTS
OF BURN INJURY IN SOLDIERS: Role of Lipid Me-
tabolism in Burn Injury

US Army Institute of Surgical Research, Brooke Army Medical Cen-
ter, Fort Sam Houston, Texas 78234-6200

PERIOD COVERED IN THIS REPORT: 1 Oct 83 through 30 Sep 84

INVESTIGATORS: David R. Strome, PhD
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Adipocytes isolated from rats 12 days after severe thermal injury showed a decreased ability to respond lipolytically to stimulation by beta-receptor agonists. Experiments were performed on adipocytes isolated from the epididymal fat of 500 gram, male Sprague-Dawley rats. Two groups were formed, normal controls and animals subjected to a 60 percent total body surface scald burn. Cells were incubated in the presence and absence of various lipolytic modifiers. Lipolysis was measured as glycerol released into the incubation medium. In unstimulated incubations, the release of glycerol by the cells was not different between the two groups. Stimulation with epinephrine or isoproterenol, as expected, increased glycerol release in both groups. However, the release of glycerol from the cells of burned animals was significantly less than that from control cells. Epinephrine dose-response curves showed that this decreased lipolytic response in cells from burned animals existed at all concentrations of epinephrine used. In addition, there was a slight shift to the right in the dose for half-maximal stimulation in the burned group, suggesting a decrease in the sensitivity of the receptor for epinephrine. Removal of adenosine from the incubation medium containing epinephrine resulted in a further augmentation of the glycerol release rate in cells from both normal and burned rats. However, the increase in the burned group was much greater, such that there was no longer any difference in the lipolytic response to epinephrine between cells from normal and burned animals.

Adipocytes
Lipolytic Stimulation
Glycerol Response
Dose-Response
Adenosine

However, the increase in the burned group was much greater, such that there was no longer any difference in the lipolytic response to epinephrine between cells from normal and burned animals. Since the adenosine concentrations in the incubation media were the same in all groups, this differential response to removing adenosine suggests that the cells respond differently to the inhibitor.

THE ROLE OF LIPID METABOLISM IN BURN INJURY

Severe thermal injury initiates a series of complex metabolic disturbances in humans. Metabolic rate increases above normal in proportion to burn size, maximizing at 10 to 15 days postburn (1). Alterations in lipid metabolism during this hypermetabolic period are reflected in increased plasma fatty acid and glycerol concentrations (2,3), increased uptake rates for infused lipid emulsions (4), and depletion of body fat stores (5,6). Changes in circulating hormones include an increase in plasma and urine catecholamines (7,8). Although the clinical manifestations of these changes are well described, the causes for many of the changes are unknown.

This protocol was designed to examine the relationship between catecholamines and the increased mobilization of fat from adipose tissue. The following report summarizes the major find-

¹Wilmore DW and Aulick LH: Metabolic changes in burn patients. *Surg Clin North Am* 58:1173-1178, 1978.

²Harris RL, Frenkel RA, Cottam GL, et al: Lipid mobilization and metabolism after thermal trauma. *J Trauma* 22:194-198, 1982.

³Birke G, Carlson LA, and Liljedahl S-O: Lipid metabolism and trauma. III. Plasma lipids and lipoproteins in burns. *Acta Med Scand* 178:337-350, 1965.

⁴Wilmore DW, Moylan JA, Helmkamp GM, et al: Clinical evaluation of a 10% intravenous fat emulsion for parenteral nutrition in thermally injured patients. *Ann Surg* 178:503-513, 1973.

⁵Reiss E, Pearson E, and Artz CP: The metabolic response to burns. *J Clin Invest* 35:62-77, 1956.

⁶Davies JW and Fell GS: Tissue catabolism in patients with burns. *Clin Chim Acta* 51:83-92, 1974.

⁷Wilmore DW, Long JM, Mason AD Jr, et al: Catecholamines: mediator of the hypermetabolic response to thermal injury. *Ann Surg* 180:653-669, 1974.

⁸Aikawa N, Caulfield JB, Thomas RJS, et al: Postburn hypermetabolism: relative evaporational heat loss and catecholamine level. *Surg Forum* 26:74-76, 1975.

ings which have been presented in full detail in earlier published accounts (9-11).

Adipocytes from normal and 60 percent total body surface burned rats were incubated in the presence and absence of several modifiers of lipolysis. Basal rates of glycerol production were never found to be different between burned and normal groups. However, the ability of adipocytes from burned animals to release glycerol in response to a single, acute, maximal hormonal stimulation was reduced when compared to normal controls. This reduced responsiveness appeared as early as the fifth and continued through the twentieth postburn day. It was present whether the adipocytes were stimulated with epinephrine or isoproterenol. When stimulated with a large range of epinephrine concentrations, adipocytes from burned rats had a consistently decreased lipolytic response with respect to normals. In addition to the decrease in stimulated rates of glycerol production, there was a slight increase in the dose for half-maximal stimulation.

Several mechanisms can theoretically affect the lipolytic response of adipocytes in altered physiological states. These include not only changes in receptor sensitivity or number but changes in the activity of phosphodiesterase (12) or of protein kinase or hormone-sensitive lipase (13,14). There can also be experiment-related factors such as feedback inhibition due to

⁹Strome DR, Goodwin CW Jr, and Mason AD Jr: The role of lipid metabolism in burn injury: alterations in adipocyte responsiveness to hormonal stimulation. *In* US Army Institute of Surgical Research Annual Research Progress Report for FY 1981, pp 387-397.

¹⁰Strome DR, Newman JJ, Goodwin CW Jr, et al: The role of lipid metabolism in burn injury: lipolytic responsiveness to epinephrine in adipocytes. *In* US Army Institute of Surgical Research Annual Research Progress Report for FY 1982, pp 338-349.

¹¹Strome DR, Newman JJ, Goodwin CW Jr, et al: The role of lipid metabolism in burn injury: mechanisms of the reduced lipolytic response. *In* US Army Institute of Surgical Research Annual Research Progress Report for FY 1983, pp 435-445.

¹²Engfeldt P, Arner P, and Ostman J: Changes in phosphodiesterase activity of brain subcutaneous adipose tissue during starvation. *Metabolism* 31:910-916, 1982.

¹³Shepherd RE, Noble EG, Klug GA, et al: Lipolysis and cAMP accumulation in adipocytes in response to physical training. *J Appl Physiol* 50:143-148, 1981.

¹⁴Oscai LB, Caruso RA, Wergeles AC, et al: Exercise and the cAMP system in rat adipose tissue. I. Lipid mobilization. *J Appl Physiol* 50:250-254, 1981.

differential inhibition of lipolysis by adenosine (15,16) or free fatty acids (15,17) in the incubation medium. The shift to higher concentrations in the dose for half-maximal stimulation in the burned group is consistent with either a decrease in receptor sensitivity for epinephrine or an increase in the sensitivity of inhibitory receptors, such as those for adenosine.

Incubation medium adenosine concentrations were not different between burned and normal groups in either the control or stimulated samples nor were the stimulated samples different from the controls in either group. Therefore, the differing responses between burned and control groups cannot be ascribed to higher concentrations of inhibitory adenosine in one group.

Adenosine deaminase (ADA) removes adenosine from the incubation medium by deamination to inosine, which is lipolytically inactive. No effect on glycerol production was observed when ADA was added to nonstimulated control samples. However, addition of ADA to the incubation medium in epinephrine-stimulated samples was accompanied by an increase in glycerol production above that found with epinephrine alone. This ability of ADA to increase stimulated lipolysis is consistent with the action of adenosine. In the presence of epinephrine, the deamination of adenosine with ADA removes the inhibition due to medium adenosine and allows full expression of the lipolytic effects of the catecholamine. In our experiments, the augmentation was greater in the burn samples, such that the normally observed difference in lipolytic response between cells from burned and normal animals was abolished. These findings in relation to adenosine suggest that the cells from the burned animals may have an altered sensitivity to adenosine inhibition through the adenosine-receptor interaction. This possibility remains unresolved.

At the beginning of the period which this report covers, the primary investigator assumed the responsibilities and duties of the Chief, Bioengineering Branch, which includes the maintenance and operation of the computer facility. The time demands of this position have precluded continuing the research protocol. It is therefore terminated.

¹⁵Hjemdahl P: Studies on the antilipolytic effect of acidosis. *Acta Physiol Scand Suppl* 434:1-40, 1976.

¹⁶Fain JN and Wieser PB: Effects of adenosine deaminase on cyclic adenosine monophosphate accumulation, lipolysis, and glucose metabolism of fat cells. *J Biol Chem* 250:1027-1034, 1975.

¹⁷Burns TW, Langley PE, Terry BE, et al: The role of free fatty acids in the regulation of lipolysis by human adipose tissue cells. *Metabolism* 27:1755-1762, 1978.

PRESENTATIONS/PUBLICATIONS

Strome DR: Mechanism of reduced lipolytic response in rat adipocytes following thermal injury. Presented at the American College of Surgeons' 1983 Clinical Congress, Atlanta, Georgia, 16-21 October 1983.

Strome DR, Newman JJ, Goodwin CW Jr, Mason AD Jr, and Pruitt BA Jr: Mechanisms of reduced lipolytic response in rat adipocytes following thermal injury. *Surg Forum* 34:103-105, 1983.

FINAL REPORT

PROJECT NUMBER: 3M161102BS10-00, BASIC RESEARCH

PROJECT TITLE: THE STUDY OF METABOLISM AND NUTRITIONAL
EFFECTS OF BURN INJURY IN SOLDIERS: An
Analysis of the Utility of Amylase Isozyme
Differentiation in the Diagnosis of
Pancreatitis in Burned Patients

US ARMY INSTITUTE OF SURGICAL RESEARCH
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1 October 1983 - 30 September 1984

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ABSTRACT

PROJECT NUMBER: 3M161102BS10-00, BASIC RESEARCH

PROJECT TITLE: THE STUDY OF METABOLISM AND NUTRITIONAL EFFECTS OF BURN INJURY IN SOLDIERS: An Analysis of the Utility of Amylase Isozyme Differentiation in the Diagnosis of Pancreatitis in Burned Patients

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PERIOD COVERED IN THIS REPORT: 1 Oct 83 through 30 Sep 84

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REPORT CONTROL SYMBOL: MEDDH-288(R1)

In one study, 30 percent of burned patients had hyperamylasemia and those patients had a higher than predicted mortality. The conclusion was that pancreatitis is a significant complication of thermal injury. However, the study did not differentiate salivary hyperamylasemia (excluding pancreatitis) from pancreatic hyperamylasemia (support evidence of pancreatitis). We are repeating the study with the addition of identification of amylase isozyme type (pancreatic or salivary) responsible for the hyperamylasemia. During the past year, eight patients were enrolled in the study. One died on the second study day and another patient was withdrawn from the study due to sample conflicts with another protocol. In the remaining six patients, hyperamylasemia (salivary type) was observed only once.

Human
Volunteer
Amylase
Isozymes
Pancreas
Salivary Glands
Burns
Thermal Injury
Pancreatitis

AN ANALYSIS OF THE UTILITY OF AMYLASE ISOZYME DIFFERENTIATION IN THE DIAGNOSIS OF PANCREATITIS IN BURNED PATIENTS

INTRODUCTION

Many severely burned patients develop ileus, abdominal distention, hypotension, and hyperglycemia. One etiology associated with these findings is pancreatitis, which has been reported to occur in 35% of autopsied burn patients (1). However, there is no laboratory test which can specifically confirm the diagnosis of pancreatitis (2-4).

Measurement of serum and/or urinary amylase is a standard laboratory test used to support the clinical diagnosis of pancreatitis. Amylase, however, is synthesized not only in the pancreas, but also in the salivary glands (2). Each organ produces multiple forms of the amylase molecule (isozymes). Both serum and urine contain a mixture of pancreatic and salivary amylase isozymes (2). Thus, the presence of hyperamylasemia and/or hyperamylasuria is not diagnostic of pancreatitis, as the amylase elevation could be secondary to salivary disease or injury (2).

Salivary and pancreatic isozymes differ in their net electric charge and can be separated by electrophoresis (2), isoelectric focusing (5), and ion exchange chromatography (6). Thus, the relative amounts of salivary and pancreatic amylase in a clinical sample can be determined. An elevation of salivary amylase isozymes in serum or urine does not support the diagno-

¹Goodwin CW, Pruitt BA Jr: Increased incidence of pancreatitis in thermally injured patients: a prospective study. *Proceedings of the American Association for the Surgery of Trauma*, 17-19 September 1981, p 106.

²Lehrner LM, Ward JC, Karn RC, Ehrlich CE, and Merritt AD: An evaluation of the usefulness of amylase isozyme differentiation in patients with hyperamylasemia. *Am J Clin Path* 66:576-587, 1976.

³Meszaros I, Goth L, and Vattay G: The value of serum catalase activity determinations in acute pancreatitis. *Am J Dig Dis* 18:1035-1041, 1973.

⁴Levitt MD and Johnson SG: Is the Cam/Ccr ratio of value for the diagnosis of pancreatitis? *Gastroenterol* 75:118-119, 1978.

⁵Levitt MD, Ellis C, and Engel RR: Isoelectric focusing studies of human serum and tissue isoamylases. *J Lab Clin Med* 90:141-152, 1977.

⁶Fridhandler L and Berk JE: Simplified chromatographic method for isoamylase analysis. *Clin Chim Acta* 101:135-138, 1980.

sis of pancreatitis. However, other intra-abdominal conditions, such as duodenal ulcer and choledocholithiasis, may rarely cause hyperamylasemia and/or hyperamylasuria (7). While the isozyme type elevated in these conditions has not been identified, it is probable that pancreatic amylase isozymes are elevated in these conditions.

Therefore, the current study was undertaken to determine if amylase isozyme differentiation can improve the diagnostic accuracy of the laboratory tests (serum amylase, amylase clearance, and timed amylase excretion) routinely used to support the clinical diagnosis of pancreatitis.

MATERIALS AND METHODS

Patients 18 years of age or older admitted to the US Army Institute of Surgical Research who met the criteria of admission to the Institute more than 48 hours postburn, admission creatinine greater than 1.5 mg/dl, and signed informed consent were eligible for inclusion in the study. Death within 24 hours of admission or withdrawal of consent were reasons for exclusion from the study.

Samples collected from study subjects included 10 milliliters serum and a timed two-hour urine sample daily for 14 days. One saliva sample was obtained from each patient. Amylase was assayed by the Clinical Biochemistry Section using a standard method. Amylase isozymes were determined by electrophoresis, isoelectric focusing, and ion exchange chromatography (8).

RESULTS

During FY 84, four patients were enrolled in the study. Patient characteristics and laboratory data are displayed in Table 1. A clinical diagnosis of pancreatitis was not made in any of these patients.

DISCUSSION

Combining data from FY 83 and FY 84, 10 patients have been analyzed. Only one hyperamylasemic sample has been observed. Electrophoresis revealed that the sample contained 60% to 70% salivary amylase type isozymes. Since all the patients have survived and pancreatic type hyperamylasemia has not been observed, the accuracy of the test has not been confirmed by pancreatic histology.

⁷Salt WB 2d and Schenker S: Amylase - its clinical significance: a review of the literature. *Medicine* 55:269-289, 1976.

⁸Chrumbach A, Hjelmeland L, and Nguyen NY: Gel electrofocusing with increased degrees of freedom. In *Electrophoresis* '79. Randola BJ (ed). Berlin: Walter de Gruyter and Company, 1980, pp 3-22.

TABLE 1

<u>Age</u>	<u>Sex</u>	<u>Percent Burn</u>	<u>Serum Amylase During Study (Units/L)</u>			<u>Urine Amylase During Study (Units/L)</u>			<u>Survived</u>
			<u>Min</u>		<u>Max</u>	<u>Min</u>		<u>Max</u>	
21	M	36.0	8	-	78	71	-	214	Y
23	F	50.0	4	-	58	58	-	156	Y
28	F	46.0	19	-	76	74	-	236	Still In Hospital
57	M	30.5	21	-	50	13	-	291	Y
NORMAL =			20	-	110	NOT ESTABLISHED			

While a prior study (1) from the Institute reported a 30% incidence of pancreatitis (hyperamylasemia) in burn patients, we have not observed a single case of pancreatitis in the studied patients. We conclude that pancreatitis is not a significant complication in burn patients during the first 14 to 16 days post-injury.

PRESENTATIONS/PUBLICATIONS

None.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY						1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
						DA OG 6968	83 10 01	DD-DRAB(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		
82 10 12	D. Change	U	U		CX			
10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER				
a. PRIMARY	61102A	3M161102BS10	BB	303				
b. CONTRIBUTING								
c. CONTRIBUTING	STOG 82/83 - 612/4							
11. TITLE (Precede with Security Classification Code)								
(U) Alteration of Host Resistance in Burned Soldiers								
12. SUBJECT AREAS								
06 05 Clinical Medicine 0615								
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING ORGANIZATION		16. PERFORMANCE METHOD		
76 10		CONT		DA		C. In-House		
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE				
a. DATE EFFECTIVE		EXPIRATION		FISCAL YEARS		a. PROFESSIONAL WORK YEARS		b. FUNDS (In thousands)
b. CONTRACT/GRANT NUMBER				84		5.0		270
c. TYPE		d. AMOUNT		85		5.0		330
e. KIND OF AWARD		f. CUM/TOTAL						
19. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION				
a. NAME				a. NAME US Army Institute of Surgical Research				
US Army Institute of Surgical Research				Microbiology Branch				
b. ADDRESS (include zip code)				b. ADDRESS				
Ft. Sam Houston, Texas 78234-6200				Ft. Sam Houston, Texas 78234-6200				
c. NAME OF RESPONSIBLE INDIVIDUAL				c. NAME OF PRINCIPAL INVESTIGATOR				
Pruitt, BA, Jr				McManus, AT				
d. TELEPHONE NUMBER (include area code)				d. TELEPHONE NUMBER (include area code)				
512-221-2720				512-221-3411				
21. GENERAL USE				f. NAME OF ASSOCIATE INVESTIGATOR (if available)				
FINA								
MILITARY/CIVILIAN APPLICATION: M				g. NAME OF ASSOCIATE INVESTIGATOR (if available)				
22. KEYWORDS (Precede EACH with Security Classification Code) (U) Tissue Spreading Factors; (U) Lab Animals; (U) Rats; (U) Infection; (U) Immunostimulants; (U) Virulence Factors; (U) Plasmids; (U) Anti-								
23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code) biotic Effects; (U) Ram II								
23. (U) To define the microbial basis of opportunistic infection in susceptible burned soliders, identify specific mechanisms of decreased host resistance that are targeted by opportunistic pathogens and develop and evaluate countermeasures.								
24. (U) The high susceptibility of burned rats to experimental infection with <u>Pseudomonas aeruginosa</u> and <u>Proteus mirabilis</u> will be investigated. The effect of <u>in vitro</u> alterations of specific microbial characteristics on infection will be investigated. Specific anti-microbial and immunostimulator therapies will be examined.								
25. (U) 8310 - 8409. A clinical trial of the experimental parenteral antibiotic Thienamycin Formamidine (Merck MK787) is in progress. The Compound was tested for <u>in vitro</u> activity against 2,978 burn patient isolates. Overall sensitivity was 96.7%. Development of resistance, however, did occur during treatment of several patients. <u>Pseudomonas aeruginosa</u> was the most common resistant species observed. Several resistant strains have also subsequently been isolated from patients who were not treated with Thienamycin. The possibility of selection of resistance by other B-Lactam antibiotics is being investigated.								

ANNUAL PROGRESS REPORT

PROJECT NO. 3M161102BS10-00, BASIC RESEARCH

REPORT TITLE: ALTERATION OF HOST RESISTANCE IN BURNED SOLDIERS

US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234

1 October 1983 - 30 September 1984

Investigators:

Albert T. McManus, Ph.D.
Arthur D. Mason, Jr., M.D.
Basil A. Pruitt, Jr., M.D., Colonel, MC
Camille L. Denton, M.A.
George T. Daye, Jr., M.A.
Virginia C. English, M.A.

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3M161102BS10-00, BASIC RESEARCH

REPORT TITLE: ALTERATION OF HOST RESISTANCE IN BURNED SOLDIERS

US Army Institute of Surgical Research, Brooke Army Medical Center, Fort Sam Houston, Texas 78234

Period covered in this report: 1 October 1983 - 30 September 1984

Investigators: Albert T. McManus, Ph.D.
Arthur D. Mason, Jr., M.D.
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Reports Control Symbol MEDDH-288(R1)

A clinical trial of the experimental parenteral antibiotic thienamycin formamidine (Merck MK0787) is in progress. The compound was tested for in vitro activity against 2,978 burn patient isolates. Overall sensitivity was 96.7%. Development of resistance, however, did occur during treatment of several patients. Pseudomonas aeruginosa was the most common resistant species observed. Several resistant strains have also subsequently been isolated from patients who were not treated with thienamycin. The possibility of selection of resistance by other beta-lactam antibiotics is being investigated.

Rat model
Infection
Immunostimulants
Virulence factors
Antibiotic effects
Humans
Sulfamylon
Vaccine
Investigational drugs

ALTERATION OF HOST RESISTANCE IN BURNED SOLDIERS

EXPERIMENTAL PARENTERAL AGENTS

Measurement of in vitro sensitivity to the investigational cephalosporin antibiotic cefsulodin sodium (Abbott) was performed on 463 isolates of Pseudomonas aeruginosa. Measurements were made by agar overlay disc (30 mcg) diffusion. A comparison of the results of the last five reporting periods is presented in Table 1. For the second consecutive year, in vitro sensitivity has improved. It is also of note that it has been 2 years since our clinical trial with this agent (1). This antibiotic is expected to be approved for use during FY 85.

Table 1. Cefsulodin Sodium Activity against Burn Patient
Pseudomonas aeruginosa

	FY 80	FY 81	FY 82	FY 83	FY 84
No. Resistant	5	8	143	49	36
No. Sensitive	76 (6.2)	556 (1.4)	655 (17.9)	355 (12.1)	463 (7.2)

% resistant in parentheses.

FY 82 vs FY 80 + FY 81, $P < .01$, FY 82 more resistant.

FY 83 vs 82, $P < .01$, FY 83 less resistant.

FY 84 vs FY 83, $P < .02$, FY 84 less resistant.

The investigational antibiotic N-formimidoyl thienamycin (Primaxin^R, Merck, Inc.) has been in clinical trial at this Institute during the reporting period. A clinical summary of patient results during FY 84 is attached in a separate report. In vitro, Primaxin was tested against 2,978 isolates representing 37 bacterial species. The distribution of organisms and sources of isolation are presented in Figures 1 and 2. Resistance has significantly increased during the trial. The overall incidence of resistance, however, was small, 3.3%, but significantly greater ($P < .01$) than the 0.38% reported in FY 83. The principal offender was Pseudomonas aeruginosa with 77 isolations from nine patients. Only five of the nine patients had been or were being treated with the drug. The distribution of zone sizes of inhibition (10 mcg disc) for gram-positive and gram-negative isolates for FY 83 and FY 84 are presented in Figures 3 and 4 respectively. The mean zone of inhibition for gram-positive organisms was 39.77 mm. This large zone presents a

1. Pruitt BA Jr, Mason AD Jr, McManus AT, McManus WF: A clinical study to assess the safety and efficacy of Abbott-46811 in the treatment of infections caused by Pseudomonas aeruginosa in burn patients. USAISP Annual Report FY 82, Ft Sam Houston, Texas, pp 137-141.

FREQUENCY OF ORGANISMS TESTED

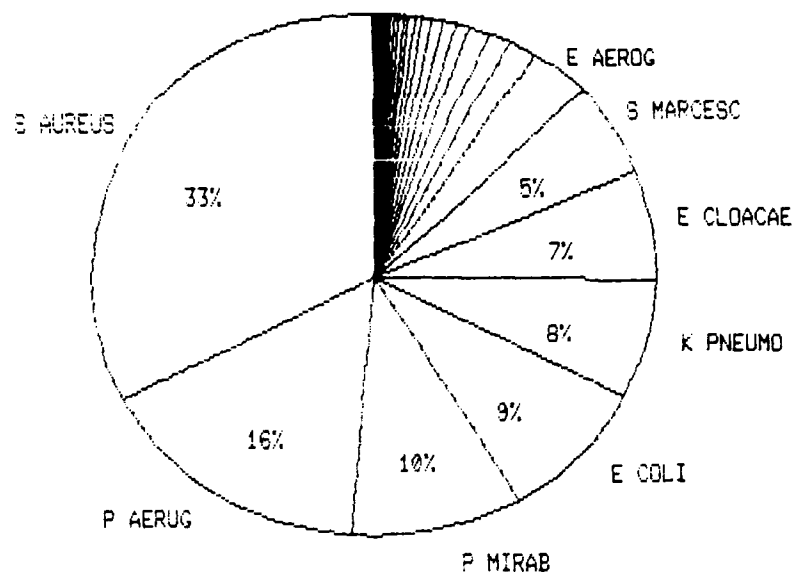


Figure 1. Distribution of organisms tested for in vitro sensitivity to N-formimidoyl thienamycin.

FREQUENCY OF ORGANISM SOURCES

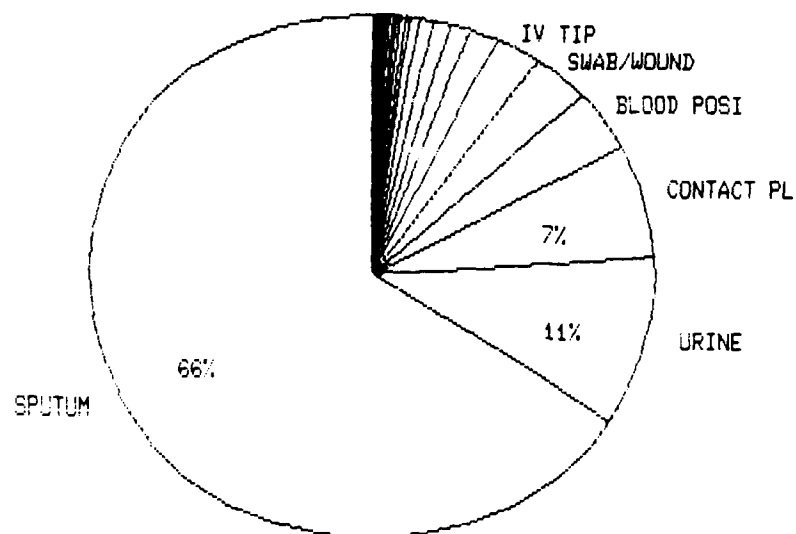


Figure 2. Distribution of sites of isolation of organisms tested for in vitro sensitivity to N-formimidoyl thienamycin.

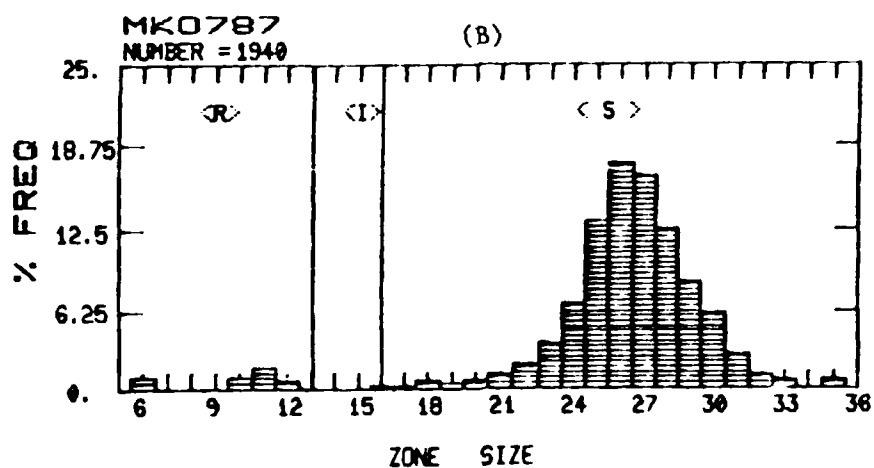
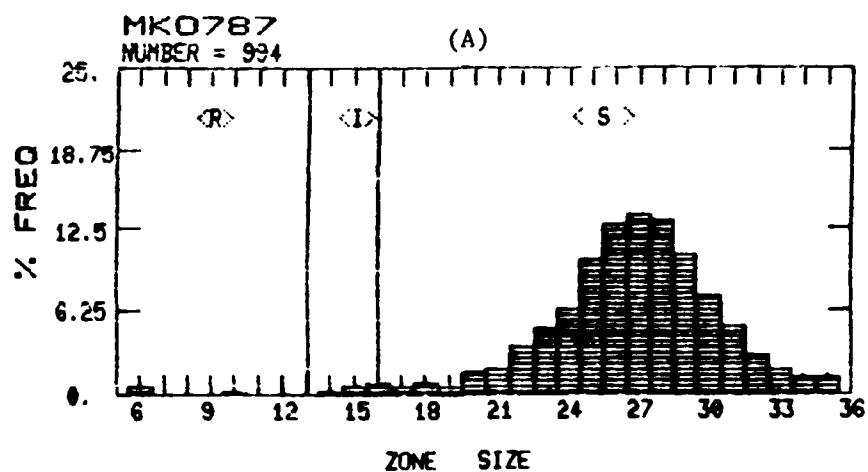


Figure 3. Comparison of the distributions of zones of inhibition of gram-negative organisms in FY 83 (A) and FY 84 (B) using N-formimidoyl thienamycin (MK0787) disc (10 mcg).

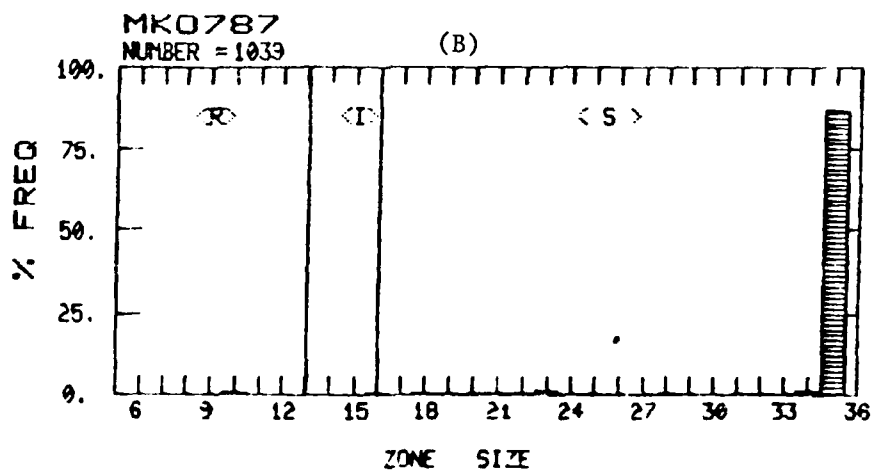
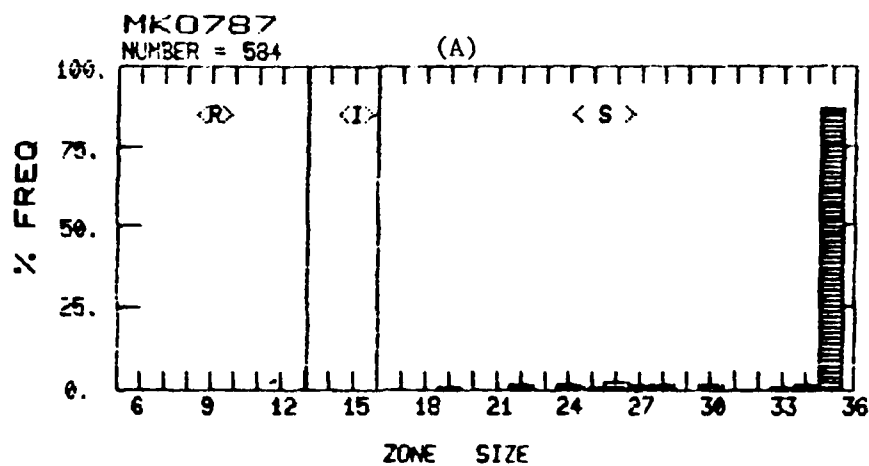


Figure 4. Comparison of the distributions of zones of inhibition of gram-positive organisms in FY 83 (A) and FY 84 (B) using N-formimidoyl thienamycin (MK0787) disc (10 mcg).

serious technical problem in that the Primaxin zone is often so large that it can hinder the reading of other drug zones of inhibition.

EXPERIMENTAL TOPICAL AGENTS

A clinical trial of chlorhexidine diphosphanilate 2% (Westwood, WP-973) was proposed to and approved by this Institute's Human Use Review Committee. The trial was not initiated because the manufacturer withdrew. Preliminary trials at other institutions revealed that the 2% formulation was painful when applied to partial-thickness wounds. Other formulations are being investigated.

IN VITRO SENSITIVITY TO SULFAMYLOX OF PSEUDOMONAS AERUGINOSA RECOVERED FROM BURN PATIENTS

In FY 84, 190 strains of P. aeruginosa isolated from 44 patients were examined by agar dilution of Sulfamylon acetate. The mean minimum inhibitory concentration (MIC) was 0.253%, which compares to 0.156% in FY 83. The median MIC in FY 84 was 0.156% which was the same as FY 83. The distribution of MIC data for FY 83 and FY 84 are presented in Figure 5. The median values for the past 11 reporting periods are reported in Table 2.

PUBLICATIONS

McManus AT: Examination of neutrophil function in a rat model of decreased host resistance following burn trauma. Rev Infect Dis 5 (Suppl 5):S898-S907, 1983.

McManus AT, Denton CL, Mason AD Jr: Topical chlorhexidine disphosphanilate (WP-93) in burn wound sepsis. Arch Surg 119:206-211, 1984.

Yurt RW, McManus AT, Mason AD Jr, Pruitt BA Jr: Increased susceptibility to infection related to extent of burn injury. Arch Surg 119:183-188, 1984.

PRESENTATIONS

McManus AT: Effective topical chemotherapy of burn wound sepsis with chlorhexidine disphosphanilate (Westwood WP-973). Twenty-third Annual Meeting of the Interscience Conference on Antimicrobial Agents and Chemotherapy, Washington, D.C., 24-26 October 1983.

McManus AT: Disappearance of endemic methicillin resistant Staphylococcus aureus: Association with vancomycin usage with four-year maintenance of sensitivity. Third International Symposium on Infections in the Immunocompromised Host, Toronto, Canada, 24-28 June 1984.

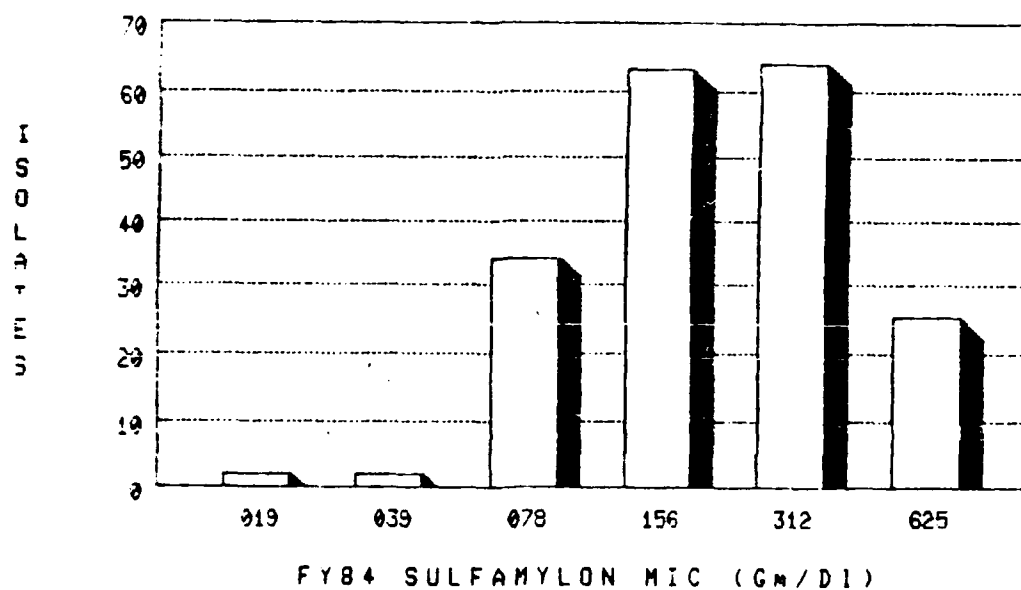
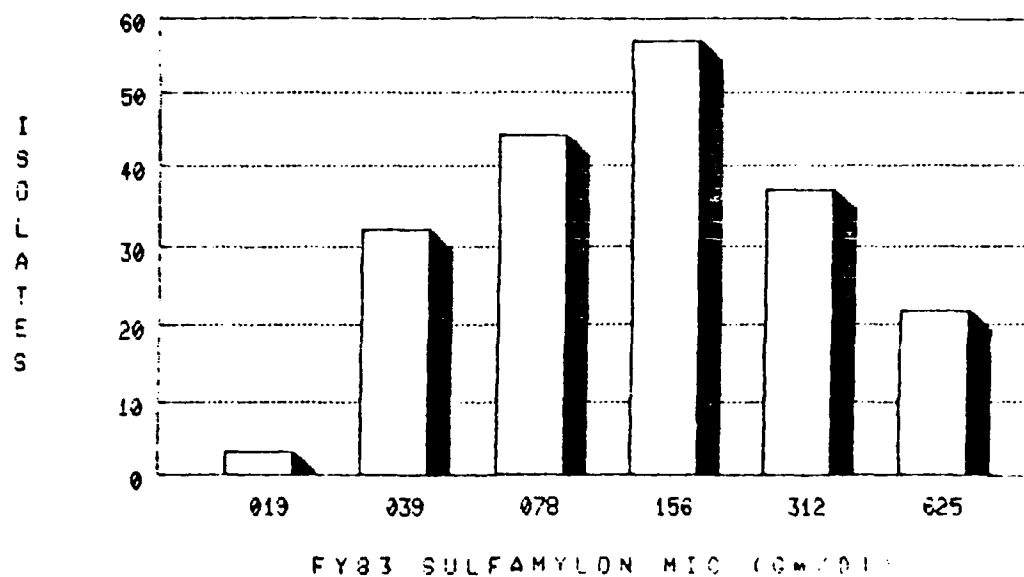


Figure 5. Comparison of the distributions of minimal inhibitory concentrations of Sulfamylon acetate between FY 83 and FY 84.

Table 2. Median Value of Pseudomonas aeruginosa Sensitivity to Sulfamylon, 1973-1984

Year	No. of Strains Tested	Median Inhibitory Level (gm/dl)
1973	285	0.111
1974	437	0.086
1975	656	0.125
FY77	698	0.117
FY78	141	0.089
FY79	715	0.324
FY80	461	0.198
FY81	468	0.253
FY82	733	0.295
FY83	195	0.156
FY84	190	0.156

ANNUAL RESEARCH PROGRESS REPORT

PROJECT NO.: 3M161102BS10-00, BASIC RESEARCH

REPORT TITLE: ALTERATION OF HOST RESISTANCE
IN BURNED SOLDIERS:
THERAPY WITH IgG AND T4 IN BURNED PATIENTS

US Army Institute of Surgical Research
Brooke Army Medical Center
Fort Sam Houston, Texas 78234

1 October 1983 - 30 September 1984

Investigators:

Khan Z. Shirani, M.D., LTC, MC
George M. Vaughan, M.D., LTC, MC
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David G. Burleson, Ph.D., MAJ, MC
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Reports Control Symbol MEDDH-288(R1)

Unclassified

ABSTRACT

PROJECT NO.: 3M161102BS10-00, BASIC RESEARCH

REPORT TITLE: ALTERATION OF HOST RESISTANCE
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THERAPY WITH IgG AND T4 IN BURNED PATIENTS

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Reports Control Symbol MEDDH-288(R1)

Despite the availability of effective systemic antimicrobial agents, the mortality of the infected burn patient remains unacceptably high and prognosis of those beyond the second postburn week seems also to be poor. It is known that the circulating levels of IgG remain suppressed for several weeks after burns. The aim of the present randomized study is to determine whether or not IgG and T4 replacement therapy in burn patients will alter the frequency or intensity of septic complications.

Within five days postburn, patients with a 20-80% probability of mortality are randomized to receive either no IgG or IgG (IGIV, Cutter Biological) in a dose of 500 mg per kg pre-burn weight, twice weekly for a 4-week or longer period until they are completely healed. Among the IgG recipients and controls, those with FT4I below 4 during the second postburn week are randomized to T4 or no T4 replacement groups. It is hoped that this form of prophylaxis in burn patients will result in improved survival by reducing the incidence and severity of post-traumatic septic complications.

Thus far, nine patients have been entered into this study - five have received IgG, four have served as controls, and one control subject with low FT4I has been given T4 replacement. This ongoing study will be concluded when approximately 100 patients have been enrolled, and at that point, the data will be analyzed to assess the efficacy of this form of therapy for the prevention of infection in burn patients.

FINAL REPORT

PROJECT NUMBER: 3M161102BS10, BASIC RESEARCH

PROJECT TITLE: ALTERATION OF HOST RESISTANCE IN BURNED SOLDIERS:
Double-Blind Study of C. parvum Vaccine in the
Prevention of Infection Following Burn Injury

US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234-6200

1 October 1983 - 30 September 1984

INVESTIGATORS

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Arthur D. Mason, Jr., MD
Basil A. Pruitt, Jr., MD, Colonel, MC

*University of Cincinnati

REPORT CONTROL SYMBOL - MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NUMBER: 3M161102BS10-00, BASIC RESEARCH

PROJECT TITLE: ALTERATION OF HOST RESISTANCE IN BURNED SOLDIERS:
Double-Blind Study of C. parvum Vaccine in the
Prevention of Infection Following Burn Injury

US Army Institute of Surgical Research, Brooke Army Medical
Center, Fort Sam Houston, Texas 78234-6200

PERIOD COVERED IN THIS REPORT: 1 Oct 83 through 30 Sep 84

INVESTIGATORS: Khan Z. Shirani, MD, Lieutenant Colonel, MC
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J. Wesley Alexander, MD
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Basil A. Pruitt, Jr., MD, Colonel, MC

REPORT CONTROL SYMBOL: MEDDH-288(R1)

Five adult male burn patients who satisfied the eligibility criteria were entered into the double-blind study of C. parvum therapy and received blinded infusions, each over a one-hour period, twice weekly during the first postburn week and once weekly thereafter. Up to July 1984, when, due to a lack of suitable candidates, this study was terminated, two patients had been treated with C. parvum and three controls had received placebo (saline) infusions.

One patient, during each of four C. parvum infusions, experienced chills, dyspnea, and mental obtundation which resolved spontaneously following completion of these infusions. Another patient, after completion of the full course of C. parvum therapy, developed thrombocytopenia and was managed with platelet replacement.

In the control group, during the first two saline infusions, one patient became nauseated and required antiemetics and another patient, one-month postburn, suffered migratory myalgia and developed a left pleural effusion, both of which responded to conservative therapy.

All patients survived, none developed bacteremia, and all had a good graft take. The small sample size does not permit any conclusions regarding the efficacy of C. parvum in the prevention of infection in burn patients.

C. parvum
Infection
Reticuloendothelial System (RES)

DOUBLE-BLIND STUDY OF C. parvum IN THE PREVENTION OF INFECTION FOLLOWING BURN INJURY

Thermal injury, by destroying physical barriers to infection and through the suppression of cellular and humoral immunity (1-3), predisposes burn patients to septic complications which increase the risk of mortality. The present study attempted to restore immunity in burn patients with the use of C. parvum, a nonspecific reticuloendothelial system stimulant known to protect against experimental infection with Gram-positive bacteria (4), Salmonella (5), and Listeria monocytogenes (6). In addition, this agent has also been reported to increase survival after experimental burns (7,8).

MATERIALS AND METHODS

After stratification according to age (18 through 39 years and 40 through 60 years) and burn size (40 to 60 percent and 60 to 80 percent), patients were randomized into this double-blind study to receive either normal saline or a formalin-fixed (5

¹Alexander JW, Nahmias AJ (ed), and O'Reilly R (ed): Infection in the patient with severe burn. In Immunology of Human Infection. New York: Plenum Medical Book Company, 1980, c1979.

²Alexander JW, Stinnett JD, Ogle CK, et al: A comparison of immunologic profiles and their influence on bacteremia in surgical patients with a high risk of infection. Surgery 86:94-104, 1979.

³Alexander JW, Ogle CK, Stinnett JD, et al: A sequential, prospective analysis of immunologic abnormalities and infection following severe thermal injury. Ann Surg 188:809-816, 1978.

⁴Halpern B, Fray A, Crepin Y, et al: Corynebacterium parvum, a potent immunostimulant in experimental infections and in malignancies. In Immunopotential: Proceedings (Ciba Foundation Symposium). Amsterdam, New York: Elsevier, 18:217-236, 1973, c1974.

⁵Collins FM and Scott MT: Effect of Corynebacterium parvum treatment on the growth of Salmonella enteritidis in mice.

⁶Ruitenberg EJ and Van Noorle Jansen LM: Effect of Corynebacterium parvum on the course of a Listeria monocytogenes infection in normal and congenitally athymic (nude) mice. Zentralbl Bakteriologie (Orig A) 231:197-205, 1975.

⁷Kirov A, Hahn H, Muller F, et al: Therapeutic effects of Corynebacterium parvum in experimental burn disease of mice. Burns 6:45-47, 1979.

⁸Stinnett JD, Alexander JW, Morris MJ, et al: Improved survival in severely burned animals using intravenous Corynebacterium parvum vaccine post injury. Surgery 89:237-251, 1981.

parts/10,000) thimerosal-preserved (0.01 percent) preparation of the Gram-positive anaerobic bacillus, C. parvum (COPRAVAX®, Burroughs Wellcome Company), twice during the first postburn week and weekly thereafter for the following two weeks. The initial dose of C. parvum was 2.5 mg/kg and the subsequent doses were 5 mg/kg. All infusions were blinded and were given over a 60-minute period through an in-line Ultrapore® blood filter. Those ineligible to enter the study were patients less than 18 years of age and those with evidence of inhalation injury, cardiovascular disease, allergic disorders, pre-existing infection, hepatic or renal impairment, or abnormal coagulation profile. Patients were monitored for the development of infection by clinical assessment; chest x-ray (daily); urine, sputum, and blood cultures (three weekly); CBC; urinalysis and SMA (twice weekly); and by a burn wound biopsy (if clinically indicated). Coagulation studies were performed prior to and four hours following each infusion.

Five patients who met eligibility criteria were studied. Nausea experienced by one patient during saline infusion was easily controlled with Benadryl®. Another patient, one month following saline therapy, experienced three episodes of migratory myalgia affecting predominantly the chest wall and anterior trunk. One week later, he developed a left pleural effusion which resolved spontaneously.

Following completion of a full course of C. parvum infusions, one patient developed thrombocytopenia and bleeding from freshly excised burn wounds. The thrombocytopenia persisted for more than two weeks, during which time he required replacement with multiple units of platelets. Because of the severity and persistence of the thrombocytopenia, the code was broken for this patient and it was found that he, in fact, did receive C. parvum. This patient was also receiving topical burn wound therapy using a sulfonamide-containing cream and was treated with amikacin and vancomycin just prior to developing thrombocytopenia. Many drugs, including C. parvum, are capable of reducing the number of circulating platelets and C. parvum cannot be specifically implicated as the cause of this episode of thrombocytopenia, but the association should be noted. This patient's recovery was otherwise uneventful.

In the second treated patient, chills, dyspnea, and mental obtundation were observed during the course of each of four C. parvum infusions; however, in each instance, his symptoms resolved spontaneously.

Blood cultures taken three times per week revealed no bacteremia in these patients and they did not become clinically septic. Coagulation profiles prior to and four hours after completion of each infusion remained normal in all five study patients. All had a good graft take and all survived.

These data are insufficient to permit any conclusions regarding the efficacy of *C. parvum* in preventing infection in burn patients. The results of the *C. parvum* study are summarized in the following table.

PRESENTATIONS/PUBLICATIONS

None.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1 AGENCY ACCESSION	2 DATE OF SUMMARY	REPORT CONTROL SYMBOL	
				DA OG 1842	84 10 01	DD-DR&E(AR) 636	
3 DATE PREV SUMMARY	4 KIND OF SUMMARY	5 SUMMARY SCTY	6 WORK SECURITY	7 REGRADING	8 DISB N INSTR N	9 LEVEL OF SUM A WORK UNIT	
83 10 01	D. Change	U	U		CX		
10 NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER			
a. PRIMARY	61102A	3M161102BS10	BB	304			
b. CONTRIBUTING							
c. CONTRIBUTING	STUG 82/83 - 6.2/4						
11. TITLE (Precede with Security Classification Code)							
(U) Roles of Thyroid Hormones in Burn Pathophysiology							
12 SUBJECT AREAS							
06 05 Clinical Medicine 06 01 Biochemistry							
13 START DATE	14. ESTIMATED COMPLETION DATE		15. FUNDING ORGANIZATION		16. PERFORMANCE METHOD		
79 08	CONT		DA		C. In-House		
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE			
a. DATE EFFECTIVE	EXPIRATION		FISCAL YEARS	a. PROFESSIONAL WORKYEARS		b. FUNDS (In thousands)	
b. CONTRACT/GRANT NUMBER			84	1.2		110	
c. TYPE	d. AMOUNT		85	1.2		115	
e. KIND OF AWARD	f. CUM/TOTAL						
19. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
a. NAME US Army Institute of Surgical Research				a. NAME US Army Institute of Surgical Research Internal Medicine Branch			
b. ADDRESS (include zip code) Ft. Sam Houston, Texas 78234-6200				b. ADDRESS Ft. Sam Houston, Texas 78234-6200			
c. NAME OF RESPONSIBLE INDIVIDUAL Pruitt, BA, Jr				c. NAME OF PRINCIPAL INVESTIGATOR Vaughan, GM			
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) L-Triiodothyronine; (U) Therapy; (U) Volunteers; (U) Hypothyroidism; (U) Burn II (U) Lab Animals; (U) Rats; (U) Thyroxine							
23 TECHNICAL OBJECTIVE 24 APPROACH 25 PROGRESS (Precede text of each with Security Classification Code)							
23. (U) To assess the potential benefit of treatment with thyroid hormones in burned soldiers.							
24. (U) Characterize the nature, extent, and significance of altered thyroid and metabolic economy in burn patients and the impact of replacement therapy with thyroid hormones.							
25. (U) 8310 - 8409. We have been able to document no adverse effects of giving triiodothyronine (T3) as replacement therapy in burn patients. We also observed no effect on metabolism or mortality. We have also seen that a deficit of blood thyroxine (T4) is what correlates best with mortality and with reduced mental status prior to death in nonsurvivors. Recently, outside laboratories, using a rat model, have determined that the brain prefers to use blood T4 rather than T3, and that brain T4 deiodinase (converting blood T4 to local brain T3) is an important marker for brain effects of blood T4. Those studies did not address burn injury. We have now demonstrated that the 60% burned rat has the critical abnormalities seen in burn patients, including suppressed free T4 concentration and a serum T4 binding defect. SWAG milestone perhaps achievable in 2-3 years will be (1) determination of the significance of the low T4, utilizing among other indices, brain T4 deiodinase activity and mortality in the rat model, and (2) assessment of the effectiveness of T4 replacement therapy in patients with high predicted mortality. The "end product" will be the ability to alter one or more metabolic variables in such a way as to prevent mortality and morbidity in injured soldiers.							

ANNUAL PROGRESS REPORT

PROJECT NO.: 3M161102BS10-00, BASIC RESEARCH

PROJECT TITLE: ROLES OF THYROID HORMONES IN BURN
PATHOPHYSIOLOGY: CENTRAL
FEATURES OF THYROID FUNCTION

US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234-6200

1 October 1983 - 30 September 1984

Investigators:

George M. Vaughan, M.D., LTC, MC
Khan Z. Shirani, M.D., LTC, MC

Reports Control Symbol MEDDH-288(R1)

ABSTRACT

PROJECT NO.: 3M161102BS10-00, BASIC RESEARCH

PROJECT TITLE: ROLES OF THYROID HORMONES IN BURN
PATHOPHYSIOLOGY: CENTRAL
FEATURES OF THYROID FUNCTION

US Army Institute of Surgical Research, Brooke Army
Medical Center, Fort Sam Houston, Texas 78234-6200

Period covered in this report: 1 October 1983 - 30 September 1984

Investigators: George M. Vaughan, M.D., LTC, MC
Khan Z. Shirani, M.D., LTC, MC

Reports Control Symbol MEDDH-288(R1)

Thyroid hormone concentrations (tetraiodothyronine, T4; triiodothyronine, T3) in the circulation of burned humans and animals are abnormally low. One basic question has been whether this results at least in part from central suppression of the entire pituitary-thyroid axis. A hallmark of such central suppression should be low or normal basal concentrations of circulating thyrotrophin (TSH) in the face of low thyroid hormones.

We compared serum hormones of control (C), sham burned (S), and 60% burned (B) rats on postburn day 14. C and S rats did not differ and were combined and compared to B values. B rats had suppressed mean testosterone (by 54%), T4 (49%), and T3 (38%) (each $p < 0.01$); and reverse T3 (rT3, by 74%) and TSH (41%) ($p < 0.05$). Previous studies had shown elevated free fractions of T4 and T3 in B rats. Thus, the burn rat model shows the same reproductive and thyroid hormone abnormalities seen in burned humans except for suppressed rT3 (normal or elevated in burned humans). Further, the rat model demonstrates central suppression of the thyroid axis in terms of basal TSH. Preliminary results indicate that we can measure T4 to T3 deiodination in rat brain homogenates using our sensitive T3 assay, and we will use this to monitor the effect of the low thyroid hormones on the brain in burn injury. A study is also underway to assess TSH, mortality and reduced mental function in burned humans and the effect of T4 replacement. So far, no patients have received T4 replacement therapy.

Key Words: Burns; thyroid; thyrotrophin; deiodination

ANNUAL RESEARCH PROGRESS REPORT

PROJECT NO.: 3M161102BS10-00, BASIC RESEARCH

REPORT TITLE: ROLES OF THYROID HORMONES
IN BURN PATHOPHYSIOLOGY:
REDUCED T4 AND T3 AND THEIR
ALTERED SERUM BINDING AFTER
BURN INJURY IN A RAT MODEL

US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
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1 October 1983 - 30 September 1984

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Arthur D. Mason, Jr., M.D.

Reports Control Symbol MEDDH-288(R1)

Unclassified

ABSTRACT

PROJECT NO.: 3M161102BS10-00, BASIC RESEARCH

REPORT TITLE: ROLES OF THYROID HORMONES
IN BURN PATHOPHYSIOLOGY:
REDUCED T4 AND T3 AND THEIR
ALTERED SERUM BINDING AFTER
BURN INJURY IN A RAT MODEL

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Period covered in this report: 1 October 1983 - 30 September 1984

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Total T4 and T3 concentrations are often suppressed after burns in humans. To investigate the significance of such changes, an animal model would be useful. We have characterized serum T4 and T3 after full-thickness scald burns (60% body surface under anesthesia) of 270 g male Sprague-Dawley rats housed in a light:dark cycle of 14:10 h. Groups (N=9-15) of BURN, SHAM (anesthesia, fur clipped, no burn) and CON (controls) were sacrificed on postburn days 8 and 14. T4 and T3 (radioimmunoassay), free indices (FT4I and FT3I = respective total T4 or T3 X in vitro charcoal T3 uptake, T3U), and free concentrations ($\overline{\text{FT4}}$ and $\overline{\text{FT3}}$ = total T4 or T3 X respective equilibrium dialyzable fraction, T4DF or T3DF) were not different between CON and SHAM. Compared to SHAM, mean T4 and FT4I (by about 48% of respective SHAM means on both days), TT3 (by 36, 43%), and FT3I (by 38, 45%) (day 8, 14) were suppressed in BURN (all $p < 0.001$). T4DF (both days) and T3DF (day 14) were significantly elevated in BURN, demonstrating a deficit in transport binding, but T3U was not. FT4 (by 26, 22%) and FT3 (by 33, 34%) (day 8, 14) were significantly lower in BURN. On either day, covariance analyses (BURN vs combined CON+SHAM) correlated FT4I or FT3I with respective FT4 or FT3 (all $p < 0.001$, slopes not different in BURN vs CON+SHAM), but the FT4I and FT3I in BURN were significantly lower (all $p < 0.001$) than predicted by the depressed respective FT4 and FT3 in BURN.

Similarly to humans, burned rats exhibit suppressed circulating total and free T4 and T3 concentrations despite elevated dialyzable (free) fractions of T4 and T3. Because of failure of the T3U to account for this transport binding abnormality, the results are most consistent with a burn-induced circulating inhibitor(s) for binding of T4 and T3 not only to transport proteins but also to in vitro charcoal, perhaps similar to inhibitors previously described in the serum of patients with various nonthyroidal illnesses. The thermally traumatized rat appears to be a good model for thyroid changes in burns and other nonthyroidal illness.

Altered serum binding
Tetratriiodothyronine (T4)
Triiodothyronine (T3)
Nonthyroidal illness
Rat Model

REDUCED SERUM T4 AND T3 AND THEIR ALTERED SERUM
BINDING AFTER BURN INJURY IN RATS

In humans, a reduction in the circulating concentrations of bioactive thyroid hormones is characteristic of nonthyroidal illness (NTI) (1-3). In burn patients, similar to patients with other NTI, serum concentrations of 3,5,3'-triiodothyronine (T3) and often tetraiodothyronine (T4), as well as the indices of free hormonal concentrations, FT3I and often FT4I (index = total hormone concentration times T3 uptake), are suppressed (4-7). Serum free T3 and often free T4 (FT3 and FT4, determined as the product of T4 or T3 times the respective dialyzable free fraction) are suppressed in patients with major burns (5,8) and FT4I and FT3I reflect the FT4 and FT3 respectively ($r > 0.93$) in a linear fashion (4,8). Thus, burns could be distinguished from controls equally well with FT4I or FT4 and with FT3I or FT3 values (8).

1. Melmed, S., Geola, F.L., Reed, A.W., et al: A comparison of methods for assessing thyroid function in nonthyroidal illness. J. Clin. Endocrinol. Metab. 54:300-306, 1982.

2. Slag, M.F., Morley J.E., Elson M.K., et al: Free thyroxine levels in critically ill patients. J.A.M.A. 246:2702-2706, 1981.

3. Wartofsky, L., and Burman, K.D.: Alterations in thyroid function in patients with systemic illness: the "euthyroid sick syndrome". Endocrine Rev. 3: 164-217, 1982.

4. Becker, R.A., Vaughan, G.M., Ziegler, M.G., et al: Hypermetabolic low triiodothyronine syndrome of burn injury. Crit. Care Med. 10:870-875, 1982.

5. Becker, R.A., Wilmore, D.W., Goodwin, C.W. Jr., et al: Free T4, free T3, and reverse T3 in critically ill, thermally injured patients. J. Trauma 20:713-721, 1980.

6. Calvano S.E., Chiao, J., Reaves, L.E., et al: Changes in free and total levels of plasma cortisol and thyroxine following thermal injury in man. J. Burn Care Res. 5:143-151, 1984.

7. Popp, M.D., Srivastava, L.S., Knowles, H.C. Jr., et al: Anterior pituitary function in thermally injured male children and young adults. Surg. Gynecol. Obstet. 145:517-524, 1977.

8. Vaughan, G.M., Mason, A.D. Jr., and Pruitt, B.A. Jr.: Mental status, T4, and survival after burn injury. (Abstract 2501) In: VIIth International Congress of Endocrinology, Quebec, Canada, Excerpta Medica, International Congress Series No. 652. Amsterdam: Elsevier Science Publishers, p. 1511, 1984.

There is a T4 and T3 transport binding abnormality in the serum of burn patients. The dialyzable fractions of T4 (T4DF) and of T3 (T3DF) and the in vitro T3 charcoal uptake (T3U) were elevated in burns (6,8-10). These elevations were similar enough between T3U and the dialyzable fractions to allow observation of a close relationship between indices and free concentrations mentioned above in human burn injury. Nevertheless, analysis of covariance indicated a statistically demonstrable excessive reduction of the FT4I and FT3I in burn patients compared to controls, indicating relatively less elevation of T3U than of T4DF or T3DF.

The present investigation seeks to determine in an animal model whether concentrations of T4 and T3 respond to burns as in humans, whether there is a serum transport binding deficit and whether such a binding abnormality exhibits the same pattern of disparity in response to burn injury between free indices and free concentrations of hormones seen in humans.

9. Vaughan G.M., and Seraile L.G.: Assessment of thyroid hormone kinetics in thermally injured patients: altered transport binding of T4 and T3 in burned soldiers. In: Annual Research Progress Report, FY 1982: U.S. Army Institute of Surgical Research. Ft. Detrick, MD: U.S. Army Medical Research and Development Command, 1982, pp. 307-316.

10. Vaughan G.M., Seraile, L.G., McManus, W.F., et al: Assessment of L-triiodothyronine therapy in thermally injured patients--the hypermetabolic low triiodothyronine syndrome in thermally injured patients. In: Annual Research Progress Report, FY 1982: U.S. Army Institute of Surgical Research. Ft. Detrick, MD: U.S. Army Medical Research and Development Command, 1982, pp. 257-264.

MATERIALS AND METHODS

Adult male Sprague Dawley rats were housed in a 14:10 hours light:dark cycle at a constant 22° C ambient temperature and fed tap water and standard laboratory chow ad libitum. During pentobarbital anesthesia, fur was clipped in the usual area of burn (SHAM) or additionally a full-thickness scald burn covering 60% of body surface was applied (BURN) in a standard manner (11). Unanesthetized and unclipped rats served as additional controls (CON). Animals were sacrificed in groups of 9-15 rats on postburn day (PBD) 8 or 14 to obtain trunk serum for analysis of T4 and T3 (radioimmunoassay) and T3U (in vitro uptake of tracer 125I-T3 from serum directly onto charcoal matrix) (Diagnostic Products kits for T4, T3, and T3U). Standard determinations were also made for equilibrium dialyzable fractions (T4DF and T3DF) of 125I-tracer hormones added to serum and crossing a protein-impermeable membrane into buffer (Nichols Institute, San Pedro, California). The indices of free hormone concentrations (FT3I and FT4I) were the product of total concentration of hormone (T3 or T4) and the in vitro charcoal T3 uptake (T3U). The concentrations of free hormones (FT3 and FT4) were the product of total hormone (T3 or T4) multiplied by respective dialyzable fraction (T4DF or T3DF). Comparisons among group means were made by t-test for several means using the Bonferroni correction for multiple comparisons, and differences in the relationship of free index to free concentration of hormones between groups were assessed with analyses of covariance.

RESULTS

The results for each group are shown in Fig. 1. Direct and derived hormonal values did not differ significantly between CON and SHAM groups. By PBD 14, mean weight gain of BURN (16%) was about half that (31%) of SHAM ($p < 0.01$). On PBD 8 and 14, burn injury produced a significant suppression in thyroid hormones. In BURN, mean T4 and FT4I were about 52% of respective SHAM means on both PBD 8 and 14, T3 was 64% (PBD 8) or 57% (PBD 14) of that in SHAM, and FT3I was 62% (PBD 8) or 55% (PBD 14) of that in SHAM (all $p < 0.001$). T4DF (both PBD 8 and 14) and T3DF (day 14) were significantly elevated in BURN, demonstrating a deficit in transport binding, but T3U was not elevated. Mean FT4 was 74% (PBD 8) or 78% (PBD 14) and FT3 67 % (PBD 8) or 66% (PBD 14) of respective mean SHAM values, all significantly lower in BURN.

11. Walker, H.L., and Mason, A.D. Jr.: A standard animal burn. J. Trauma 8:1049-1051, 1968.

Figure 2 shows the relationship of FT4I to FT4 and FT3I to FT3 in BURN compared to the combined CON and SHAM groups. On either PBD 8 or 14, covariance analyses showed significant correlation (all $p < 0.001$) of FT4I or FT3I with respective FT4 or FT3, and the slopes were not different in BURN versus CON+SHAM. However, the excessively lower FT4I and FT3I in BURN significantly underestimated the respective FT4 and FT3 in BURN, with all vertical positional (intercept) differences at $p < 0.001$ versus CON+SHAM. The figures depict T3U and the free indices (FT4I and FT3I) calculated on the conventional basis of the T3U expressed as the fraction of total in vitro tracer ^{125}I -T3 binding to the matrix after incubation. It has been suggested that expressing the T3U as the tracer binding ratio (counts in matrix/counts in serum) might theoretically enhance the correspondence between FT4I and FT4 (see 3). However, reanalysis of the data with use of this modification of the T3U did not improve this correspondence or change the results.

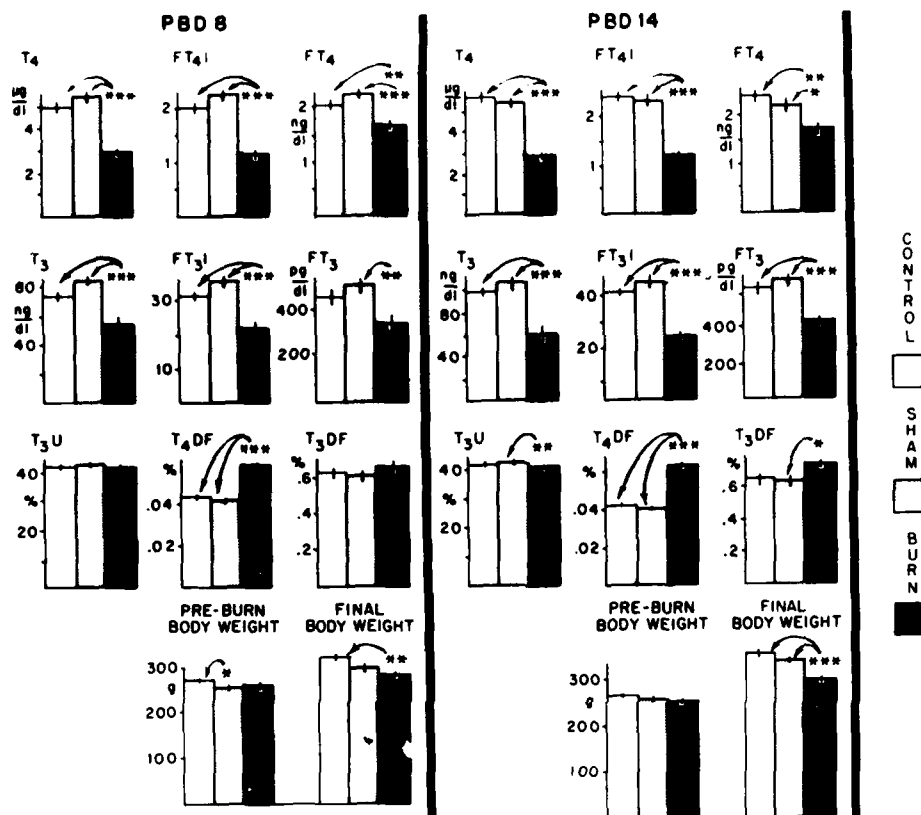


Fig. 1: Group means ($\pm$ SE) for the measured variables in the three groups of rats on postburn day (PBD) 8 or 14. See text (Materials and Methods) for explanations of the symbols for the hormonal measurements.

* $p < 0.05$.
 ** $p < 0.01$.
 *** $p < 0.001$.

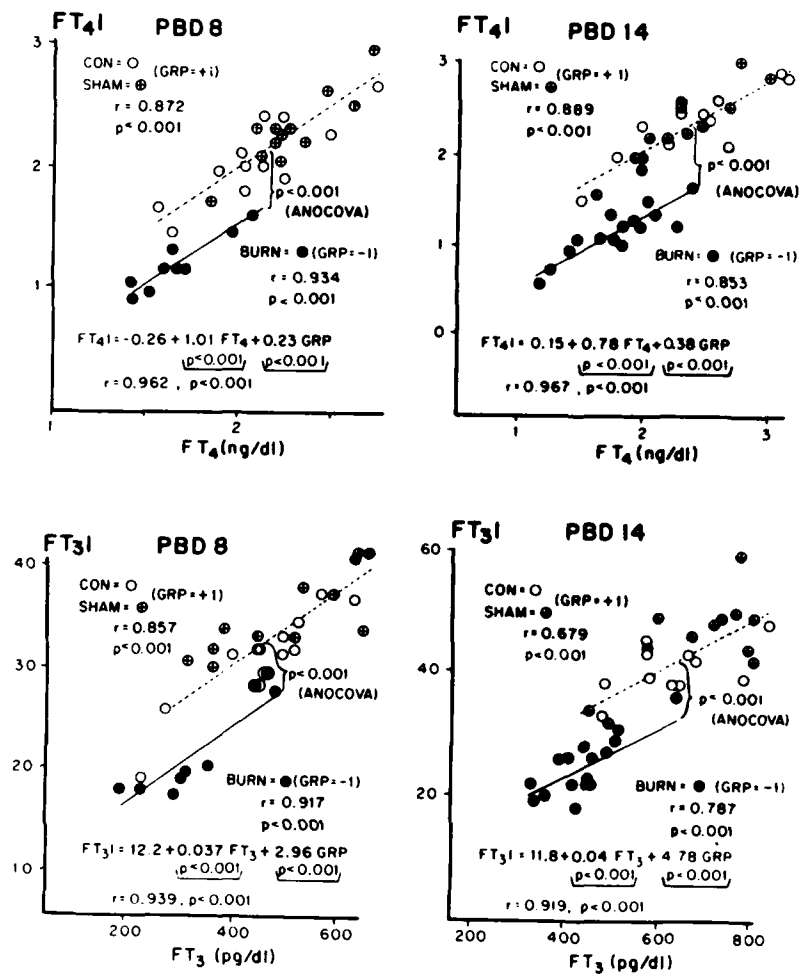


Fig. 2: Analysis of covariance (ANOCOVA) comparing the relationship of the free hormonal indices (FT4I, FT3I) to the respective free concentrations (FT4, FT3) between the combined CON+SHAM (dashed line) and the BURN (solid line) groups. GRP, grouping variable.

DISCUSSION

Burn injury in rats, as in humans, is capable of suppressing the total and free concentrations of circulating T3 and T4 and reducing the extent of serum binding of these hormones as reflected by increased dialyzable fractions. The overall response is similar in these two species in that there is reduction of total T3 and T4 concentrations in burns, with reduction of their bound fractions (elevation of their dialyzable fractions) which may not restore their free concentrations (the biologically active forms) to normal. In this condition, burned humans (12) and rats (13) are not hypometabolic but have elevated O2 consumption. Mainly on the basis of work in humans, this apparently paradoxical situation has tentatively been ascribed to a switch in control of resting metabolic rate from predominantly thyroidal influence to influence by augmented function of a composite hormonal system including sympathetic activity (12-17), and plasma cortisol and glucagon concentrations (18) in burn injury and other hypermetabolic (febrile) non-thyroidal illnesses (NTI). Numerous questions which arise as to the nature of the interactions among thyroid hormone binding, free thyroid hormone concentrations, thyroid hormone production and degradation, peripheral

12. Vaughan, G.M., and Becker, R.A.: Thyroid hormones and catecholamines in burn patients: a hypermetabolic low T3 syndrome. In: Ziegler MG, and Lake CR (eds), Norepinephrine. Front Clin Neurosci, Vol 2. Baltimore: Williams and Wilkins Co., 1984, pp. 450-470.

13. Herndon, D.N., Wilmore, D.W., Mason, A.D. Jr., et al.: Humoral mediators of nontemperature-dependent hypermetabolism in 50% burned adult rats. Surg. Forum 28:37-39, 1977.

14. Becker, R.A., Vaughan, G.M., Goodwin, C.W. Jr., et al: Plasma norepinephrine, epinephrine, and thyroid hormone interactions in severely burned patients. Arch. Surg. 115:439-443, 1980.

15. Felicetta, J.V., Goodner, C.J., and Green, W.L.: Hypermetabolism in burned patients: Role of thyroid hormones. (Abstract) Clin. Res. 28:23A, 1980.

16. Harrison, T.S., Seaton, J.F., and Feller, I.: Relationship of increased oxygen consumption to catecholamine excretion in thermal burns. Ann. Surg. 165:169-172, 1967.

17. Wilmore, D.W., Long, J.M., Mason, A.D. Jr., et al: Catecholamines: Mediator of the hypermetabolic response to thermal injury. Ann. Surg. 180:653-669, 1974.

18. Vaughan, G.M., Becker, R.A., Unger, R.H., et al., Non-thyroidal control of metabolism after burn injury: Possible role of glucagon. Metabolism, 1984 (in press).

conversion of T₄ to T₃, and activation of elements of sympathetic and other hormonal systems in NTI, might now profitably be addressed using the rat burn model.

The mechanism of the reduced iodothyronine binding in NTI is not well understood, although the observation is thought not to be explainable by reductions in serum thyronine-binding protein levels, but to involve a circulating inhibitor for binding of thyroid hormones to plasma proteins in many of these patients. The inhibitor has appeared variously to be non-ultrafilterable (19), an immunoglobulin (20), a heat labile nondialyzable factor(s) possibly leaking into the circulation from injured tissues (21), a nondialyzable, non-immunoglobulin factor(s) (22), or an ether-extractable fat or fatty acid(s) (23). Particularly interesting is the factor(s) described by Oppenheimer et al. (22) in serum of some NTI patients which appeared to inhibit iodothyronine binding not only to serum proteins, but also to charcoal matrix and to incubated rat hepatocytes, suggesting a broad range of action of the binding-inhibitor and its potential for pathophysiological importance. Kaptein et al. (24) provided indirect evidence supporting decreased thyronine binding to tissues of NTI patients by demonstrating a slowed distribution (early-phase

19. Woeber, K.A., and Maddux, B.A.: Thyroid hormone binding in non-thyroidal illness. Metabolism 30:412-416, 1981.

20. Chopra, I.J., Teco, G.N.C., Nguyen, A.H., et al.: In search of an inhibitor of thyroid hormone binding to serum proteins in nonthyroidal illnesses. J. Clin. Endocrinol. Metab. 49:63-68, 1979.

21. Chopra, I.J., Soloman, D.H., Teco, G.N.C., et al.: An inhibitor of the binding of thyroid hormones to serum proteins is present in extrathyroidal tissues. Science 215:407-409, 1982.

22. Oppenheimer, J.H., Schwartz, H.L., Mariash, C.N., et al.: Evidence for a factor in the sera of patients with nonthyroidal disease which inhibits iodothyronine binding by solid matrices, serum proteins, and rat hepatocytes. J. Clin. Endocrinol. Metab. 54:757-766, 1982.

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disappearance from serum) after injection of radiotracer T4 or T3. Studies showing decreased T3 receptor binding in a number of conditions and decreased T3 content in tissues from sick patients have been reviewed (3). It is thus possible that a binding inhibitor in NTI could further diminish the biologic effectiveness of the already reduced concentrations of free circulating thyroid hormones. The extensiveness of the binding defect and its metabolic significance might now be investigated in the rat burn model.

Whereas the rise in T4DF and T3DF was reflected by a less than proportional (though still quite significant) rise of mean T3U in burned humans (6,8), this disparity was even greater in the rats which showed no elevation of T3U after burn injury (Fig. 1). This disparity of response between T3U and the dialyzable fractions is further demonstrated by the positional difference in the vertical dimension between the broken (CON+SHAM) and solid (BURN) line in each panel of Fig. 2, correlating the free indices (based on T3U) with the respective free concentrations (based on the free fractions). We do not have independent measurements of serum thyronine binding proteins, though the content of binding proteins are the same whether the serum is subjected to the T3U test or to dialysis.

Whatever the mechanism for the disproportionately lower free indices compared to free concentrations, it involves blunting of the rise in the T3U expected from the rise in free fractions of T4 and T3. One explanation for this might be a burn-induced reduction in concentration of one of the serum proteins (thyroxine-binding prealbumin, TBPA) that binds a minor fraction of T4 but not T3, since burned humans exhibited a reduction in this protein (25). As discussed in a recent review (26), the T3U is routinely

25. Moody, B.J.: Changes in the serum concentrations of thyroxine-binding prealbumin and retinol-binding protein following burn injury. Clin Chim Acta 118:87-92, 1982.

26. Engler, D., and Burger, A.G.: The deiodination of the iodothyronines and of their derivatives in man. Endocrine Rev. 5:151-184, 1984.

performed under conditions in which the binding of T4 to TBPA may be inhibited, and the resultant additional unbound T4 might displace some of the labelled T3 from the serum and augment the T3U value. This would have occurred more in the controls than in the burns resulting in apparent suppression of T3U in the burns, if burned rats also have reduced TBPA. However, such an explanation seems inadequate, since it would not account for the disproportionality between the FT3I and the FT3 also seen in our burned rats compared to controls. Reported inability to detect any TBPA in rats (27) also suggests the need for another explanation. The pattern of response as related to the binding abnormality in our rats and in NTI could be explained by the presence of a binding-inhibitor (22) which not only reduces the binding of iodothyronines to serum proteins (permitting greater dialysis of hormone through a membrane into buffer and greater binding of hormone to matrix in the T3U test), but also reduces binding of iodothyronines to charcoal (preventing a rise in T3U that otherwise would have resulted from decreased hormone binding to serum protein). This hypothesis could be further tested using the rat burn model.

The present findings of inability of the T3U to represent the changes in T4DF or T3DF and the consequent disparity between FT4I and FT4 as well as between FT3I and FT3 in burned rats are similar (though apparently of greater magnitude) to those in burned humans, are also similar to those in other human NTI which often produce

27. Refetoff, S., Robin N.I., and Fang, V.S.: Parameters of thyroid function in serum of 16 selected vertebrate species: a study of PBI, serum T4, Free T4, and the pattern of T4 and T3 binding to serum proteins. *Endocrinol.* 86:793-805, 1970.

great disparity between FT4I and FT4 (19,22,28), and are compatible with a circulating factor inhibiting T4 and T3 binding to charcoal as suggested in humans with NTI (22). Thus, the burned rat may provide a good model for study of the alterations of iodothyronine binding in NTI, as well as the pathophysiologic significance of reduced free thyronine concentrations in illness. Such studies would have clinical relevance in that suppressed free T4 and T3 have been correlated with deficient mental status and increased mortality in burn patients (8).

28. Chopra, I.J., Solomon, D.H., Hepner, G.W., et al.: Misleadingly low free thyroxine index and usefulness of reverse triiodothyronine measurement in nonthyroidal illnesses. Ann. Intern. Med. 90:905-912, 1979.

PRESENTATIONS/PUBLICATIONS

Presented to the 44th Annual Meeting of The American Association for the Surgery of Trauma Annual Meeting, September 20, 1984, New Orleans, LA.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION DA 304527	2. DATE OF SUMMARY 84 10 01	REPORT CONTROL SYMBOL DD-DR&B(AR) 636	
3. DATE PREV SUMRY 84 07 17	4. KIND OF SUMMARY D. CHANGE	5. SUMMARY SCTY U	6. WORK SECURITY U	7. REGRADING	8. DISB'N INSTR'N CX	9. LEVEL OF SUM A WORK UNIT ISR	
10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER		WORK UNIT NUMBER		
a. PRIMARY	61101A	61101A91C	00		075		
b. CONTRIBUTING							
c. CONTRIBUTING	None						
11. TITLE (Precede with Security Classification Code) (U) Cardiovascular and Endocrine Sequellae of Burn Resuscitation							
12. SUBJECT AREAS 06 16 Physiology 0609							
13. START DATE 84 08		14. ESTIMATED COMPLETION DATE CONT		15. FUNDING ORGANIZATION DA		16. PERFORMANCE METHOD C. In-House	
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE			
a. DATE EFFECTIVE		EXPIRATION		FISCAL YEARS		a. PROFESSIONAL WORKYEARS	
b. CONTRACT/GRANT NUMBER				84		0.1	
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e. KIND OF AWARD		f. CUM/TOTAL				b. FUNDS (In thousands) 45 10	
19. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
a. NAME US Army Institute of Surgical Research				a. NAME US Army Institute of Surgical Research Surgical Study Branch			
b. ADDRESS (include zip code) Ft. Sam Houston, Texas 78234-6200				b. ADDRESS Ft. Sam Houston, Texas 78234-6200			
c. NAME OF RESPONSIBLE INDIVIDUAL Pruitt, BA, Jr				c. NAME OF PRINCIPAL INVESTIGATOR Shirani, KZ			
d. TELEPHONE NUMBER (include area code) 512-221-2720				d. TELEPHONE NUMBER (include area code) 512-221-4652			
21. GENERAL USE FINA M MILITARY/CIVILIAN APPLICATION				f. NAME OF ASSOCIATE INVESTIGATOR (if available)			
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22. KEYWORDS (Precede EACH with Security Classification Code) (U) Resuscitation Fluids; (U) Burn Injury; (U) Hormones; (U) Cardiovascular Hemodynamics; (U) Lab Animals; (U) Rats							
23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)							
23. (U) This study is designed to assess the resuscitation needs in rats with 50% total body surface area burns. Central arterial pressure will serve as a guide to adjustments in infusion rates of fluids containing varying amounts of crystalloid and colloid solution. Measurements of various hemodynamic indices, blood chemistries, vasopressin and renin angiotensin concentrations will be carried out at specific postburn intervals. 24. (U) Analysis of infusion rates for various resuscitation regimens required to achieve hemodynamic stability and the endocrine alterations subsequent to such therapy will provide key information for the assessment of successful resuscitation in this extensive thermal injury model. Acquisition of such information is vital to the better understanding of critical resuscitation needs in burned soldiers. 25. (U) 8310 - 8409. Cardiomax II, the equipment for the measurement of central arterial pressure and for the determination of cardiac output by thermodilution technique, has been recently acquired and is currently being evaluated. Initial observations suggest that the F#1.5 thermodilution probes when placed in the aortic arch interfere with the blood flow and the pressure measurements. This problem is being addressed by ordering F#1.0 probes, the smallest available thermodilution probes.							

ANNUAL PROGRESS REPORT

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

PROJECT TITLE: CARDIOVASCULAR AND ENDOCRINE SEQUELLAE OF
BURN RESUSCITATION

US Army Institute of Surgical Research
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Fort Sam Houston, Texas 78234-6200

1 October 1983 - 30 September 1984

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ABSTRACT

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

PROJECT TITLE: CARDIOVASCULAR AND ENDOCRINE SEQUELLAE OF
BURN RESUSCITATION

Period Covered In This Report: 1 Oct 83 - 30 Sep 84

INVESTIGATORS: Khan Z. Shirani, M.D., LTC, MC
Arthur D. Mason, Jr., M.D.
George M. Vaughan, M.D., LTC, MC

Reports Control Symbol - MEDDH-288 (R1)

The primary goal of fluid therapy in the resuscitation phase of burn injury is to maintain hemodynamic stability without overloading the circulation. Current methods in clinical practice often do not allow administration of adequate volume without simultaneous signs of overload such as pulmonary edema. Such patients often succumb to infection after resuscitation.

In a standard murine burn model, resuscitation regimens consisting of fluids of varying composition will be compared for their effectiveness in restoring intravascular volume as judged by the maintenance of central arterial pressure, cardiac output, and other derived indices of cardiovascular performance. Because of the small blood volume, the murine burn model is expected to be extremely sensitive to the volume shifts which accompany burns and resuscitation. However, the necessary methodology must be assessed.

The techniques for placement of a retrograde intra-aortic combined thermistor probe and central arterial pressure catheter, inserted via the internal carotid artery, and the transvenous intracardiac catheter required for fluid therapy and cardiac output determinations have been accomplished and measurements are consistent and agree with published results. Further work on evaluating various fluid regimens and longitudinal study of the effects of burn injury on endocrine responses of the organism have been planned.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
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3. DATE PREV SUMMARY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISB'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
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a. PRIMARY	61101A	JA161101A91C	00	076		
b. CONTRIBUTING						
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11. TITLE (Precede with Security Classification Code)						
(U) Mechanisms of Opportunistic Infection in Burned Soldiers						
12. SUBJECT AREAS						
06 15 Microbiology 06 14 Pharmacology						
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING ORGANIZATION		16. PERFORMANCE METHOD
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17. CONTRACT/GRANT				18. RESOURCES ESTIMATE		
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a. NAME				a. NAME		
US Army Institute of Surgical Research				US Army Institute of Surgical Research		
b. ADDRESS (include zip code)				b. ADDRESS		
Ft. Sam Houston, Texas 78234-6200				Ft. Sam Houston, Texas 78234-6200		
c. NAME OF RESPONSIBLE INDIVIDUAL				c. NAME OF PRINCIPAL INVESTIGATOR		
Pruitt, BA, Jr				McManus, AT		
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22. KEYWORDS (Precede EACH with Security Classification Code) (U)Virulence Factors;(U)Plasmids;(U)Infection;(U)Toxins;(U)Antibiotics;(U)Pharmacologic Modulation;(U)Vaccines;(U)Lab Animals;(U)Rats;						
23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code) (U)Mice						
23. (U) Define mechanisms of microbial pathogenicity in burned soldier. Develop methods to combat specific virulence factors of opportunistic pathogens. Identify specific defects in immune defenses targeted by opportunistic pathogen. Develop methods to increase host resistance of burned soldiers to opportunistic infection. 24. (U) This project will examine both bacterial and host factors relating to opportunistic infection. A genetic approach will be used to investigate virulence mechanisms of bacteria taken from human burn infections. Isolates will be examined for the presence of extra chromosomal elements (plasmids) that might explain differences in strain virulence. Specific hypothesis about plasmids or chromosomal gene products as virulence factors will be investigated. Virulence mechanisms will be investigated in animal models. Knowledge of specific virulence mechanisms will be used to develop pharmacological, biological or physical means to disrupt microbial virulence. 25. (U) 8310 - 8409. Computerized surveillance has found that more than 50% of infecting gram negative organisms contain sulfonamide resistance mechanisms. The types of organisms causing these infections were distinct from the previous reporting period. The plasmid epidemic previously described as originating in Providencia Stuartii was followed through infection of four bacterial genera until it was eliminated during the reporting period. Two distinct plasmid borne sulfonamide resistance dihydropteroate syntase genes are under examination for possible DNA probe preparation. These probes will be used to identify these genes in infecting organisms.						

ANNUAL PROGRESS REPORT

PROJECT NO. 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: MECHANISMS OF OPPORTUNISTIC INFECTION IN BURNED SOLDIERS

US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234

1 October 1983 - 30 September 1984

Investigators:

Albert T. McManus, Ph.D.
Camille L. Denton, M.A.
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Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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After 11 months' absence, multiply resistant strains of Enterobacter cloacae have returned to the intensive burn care area. A total of 24 patients were colonized. The outbreak is characterized by two plasmid types of equal molecular size (80 megadaltons), distinguishable by restriction endonuclease mapping. A significant increase in Serratia marcescens colonization occurred during the reporting period. The reason for the occurrence could not be identified on the basis of antibiotic resistance selection.

Plasmids
Infection
Virulence factors
Antibiotics
Humans

MECHANISMS OF OPPORTUNISTIC INFECTION IN BURNED SOLDIERS

MULTIPLY RESISTANT ENTERIC ISOLATES

We reported in our last Annual Report (1) that the plasmid epidemic originating in Providencia stuartii in FY 81 ended in September 1983. Evidence of endemic spread of multiply resistant enteric species was absent until March of 1984. This was 11 months after the opening of the new intensive care unit. A gentamicin resistant Enterobacter cloacae was isolated from the urine of a patient on the 58th postburn day. Subsequent to this index case, 24 patients were colonized with E. cloacae with essentially the same antibiotic resistance pattern. The resistance pattern of the index case and the first isolate of each of the other 23 colonized patients is presented in Table 1. The distribution of cases of colonization is presented in Figure 1. A total of 146 isolations were made.

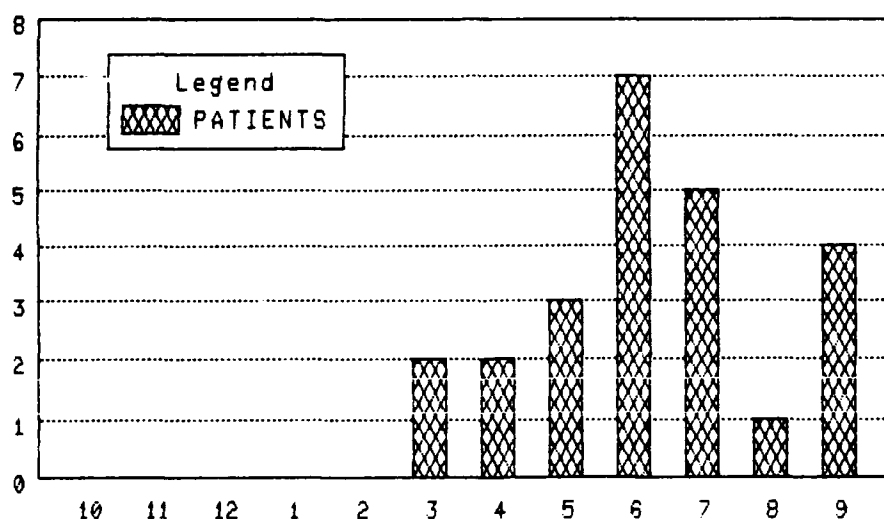


Figure 1. Distribution of patient colonization with multiply resistant E. cloacae during FY 84. Each of the 24 cases is noted in the month in which colonization first occurred.

Selected isolates were examined for transferable antibiotic resistance. The E. cloacae were found to transfer all resistances to Escherichia coli K-12. Examination of plasmid content was done on

1. McManus AT, Denton CL, English VC, Daye GT Jr, Mason AD Jr: Mechanisms of opportunistic infection in burned soldiers. USAISR Annual Report FY 1983, Ft Sam Houston, Texas, pp. 328-334.

TARCE 1. MULTIPLY RESISTANT ENTEROBACTER CLOACAE STRAINS BY PATIENT, DATE OF FIRST ISOLATION PER PATIENT, PATIENT'S POST BURN DAY, SOURCE OF ISOLATION, ANTIBIOTIC RESISTANCE PATTERN (GM=GENTAMICIN, NN=TOBRAMYCIN, NET=NETILMICIN, K=KANAMYCIN, C=CHLORAMPHENICOL, AMP=AMPICILLIN, TIC=TICARCILLIN, MZ=MEZLOCILLIN, PIP=PIPERACILLIN, SD=SULFADIAZINE), WARD(1=14A, 2=14B) AND QUANTITY OF GROWTH.

CASE	YEAR	MONTH	DAY	POST BURN DAY	SOURCE	GM	NN	NET	K	C	AMP	TIC	MZ	PIP	SD	WARD	GROWTH	
																	LOG	10
1	84	03	05	58	URINE		R	R	R	R	R	R	R	R	R	R	1	5
2	84	03	23	87	URINE		R	R	R	R	R	R	R	R	R	R	1	5
3	84	04	23	16	CONTACT PL		R	R	S	R	R	R	R	R	R	R	1	
4	84	04	23	37	CONTACT PL		R	R	R	R	R	R	R	R	R	R	1	
5	84	05	03	67	SWAB/MISC		R	R	R	R	R	R	R	R	R	R	1	
6	84	05	27	109	PORCINE GR		R	R	R	R	R	R	R	R	R	R	1	
7	84	05	30	14	CONTACT PL		R	R	R	R	R	R	R	R	R	R	1	
8	84	06	01	15	BIOPSY #1		R	R	R	R	R	R	R	R	R	R	1	8
9	84	06	06	123	URINE		R	R	R	R	R	R	R	R	R	R	2	5
10	84	06	13	59	URINE		R	R	R	R	R	R	R	R	R	R	1	5
11	84	06	14	61	URINE		R	R	R	R	R	R	R	R	R	R	1	5
12	84	06	15	26	URINE		R	R	R	R	R	R	R	R	R	R	1	5
13	84	06	15	5	URINE		R	R	R	R	R	R	R	R	R	R	1	5
14	84	06	22	8	URINE		R	R	R	R	R	R	R	R	R	R	1	5
15	84	07	03	73	PORCINE GR		R	R	R	R	R	R	R	R	R	R	1	5
16	84	07	04	34	URINE		R	R	R	R	R	R	R	R	R	R	2	5
17	84	07	06	2	URINE		R	R	R	R	R	R	R	R	R	R	2	5
18	84	07	17	20	SPUTUM		R	R	R	R	R	R	R	R	R	R	1	8
19	84	07	20	38	BLOOD POSI		R	R	R	R	R	R	R	R	R	R	1	
20	84	08	24	17	SPUTUM		R	R	R	R	R	R	R	R	R	R	1	6
21	84	09	03	2	SPUTUM		R	R	R	R	R	R	R	R	R	R	1	8
22	84	09	10	15	SPUTUM		R	R	R	R	R	R	R	R	R	R	1	7
23	84	09	17	19	SWAB/WOUND		R	R	R	R	R	R	R	R	R	R	1	
24	84	09	24	12	SPUTUM		R	R	R	R	R	R	R	R	R	R	1	7

transconjugants by agarose gel electrophoresis. The E. cloacae strains examined showed a transferable plasmid of about 80 megadaltons. This size was the same as the Providencia plasmid previously reported. Attempts were made to characterize the plasmids further. Figure 2 shows a gel electropherogram of crude cell lysates of the FY 81 P. stuartii (C5, lane 1) and E. cloacae from the FY 84 outbreak (lanes 2 and 3). The three strains contain at least one large plasmid of about 80 megadaltons. Lanes 1a-3a contain the plasmids of lanes 1-3 which have been digested with the endonuclease ECO RI. Each band in the gel represents a piece of DNA that has been cut from its original plasmid. Smaller pieces move faster during electrophoresis. The P. stuartii plasmid showed a distinct 13-band pattern. The FY 84 E. cloacae show two distinct patterns with this enzyme. It appears that FY 84 isolates contain similar but distinct plasmids. Figure 3 shows the patterns of other E. cloacae K-12 transconjugants. Lane 1 is the K-12 C600 host which contains no plasmid DNA. Lanes 2-6 contain plasmids mated into E. coli K-12 C600 from FY 84 E. cloacae isolates. Again, a consistent single plasmid of 80 magadaltons is present. Lanes 2a-6a contain the ECO RI digests of lanes 2-6. Two digestion patterns as noted for FY 84 E. cloacae in Figure 2 are present in Figure 3. Digests in lanes 2a, 3a and 5a are distinct from lanes 4a and 6a. These data indicate the endemic multiply resistant E. cloacae isolates may represent two plasmid types. These plasmids have similar size but have distinct ECO RI digestion sites. These plasmids and isolates from the continuing endemic will be further examined and reported in the next reporting period.

SERRATIA MARCESCENS COLONIZATION

A significant increase in the incidence of patients colonized with Serratia marcescens occurred during FY 84. Colonization occurred in 26 patients of the 195 patients sampled. This compares to two patients out of 186 sampled in FY 83 ($P < .001$). The index case occurred in December 1983. A culture of this patient's sputum yielded S. marcescens on the 16th PBD. Twenty-five patients were colonized during the year (Figure 4); the average day of colonization was 21 days postburn. No evidence of antibiotic resistance pressure was observed. For example, 95% of tested strains were sensitive to sulfonamides. This may be the first example of a persistent organism whose persistence could not be explained on the basis of antibiotic resistance selection.

SEROLOGIC TYPES OF PSEUDOMONAS AERUGINOSA ISOLATED FROM BURN PATIENTS IN FY 84.

O-serotyping was performed on 197 isolates from 46 burned patients. Difco International Typing Sera were used on autoclaved suspensions. Typing was possible for 96% of isolates, which is a significant improvement from FY 83's 82% ($P < .01$). In addition, 19 distinct serotypes were observed in FY 84. This compares to 13 in FY 82. The distributions of serotypes, number of isolates and patients per serotype are presented in Table 2 and Figure 5. Types 11 and 1 were the most common types colonizing patients.

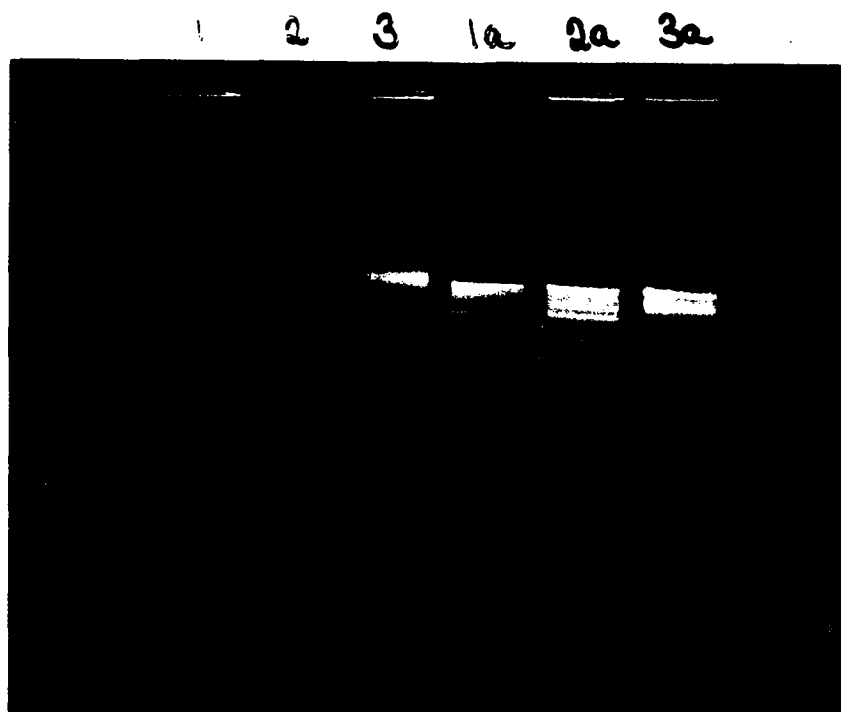


Figure 2. 1% agarose plasmid profile of organisms carrying a gentamicin resistant marker. Lane 1: *P. stuartii* (C5); lane 2: *E. cloacae* 840606042; lane 3: *E. cloacae* 84101050; lane 4: *P. stuartii* (C5) cut with *ECO* RI; lane 5: *E. cloacae* 840606042 cut with *ECO* RI; lane 6: *E. cloacae* 84101050 cut with *ECO* RI.

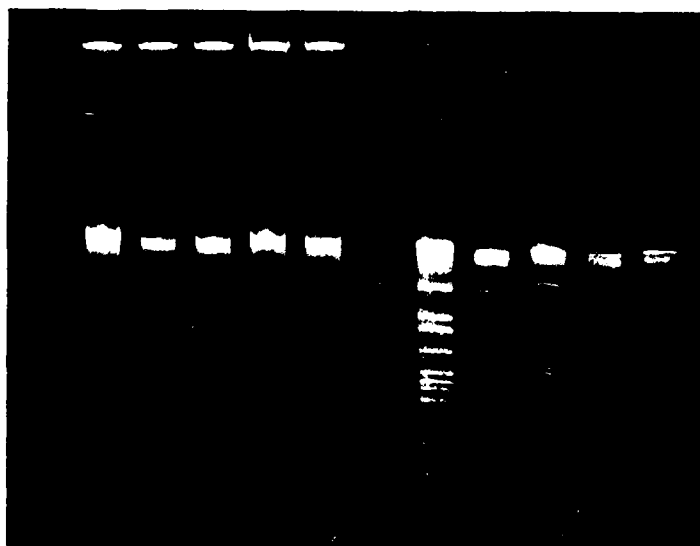


Figure 3. Agarose gel electrophoresis of crude lysates. Lane 1: *E. coli* K-12 C600; lanes 2-6: FY 84 *E. cloacae* multiply resistant isolates. Lanes 1a-6a are digests of lanes 1-6 using DNA endonuclease *ECO* RI.

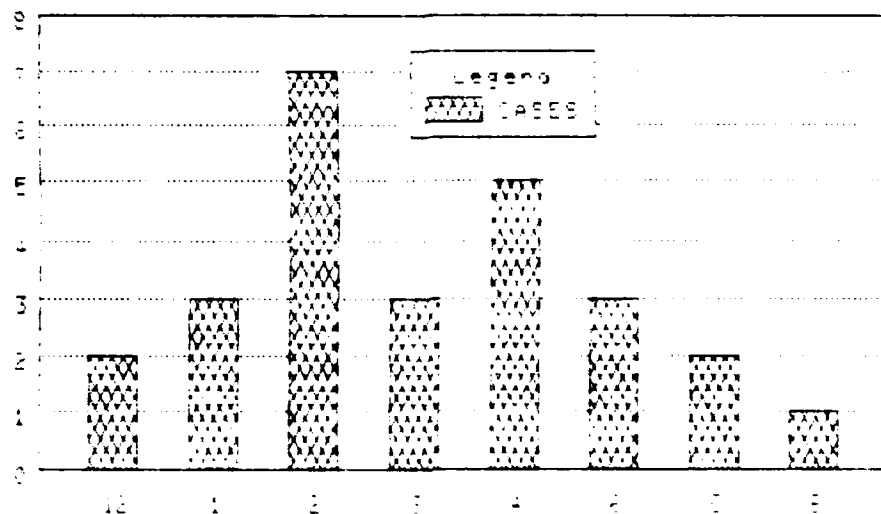


Figure 4. Distribution of patients colonized with *Serratia marcescens* during FY 84. Each of the 26 cases is noted in the month in which colonization first occurred.

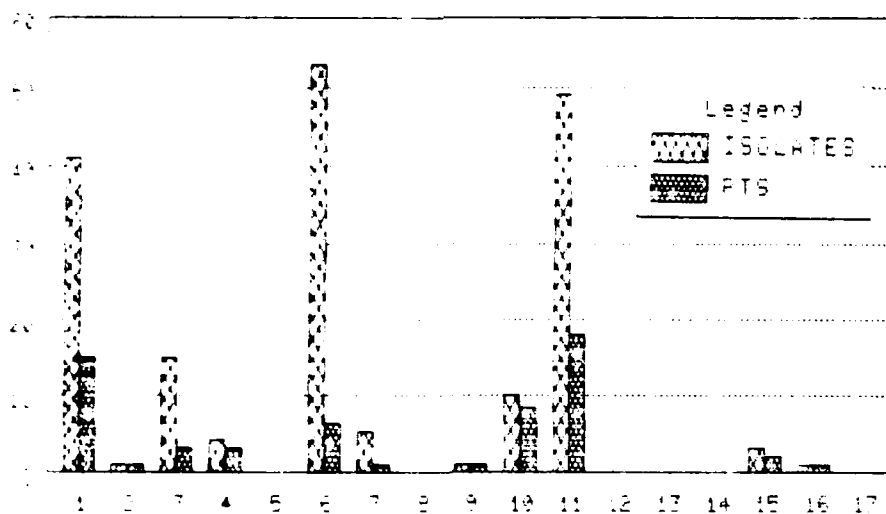


Figure 5. Distribution of *P. aeruginosa* serotypes during FY 84 by numbers of isolates and patients.

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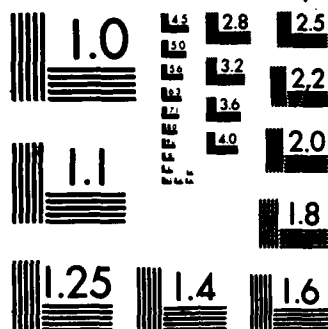
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Table 2. Serotypes of Pseudomonas aeruginosa
Isolates from 46 Burned Patients in FY 84

Type	No. of Strains	No. of Patients
1	41	15
1,7	1	1
2	1	1
3	15	3
4	4	3
5	-	-
6	53	6
7	5	1
8	-	-
9	1	1
10	10	8
10,11	1	1
11	49	18
12	-	-
13	-	-
14	-	-
15	3	2
16	1	1
17	-	-
Not typable	12	7

PUBLICATIONS/PRESENTATIONS

None.

ANNUAL PROGRESS REPORT

PROJECT NO. 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: PRELIMINARY STUDIES OF LYMPHOID SUBPOPULATIONS IN A
BURNED ANIMAL MODEL

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BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234

1 October 1983 - 30 September 1984

Investigators:

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ABSTRACT

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The decreased host resistance to infection that occurs after severe burn injury suggests an impaired immunological capacity in burn victims. The ability to measure the immunological competence of burn patients would have clinical value for monitoring immunological integrity and assessing the effectiveness of therapeutic intervention. A standard animal model of burn injury and infection was used to evaluate the feasibility of measuring immunocompetence by analyzing the lymphocyte populations after burn injury and burn injury complicated by infection. Burned-infected rats had severely decreased absolute lymphocyte counts and decreased levels of lymphocytes relative to other circulating leukocytes in whole blood. Blood lymphocyte subpopulations purified on Ficoll-Hypaque gradients from control, burned and burned-infected rats were measured by flow cytometry to determine changes in the subpopulations induced by burn injury and complicating infection. Burned rats had slight but significant decreases in the relative number of T cells and suppressor cells on postburn days 2 and 4 compared to control. Burned-infected animals, however, had a substantial decrease in the relative number of T cells, helper cells and helper:suppressor ratios at postburn days 2, 4 and 7. The magnitude of the changes grew progressively larger with time postburn. Use of light scatter to select abnormal leukocytes to be eliminated from subpopulation analysis did not significantly change the relative ratio of helper cells to suppressor cells determined for each group. Light scatter analysis and morphological analysis revealed increasing numbers of non-lymphoid and abnormal lymphoid cells contaminating Ficoll-Hypaque preparations of lymphocytes from burned-infected rats. The proportion of contaminating cells increased with time postburn and postinfection. Present techniques of lymphocyte separation and flow cytometric analysis are inadequate to provide unequivocal measurement of lymphocyte subpopulations from burned-infected rats. New techniques to accomplish this are now under development.

Thermal injury
Immunological model
Flow cytometry
Lymphocyte subpopulations

Helper:suppressor ratio
Lab animals
Rats

PRELIMINARY STUDIES OF LYMPHOID SUBPOPULATIONS IN A BURNED ANIMAL MODEL

Severe thermal injury results in increased susceptibility to sepsis, which is a major contributor to mortality in burn patients. Host defense against infection is a complex combination of many different specific and non-specific antimicrobial processes. It is not known which of the many changes that occur in these processes in the burn patient may contribute to increased infection risk (1-3). One change that has been reported in burn patients is a shift in the relative numbers of regulatory lymphocytes, and this change was correlated with mortality (4-6). The relative proportions of immunoregulatory cells were determined after the purified cells were treated with appropriate fluorescent monoclonal reagents; the relative numbers of cells binding the fluorescent label were determined by fluorescent microscopy or flow cytometry. A change in the ratio of helper and suppressor subpopulations has been associated with immunosuppression in conditions other than burn injury which are characterized by increased susceptibility to infection (7).

The hypothesis that has developed from these findings is that increased numbers of "suppressor" lymphocytes compared to "helper" lymphocytes work to suppress the immune system and make the patient susceptible to sepsis. The underlying assumption is that the mere presence of a higher proportion of cells bearing the suppressor surface antigen results in suppression of the immune response in vivo. There is no direct evidence to establish this theory. The opposing hypothesis that a relative increase in suppressor cells may be the result of sepsis and not a

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1. Kulick MI, Lopez RR: Immunological aspects of thermal injuries. *Ann Plast Surg* 7:252-255, 1981.
 2. Alexander JW, Ogle CK, Stinnett JD, MacMillan BG: A sequential, prospective analysis of immunologic abnormalities and infection following severe thermal injury. *Ann Surg* 188:809-816, 1978.
 3. Munster AM, Winchurch RA, Birmingham WJ, Keeling P: Longitudinal assay of lymphocyte responsiveness in patients with major burns. *Ann Surg* 192:772-775, 1980.
 4. McIrvine AJ, O'Mahony JB, Saporoschetz I, Mannick JA: Depressed immune response in burn patients: Use of monoclonal antibodies and functional assays to define the role of suppressor cells. *Ann Surg* 196:297-304, 1982.
 5. Rodrick ML, O'Mahony J, McIrvine A, Collins K, Mannick J: Comparison of fluorescence, microscopic and flow cytometric analysis of T cell subsets in burn patients. *Fed Proc* 42:950, 1983.
 6. Antonacci AC, Reaves LE, Calvano SE, Amand R, De Riesthal HF, Shires GT: Flow cytometric analysis of lymphocyte subpopulations after thermal injury in human beings. *Surg Gynecol Obstet* 159:1-8, 1984.
 7. Goldstein G, Lifter J, Mittler R: Immunoregulatory changes in human disease detected by monoclonal antibodies to T lymphocytes. In: *Monoclonal Antibodies in Clinical Medicine*, A. McMichael (ed), Academic Press, London, p. 39, 1982.

contributing cause is just as reasonable. In fact, continued reports of the heterogeneous nature of lymphocyte subsets and the demonstration that certain "helper" cells may participate in inducing suppressor cells make the first hypothesis far less reasonable (8).

We have measured changes in lymphocyte subpopulations in an established animal burn and infection model (9) in an effort to determine the relationship of subpopulation changes to the increased susceptibility to infection seen in this model after thermal injury.

METHODS

Male albino rats were randomly assigned to one of three groups, an unburned control group, a burned group and a burned group with infection. All rats were anesthetized with pentobarbital (1 mg/100 g body weight, IP), and those in the burned group were shaved, placed in a plastic mold and subjected to a 30% total body surface full-thickness burn by a 10-second immersion in boiling water. Infection was induced by seeding 1 ml of a 16-hour broth culture containing approximately 10^8 *Pseudomonas aeruginosa* (strain 12-4-4) on the rat dorsum within 1 hour of scalding. Rats were sacrificed at 48, 96 and 168 hours after burning and blood was taken for culture and cell analysis. The rats were anesthetized with pentobarbital and exsanguinated by opening the body cavity and bleeding from the hepatic vein. A portion of the blood sample (1 ml) from burned and burned-infected rats was cultured in trypticase soy broth to assess the presence of bacteria.

Total leukocyte counts were made on whole blood using a Coulter Counter and a differential slide was prepared. The remaining blood was used in the isolation of lymphoid cells on a Ficoll-Hypaque gradient. The isolated cells were washed and a portion used to prepare a slide for differential analysis using Wright's stain; a second portion was used for flow cytometry analysis. Purified cells were prepared for differential staining by centrifugation onto a microscope slide in a cyto-centrifuge (Shandon). After drying, the cells were fixed and stained in an automated slide stainer (Fisher). Cells for flow cytometry analysis were stained with appropriately diluted fluorescein (FITC) labeled monoclonal reagents (Pel-Freez). The stained cells were analyzed for relative numbers of positive and negative cells by a Fluorescence Activated Cell Sorter (Becton Dickinson model 400 modified with the addition of a consort 40 data analysis system and 4th parameter PMT). FITC was excited using the 488 nm spectral line of a 5-watt argon ion laser. In each experiment, the instrument was standardized with 4.3 micron fluorescent labeled microspheres (Becton Dickinson) initially and after

8. Green DR, Chue B, Gershon RK: Discrimination of two types of suppressor T cells by surface phenotype and by function: The ability to regulate the contrasuppressor circuit. *J Mol Cell Immunol* 1:19-25, 1983.

9. Walker HL, Mason AD Jr: A standard animal burn. *J Trauma* 8: 1049-1051, 1968.

every 12 samples. For each surface antigen, 5000 cells were analyzed and the numbers of cells positively stained for T lymphocyte (clone W3/13), suppressor/cytotoxic (clone OX-8), and helper/inducer (clone W3/25) antigen were determined by a locally developed program on the Consort 40 data analysis system. Background fluorescence due to non-specific binding and autofluorescence was quantified by reacting a portion of the cells with a FITC labeled mouse monoclonal antibody against a human antigen. The threshold used to determine the number of positively staining cells was defined such that 5% or less of the cells in the background control fell above the threshold. Cells were initially gated on forward scatter to eliminate contaminating cell debris, red blood cells and platelets from the analysis.

Statistical analyses (t tests) were performed using BMDP (UCLA, CA) program P7D on a minicomputer (DEC VAX 11/780). Bonferroni probabilities were determined for each of the possible pairwise comparisons for the three possible pairs of groups (i.e., control vs. burned, burned vs. infected and control vs. infected). In the Bonferroni test the significant p value is adjusted for the multiple comparison of all pairs of means. Thus, to be significant at the .05 level the p value obtained for the t test must be less than .016667, .00333 at the .01 level and .000333 at the .001 level. The significance of the probabilities was verified by the Tukey studentized range method and the Scheffe method.

RESULTS

At each designated time period, whole blood from control, burned and burned-infected rats was fractionated on Ficoll-Hypaque gradients. Cells taken from the gradient interface were stained with monoclonal reagents for analysis by flow cytometry. The results (shown in Figure 1A-1C) demonstrate that there is a progressive decrease from postburn day (PBD) 2 to PBD 7 in the relative number of T cells from the blood of burned-infected animals compared to control ($p = .0012$ or less). There were also slight but significant decreases in T cells in burned animals on PBD 2 and 4 ($p = .01$ or less). Cells staining for helper lymphocyte antigen were sharply decreased in burned-infected animals compared to control ($p = .0018$ or less). Again the decrease was progressive over time. Animals that were burned only had no significant decrease in the proportion of helper cell positives.

Although the mean for the percentage of cells staining positively for suppressor cell antigen was greater than that of control on PBD 4 and 7, the increase was not statistically significant. There was a significant decrease, however, in the percentage of cells staining for suppressor antigen from burned animals on PBD 2 and 4 ($p = .01$ or less).

The mean difference in the ratio of helpers to suppressors (shown in Figure 2) was also significantly decreased in the burned-infected group on PBD 2 through 7 ($p = .0143$ or less) and the decrease was progressively greater at later times. The helper:suppressor ratio in burned animals was never significantly different from control at the times tested.

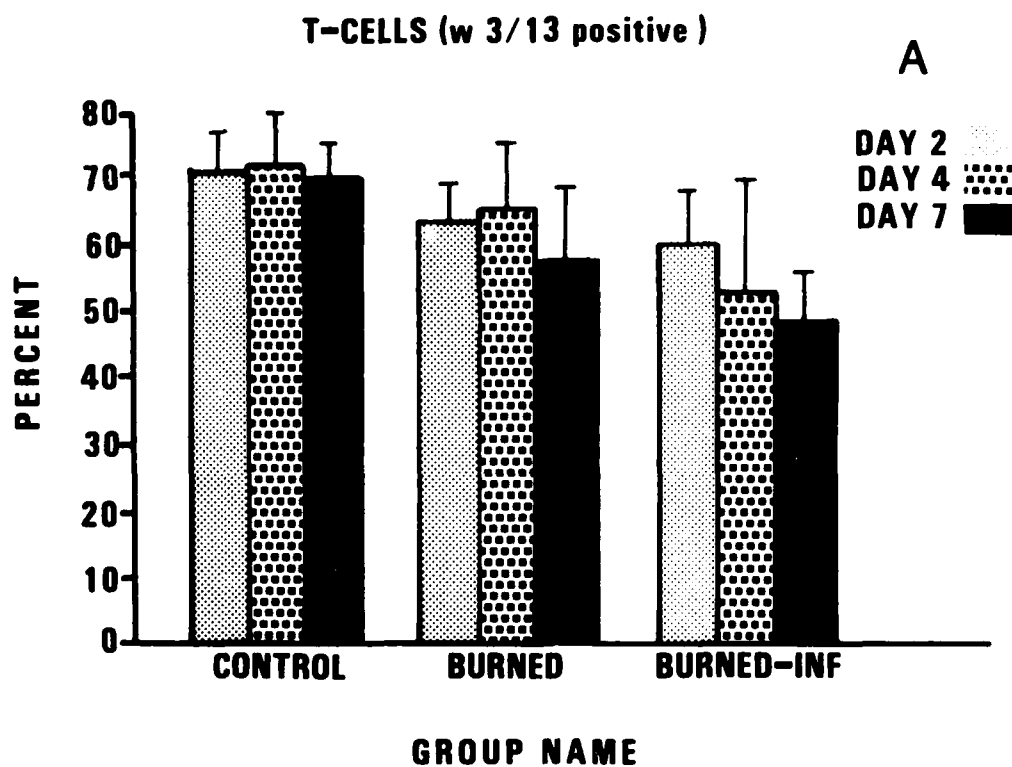


Figure 1A

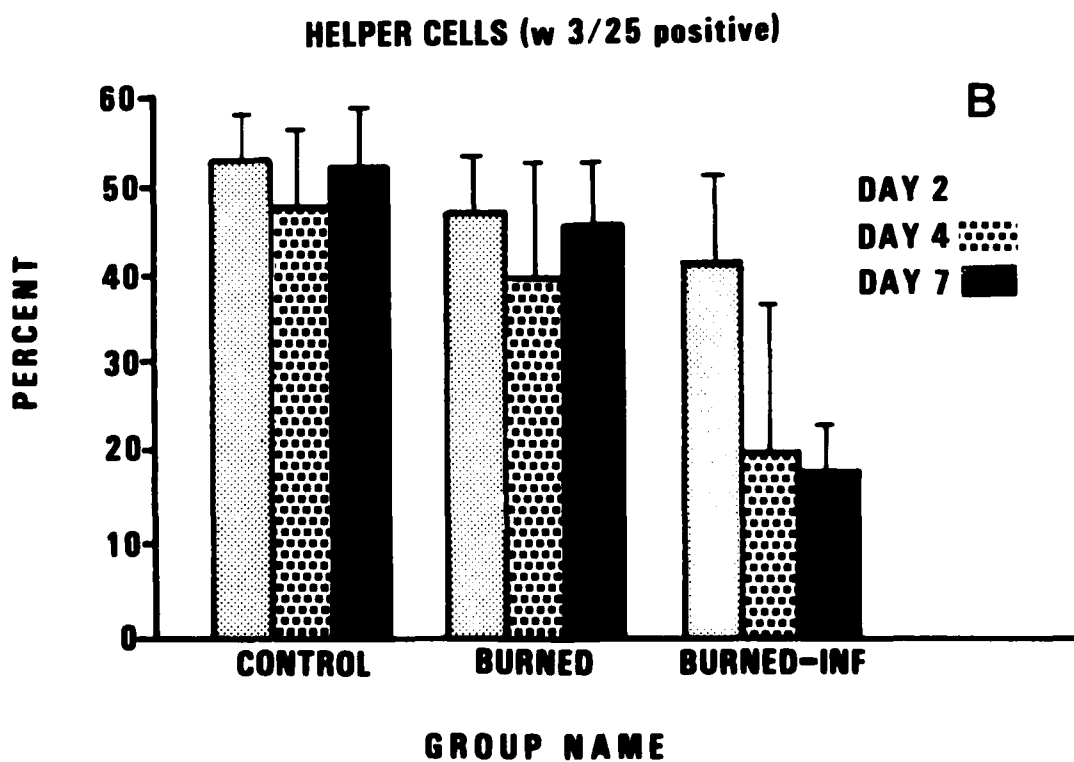


Figure 1B

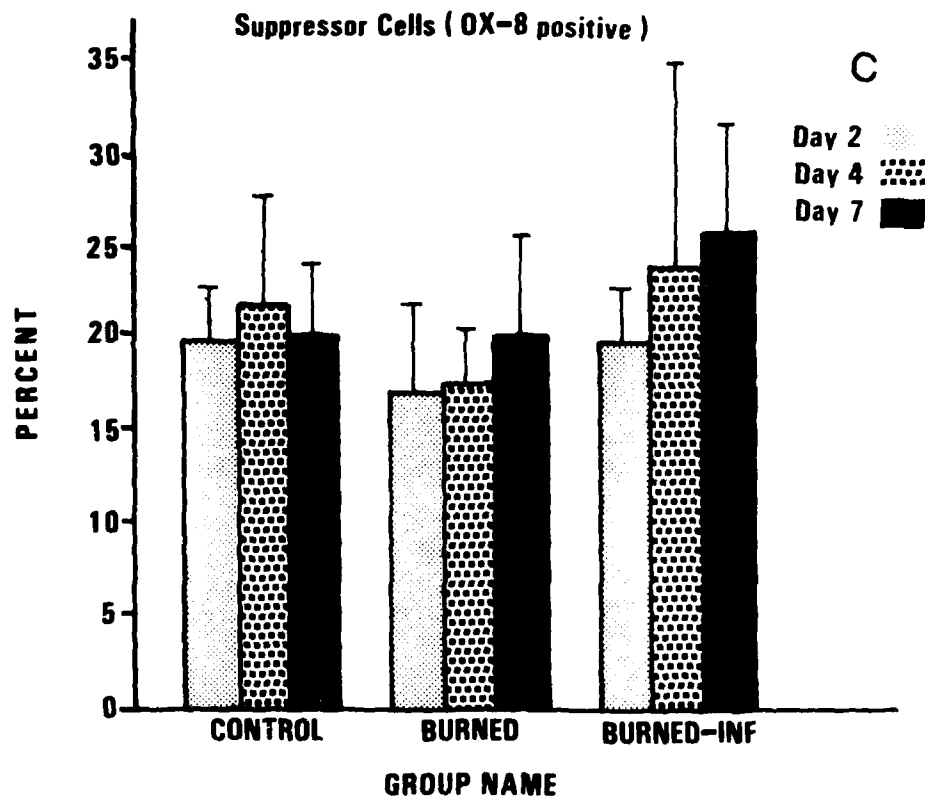


Figure 1C

Figure 1. Flow cytometry analysis of the cells from the Ficoll-Hypaque mononuclear fraction staining positively for T cell antigens. Determinations were made at three separate postburn days and comparisons made between control, burned and burned-infected for each day. Bars represent the mean of the positives represented as a percent of the total cells analyzed $\pm$ SD.

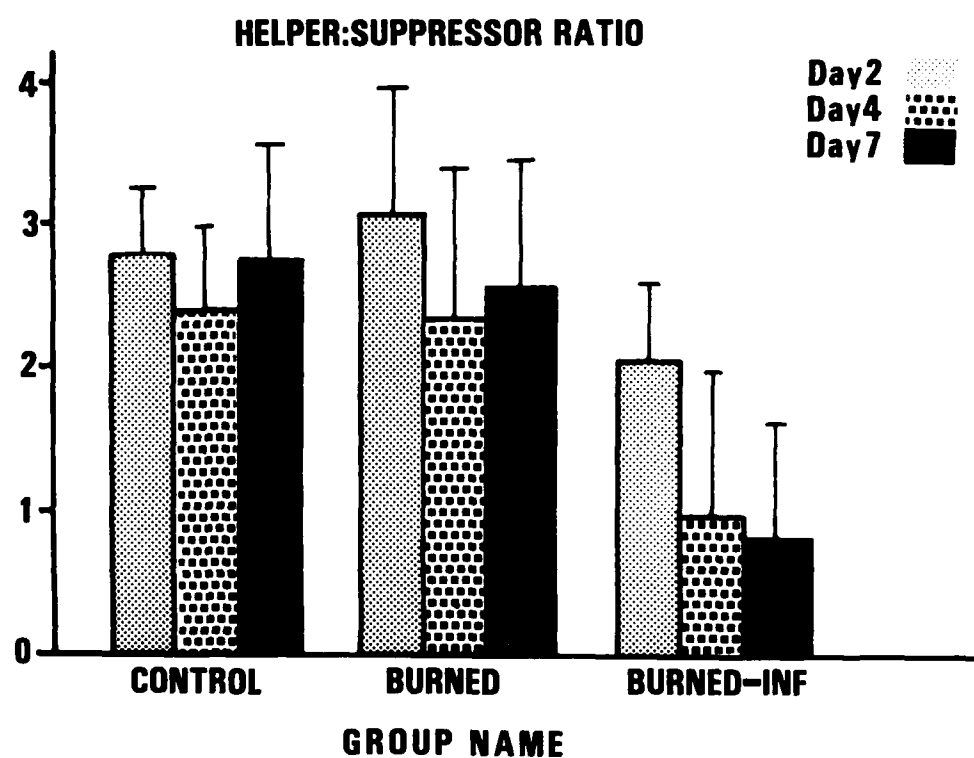


Figure 2. Ratio of the percentage of helper (W3/25 positive) cells to the percentage of suppressor (OX-8 positive) cells from the mononuclear cell fraction of the Ficoll-Hypaque gradient.

The decrease in the helper:suppressor ratio seen in the infected animals is accompanied by a sharp decrease in the absolute circulating lymphocyte count. Blood smears were prepared from whole blood, stained with Wright's stain and differentiated under the light microscope. The results shown in Figure 3 demonstrate that there is a decrease in the proportion of blood lymphocytes relative to non-lymphoid cells in the blood from infected rats, reaching a low of approximately 50% by PBD 7 compared to 79% for control animals ($p = .0001$ or less). The fraction of lymphocytes determined from the blood smear was multiplied by the whole blood leukocyte count to determine the absolute lymphocyte count. On PBD 4, the mean absolute lymphocyte counts were 3860 990 (control), 3520 1827 (burned) and 1730 381 (infected). This large decrease in the count for infected animals ($p = \text{less than } .00005$) was similar to that seen on PBD 2 and 7.

Ficoll-Hypaque preparations from the blood of control animals usually contain more than 95% lymphocytes. Red blood cells, polymorphonuclear leukocytes and many monocytes pass through the Ficoll-Hypaque layer which has a density of 1.07 g/ml. The lymphocytes, which are generally lighter than 1.07 g/ml, stay at the gradient:plasma interface. Results from the differentiation of slides made from gradient interface cells are shown in Figure 4. Compared to control, there was little change in the cell types from burned only animals but cell preparations from infected animals contained a much smaller proportion of lymphocytes and a corresponding increase in non-lymphoid cells at PBD 4 and 7. The non-lymphoid cells are principally macrophages and immature granulopoietic cells that have a density closer to that of lymphocytes than mature forms. By PBD 7, these cells comprised more than 60% of the cells isolated from the gradient.

This drastic change in cell morphology can also be demonstrated by light scatter analysis with the flow cytometer. The flow cytometer can analyze light scattered in the forward direction, i.e., the same direction that the incident laser light is traveling. This light is collected at about $18-22^\circ$ from the incident beam. For cells of the same refractive index quantitation of the forward light scatter is a relatively reliable indicator of cell size. Thus an increase in the amount of light scattered represents a proportional increase in cell volume. Light scattered at right angles (90° scatter) to the direction of the incident laser light can also be measured. The amount of right angle light scattered depends not only on cell size but also the amount of internal structure of the cell. Thus granular cells such as PMN or actively growing blast cells with a substantial amount of rough endoplasmic reticulum scatter much more 90° light than resting lymphocytes.

Figure 5 is a two-parameter histogram displayed in three dimensions showing forward scatter on the X axis, 90° scatter on the Y axis and cell number on the Z axis. The drastic change in cell morphology is reflected in a corresponding change in light scatter by cells from infected rats on PBD 4. In order to determine the effect of the presence of these highly refractive cells on the calculation of helper:suppressor cell ratios determined for the three groups of rats, the data from several experiments were reanalyzed on the computer with the cells scattering

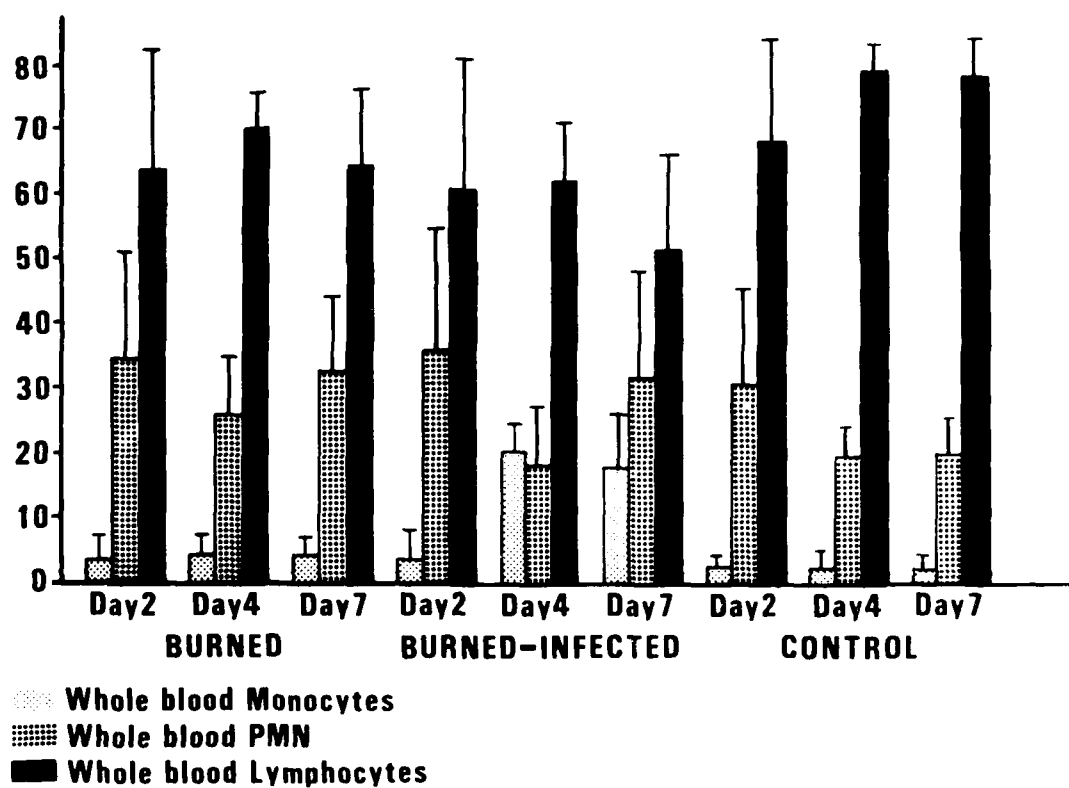


Figure 3. Differential analysis of whole blood leukocytes from control, burned and burned-infected rats. Cells were differentiated by morphology after smears were prepared from blood taken from all animals in each group on postburn days 2, 4 and 7 and stained with Wright's stain. Cells were designated as monocyte, granulocyte or lymphocyte. The bars represent the mean of the percent of total cells counted.

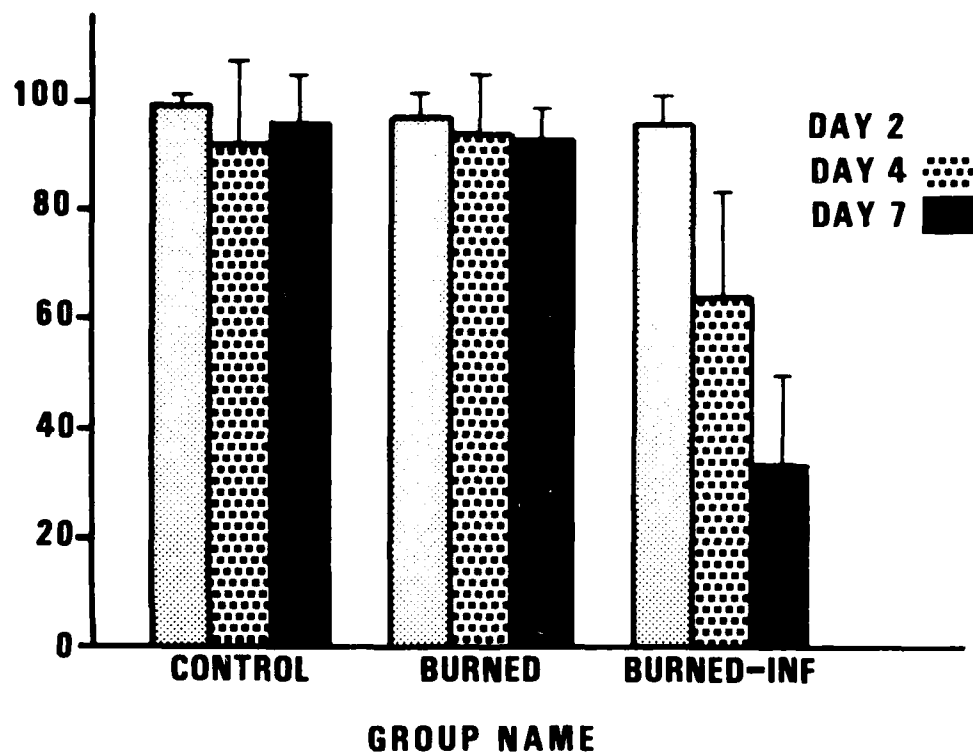


Figure 4. The proportion of lymphocytes in the mononuclear cell fraction of Ficoll-Hypaque gradients from control, burned and burned-infected rats. Differential analysis was performed on cytocentrifuge slides preps of cells taken from the gradient and stained with Wright's stain. Bars represent the mean percent lymphocytes of 200 cells counted $\pm$ SD.

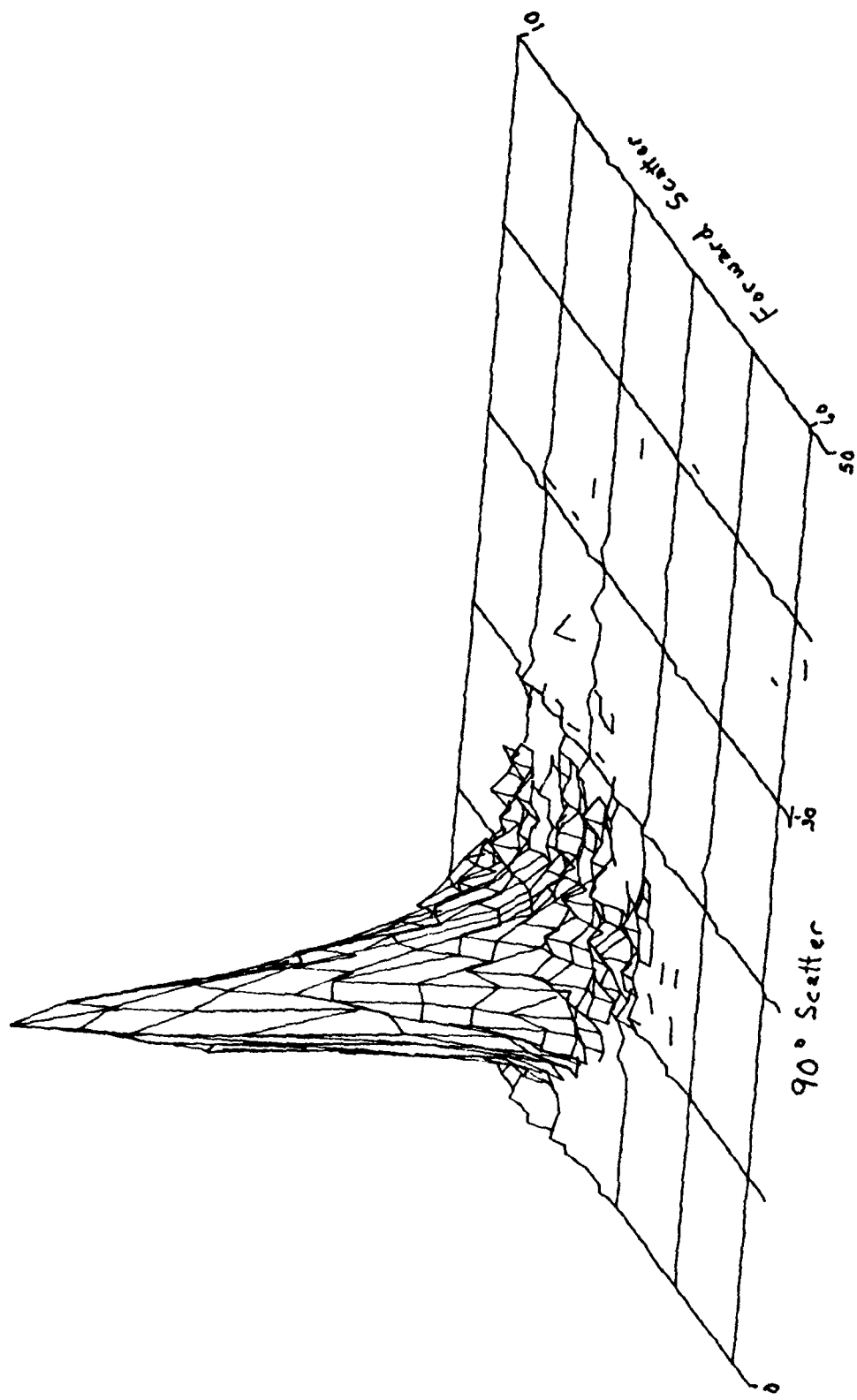


Figure 5A. Cells from a control rat.

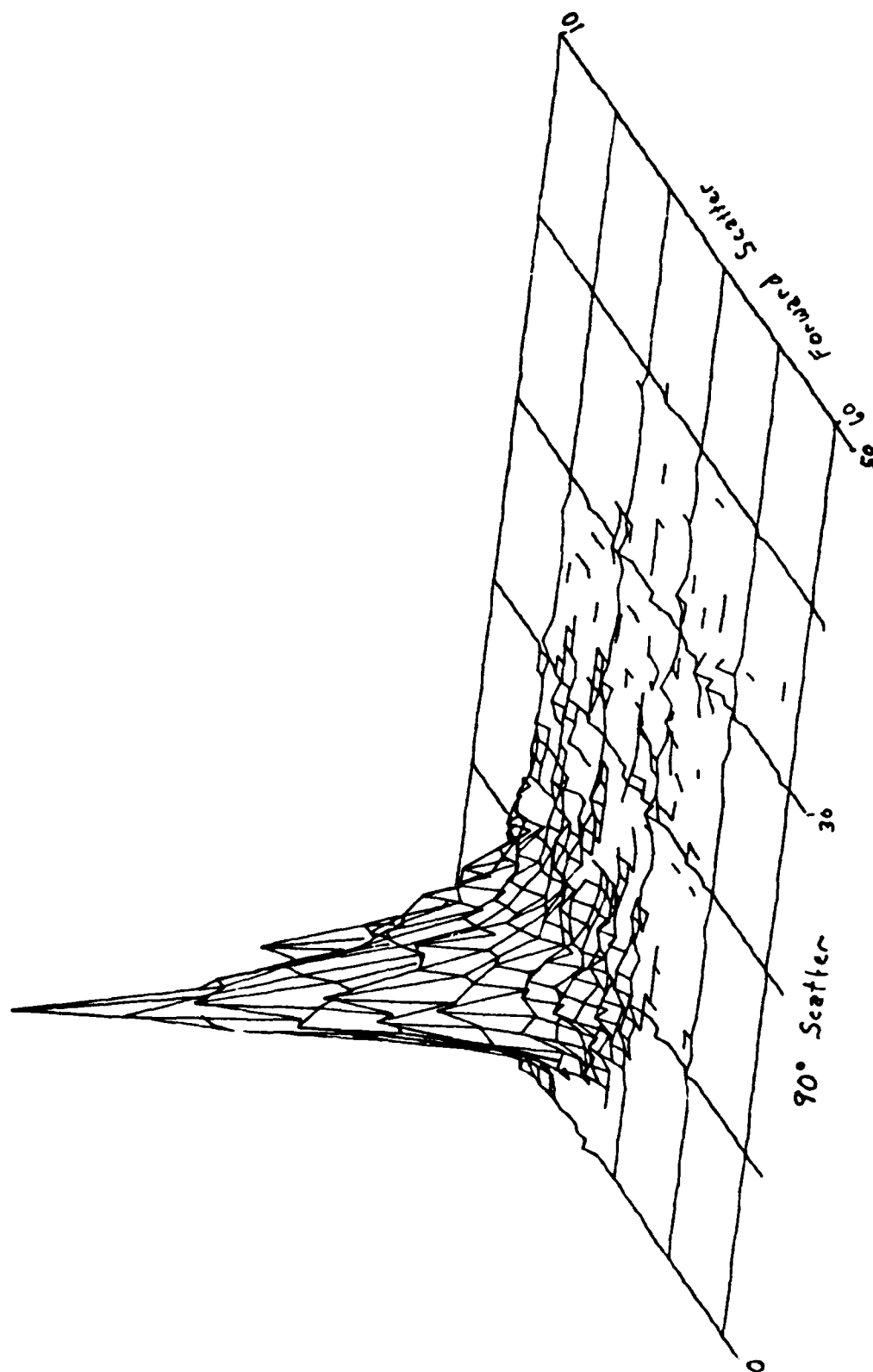


Figure 5B. Cells from a burned rat.

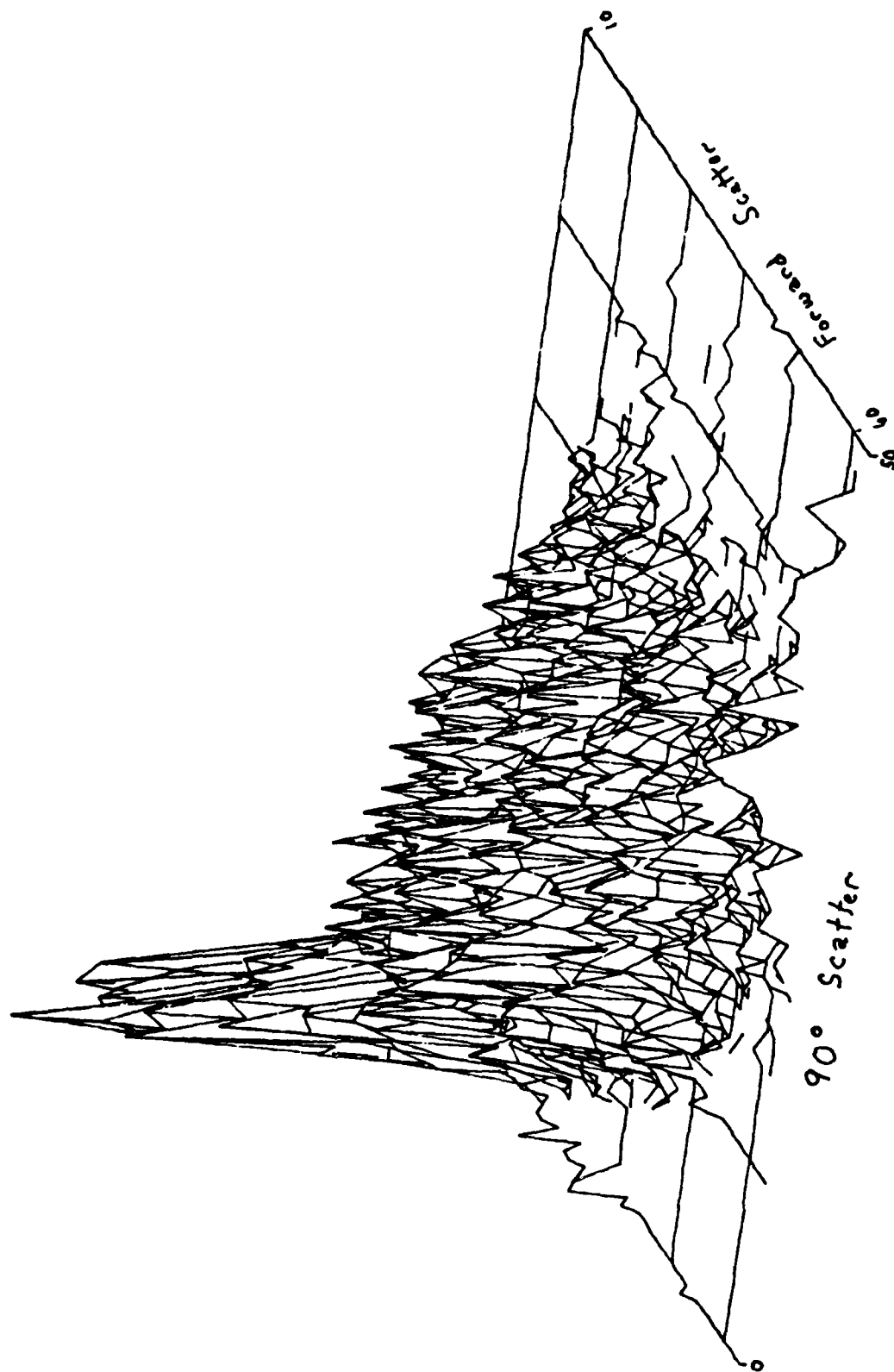


Figure 5C. Cells from a burned-infected rat.

Figure 5. Light scatter analysis of rat blood cells purified on Ficoll-Hypaque gradients on PBD 4. The forward and 90° light scatter intensities of 5000 cells were obtained by flow cytometry. The data obtained was normalized with respect to peak height on the Z axis and displayed as a 3-dimensional representation on a 2-parameter histogram. Forward light scatter intensity is plotted on the X axis, 90° light scatter intensity is plotted on the Y axis and cell number is represented on the Z axis.

more 90° light than control lymphocytes electronically removed from the determination of the number of fluorescent positive cells. Helper: suppressor ratios determined by the reanalysis are shown in Figure 6. The postburn day averages for all animals changed slightly after gated analysis but the relative ratio for infected animals compared to non-infected animals was similar in both the gated and ungated analyses. This indicates that a significant number of the highly refractive cells were positive for both helper and suppressor antigen.

In one experiment, the cells from several animals that scattered larger amounts of 90° light and would have been gated out of the reanalysis described above were sorted into a test tube, used to prepare a slide, and stained with Wright's stain for differentiation under the light microscope. The highly refractive population consisted of immature granulocytes and macrophages as well as blastic lymphocytes, all of which are larger and have more internal structure than resting lymphocytes. A more quantitative differential analysis of these cells is currently under way.

DISCUSSION

There are an increasing number of reports in the literature purporting to show decreases in helper:suppressor ratios as an indicator of immunosuppression in clinical conditions where infection or sepsis is a characteristic problem. Recent reports, however, are beginning to question the utility of this test as a measure of immunological capacity (10,11), except in a few well-defined cases such as Acquired Immune Deficiency Syndrome. The range of the ratio in normal subjects can be quite large, making analysis of an individual patient hard to interpret. In severe congenital immunodeficiency diseases, there appears to be no correlation between helper:suppressor ratios and the capacity of the host to combat infection. In the reports relating specifically to burns (4,6), there has been no attempt to address the problem of the drastic changes in the hematological profile of burn patients that might effect these measurements. No indication was given of the purity of the Ficoll-Hypaque cell preparations used in the analysis. If a change in helper:suppressor ratio induces a state of immunosuppression that predisposes a burned individual to infection, then the change in ratio should coincide with the presence of the burn and should precede the onset of infection. In the human patient, it is difficult to clearly

10. Buckley RH: Studies of patients with severe cellular and humoral immuno-deficiency diseases using monoclonal antibodies. In: Monoclonal Antibodies: Probes for the Study of Autoimmunity and Immunodeficiency, B.F. Haynes (ed), Academic Press, New York, pp. 83-95, 1983.

11. Lovett EJ III, Schnitzer B, Keren DF, Flint A, Hudson JL, McClatchey KD: Application of flow cytometry to diagnostic pathology. *Lab Invest* 50:115-140, 1984.

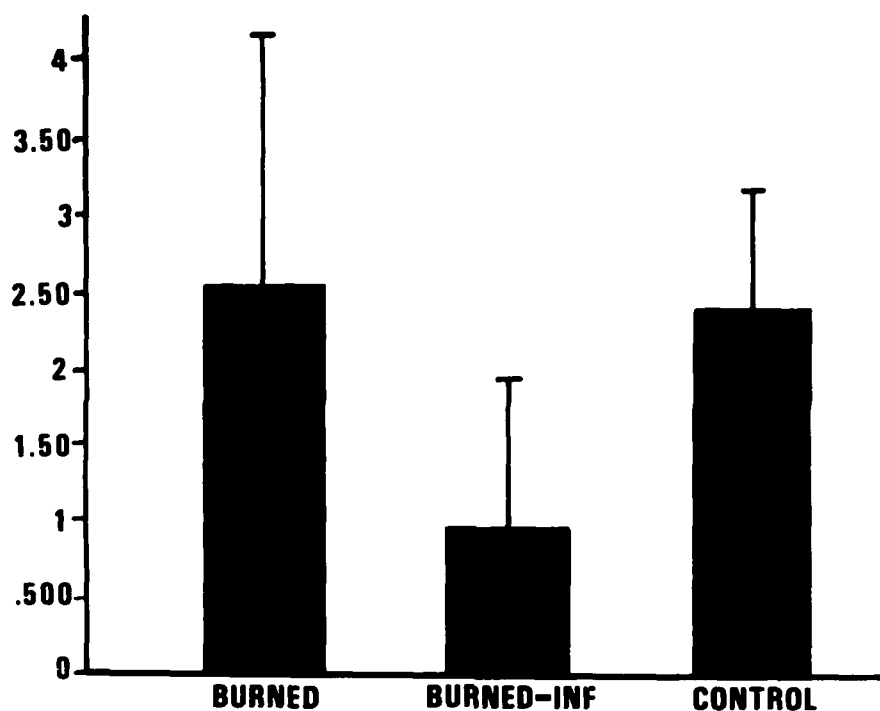


Figure 6A. Analysis of ungated data.

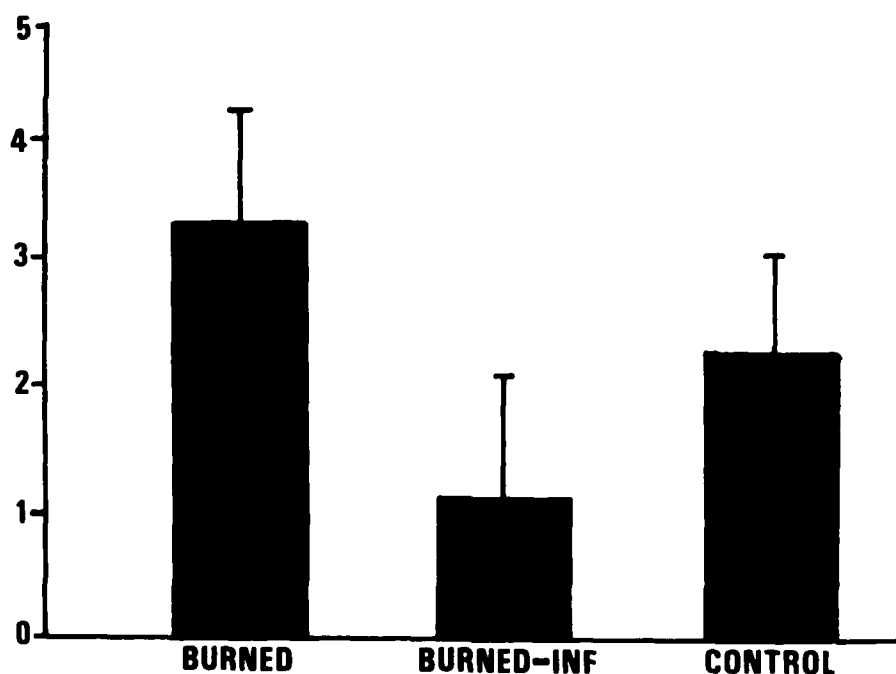


Figure 6B. Analysis of gated data.

Figure 6. Comparison of helper:suppressor ratios determined before and after 90° light scatter gating. Cells were taken from animals on post-burn day 4 and purified on Ficoll-Hypaque gradients. The cells were analyzed for helper and suppressor T lymphocyte antigen and the helper:suppressor ratio determined. For gated analysis, data from cells with high intensity 90° light scatter were eliminated from calculations by setting a maximum 90° threshold before the ratios were determined.

distinguish the point at which sepsis occurs and set a point at which a change in ratio can be measured that might be indicative of immunosuppression induced by thermal injury and not a result of infection or treatment.

The burned rat provides a model where this distinction can be made. Burned rats are susceptible to infection by *P. aeruginosa* strain 12-4-4. Uninfected burned rats have a greater than 90% survival rate at 21 days. Infected rats have lower than 10% survival. If the burn induces a change in helper:suppressor ratios that predisposes the rats to infection, then that change in ratio should correspond to the presence of the burn.

This study clearly shows that a change in ratio does not accompany the increased susceptibility to infection induced by a 30% total body surface burn. The change in ratio for burned rats is not significantly different than for unburned controls. Whether the change in ratio seen in infected animals is due to an actual shift in the relative proportion of helper and suppressor cells or to artifacts introduced by the drastic change in the morphology of the leukocytes isolated from these animals cannot be determined at this time. A more detailed analysis of the cells from the infected animals is currently under way.

A definitive analysis of cells from these animals will require the development of new techniques. One approach is to obtain homogeneous lymphocytes by developing new methods of cell purification based on parameters other than buoyant density. Present techniques used to isolate lymphocytes by buoyant density are not adequate to separate lymphocytes from non-lymphoid cells in the burned-infected rat. Sedimentation velocity techniques show promise in this area. Alternatively, techniques might be devised that will allow lymphocytes to be distinguished from non-lymphocytes by flow cytometry. Light scatter can be used to accomplish this in normal individuals but our experience shows that blastic lymphocytes cannot be separated from immature granulocytes or macrophages by light scatter alone. Theoretically, dual labeling of cells by monoclonal antibodies with different specificity and different chromophores should allow specific determination of the number of lymphocytes in an impure preparation. For instance, the first antibody and chromophore could be directed at T lymphocytes and the second at a subpopulation of T lymphocytes such as helper cells.

There are several obstacles to development of the dual chromophores approach. First, it must be established that the monoclonal antibodies used to identify the lymphocyte subpopulations do not cross react with other cell types present. There are recent reports in the literature that W3/13 (the rat T-cell marker) cross reacts with mature granulocytes (12) and W3/25 (the rat T helper marker) reacts with some macrophages

12. Williams AF, Galfre G, Milstein C: Analysis of cell surfaces by xenogeneic myeloma-hybrid antibodies: Differentiation antigens of rat lymphocytes. *Cell* 12:663-673, 1977.

(13). There is as yet no information on cross-reactivity of these antibodies with immature hemopoietic cells. This specificity will need to be determined to obtain accurate measurements on cells from burned-infected rats.

Additional difficulties to be overcome include the tendency for macrophages and blast cells to non-specifically absorb fluorescent antibody and mask true specific binding by increasing background levels. It may be possible to overcome this tendency by using unlabeled monoclonal reagents followed by chromophore labeled Fab fragments of goat anti-mouse antibodies as second step reagents instead of using directly labeled monoclonal antibodies as single step reagents. The increased autofluorescence of blast cells (possibly due to increased levels of NAD[P]H) also increases background fluorescence levels and limits the resolution of stained populations of cells from unstained populations. This problem will be addressed by maximizing the amount of second-step chromophore retained by the stained cells and electronic gating techniques.

These problems must be resolved before accurate lymphocyte subpopulation measurements can be made in burned individuals. The emphasis of this study has shifted as outlined above to develop new techniques in cell separation and flow cytometry that will make it possible to accurately measure lymphocyte populations in burned individuals.

13. Barclay AN: The localization of populations of lymphocytes defined by monoclonal antibodies in rat lymphoid tissues. *Immunology* 42:593-600, 1981.

PRESENTATIONS

Burleson DG: Inhibition of the oxygenation activity of high buoyant density granulocytes and low buoyant density macrophages by cells of intermediate buoyant density. 20th Annual Meeting of the Reticuloendothelial Society, Portland, Oregon, 11 October 1983.

Burleson DG: Changes in lymphocyte subpopulations after burn injury and burn injury with infection. Accepted for presentation, 21st Annual Meeting of the Reticuloendothelial Society, Montreal, Canada, 16 October 1984.

PUBLICATIONS

Burleson DG, Vaughn GK, Mason AD Jr: Changes in lymphocyte subpopulations after burn injury and burn injury with infection. *J Leukocyte Biol* 36:433-434, 1984.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1 AGENCY ACCESSION DA 305253	2 DATE OF SUMMARY 84 10 01	REPORT CONTROL SYMBOL DD-DR&B(AR) 636	
3 DATE PREV SUMMARY None	4 KIND OF SUMMARY A. New	5 SUMMARY SCTY U	6 WORK SECURITY U	7 REGRADING	8 DISB'N INSTR'N CX	9 LEVEL OF SUM A. WORK UNIT ISR	
10 NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER			
a. PRIMARY	61101A	3A161101A91C	00	078			
b. CONTRIBUTING							
c. CONTRIBUTING	None						
11. TITLE (Precede with Security Classification Code) (U) Preliminary Studies on Zinc Homeostatic Control and Immunocompetence in a Burned Animal Model							
12 SUBJECT AREAS 06 01 Biochemistry 06 13 Microbiology							
13 START DATE 84 09	14. ESTIMATED COMPLETION DATE CONT	15. FUNDING ORGANIZATION DA	16 PERFORMANCE METHOD C. In-House				
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE			
a. DATE EFFECTIVE	EXPIRATION	FISCAL YEARS	a. PROFESSIONAL WORK YEARS	b. FUNDS (In thousands)			
b. CONTRACT/GRANT NUMBER		84	0.1	3			
c. TYPE	d. AMOUNT	85	1.5	50			
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19. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
a. NAME US Army Institute of Surgical Research				a. NAME US Army Institute of Surgical Research Biochemistry Branch			
b. ADDRESS (include zip code) Ft. Sam Houston, Texas 78234-6200				b. ADDRESS Ft. Sam Houston, Texas 78234-6200			
c. NAME OF RESPONSIBLE INDIVIDUAL Pruitt, BA, Jr				c. NAME OF PRINCIPAL INVESTIGATOR Shippee, RL			
d. TELEPHONE NUMBER (include area code) 512-221-2720				d. TELEPHONE NUMBER (include area code) 512-221-7738			
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) Thermal Injury; (U) Zinc Homeostasis; (U) Immunocompetence; (U) Lab Animals; (U) Rats							
23. TECHNICAL OBJECTIVE 24 APPROACH 25 PROGRESS (Precede text of each with Security Classification Code)							
23. (U) To determine the effect of thermal injury with and without the complication of infection on the homeostatic control of zinc metabolism. To determine the interrelationship of the changes in zinc metabolism with host resistance. To develop a rational basis of dietary or pharmacological manipulation of zinc nutritional support during thermal injury. 24. (U) The excretion of zinc into the intestinal lumen has been shown to be, quantitatively, an important homeostatic control mechanism of zinc metabolism. The effect of thermal injury with and without the complication of infection on the endogenous excretion of zinc will be investigated. The rat burn model developed by Walker and Mason will be used. A zinc-free, semi-purified ration will be fed to rats that are being administered zinc as a daily subcutaneous injection. Animals will be housed in stainless steel metabolic cages to facilitate daily monitoring of food and water intake and collection and subsequent analysis of total fecal and urine output. Initial experiments will be aimed at developing the proper level of injected zinc needed to provide adequate zinc nutriture. The basis for this determination will be the comparison on tissue zinc levels, body weight maintenance, and zinc metalloenzyme activity between zinc-injected and zinc-fed rats. Once the nutritional aspects of the model are defined, the							

CONTINUATION OF DD FORM 1498 FOR "PRELIMINARY STUDIES ON ZINC HOMEOSTATIC CONTROL AND IMMUNOCOMPETENCE IN A BURNED ANIMAL MODEL"

effect of thermal injury on endogenous excretion of zinc will be studied. Concurrently, numerous immunological parameters will be assessed, i.e., in vitro mitogenic stimulation, qualitative and quantitative measurements of immunoglobulin secretion, and changes in lymphoid subpopulations.

25. (U) 8410 - 8409. An initial pilot study has been completed that has verified the subcutaneous injection method of zinc to maintain zinc nutriture in rats fed a zinc-deficient diet. This determination was based on comparison of body weight maintenance, tissue zinc concentration, and voluntary caloric intake between zinc-injected and zinc-fed rats. Further studies await the receipt of stainless steel metabolic cages.

ABSTRACT

PROJECT NO. 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: PRELIMINARY STUDIES ON ZINC HOMEOSTATIC CONTROL AND
IMMUNOCOMPETENCE IN A BURNED ANIMAL MODEL

US Army Institute of Surgical Research, Brooke Army Medical Center, Fort
Sam Houston, Texas 78234

Period covered in this report: 1 September 1984 - 30 September 1984

Investigator: Ronald L. Shippee, Ph.D., Captain, MSC

Reports Control Symbol MEDDH-288(R1)

The overall purpose of this investigation is to study the effects of burn injury, with or without the complication of infection, on the homeostatic control of zinc metabolism. An understanding of zinc homeostatic control during recovery from thermal injury will lead to a better rationale for dietary and/or pharmacological zinc supplementation of burned patients. The initial approach is to study the endogenous excretion of zinc in the rat by feeding a zinc-free semi-purified ration with concomitant daily subcutaneous (s.c.) injections of zinc. This report presents the findings of a preliminary experiment to test the efficacy of this modality to: (a) determine endogenous excretion and (b) supply maintenance zinc requirements of the adult rat. Based on body weight maintenance, voluntary caloric intake, plasma alkaline phosphatase activity, and plasma, liver, and kidney zinc concentration, it was determined that between 0.5 and 1.5 mg zinc/kg body weight/day is sufficient to maintain the zinc status of the adult rat.

Zinc homeostasis
Endogenous excretion
Rat model
Thermal injury

ANNUAL PROGRESS REPORT

PROJECT NO. 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: PRELIMINARY STUDIES ON ZINC HOMEOSTATIC CONTROL AND
IMMUNOCOMPETENCE IN A BURNED ANIMAL MODEL

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FORT SAM HOUSTON, TEXAS 78234

1 September 1984 - 30 September 1984

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UNCLASSIFIED

PRELIMINARY STUDIES ON ZINC HOMEOSTATIC CONTROL AND IMMUNOCOMPETENCE IN A BURNED ANIMAL MODEL

RATIONALE FOR EXPERIMENTAL APPROACH

Indirect evidence of altered zinc homeostatic control in humans resulting from thermal injury has been suggested by a number of studies (1-3). Davies and Fell reported that zinc is excreted by patients with 10-30% body surface burn at twice the rate observed in normal individuals, while excretion rates approaching five times normal were observed in patients with more extensive burns (34-75%).

Powanda *et al.* (4) have reported the effect of thermal injury with and without the complication of infection on zinc redistribution in serum and liver using a burned rat model. To obviate differences in food intake caused by burning and/or infection, rats were fasted following burning and/or seeding with *Pseudomonas aeruginosa*. Control-fasted rats had a gradual decrease in serum zinc with no accumulation of zinc in the liver over the 6 days of the experiment. The serum levels of zinc in both burn-fasted (BF) and burn-fasted-infected (BFI) rats decreased sharply 24 hours after thermal injury, while levels of zinc in the liver increased. During the next 5 days, however, serum zinc levels decreased in the BFI rats and remained constant in BF rats, while levels of liver zinc concentration increased with time in BFI rats and decreased in BF animals. This study suggested distinctly different homeostatic changes in zinc metabolism when cumulative levels of stress were placed on the animals. However, it was impossible to determine how responses may have been modified by zinc deficiency alone due to the fasted condition of all the animals.

Although the human and animal studies suggest that thermal injury causes a disturbance in homeostatic control of zinc, no direct evidence of changes in absorption and/or endogenous intestinal excretion exists. Because excretion into the intestinal lumen has been shown to be a quantitatively important homeostatic control mechanism of zinc metabolism (5) it would be of interest to study the effect of thermal injury on this route of excretion.

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1. Davies, JW: Physiological Responses to Burning Injury. Academic Press, New York, NY, pp 240-244, 1982.
 2. Davies, JW and Fell, GS: Tissue catabolism in patients with burns. Clin Chim Acta 51:83, 1974.
 3. Carr, G and Wilkinson, AW: Zinc and copper urinary excretions in children with burns and scalds. Clin Chim Acta 61:199, 1975.
 4. Powanda, MC, Villarreal, Y, Rodriguez, E, Braxton, G and Kennedy, CR: Redistribution of zinc within burn and burn-infected rats. Proc Soc Exp Biol Med 163:286, 1980.
 5. Weigand, E and Kirchgessner, M: Model study on the factorial derivation of the requirement of trace elements - zinc requirement of the growing rat. Z Tierphysiol Tiernahr Futtermittelkd 39:84, 1977.

Given the ubiquity of zinc among animal tissues and its essentiality for numerous metalloenzymes and nucleic acid metabolism, it is not difficult to understand possible ramifications of altered zinc metabolism due to thermal injury. For example, Davies (2) has recently reviewed the literature concerning zinc supplementation and rate of wound healing in burn patients. Numerous controlled clinical studies can be found that have tested the effect of both oral supplementation and topical application of zinc on rate of wound healing. Based on available evidence, it is difficult to state conclusively that either route of zinc treatment directly increases rate of wound healing. However, one study has recently reported that an additional benefit may accrue from the fact that zinc (as in zinc oxide tape) may possess an antibacterial effect against many strains of Pseudomonas aeruginosa, Staphylococcus aureus and Streptococcus pyogenes (6).

Epidemiologic studies, clinical investigations and laboratory animal studies have shown that zinc nutriture can modulate immunocompetence (7-9). It has been a consistent finding that cell-mediated responses are affected by zinc deficiency (9,10). Considering that it is well established that burn patients are highly susceptible to infection by normal nonpathogenic organisms, it appears justified to investigate interrelationships between zinc metabolism and immunocompetence during recovery from thermal injury.

A number of questions remain to be resolved concerning zinc metabolism under the stress of trauma and/or infection. What are the homeostatic adjustments of zinc metabolism in response to trauma and/or infection? To what extent does the homeostatic adjustment of zinc metabolism affect immunocompetence? Most importantly, can zinc nutriture modulate the immune response during the recovery from thermal injury?

RESEARCH OBJECTIVE

It is the objective of this research to investigate the homeostatic control of zinc metabolism of the burned rat under controlled nutrient

6. Hallmans, G and Elmros, T: Zinc tape treatment of burns infected by Pseudomonas aeruginosa. An experimental study on guinea pigs. CITED IN: Davies, JW: Physiological Responses to Burning Injury. Academic Press, New York, NY, p 244, 1982.

7. Chavapil, M: The role of zinc in the function of some inflammatory cells. CITED IN: Zinc Metabolism: Current Aspects in Health and Disease. Alan R. Liss, Inc., New York, NY, pp 103-122, 1977.

8. Chandra, RK and Newberne, PM: Nutrition, Immunity and Interactions of Infection Mechanisms. Plenum Press, New York, NY, 1977.

9. Good, ES, Fernandes, G and West, A: Nutrition immunity and cancer - Part I: Influence of protein or protein calorie malnutrition and zinc deficiency on immunity. Clin Bull 9:3, 1979.

10. Lueke, RM and Fraker, PJ: The effect of varying dietary zinc levels on growth and antibody mediated response in two strains of mice. J Nutr 109:1373, 1979.

intake. Concurrently, various indices of immunocompetence will be assessed in an attempt to relate zinc nutriture and homeostasis to immunocompetence in the burned rat.

PRELIMINARY EXPERIMENTAL PROTOCOL

To study the dynamics of mineral metabolism in whole animals, it is common practice to conduct a combination of mineral balance and radioactive isotope tracer kinetic studies. As an alternative to using radioactive isotopes, Flanagan (11) has recently reported using zinc deficient semi-purified diets fed to rats supplemented with daily subcutaneous injections of zinc. Conducted in this manner, analysis of daily fecal collections for zinc concentration would reflect endogenous fecal zinc excretion.

This report presents the results of a preliminary study to determine the efficacy of this approach and the level of zinc supplementation by subcutaneous injection needed to maintain the zinc status of the adult rat.

Animals and Housing: Eight male albino rats, with a mean body weight of 403 g, were housed individually in stainless steel wire bottom cages. Each cage was modified to collect the feces in such a manner as to prevent exogenous zinc contamination. A lighting schedule of 12 hours off and 12 hours on was maintained throughout the course of the experiment. Animals were weighed daily prior to feeding.

Diet: A zinc-free semi-purified diet (Zeigler, P.O. Box 95, Gardners, PA 17324) was purchased and analyzed by atomic absorption spectrophotometry for zinc concentration. The diet was determined to contain < 0.05 ppm zinc on an as fed basis. The diet was fed in acid washed glass bowls.

Methods: Two animals were assigned to each of the following four treatments:

- Treatment A = fed the semi-purified diet supplemented to 50 ppm Zn, daily s.c. injection of sterile saline (zinc as zinc acetate)
- Treatment B = fed the zinc deficient semi-purified diet, daily s.c. injection of sterile saline
- Treatment C = fed the zinc deficient semi-purified diet, daily s.c. injection with 0.5 mg Zn/kg body weight in sterile saline (zinc as zinc acetate)
- Treatment D = fed the zinc deficient semi-purified diet, daily s.c. injection with 1.5 mg Zn/kg body weight in sterile saline

11. Flanagan, P: A model to produce pure zinc deficiency in rats and its use to demonstrate that dietary phytate increases the excretion of endogenous zinc. J Nutr 114:493, 1984.

The animals were maintained on their respective treatments for 12 days. Three days after initiation of the treatments, body weight and feed intake were determined and fecal collection performed on a daily basis. On day 12, all animals were sacrificed and heparinized blood, kidney and liver removed.

Plasma was added to 20% trichloroacetic acid (1:4), vortexed, and centrifuged at 2000 x g for 30 minutes. The supernatant was then aspirated directly into the flame of an atomic absorption spectrophotometer (Perkin-Elmer Mdl 5000, Norwalk, CT).

Duplicate 1 g samples of liver and kidney were dried at 38° C for 12 hours, followed by the addition of 10 ml concentrated nitric acid and then condensed on a hot plate to an approximate volume of 2 ml. An additional 10 ml of concentrated nitric acid was added and again condensed to approximately 2 ml. The samples were then diluted to an appropriate volume and aspirated into the flame of an atomic absorption spectrophotometer. Using a standard reference material (Standard Reference Material 1577a [Bovine Liver], National Bureau of Standards, Washington, D.C. 20234), this method has been shown to give a zinc recovery within the certified range of the standard.

Daily fecal collections were dried at 38° C for 12 hours and then dry ashed at 800° C for 12 hours. The ash was then dissolved in concentrated nitric acid, diluted appropriately and aspirated into the flame of an atomic absorption spectrophotometer. This dry ashing method was found to give a recovery of zinc within the certified range for the standard reference material.

Alkaline phosphatase activity was quantified by the hydrolysis of p-nitrophenyl phosphate as assayed using an autoanalyzer (Instrumentation Laboratory Mdl 508, 113 Hartwell Avenue, Lexington, MA 02173).

RESULTS AND DISCUSSION

Table 1 shows mean body weight change, caloric intake, endogenous zinc excretion and plasma, kidney and liver concentrations.

Based on tissue zinc concentration, the s.c. injection of 0.5 and 1.5 mg Zn/kg body weight/day (treatments C and D respectively) maintained zinc levels equivalent to those of the zinc fed controls (treatment A), while the animals receiving no zinc supplementation (treatment B) showed a reduction in all three tissues.

Reduction of serum alkaline phosphatase activity has been shown to be attributable to zinc deficiency (12). Plasma alkaline phosphatase

12. Kirchgessner, M, Roth, HP and Weigand, E: Biochemical changes in zinc deficiency. CITED IN: Trace Elements in Human Health and Disease (A.S. Prasad, ed). Academic Press, Inc., New York, NY, pp 189-225, 1976.

Table 1

	Treatment ^{1,2}			
	A	B	C	D
Body weight change (g)	+29	+23	+24	+27
Calories/g body weight/day	0.18	0.15	0.20	0.19
Zinc excreted (µg/day)	780	12	84	293
Plasma alkaline phosphatase (IU)	143	142	150	151
Plasma zinc (µg/ml)	1.4	0.5	1.3	1.4
Kidney zinc (µg/g dry tissue)	129	72	114	98
Liver zinc (µg/g dry tissue)	124	73	127	129

- ¹ A=fed semi-purified diet supplemented to 50 ppm zinc, daily s.c. injection of sterile saline.
 B=fed semi-purified diet, daily s.c. injection of sterile saline.
 C=fed semi-purified diet, daily s.c. injection of 0.5 mg Zn/kg body weight in sterile saline.
 D=fed semi-purified diet, daily s.c. injection of 1.5 mg Zn/kg body weight in sterile saline.

- ² Values represent the mean of two animals.

activity did not appear to be affected by any of the treatments in this study.

The rate of endogenous zinc excretion was related directly to the amount of zinc injected. The rate of 12 $\mu\text{g/day}$ for the rats receiving no zinc supplementation represents the inevitable loss, that is the excretion of zinc that is lost despite a deficient zinc intake (i.e. zinc lost in the sloughing of intestinal cells). The rates of 84 and 293 $\mu\text{g/day}$ in groups C and D represent endogenous excretion in excess of the inevitable loss, and appear to represent a homeostatic control which decreases the retention of the administered zinc.

Based on this preliminary data, it is planned to use this methodology to study the effect of thermal injury with or without the complication of infection on endogenous excretion of zinc.

PRESENTATIONS/PUBLICATIONS

None.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
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
83 10 04	H. TERM	U	U		CX	
10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER		
a. PRIMARY	61101A	3A161101A91C	00	083		
b. CONTRIBUTING						
c. CONTRIBUTING	None					
11. TITLE (Precede with Security Classification Code) (U) The Study of Metabolism and Temperature Regulation in Animal Models of Human Burn Injury						
12. SUBJECT AREAS						
06 05 Clinical Medicine 06 16 Physiology						
13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD			
83 01	8409	DA	C. In-House			
17. CONTRACT/GRANT			18. RESOURCES ESTIMATE			
a. DATE EFFECTIVE	EXPIRATION	FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)		
b. CONTRACT/GRANT NUMBER		84	1.0	8		
c. TYPE	d. AMOUNT	85	0	0		
e. KIND OF AWARD	f. CUM/TOTAL					
19. RESPONSIBLE DOD ORGANIZATION			20. PERFORMING ORGANIZATION			
a. NAME US Army Institute of Surgical Research			a. NAME US Army Institute of Surgical Research Surgical Study Branch			
b. ADDRESS (include zip code) Ft. Sam Houston, Texas 78234-6200			b. ADDRESS Ft. Sam Houston, Texas 78234-6200			
c. NAME OF RESPONSIBLE INDIVIDUAL Pruitt, BA, Jr			c. NAME OF PRINCIPAL INVESTIGATOR Aulick, LH			
d. TELEPHONE NUMBER (include area code) 512-221-2720			d. TELEPHONE NUMBER (include area code) 512-221-4264			
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) Burn Injury; (U) Temperature Regulation; (U) Environmental Control; (U) Animals; (U) Energy Metabolism; (U) Lab Animals; (U) Rats						
23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)						
23. (U) To assess the validity and utility of a small animal model of post burn metabolism. To identify the etiology of postinjury hypermetabolism in this model and to compare this with the human metabolic response to injury. To establish the relationship between heat production and body temperature regulation in this model. 24. (U) Long term measurement of respiratory gas exchange of groups of small animals (rats) is obtained through the use of a fully automated, open and closed respiration chamber. This instrument provides excellent control of the thermal environment, humidity, lighting, air flow and noise level. With such control one can separate the basic energy cost of the injury from that caused by other extrinsic metabolic stimuli. 25. (U) 8310 - 8409. Previous work (7910-8009, 8010-8109, 8301-8309) established the degree of hypermetabolism of 20-40 kg goats following a 25% total body surface (TBS) burn. There were some technical difficulties with this model, so we studied 40-80 kg pigs with the same injury. Resting metabolism in both animals appears to increase 20-40% above normal following injury. We are currently studying 400-600 gram rats and comparing their metabolic and thermoregulatory responses to that of patients and large animals. This research has demonstrated infection causes marked increases in the temperature and metabolism of a burned rat. The metabolic effects of infection increase with severity. Animals with minimal infection reach metabolic rates 10-15% above normal resting levels, while the metabolic rate in bacteremic animals increases 45% prior to death. Effective treatment of the burn wound with commonly used antimicrobial creams decrease the change of infection and reduces the rise in metabolic rate.						

ANNUAL PROGRESS REPORT

PROJECT NO. 3A16110A91C-00, INHOUSE LABORATORY
INDEPENDENT RESEARCH

REPORT TITLE: THE STUDY OF METABOLISM AND
TEMPERATURE REGULATION IN ANIMAL
MODELS OF HUMAN BURN INJURY:
THE EFFECTS OF LOCALIZED AND
SYSTEMIC INFECTION ON AEROBIC
METABOLISM AND CORE TEMPERATURE
OF THE BURNED RAT

US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234

1 October 1983 - 30 September 1984

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ABSTRACT

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Medical Center, Fort Sam Houston, Texas 78234

Period covered in this report: 1 October 1983 - 30 September 1984

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Resting oxygen consumption ($\dot{V}O_2$) and colonic temperatures (T_c) were measured in groups of 400-600 gm, male rats (13-30/group) at thermoneutrality, before and for 24 days after 30% total body surface, full thickness burns. Burns were divided into three bacteremic and nine non-bacteremic groups. Three of the non-bacteremic groups were untreated), two were treated with Sulfamylon, two had their wounds seeded with non-virulent Pseudomonas aeruginosa, and two were seeded and treated with the burn cream. Three control groups were shaved but not burned. Localized and systemic infection raised $\dot{V}O_2$ above that caused by the burn alone. Sulfamylon treatment did not diminish the response to burn alone but did diminish the metabolic response to localized infection with nonvirulent P. aeruginosa. Hypermetabolic, non-bacteremic animals appeared to be hyperthermic rather than febrile. At some point the infected rats became febrile but the rise in T_c was only moderate until the bacteremic animal began to show other signs of sepsis. The data suggest that bacteria confined to the burn wound have systemic metabolic effects.

THE EFFECTS OF LOCALIZED AND SYSTEMIC INFECTION ON AEROBIC METABOLISM AND CORE TEMPERATURE OF THE BURNED RAT

Metabolic and neuroendocrine responses to severe thermal injury are so similar to those of the infected patient that it is frequently difficult to recognize when the burn patient becomes infected (1,2). Studies designed to identify the effects of systemic infection in these patients commonly place subjects into bacteremic and non-bacteremic groups. While this is an objective way to classify different stages of infection, bacteria produce systemic effects prior to their entrance into the bloodstream. It has been shown, for example, that the sera of non-bacteremic burn patients contain an endogenous pyrogen (3), which presumably affects total body heat production and circulation. The question then becomes whether there are metabolic responses to injury which are separate and distinct from those of superimposed infection. In this study an attempt was made to distinguish metabolic and thermoregulatory responses to bacterial colonization of the burn wound from those developing secondary to systemic infection.

MATERIALS AND METHODS

The animals selected for study were male, Sprague Dawley rats, ranging in age from three to seven months and weighing between 400 and 600 grams at the beginning of the study. They were housed in individual cages at an ambient temperature of 28 to 30°C and had access to food (Purina laboratory chow) and water throughout all phases of the study. Controlled lighting maintained a 12/12-hr light/dark cycle with the lights coming on at 0600.

1. Beisel, W.R. Metabolic response to infection. *Ann. Rev. Med.* 26:9-20, 1975.
2. Wilmore, D.W. and L.H. Aulick. Thermoregulatory responses and metabolism. Part IV: Systemic responses to infection. In: *Surgical Infectious Diseases*, ed. R.L. Simmons and R.J. Howard, Appleton-Century-Croft, New York, 1982, pp 297-312.
3. Wilmore, D.W., L.H. Aulick and B.A. Pruitt, Jr. Metabolism during the hypermetabolic phase of thermal injury. In: *Advances in Surgery*, Vol. 12, ed. C. Rob, Year Book Medical Publishers, Inc., Chicago, 1978, pp. 193-225.

Oxygen consumption was determined in groups of rats through the use of a variable volume, open and closed respiration chamber (4). Chamber temperature, humidity and air flow were held constant, while volume varied with changes in barometric pressure or respiratory exchange of the animals. Chamber temperature was set in the thermoneutral zone for the rat; 30°C for the control animals and 32 to 34°C for the burned animals. Average chamber temperature (calculated as the mean of four air temperatures and one wall temperature) never varied more than $\pm 0.3^\circ\text{C}$. Relative humidity ranged from 40 to 50% and air velocity averaged 80 ft/min in the center of the chamber.

Groups of animals (13-30 rats/group) were placed in the chamber at the same time. Each rat, housed in its own cage, was left undisturbed for at least one hour before the measurement period began. At the end of this equilibration period, valves on the chamber closed and oxygen consumption ($\dot{V}\text{O}_2$) of the group was calculated from measured changes in oxygen concentration while the chamber was hermetically sealed. Oxygen and carbon dioxide measurements were repeated at 15-minute intervals until the CO_2 concentration exceeded 0.85%. The average $\dot{V}\text{O}_2$ was then calculated for the entire closed period.

Body weights and colonic temperatures (at a depth of 6 cm) were recorded for all animals at the end of each study. Colonic temperatures were taken within one minute after the animal left the chamber, and there was no evidence of any time effect. Animal location in the chamber and the order of measurement were also randomized in an effort to reduce any unrecognized systematic errors. Oxygen consumption was calculated for the average rat and expressed in milliliters (STPD) per hour per gram body weight.

All groups were subjected to daily chamber confinement and handling until they were accustomed to experimental procedures. They were then anesthetized (sodium pentobarbital, 5 mg/100 g body weight, intraperitoneally) and the hair clipped from their backs and flanks. While anesthetized, each rat was placed in a mold exposing 30% of the total body surface and a full-thickness burn was created on the back by immersing the area in 98°C water for nine seconds. Control groups were anesthetized and shaved but not burned. Burn wounds of some groups were seeded with one of two strains of Pseudomonas aeruginosa. One group

4. Aulick, L.H., H. Arnhold, E.W. Hander, and A.D. Mason, Jr. A new open and closed respiration chamber. Quart. J. Exp. Physiol. 68:351-357, 1983.

received a virulent (ISR 59-12-4-4) strain (5,6) and four other groups were seeded with a non-virulent strain. Seeding cultures contained 10 organisms per milliliter and one milliliter of this culture was spread over the entire wound surface. All rats were allowed to recover spontaneously without fluid resuscitation.

Studies were conducted between the 7th and the 24th days after injury or sham burn. The topical antimicrobial cream, Sulfamylon (an 11.1% suspension of mafenide acetate in a water dispersible base), was applied daily to the burn wounds of two groups with and two groups without non-virulent bacterial seeding. One group of unburned controls also received daily Sulfamylon treatment. Treated wounds were bathed weekly.

During the postburn course, chamber temperature was set at different levels (30-36°C) to determine the thermal environment where O₂ uptake was minimal. Resting or minimal V_{O2} refers to the level of aerobic metabolism occurring within the thermoneutral zone.

The animals were screened daily for clinical signs of sepsis. If they were markedly febrile, losing weight rapidly and had developed a reddish brown discharge from the eyes and nose, they were dropped from the study. (Subsequent autopsies on these animals always confirmed the clinical diagnosis of systemic infection.) All rats were sacrificed after the final experiment. Blood and spleen cultures and tissue samples were obtained from one-third to one half of the animals in each group (selected at random) to establish the depth of burn and the incidence of bacterial wound invasion and systemic infection. Clinically bacteremic animals had positive blood and/or spleen cultures and histologic evidence of wound invasion. Only those groups which demonstrated at least 90% homogeneity (bacteremic or non-bacteremic) were included in this report.

RESULTS

Fifteen groups of animals were studied. (Table 1) An average of 90% of the burned non-bacteremic animals survived the burn as compared to 55% in the bacteremic groups. None of the bacteremic survivors had exhibited exceptional weight loss, developed abnormal colonic temperature or manifested other clinical signs of sepsis.

5. Walker, H.L., A.D. Mason, Jr. and G.L. Raulston. Surface infection with Pseudomonas aeruginosa. Ann. Surg. 160:297-305, 1964.

6. McManus, A.T., E.E. Moody and A.D. Mason, Jr. Bacterial motility: a component in experimental Pseudomonas aeruginosa burn wound sepsis. Burns 6:235-239, 1980.

TABLE 1. Group characteristics before (B) and after (A) injury.*#

Group	Number	Weight (gm) B/A	VO2 (ml/hr.gm) B30/A30**	Colonic Temperature
				(°C) B30/A30**
Unburned				
1. UB@	30/30	477+3/480+6	0.82±.01/0.75	36.9/36.8
2. UB	30/30	522+6/554+8	0.82±.01/0.79	37.1/37.1
3. UB,Su#	30/30	515+5/509+6	0.76±.02/0.77	36.9/36.9
Burned Non-bacteremic			B30/A32/A34**	B30/A32/A34**
4. Bu^	30/24	521+4/494+5	0.82±.01/0.99/0.90	37.3/37.6/38.0
5. Bu	30/26	512+6/490+11	0.80±.01/ /1.03	37.0/ /37.9
6. Bu	27/25	547+7/544+8	0.77±.01/0.99/0.92	37.0/37.2/37.6
7. Bu,Su	30/27	520+7/486+11	0.81±.02/ /1.02	36.8/ /37.4
8. Bu,Su	30/24	518+5/472+5	0.79±.01/1.17/1.04	37.1/37.3/37.2
9. Bu,NVP*	30/29	477+3/461+6	0.84±.01/1.04/1.04	37.1/37.4/37.6
10. Bu,NVP	30/29	503+4/486+6	0.84±.01/1.07/1.02	37.1/37.1/37.9
11. Bu,NVP,Su	29/24	511+4/488+6	0.82±.00/1.05/0.93	37.1/37.2/37.6
12. Bu,NVP,Su	30/30	535+5/516+6	0.80±.02/0.97	36.8/37.5
Burned Bacteremic				
13. BB"	30/18	537+8/494+5	0.85±.01/1.19	36.6/37.4
14. BB	30/18	485+6/470+8	0.80±.01/1.18	36.7/37.7
15. BB,+VP	30/13	552+5/466+6	0.83±.01/1.22	37.1/37.9

*# postburn day 8 for group 15 and 18-24 for all other groups.

** refers to ambient temperature before and after injury or sham burn.

@ UB, unburned; Su, Sulfamylon; Bu, burned, * NVP,
seeded with non-virulent P. aeruginosa; BB ",
burned bacteremic;

+ VP, seeded with virulent P. aeruginosa.

Under these experimental conditions, thermoneutrality for the burned rats rose from 32 to 34°C over the three-week period of observation. (Figure 1) Minimal or resting $\dot{V}O_2$ was best achieved at 32°C at the end of the first week postinjury, but two weeks later, five of six groups (#4, #6, #8-#11) studied at both temperatures were less hypermetabolic in the 34°C environment. At this time, $\dot{V}O_2$ fell from 1.05 ± 0.03 ml/hr.gm (mean $\pm$ SE) to 0.98 ± 0.02 when chamber temperature was raised from 32 to 34°C ($p < .05$).

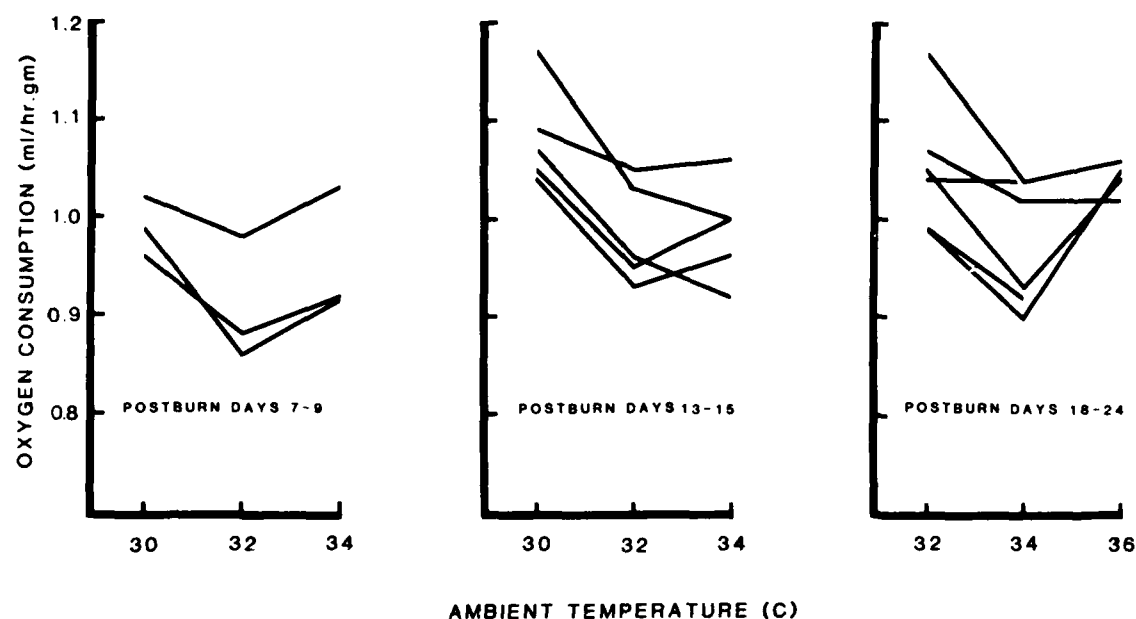


Fig. 1. The effects of ambient temperature on the average oxygen consumption of different groups of burned, non-bacteremic rats at different times postinjury. While 32°C was apparently within the thermoneutral zone at the end of the first week postinjury, sometime during the second or third week minimal $\dot{V}O_2$ was most consistently achieved in the 34°C environment.

Colonic temperature of the burned rats followed changes in ambient temperature (Figure 2). Consequently, minimal or resting $\dot{V}O_2$ following injury developed only when the rats were hyperthermic. At three weeks post injury, there was no clear relationship between core temperature and O_2 uptake (Figure 3).

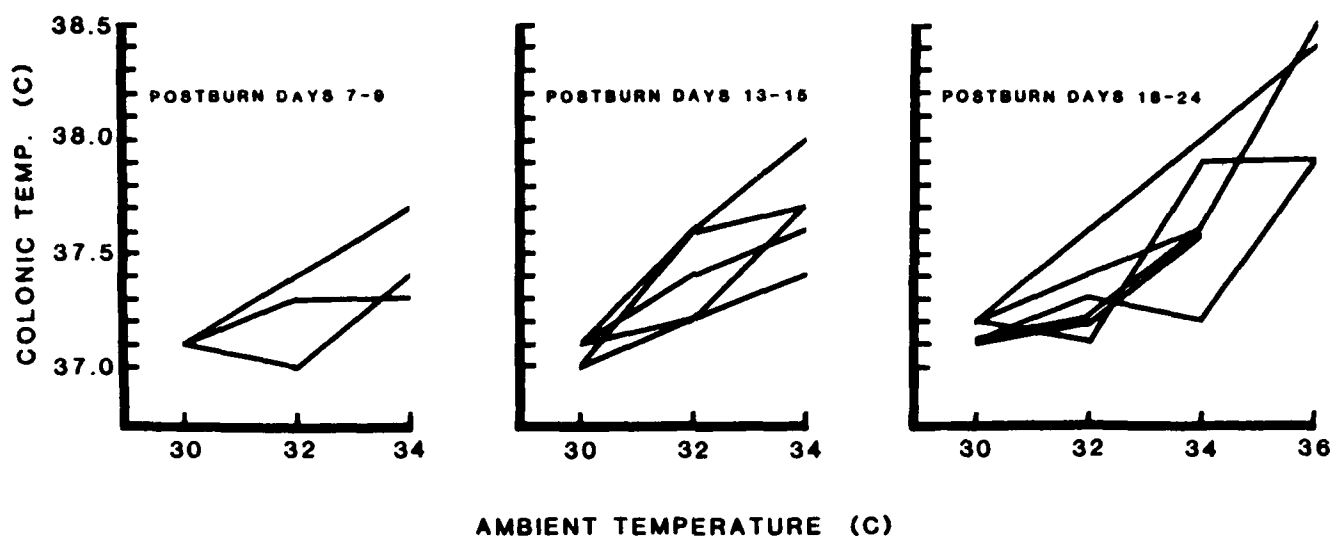


Fig. 2. At the end of the first, second and third weeks postburn, average core temperature of non-bacteremic groups rose with increasing ambient temperature. Standard error of all group means seldom exceeded 0.1°C.

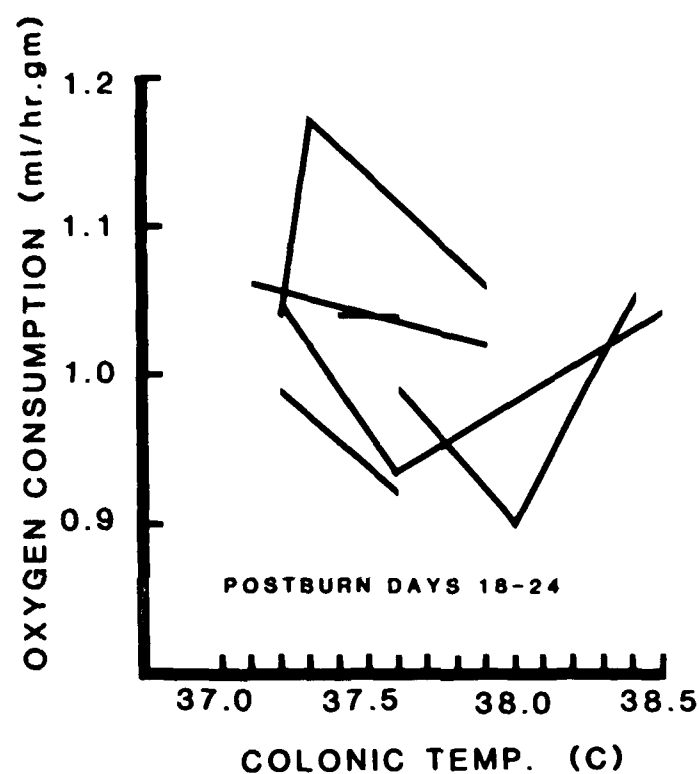


Fig. 3. At three weeks postburn, the oxygen consumption of six non-bacteremic groups bore no consistent relationship to core temperature.

The magnitude and rate of rise in aerobic metabolism was greatly increased when burned animals became bacteremic (Figure 4). At three weeks postinjury, resting $\dot{V}O_2$ of the non-bacteremic burn groups, ranged from 10 to 32% above their respective preburn levels (Table 1). Oxygen uptake of the bacteremic groups increased 40 to 48%. Since the latter groups were studied at 32°C, a portion of their postburn hypermetabolism was the result of a mild cold stress. At this temperature, the burn alone produced little or no change in colonic temperature (Figure 5). The bacteremic groups, however, developed a mild fever. (The full febrile effects of systemic infection were blunted, however, because as these animals became progressively more febrile, they usually developed other signs of sepsis and were dropped from the study.) Ultimately, there were no changes in core temperature of the non-bacteremic groups which could not be explained by differences in the thermal environment (Table 1).

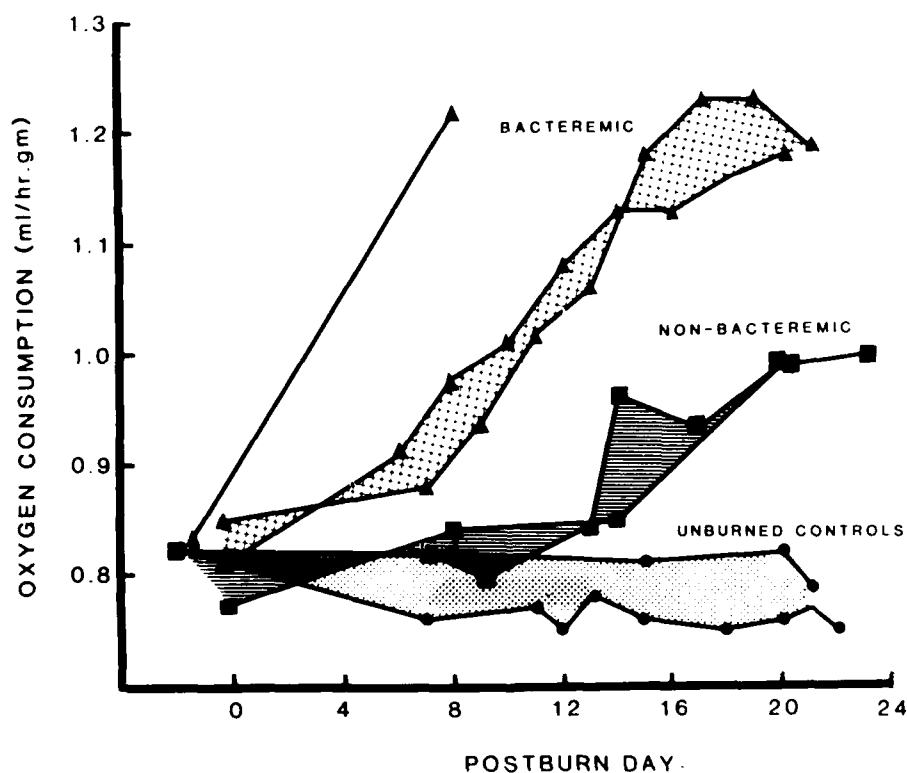


Fig. 4. The effects of different levels of infection on oxygen uptake of burned rats. Ambient temperature was 30°C for the unburned controls and 32°C for the burned animals. The zero postburn day represents an average of the last three studies prior to injury.

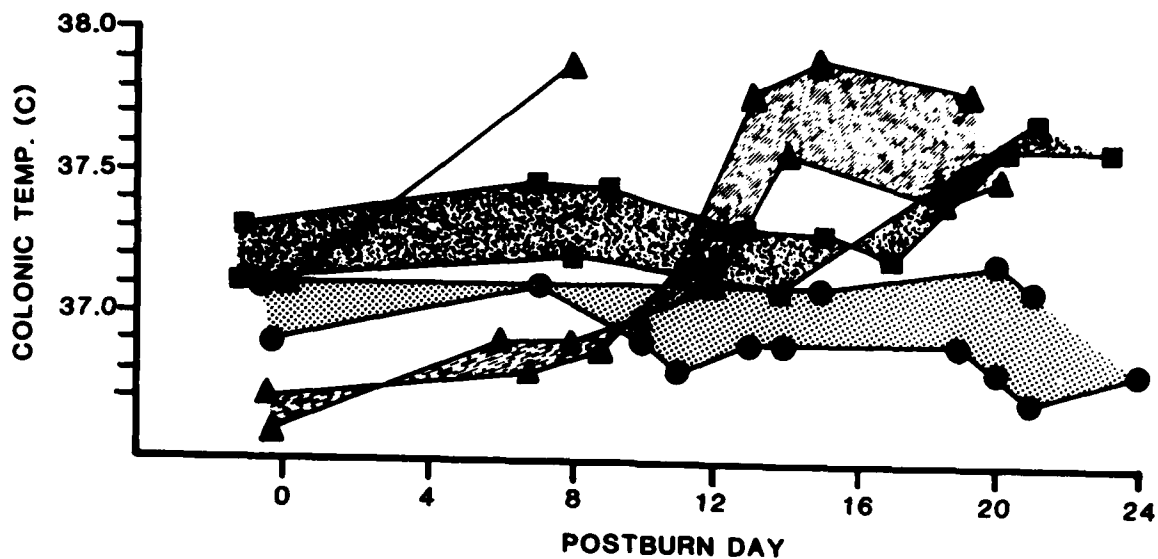


Fig. 5. The effects of different levels of infection on colonic temperature of burned rats - bacteremic groups (▲), non-bacteremic groups (■), and unburned controls (●). Ambient temperature was 30°C for controls and 32°C for the burned groups. Standard error was seldom above 0.1°C.

Seeding the burn wound with a non-virulent strain of P. aeruginosa did not increase the incidence of systemic infection or mortality (Table 1). Oxygen uptake of the seeded, untreated groups rose to equal that of the other untreated, non-bacteremic burned animals (Figures 4 and 6). While Sulfamylon treatment had no apparent effect on $\dot{V}O_2$ of burned, unseeded or unburned animals (Figure 7), it did limit the rise in postburn $\dot{V}O_2$ of the seeded groups** (Figure 6).

** A non-linear regression was determined expressing $\dot{V}O_2$ as a function of postburn day (PBD). $\dot{V}O_2$ (ml/hr.gm) of the untreated groups 9 and 10 was $1.02979 - 0.189489e-0.151106$ PBD as compared to $0.928852 - 0.116472e-0.91194$ PBD for the treated groups 11 and 12. There was no difference in treated group means between PBD's 7-21 (one way analysis of variance and covariance), but mean $\dot{V}O_2$ for the untreated group 9 at 1.02 ml/hr.gm exceeded the 0.97 ml/hr.gm achieved by the other untreated group ($p < 0.05$). Over this two week period, the mean $\dot{V}O_2$ of the two untreated groups (0.99 ml/hr.gm) was significantly above the average of the two treated groups (0.92 ml/hr.gm, $p < 0.001$).

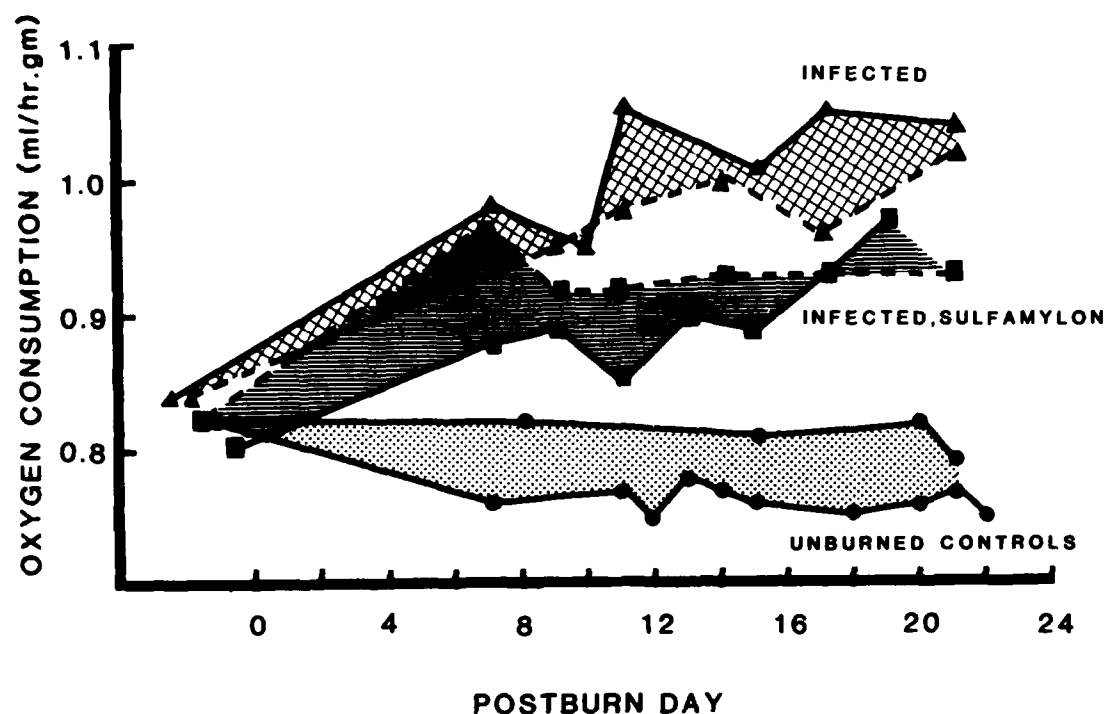


Fig. 6. The effects of daily application of Sulfamylon burn cream on oxygen consumption of groups of burned, non-bacteremic rats whose wounds had been seeded with a non-virulent strain of *P. aeruginosa*. Ambient temperature was 30°C for the unburned controls and either 32°C (solid lines) or 34°C (dashed lines) for the burned animals.

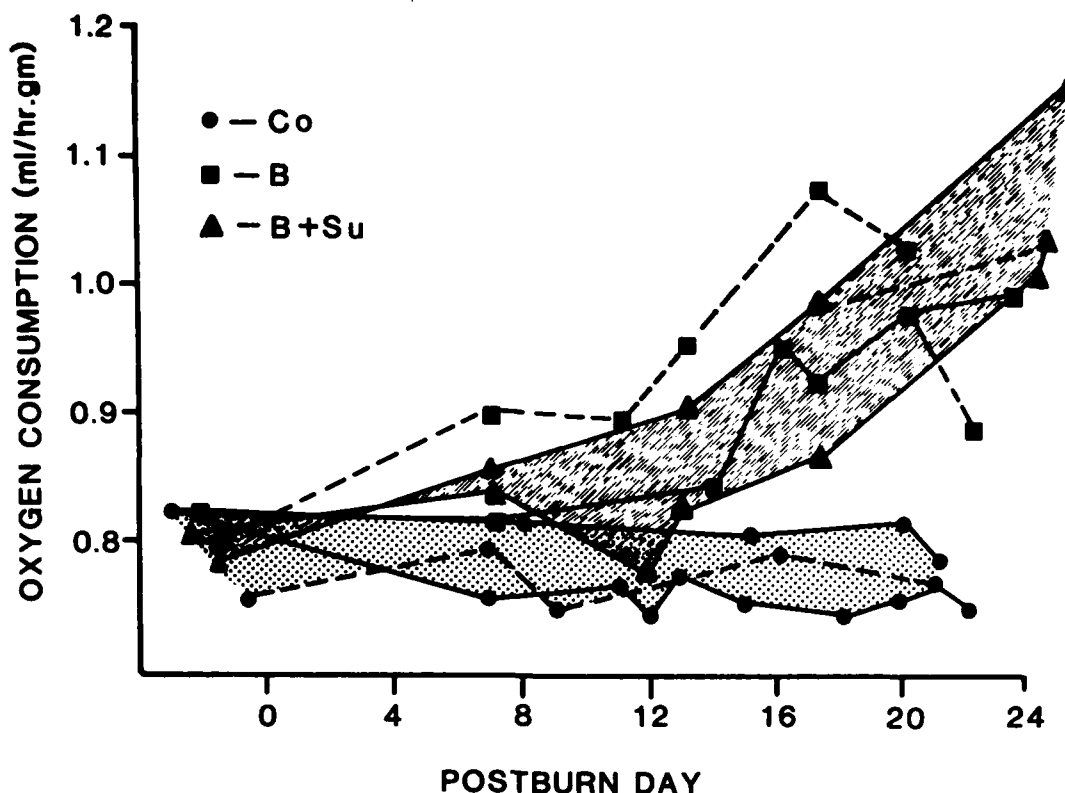


Fig. 7. The absence of an effect of Sulfamylon on oxygen consumption of burned, non-bacteremic and unburned, control groups of rats. Burned animals (Δ - treated, $\blacksquare$ - untreated) were studied at 32°C (solid lines) and 34°C (dashed lines) while the controls ($\bullet$) were studied at 30°C. Control rats treated with Sulfamylon are represented by the dashed line.

DISCUSSION

Bacterial infection has been defined as "the composite effect of a dynamic interplay between microorganisms (including their metabolic products) and the host organism" (7). Since the burn wound is sterile for only a brief time immediately after injury, the patient can be considered "infected" as soon as the host begins to respond to these bacteria.

The results of this study suggest that such a "dynamic interplay" begins while bacteria are still confined to the burn wound. This was best demonstrated when the application of an antimicrobial cream substantially reduced $\dot{V}O_2$ of the

7. Hopps, H.C. Bacterial Diseases. In: Pathology, Vol. 1, ed. W.A.D. Anderson, C.V. Mosby Co., St. Louis, 1971, p. 273.

whose wounds had been seeded with non-virulent pathogens (Figure 6). Since the $\dot{V}O_2$ of other untreated, non-bacteremic groups fell within the range of the NVP seeded, untreated animals (Figures 4 and 6), variations in aerobic metabolism among the non-bacteremic groups may be a function of differences in the extent/type of bacterial growth in the wound.

Since the burned area is not sterile and wound bacteria can raise $\dot{V}O_2$, it is legitimate to ask if there is any non-infective component in postburn hypermetabolism. While it is difficult to separate infective and non-infective components, one can make a reasonable estimate by selecting groups with minimal changes in $\dot{V}O_2$ and assuming that the infective component is minimal or non-existent. Two such groups were #4 and #11 where O_2 uptake rose only 10 and 13%, respectively. If wound bacteria exerted no metabolic impact on these animals, what other factors might contribute to the increased $\dot{V}O_2$? Since these animals were hyperthermic, the first consideration must be the effect of elevated body temperature on metabolic rate (Q_{10} effect). Colonic temperature rose from 37.3 to 38.0°C and from 37.1 to 37.6°C for groups 4 and 11, respectively. Since the Q_{10} effect can increase $\dot{V}O_2$ anywhere from 10-15% for every degree centigrade rise in core temperature (8-11), most, if not all, of the observed changes in aerobic metabolism could be attributed to the elevated body temperatures. And, since most of the increase in core temperature was the result of external heating, it would appear that with a 30% total body surface burn, the rat produced little or no "temperature independent hypermetabolism", unless the animal became infected.

8. DuBois, E.F. The basal metabolism in fever. JAMA 77:325-XXX, 1921.

9. Kinney, J.M. and C.F. Roe. Caloric equivalent of fever: I. Patterns of postoperative response. Ann. Surg. 156:610-622, 1962.

10. Roe, C.F. and J.M. Kinney. The caloric equivalent of fever. II. Influence of major trauma. Ann. Surg. 161:140-147, 1965.

11. Wilmore, D.W. The Metabolic Management of the Critically Ill. Plenum Medical Book co., New York, 1977, p. 29.

Herndon et al (12) have reported that Sulfamylon increased $\dot{V}O_2$ of burned and uninjured rats. There was no evidence of this in our study, however, since the metabolic response of the treated animals fell within the range of the untreated, non-bacteremic groups and there was no difference in $\dot{V}O_2$ of treated and untreated control groups (Figure 7). The absence of a drug effect may be a result of a reduced dose, since the previous work was conducted on animals with larger burns (50% total body surface) and the cream was applied twice daily. In the current study, topical antimicrobial treatment reduced the metabolic cost of injury, appearing to limit the effects of infection.

12. Herndon, D.N., D.W. Wilmore, A.D. Mason, Jr. and B.A. Pruitt, Jr. Increases in postburn hypermetabolism caused by application of topical ointments. Surg. Forum 29:49-51, 1978.

Non-bacteremic burned rats did not defend their elevated colonic temperatures in different thermal environments, and, as such, they must be considered hyperthermic rather than febrile (13). The absence of fever has also been reported in rats (14), goats (15), and pigs (16) with similar size burns. Non-bacteremic patients with 30% total body surface burns (17-19) and rats with larger burns (20) are febrile, however, suggesting that there are both species and burn size determinants of the thermoregulatory response to burn injury. The question raised by this study is whether these species differences reflect differences in response to bacteria or their products.

13. Stitt, J.T. Fever versus hyperthermia. Fed. Proc. 38:39-43, 1979.

14. Caldwell, F.T., H.T. Hammel and F. Dolan. A calorimeter for simultaneous determination of heat production and heat loss in the rat. J. Appl. Physiol. 21(5):1665-1671, 1966.

15. Aulick, L.H., W.B. Baze, A.A. Johnson, D.W. Wilmore and A.D. Mason, Jr. A large animal model of burn hypermetabolism. J. Surg. Res. 31:281-287, 1981.

16. Unpublished data.

17. Aulick, L.H., D.W. Wilmore, A.D. Mason, Jr., and B.A. Pruitt, Jr. Influence of the burn wound on peripheral circulation in thermally injured patients. Am. J. Physiol. 233(4):H520-H526, 1977.

18. Wilmore, D.W., L.H. Aulick, A.D. Mason, Jr. and B.A. Pruitt, Jr. Influences of the burn wound on local and systemic responses to injury. Ann. Surg. 186:444-458, 1977.

19. Aulick, L.H., E.W. Hander, D.W. Wilmore, A.D. Mason, Jr. and B.A. Pruitt, Jr. The relative significance of thermal and metabolic demands on burn hypermetabolism. J. Trauma 19:559-566, 1979.

20. Strome, D.R., L.H. Aulick, A.D. Mason, Jr. and B.A. Pruitt, Jr. Postburn hypermetabolism in the rat. The Physiologist 26:A78, 1983.

As mentioned earlier, an endogenous pyrogen has been identified in the sera of non-bacteremic burn patients (3). There was little evidence of a pyrogen in the non-bacteremic rats, but plasma zinc and iron concentrations were depressed in these animals at three weeks postinjury (21). Since such changes in trace metals suggest the presence of endogenous mediators, phagocytic cells at the wound interface may serve as the link between resident pathogens and the observed changes in host energy turnover.

The bioenergetic consequences of wound invasion were readily apparent in this burn model (Figure 4). At an ambient temperature of 32°C, the average increase in $\dot{V}O_2$ of the three bacteremic groups was 45% as compared to 26% for the non-bacteremic groups (Table 1). Since the 32°C environment eventually dropped below the thermoneutral zone for the burned animals, [presumably a result of eschar separation (14)] some of the difference in metabolic heat production of bacteremic and non-bacteremic groups may reflect differences in thermoregulation. Specifically, as the bacteremic animal became febrile and began thermoregulating around an elevated setpoint temperature, the 32°C environment would provoke a greater metabolic response than that of the afebrile, non-bacteremic rat.

The results of this study also clearly indicate that bacteria within the burn wound begin to alter host energy metabolism long before the rat becomes bacteremic or septic. From a metabolic standpoint, this has several important implications. First, it suggests that separation of patients into bacteremic and non-bacteremic groups is misleading if, by so doing, one implies a fundamental difference in basic metabolic determinants between groups. Second, it suggests a logical set of afferent signals linking the wound with metabolic alterations. Accordingly, bacterial contamination of the wound initially affects the host by activating various cells at the wound interface. They, in turn, release endogenous mediators which travel throughout the body affecting central controllers as well as peripheral tissues. The evolution of these responses would then vary with wound size, characteristics of the local bacterial population (strain, extent of proliferation and virulence) and the body's capacity to respond. Thirdly, fever and other

21. Unpublished data.

thermoregulatory adjustments then become separate manifestations of the infection process rather than the basis for the increased heat production.

PRESENTATIONS/PUBLICATIONS

Presented to the American Physiological Society Annual Meeting, 26-31 August 1984, Lexington, Kentucky.

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REDUCES POSTBURN HYPERMETABOLISM
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ABSTRACT

PROJECT NO. 3A161102A91C, INHOUSE LABORATORY
INDEPENDENT RESEARCH

REPORT TITLE: THE STUDY OF METABOLISM AND
TEMPERATURE REGULATION IN ANIMAL
MODELS OF HUMAN BURN INJURY:
TOPICAL ANTIMICROBIAL THERAPY
REDUCES POSTBURN HYPERMETABOLISM
OF THE BURNED INFECTED RAT

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Period covered in this report: 1 October 1983 - 30 September 1984

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Reports Control Symbol MEDDH-288(R1)

This study investigates the effects of Sulfamylon and Silvadene burn creams on resting oxygen uptake ($\dot{V}O_2$) and colonic temperature (T_c) in groups of unburned, burned (30% total body surface, full-thickness), and burned infected rats. Daily application of these standard antimicrobial agents had no effect on $\dot{V}O_2$ or T_c of the unburned animals. Two groups (30 rats/group) of burned untreated rats became hyperthermic (average T_c increased $0.6 - 0.9^\circ\text{C}$) and hypermetabolic ($\dot{V}O_2$ increased by 24%) over the three-week period of observation. Neither burn cream increased $\dot{V}O_2$ or T_c above that observed in these two burned, untreated groups. The initial metabolic response to wound colonization was greater than that to the burn alone and varied with bacterial virulence. Sulfamylon treatment reduced postburn hypermetabolism of rats whose wounds had been seeded with either virulent or non-virulent strains of *P. aeruginosa* such that by the 11th postburn day, $\dot{V}O_2$ of both untreated infected groups had returned to levels produced by the burn alone. Silvadene was more effective than Sulfamylon in reducing the metabolic response to *S. epidermidis* infection. The capacity of standard topical antimicrobial agents to reduce the metabolic cost of burn injury suggests that infection plays an important role in the etiology of postburn hypermetabolism of the rat.

TOPICAL ANTIMICROBIAL THERAPY REDUCES POSTBURN HYPERMETABOLISM OF THE BURNED INFECTED RAT

INTRODUCTION

The rise in energy metabolism following thermal injury has been well documented in both patients and animals, but the signals initiating and sustaining this hypermetabolism remain poorly defined. In 1978 Herndon et al. (1) reported that two commonly used antimicrobial creams, Sulfamylon and Silvadene, increased oxygen consumption of burned (50% total body surface, TBS) and unburned rats and suggested that some of postburn hypermetabolism may be a function of treatment rather than the burn itself. In a study just completed, we were unable to identify any such Sulfamylon effect in 30% TBS burned rats or uninjured controls (2). In fact, just the opposite occurred as this particular topical antimicrobial agent reduced the hypermetabolism of burned infected animals.

The purpose of this study was to extend these observations by surveying the metabolic and thermoregulatory consequences of both Sulfamylon and Silvadene in 30% TBS burned infected rats. We found no evidence that either Sulfamylon or Silvadene increased

1. Herndon, D.N., D.W. Wilmore, A.D. Mason, Jr. and B.A. Pruitt, Jr. Increases in postburn hypermetabolism caused by application of topical ointments. Surg. Forum 29:49-51, 1978.

2. Aulick, L.H., A.T. McManus, A.D. Mason, Jr., B.A. Pruitt, Jr. The effects of localized and systemic infection on aerobic metabolism and core temperature of the burned rat. Annual Research Progress Report, US Army Institute of Surgical Research, Fort Sam Houston, 1984.

resting oxygen consumption of burned or uninjured animals. What was apparent, however, was that microorganisms residing in the wound beneath the cream increased postburn hypermetabolism and that effective antimicrobial therapy reduced the metabolic effects of burn wound contaminants.

MATERIALS AND METHODS

The animals selected for study were male, Sprague Dawley rats, ranging in age from three to seven months and weighing between 400 and 600 grams at the beginning of the study. They were housed in individual cages at an ambient temperature of 28 to 30°C and had access to food (Purina laboratory chow) and water throughout all phases of the study. Controlled lighting maintained a 12/12-hr light/dark cycle with the lights coming on at 0600.

Oxygen consumption was determined in groups of rats through the use of a variable volume, open and closed respiration chamber (3). Chamber temperature, humidity and air flow were held constant, while volume varied with changes in barometric pressure or respiratory exchange of the animals. Chamber temperature was set in the thermoneutral zone for the rat; 28 to 30°C for the control animals and 32 to 34°C after injury. Average chamber temperature (calculated as the mean of four air temperatures and one wall temperature) never varied more than $\pm 0.3^{\circ}\text{C}$. Relative humidity ranged from 40 to 50% and air velocity averaged 80 ft/min in the center of the chamber.

Groups of animals (13-30 rats/group) were placed in the chamber at the same time. Each rat, housed in its own cage, was left undisturbed for at least one hour prior to study. At the end of this equilibration period, valves on the chamber closed and oxygen consumption ($\dot{V}\text{O}_2$) of the group was calculated from measured changes in oxygen concentration while the chamber was hermetically sealed. Oxygen and carbon dioxide measurements were repeated at 15-minute intervals until the CO_2 concentration exceeded 0.85%. The average $\dot{V}\text{O}_2$ was then calculated for the entire closed period.

3. Aulick, L.H., H. Arnhold, E.W. Hander and A.D. Mason, Jr. A new open and closed respiration chamber. Quart. J. Exp. Physiol. 68:351-357, 1983.

Body weights and colonic temperatures (at a depth of 6 cm) were recorded for all animals at the end of each study. Colonic temperatures were taken within one minute after the animal left the chamber, and there was no evidence of any time effect. Animal location in the chamber and the order of measurement were also randomized in an effort to reduce any unrecognized systematic errors. Oxygen consumption was calculated for the average rat and expressed in milliliters (STPD) per hour per gram body weight.

Rats were subjected to daily chamber confinement and handling until they became well accustomed to experimental procedures. After baseline measurements they were anesthetized (sodium pentobarbital, 5 mg/100 gm body weight, intraperitoneally) and the hair clipped from their backs and flanks. While still anesthetized, each rat was placed in a mold exposing 30% of the total body surface and a full-thickness burn created on the back by immersing the area in 98°C water for nine seconds. Control groups were anesthetized and shaved but not burned.

Groups of rats were divided into eleven categories. They were:

1. controls (Co)
2. controls, Sulfamylon applied to shaved back (Co+Su)
3. controls, Silvadene applied to shaved back (Co+Ag)
4. burned, (B)
5. burned, Sulfamylon (B+Su)
6. burned, Silvadene (B+Ag)
7. burned, wound seeded with virulent P. aeruginosa (B+VP)
8. burned, wound seeded with virulent P. aeruginosa, Sulfamylon (B+VP+Su)
9. burned, wound seeded with non-virulent P. aeruginosa, Sulfamylon (B+NVP+Su)
10. burned, wound seeded with S. epidermidis, Sulfamylon (B+SE+Su)
11. burned, wound seeded with S. epidermidis, Silvadene (B+SE+Ag)

The virulent P. aeruginosa organisms were of the ISR 59-12-4-4 strain (4,5). The non-virulent P. aeruginosa bacteria are currently being characterized. The S. epidermidis strain was ATCC# 12228. Seeding cultures contained 10^8 organisms per milliliter and one milliliter of this culture was spread over the entire wound surface on the day of injury. Antimicrobial creams were applied once daily and always after metabolic and temperature measurements. Treated wounds were bathed weekly.

All animals were screened daily for clinical signs of sepsis. If they were markedly febrile, losing weight rapidly and/or had developed a reddish brown discharge from the eyes and nose, they were dropped from the study. (Subsequent autopsies on these animals always confirmed the clinical diagnosis of systemic infection.) All rats were sacrificed after the final experiment. Blood and spleen cultures and tissue samples were obtained from one-third to one half of the animals in each group (selected at random) to establish the burn depth and the incidence of wound invasion and systemic infection. Clinically bacteremic animals had positive blood and/or spleen cultures and histologic evidence of wound invasion. Only those groups which demonstrated at least 90% homogeneity (bacteremic or non-bacteremic) were included in this report.

RESULTS

Four control and 12 burned groups were studied. One burn group became bacteremic (B+VP). By the eighth postburn day 57% of these animals had either died or were clearly septic. Average mortality in the other 11 non-bacteremic burn groups was 13% (range 0-40) over the three-week period of observation.

4. Walker, H.L., A.D. Mason, Jr. and G.L. Raulston. Surface infection with *Pseudomonas aeruginosa*. Ann. Surg. 160:297-305, 1964.

5. McManus, A.T., E.E. Moody and A.D. Mason, Jr. Bacterial motility: a component in experimental *Pseudomonas aeruginosa* burn wound sepsis. Burns 6:235-239, 1980.

Septic animals became markedly febrile with colonic temperatures frequently exceeding 39°C. By the end of three weeks, core temperature of the non-bacteremic groups ranged from 0.3°C below to 0.7°C above their respective preburn levels. (Table 1) The seven groups studied at 32°C had an average increase of 0.2 ± 0.1 °C (mean $\pm$ SE) as compared to 0.6 ± 0.1 °C for five groups studied at 34°C ($p < 0.05$).

Table 1. Colonic temperature of rats before and after thermal injury.

GROUP	COLONIC TEMPERATURE (°C)			Ambient Temp. (°C)
	Before	After	PBD Studied	
Unburned				
1A. Co	36.8±0.1	36.8±0.1	-	30
1B. Co	36.8±0.1	36.7±0.1	-	30
2. Co+Su	36.9±0.1	36.9±0.1	-	30
3. Co+Ag	36.8±0.1	36.8±0.1	-	30
Burned				
4A. B	37.3±0.1	37.6±0.1	20	32
4B. B	37.0±0.1	37.9±0.1	20	34
4C. B	37.0±0.1	37.6±0.1	20	34
Burned, treated				
5A. B+Su	37.1±0.1	37.0±0.1	17	32
5B. B+Su	36.8±0.1	37.4±0.1	20	34
6. B+Ag	36.7±0.2	37.1±0.1	19	32
Burned, seeded				
7. B+VP	37.1±0.1	37.9±0.1	8	32
Burned, seeded, and treated				
8. B+VP+Su	37.1±0.1	37.2±0.1	13	32
9A. B+NVP+Su	37.1±0.1	37.6±0.1	21	34
9B. B+NVP+Su	36.8±0.1	37.5±0.1	19	32
10. B+SE+Su	36.8±0.1	37.3±0.1	20	32
11. B+SE+Ag	37.3±0.1	37.0±0.1	20	32

Figure 1 illustrates the rise in resting $\dot{V}O_2$ of burned rats and how this increase was not different from that of burned animals treated with Sulfamylon.*

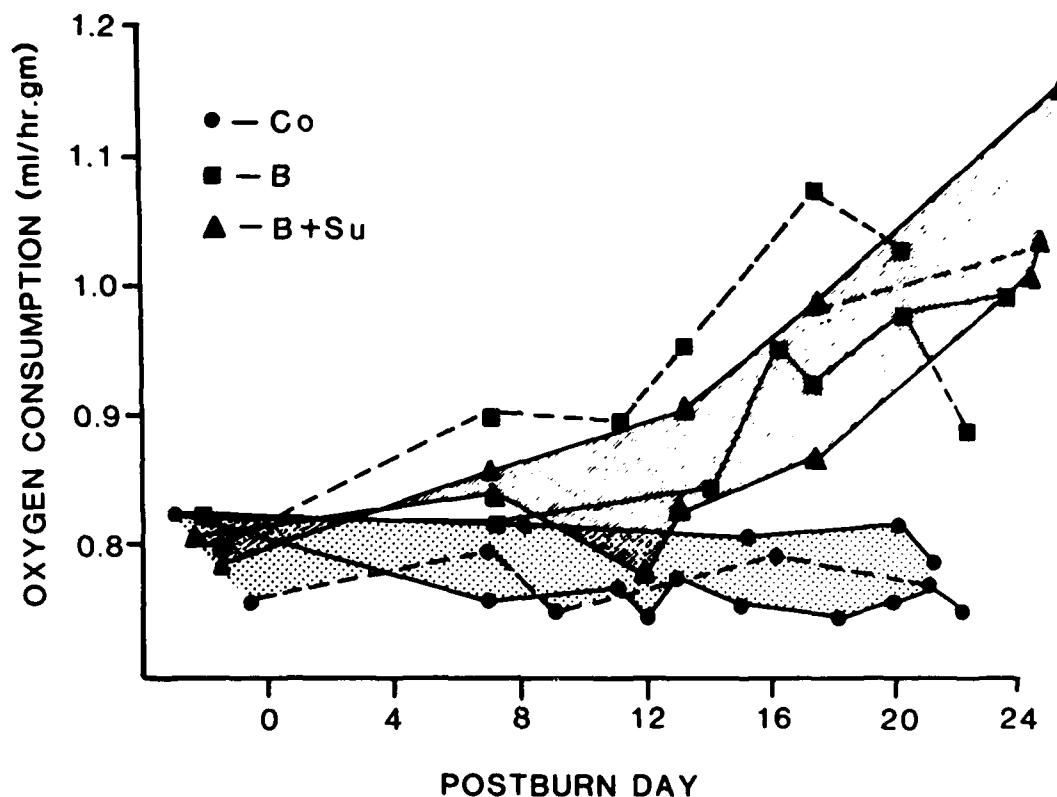


Fig. 1. Effects of daily Sulfamylon treatment on oxygen consumption of groups of unburned controls and non-bacteremic burned rats. Control studies were conducted at 30°C (●, solid lines for shaved rats and dashed line for shaved, Sulfamylon treated rats). Treated (▲) and untreated (■) burned rats were studied at 32°C (solid lines) and 34°C (dashed lines).

* These data were reported previously (2) and are presented here solely to re-emphasize that Sulfamylon, applied once daily to a 30% TBS burn, has no measurable effect on resting $\dot{V}O_2$ of the rat.

Likewise, daily application of Silvadene had no effect on resting O₂ uptake (Figure 2).

Burned animals whose wounds were seeded with the virulent pseudomonas strain and not treated with Sulfamylon (B+VP) became bacteremic and very

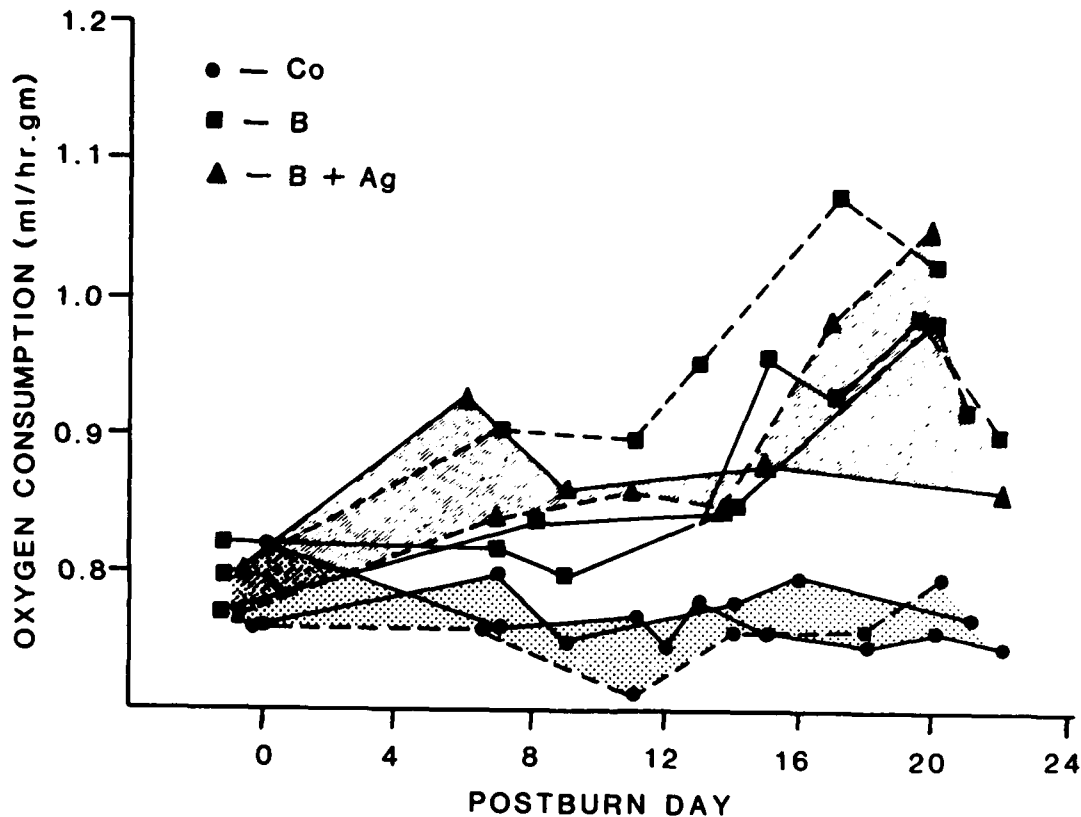


Fig. 2. Effects of daily Silvadene treatment on oxygen consumption of groups of unburned controls and non-bacteremic burned rats. Control studies were conducted at 30°C (●, solid lines for shaved rats and dashed line for Silvadene treated rats). Treated (▲) and non-treated (■) burned animals were studied at 32°C (solid lines) and 34°C (dashed lines).

hypermetabolic (Figure 3). Rats whose wounds were seeded with the same organism and treated with Sulfamylon (B+VP+Su) initially also became very hypermetabolic, but by the end of the second week postburn resting $\dot{V}O_2$ returned to a level comparable to that of burned, unseeded animals. None of the (B+VP+Su) group died or were bacteremic at the end of study. Bacterial virulence affected postburn hypermetabolism of the Sulfamylon treated animals. Rats infected with a non-virulent strain of P. aeruginosa (B+NVP+Su) did not become as hypermetabolic as those seeded with the virulent strain (B+VP+Su) but were slightly (at least initially) more hypermetabolic than burned unseeded animals (B+Su).

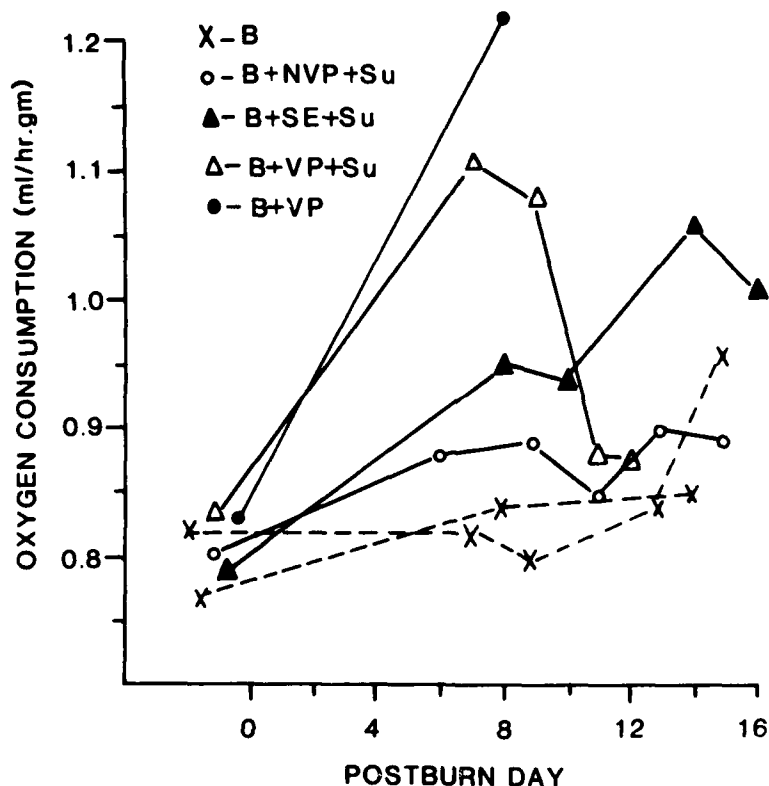


Fig. 3. Effects of Sulfamylon treatment on oxygen consumption of groups of burned rats whose wounds were seeded with different bacteria. All studies were conducted at 32°C. (x = burn, o = burn + non-virulent P. aeruginosa + Sulfamylon, $\blacktriangle$ = burn + S. epidermidis + Sulfamylon, $\blacktriangledown$ = burn + virulent P. aeruginosa + Sulfamylon, and $\bullet$ = burn + virulent P. aeruginosa).

The burn wounds of two other groups were seeded with S. epidermidis. Sulfamylon treatment appeared to be less effective in reducing the metabolic effects of this microbe (B+SE+Su) than with P. aeruginosa. None of these animals died within the 16-day period of observation.

Figure 4 offers a comparison of the relative effectiveness of Sulfamylon and Silvadene in minimizing the metabolic effects of S. epidermidis. The initial metabolic responses were comparable, but $\dot{V}O_2$ of the Silvadene treated group (B+SE+Ag) appeared to stabilize in the range maintained by non-seeded burned groups (B) while that of the Sulfamylon treated animals (B+SE+Su) continued to rise.

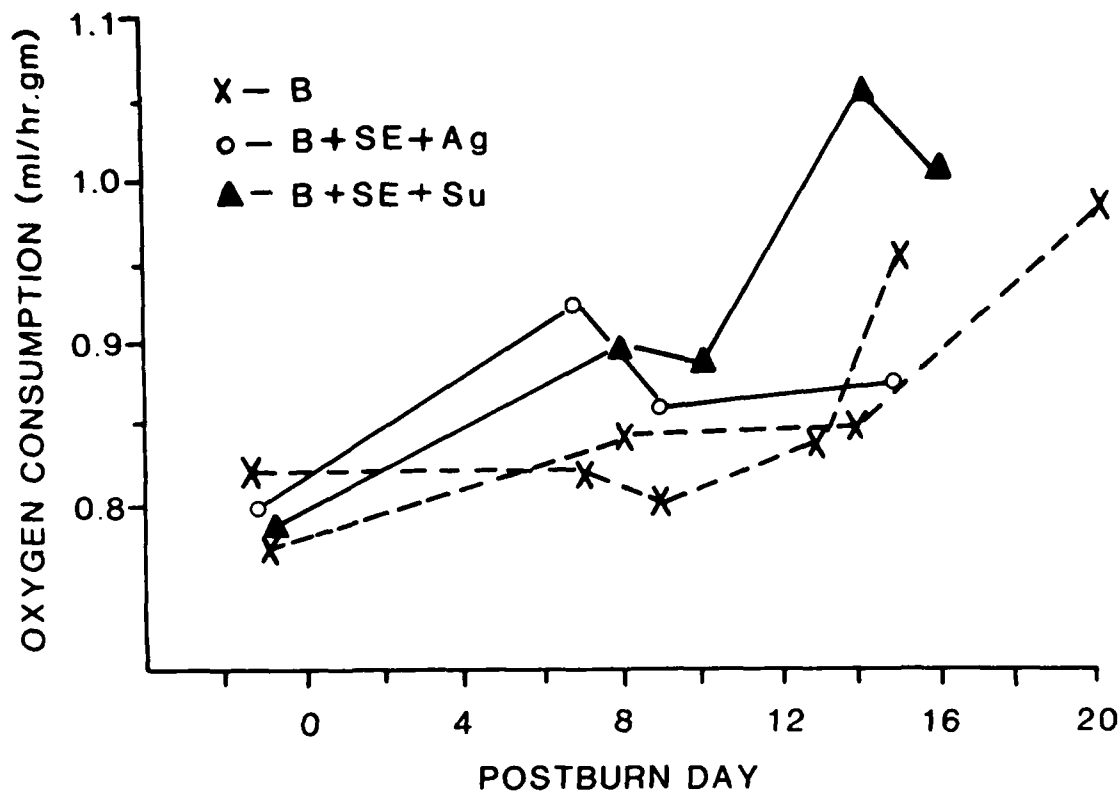


Fig. 4. Effects of Sulfamylon (▲) and Silvadene (O) treatment on the oxygen consumption of groups of non-bacteremic burned rats whose wounds were seeded with S. epidermidis. All studies were conducted at 32°C. (x = burn, no treatment).

DISCUSSION

Under these experimental conditions, postburn hypermetabolism was not accelerated by treatment with antimicrobial creams. Rather it appeared that bacteria residing in the wound beneath the cream were responsible for much of the rise in resting $\dot{V}O_2$ after injury. This interpretation is based on several observations. First, burn creams do not sterilize the wound but only reduce bacterial proliferation (6). Second, postburn hypermetabolism in this rat model is a graded response which reaches upper limits with systemic infection and is reduced by effective antimicrobial therapy (2). Third, the metabolic impact of burn wound flora appeared to vary with bacterial virulence since the initial response to the virulent P. aeruginosa infection was much greater than to its non-virulent counterpart (Figure 3). Fourth, the organism's resistance to the burn cream also determined its capacity to affect resting $\dot{V}O_2$. Sulfamylon, which has broad spectrum anti-gram-negative activity, reduced the metabolic impact of P. aeruginosa but was relatively ineffective against the staphylococcal infection. Silvadene, on the other hand, was more effective in blocking the metabolic consequences of S. epidermidis (Figure 4).

These findings are consistent enough, however, to question why they differ so radically with those of Herndon and collaborators (1). There are a number of factors which may explain this difference, but the principle one is administered dose. In the earlier work, the burns were larger (50 versus 30% TBS) and the creams were applied twice daily instead of once as was the case in this study. The reason for selecting smaller burns and reducing treatment was to avoid the high mortality reported previously in Sulfamylon treated animals with 50% burns. This smaller dose of Sulfamylon or Silvadene may have been below a threshold for metabolic stimulation.

6. Pruitt, B.A. Jr. Infections of burns and other wounds caused by *Pseudomonas aeruginosa*. In: *Pseudomonas aeruginosa: the organism, diseases it causes and their treatment*. Ed. by L.D. Sabath, Hans Huber Publishers, Bern, 1980, pp.55-70.

This and other studies (2) have shown that the core temperature of non-bacteremic, burned rats rises with thermal environment while $\dot{V}O_2$ falls toward minimal hypermetabolic levels. The apparent dependence of core temperature on ambient temperature suggests that non-bacteremic animals are hyperthermic rather than febrile. The distinction is that hyperthermia represents an increase in body temperature due to an imbalance between heat production and loss while fever is a centrally regulated response designed to increase body heat content. At some point bacterial proliferation did reach a level which had systemic thermoregulatory effects and the animals became clearly febrile. Just when this occurred could not be established in the present study, but such an associated increase in heat production would contribute to the hypermetabolism of infection.

The results of this study indicate that bacterial colonization of the burn wound raises resting $\dot{V}O_2$ in the rat, and that postburn hypermetabolism, is an expression of quantitative and qualitative differences in wound flora. Metabolic effects of infection can be reduced, but not eliminated by using the commonly employed topical burn creams, Sulfamylon and Silvadene.

PRESENTATIONS/PUBLICATIONS

None.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION DA302498	2. DATE OF SUMMARY 84 10 01	REPORT CONTROL SYMBOL DD-DR#5(AR) 636	
3. DATE PREV SUM'RY 83 10 01	4. KIND OF SUMMARY D. Change	5. SUMMARY SCTY U	6. WORK SECURITY U	7. REGRADING	8. DISB'N INSTR'N CX	9. LEVEL OF SUM A WORK UNIT	
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a. PRIMARY	61101A	3A161101A91C	00		089		
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c. CONTRIBUTING	None						
11. TITLE (Precede with Security Classification Code) (U) Inequality of VA/Q Ratios Following Smoke Inhalation Injury and the Effect of Angiotension Analogues							
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17. CONTRACT/GRANT				18. RESOURCES ESTIMATE			
a. DATE EFFECTIVE		EXPIRATION		FISCAL YEARS	a. PROFESSIONAL WORKYEARS		b. FUNDS (In thousands)
b. CONTRACT/GRANT NUMBER				84	0.5		22
c. TYPE		d. AMOUNT		85	0.5		15
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a. NAME US Army Institute of Surgical Research				a. NAME US Army Institute of Surgical Research Surgical Study Branch			
b. ADDRESS (include zip code) Ft. Sam Houston, Texas 78234-6200				b. ADDRESS Ft. Sam Houston, Texas 78234-6200			
c. NAME OF RESPONSIBLE INDIVIDUAL Pruitt, BA, Jr				c. NAME OF PRINCIPAL INVESTIGATOR Mason, AD			
d. TELEPHONE NUMBER (include area code) 512-221-2720				d. TELEPHONE NUMBER (include area code) 512-221-7832			
21. GENERAL USE FINA M MILITARY/CIVILIAN APPLICATION				f. NAME OF ASSOCIATE INVESTIGATOR (if available) Shimazu, I			
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23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code) 23. (U) To evaluate the effect of smoke inhalation on pulmonary ventilation and perfusion. To study the efficacy of angiotensin analogues in the amelioration of the cardiopulmonary effects of inhalation injury. 24. (U) Ventilation-perfusion ratios will be measured utilizing the six-inert gas technique. These pulmonary variables will be correlated with standard cardiopulmonary variables before and after the introduction of inhalation injury and subsequent treatment with [Sar ¹ , Ile ⁸]-Angiotensin II. Postmortem lung histology and water weight will be correlated with the in vivo measurement of lung water as determined by the double indicator dilution technique. 25. (U) 8310 - 8409. An inhalation injury technique has been standardized in adult sheep. A satisfactory, non-lethal smoke injury with predictable impact on respiratory function can be regularly produced. Preliminary studies of six-gas determination in the mass spectrometer have been carried out and several technical problems have been resolved. Studies of the interaction of smoke inhalation and broncho-pulmonary infection will be added to the protocol.							

ANNUAL PROGRESS REPORT

PROJECT NO. 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: INEQUALITY OF VA/Q RATIOS FOLLOWING SMOKE INHALATION
INJURY AND THE EFFECT OF ANGIOTENSIN ANALOGUES: A LARGE
ANIMAL MODEL OF SMOKE INHALATION INJURY WITH GRADED
SEVERITY

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Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

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Reports Control Symbol MEDDH-288(R1)

Inhalation injury is one of the primary determinants of survival following major burns. Such injury is influenced by the temperature and components of the inhaled smoke, tidal volume and contact time with the smoke. We have developed a large animal model of inhalation injury in which graded severity is achieved by changing the contact time. PaO_2 and mean blood pressure 24 hours after smoke inhalation were inversely related to the extent of smoke exposure ($r^2 = 0.451$ and 0.697 , respectively). This model of inhalation injury exhibits dose response and reproducibility. The animal (sheep) is large enough to allow detailed control and physiological measurement during experimentation. The model is suitable for studies of mortality with or without cutaneous burns as well as pathophysiologic studies of the mechanism of such injury.

Inhalation injury
Cardiac output
Lab animal
Sheep

INEQUALITY OF VA/Q RATIOS FOLLOWING SMOKE INHALATION INJURY AND THE
EFFECT OF ANGIOTENSIN ANALOGUES: A LARGE ANIMAL MODEL OF SMOKE
INHALATION INJURY WITH GRADED SEVERITY

INTRODUCTION

It was not until the 1970s that the significance of pulmonary injury due to smoke inhalation in burn patients was widely realized, though antecedent work by Phillips had indicated the likelihood that such injury was of consequence [1-3]. Only in recent years have early diagnosis of smoke inhalation and evaluation of its severity achieved wide clinical interest and even now the pathophysiologic mechanisms of the injury are not clear. Although several animal models have been developed to study such mechanisms, they have either been small animal models [4,5] or not controllable with respect to severity [6]. We have developed a large animal model of smoke inhalation which is reproducible and exhibits dose response.

MATERIALS AND METHODS

One- to 2-year-old male 25-40 kg, castrated random source sheep were obtained from a commercial source for use in this study. The sheep were housed in conventional outdoor runs and fed commercial chows and water ad libitum. Baseline CBC's, total proteins and dewormings were done 2 weeks prior to experimental use. Fifty-three sheep were divided into control (6), sham smoke (6) and smoke injury (41) groups.

Smoke was produced by burning 10 disposable underpads (45/52.5 cm, 40 g each; Hosposable Inc.) in a smoke generator. This generator is a 32-gal metallic can equipped with an air inlet, a chimney with a damper, a window and a smoke outlet. The disposable underpad is made of polyethylene, wood pulp and non-woven cellulose fabric. The smoke is

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1. DiVincenti FC, Pruitt BA Jr, and Reckler JM: Inhalation injuries. J Trauma 11:109-117, 1971.
 2. Moylan JA Jr, Wilmore DW, Mouton DE, and Pruitt BA Jr: Early diagnosis of inhalation injury using ¹³³Xenon lung scan. Ann Surg 176: 477-484, 1972.
 3. Phillips AW, and Cope O: Burn therapy. III: Beware the facial burn! Ann Surg 156:759-766, 1962.
 4. Potkin RT, Robinson NB, Hudson LD, Howard ML, Thorning DR, and Schumacher RL: An animal model of smoke inhalation. Am Rev Resp Dis 121:178, 1980.
 5. Zawacki BE, Jung RC, Joyce J, and Rincon E: Smoke, burns and the natural history of inhalation injury in fire victims: A correlation of experimental and clinical data. Ann Surg 185:100-110, 1977.
 6. Walker HL, McLeod CG Jr, and McManus WF: Experimental inhalation injury in the goat. J Trauma 21:962-964, 1981.

introduced through a 30-cm copper pipe (1.6-cm diameter) into a volume adjustable delivery system which permits delivery of either smoke or air. The smoke reaches ambient temperature during passage through the pipe and delivery system and contains 10 to 14% oxygen, 3 to 8% carbon dioxide and 0.7 to 2.2% carbon monoxide.

The sheep subjected to smoke inhalation were exposed to 6, 9, 12, 15 or 18 units of smoke. One unit of smoke consists of three successive exposures to smoke with a tidal volume of 30 ml/kg and breath hold of 5 seconds followed by 10 successive ventilations with room air. The time required per unit is about 50 seconds. This procedure is done under general anesthesia; the animals were extubated after smoke inhalation.

Animals were studied 24 or 72 hours after smoke inhalation. On the day of experiment, arterial and central venous lines, a Swan-Ganz catheter and a lung water catheter were inserted following general anesthesia. Blood gas, blood chemistry and cardiopulmonary variables were measured every 30 minutes. Ventilation was controlled by a volume-limited ventilator at a tidal volume of 15 ml/kg. Two consecutive sigh ventilations of 22 ml/kg were introduced every 3 minutes to prevent atelectasis. Lactated Ringer's solution was continuously infused at the rate of 1.5 ml/kg/hr during the experiment. Complete necropsies were performed on all animals sacrificed at the end of the experiments or dying spontaneously. Lung samples were taken for light microscopy and transmission and scanning electron microscopy.

RESULTS

No significant differences were observed between the control ($n = 6$) and the sham ($n = 6$) smoke groups. After 24 hours, mortalities in groups subjected to 6, 9, 12, 15 or 18 units of smoke exposure were 0% (0/7), 0% (0/8), 20% (1/5), 50% (1/2) and 100% (2/2) respectively. At 72 hours, exposures to 6, 9 and 12 units of smoke produced mortalities of 0% (0/7), 33% (2/6) and 100% (4/4) respectively. Cardiopulmonary data of each group are listed in Table 1. Statistical analysis was done by Student-Newman-Keuls multiple range test and differences were considered significant at $P < 0.05$.

- 1) CO-Hb level: Venous CO-Hb levels immediately after smoke exposure were 55.6 ± 5.2 (%), 63.7 ± 2.4 and 71.7 ± 2.0 for 6-unit ($n = 7$), 9-unit ($n = 8$) and 12-unit ($n = 4$) exposures.
- 2) pH: After 24 hours, only the 12- and 15-unit smoke exposures produced significant depression of plasma pH. After 72 hours, surviving sheep exhibited no acidosis.
- 3) Arterial PO_2 : After 24 hours, PaO_2 ($y = \text{torr}$) was negatively related to the number of units of smoke exposure ($x = \text{units}$): $y = -5.44x + 112.3$ ($r^2 = 0.451$, $n = 20$). PaO_2 was significantly depressed after 72 hours but regression was not significant.
- 4) Arterial PCO_2 : After 24 hours, some of the animals exposed to 9 units or more had elevated plasma PCO_2 , but this finding was inconsistent.

Table 1. Mean and Standard Error of Each Group

	Control + Sham	24 hr 6 U	24 hr 9 U	24 hr 12 U	24 hr 15 U	24 hr 18 U	72 hr 6 U	72 hr 9 U	72 hr 12 U
Number	12	7	8	5	2	2	7	6	4
Mortality	0/12	0/7	0/8	1/5	1/2	2/2	0/7	2/6	4/4
pH	7.475 0.016	7.523 0.025	7.473 0.031	7.312 0.062	7.332 -	- -	7.541 0.014	7.440 0.029	- -
PO ₂	88.8 1.42	75.7 6.1	70.8 5.6	38.5 7.4	34.0 -	- -	76.4 6.4	56.3 12.1	- -
PCO ₂	29.8 1.3	28.7 0.99	30.1 2.2	39.0 4.5	41.0 -	- -	29.6 1.5	32.5 3.6	- -
mBP	110 4.2	110 2.1	100 4.4	72 6.2	60 -	- -	108 5.6	105 6.2	- -
mPAP	6.7 1.0	9.3 1.4	8.4 0.93	18.8 3.3	19.0 -	- -	7.3 2.0	11.6 0.72	- -
CI	3.25 0.19	4.32 0.30	3.36 0.24	2.54 0.52	3.98 -	- -	3.14 0.25	3.53 0.36	- -
LVS WI	34.2 3.4	38.0 3.6	28.4 2.2	14.7 2.8	27.5 -	- -	24.9 2.7	30.2 4.4	- -
TPRI	3013 288	2073 119	2445 224	2541 568	1395 -	- -	2835 256	2395 260	- -
PVRI	145 18.1	138 26.2	117 13.4	563 125	279 -	- -	162 28.0	225 30.4	- -
SC	114 7.8	98.6 10.2	91.1 13.3	41.0 9.0	84 -	- -	110 14.4	80.8 22.8	- -
PR	13.3 1.0	16.5 2.0	20.4 2.0	51.2 14	24.0 -	- -	16.3 2.3	38.0 17	- -
EVLWV	9.10 0.64	11.4 0.59	10.6 0.62	13.4 2.8	8.4 -	- -	11.3 0.68	10.9 1.4	- -

PO₂, arterial oxygen tension (torr); PCO₂, arterial CO₂ tension (torr); mBP, mean blood pressure (torr); mPAP, mean pulmonary artery pressure (torr); CI, cardiac index (l/min/m²); LVS WI, left ventricular stroke work index (g·m/m²); TPRI, total peripheral resistance index (dynes·sec·m²/cm⁵); PVRI, pulmonary vascular resistance index (dynes·sec·m²/cm⁵); SC, static compliance (ml/cm H₂O); PR, pulmonary resistance (cm H₂O·sec/l); EVLWV, extravascular lung water volume (ml/kg).

- 5) Mean blood pressure: After 24 hours, mean blood pressure ($y = \text{torr}$) showed a relationship similar to PO_2 : $y = -5.93x + 148.4$ ($r^2 = 0.697$, $n = 20$). This regression was not significant after 72 hours.
- 6) Mean pulmonary arterial pressure: Animals exposed to 12 units or more showed significantly increased PAP after 24 hours.
- 7) Cardiac index: After 24 hours, cardiac index was significantly higher in the 6-unit group than in the control and sham groups. At doses above 6 units, cardiac index ($y = \text{l/min}\cdot\text{m}^2$) decreased progressively with smoke exposure ($x = \text{units}$) between 6 and 12 units: $y = -0.30x + 6.10$ ($r^2 = 0.454$, $n = 19$), but the cardiac index of the 12-unit group did not differ significantly from the control and sham groups.
- 8) Left ventricular stroke work index: After 24 hours, LVSWI ($y = \text{g}\cdot\text{m}/\text{m}^2$) was negatively related to the number of units of smoke exposure ($x = \text{units}$) between 6 and 12 units: $y = -3.81x + 61.5$ ($r^2 = 0.599$, $n = 19$). LVSWI of the 12-unit group was significantly lower than that of the sham, control and 6-unit groups.
- 9) Total peripheral resistance index: No significant differences were observed among surviving sheep.
- 10) Pulmonary vascular resistance index: In the 12 unit group, PVRI was significantly elevated 24 hours after injury.
- 11) Pulmonary resistance: After 24 hours, the 12 unit group had significantly increased pulmonary resistance.
- 12) Static compliance: Compliance was significantly lower than that in controls in animals exposed to 12 units of smoke.
- 13) Extravascular lung water volume: After 24 hours, some of the animals exposed to smoke had elevated EVLWV, but this finding was inconsistent.

DISCUSSION

In the exposed group, dose response was observed both 24 and 72 hours after smoke inhalation. However, the data 24 hours after exposure were more distinct and suitable for physiological study and the higher doses of smoke caused death by 72 hours. Eighteen units of smoke were fatal within 24 hours, while a 12-unit dose was fatal by 72 hours. In this series there was only one observation for 15-unit exposure at 24 hours, and the changes occurring with 15-unit exposure are therefore poorly defined. Changes of most of the cardiopulmonary parameters became significant only after 12-unit exposure. This suggests that the dose response curve is sigmoid in shape, rather than linear, with the 12-unit exposure located beyond the steep portion of the sigmoid. With the present data, we cannot derive the exact shape of the dose response curve, but distinct dose response was observed in mean blood pressure, PAP,

cardiac index and LVSWI after 24 hours, which suggests that the model is suitable for pathophysiologic study of such injury. This large animal model will also allow sophisticated physiologic techniques such as measurement of lung lymph flow [7] and VA/Q ratio distribution [8] as well as serial blood sampling. It is also suitable for studies of mortality with and without cutaneous burns.

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Pruitt, BA Jr: The Regional Burn Center and Advances in Burn Care. Burn Center Dedication, Oklahoma City, OK, 4 October 1983.

Pruitt, BA: The Regional Burn Center and Advances in Burn Care of the Extensively Burned Patient. Department of Surgery, University of Texas Health Science Center, San Antonio, TX, 7 October 1983.

McManus, WF: Emergency Care of Thermal, Chemical and Electric Injuries. Regional EMS Meeting, Seguin, TX, 6 October 1983.

Pruitt BA Jr: Care of the Extensively Burned Patient. Department of Surgery, University of Texas Health Science Center, San Antonio, TX, 7 October 1983.

Robertson KE: Emergency Management of Thermal Injuries. University of Texas Undergraduate Nurses, University of Texas at San Antonio, TX, 7 October 1983.

Burleson DG: Inhibition of the Oxygenation Activity of High Buoyant Density Granulocytes and Low Buoyant Density Macrophages by Cells of Intermediate Buoyant Density. The 20th Annual Meeting of the Reticuloendothelial Society, Portland, OR, 11 October 1983.

Robertson, KE: Emergency Management of Thermal Injuries. Visiting Egyptian Military Nurses from Wilford Hall Medical Center, Institute of Surgical Research, Fort Sam Houston, TX, 11 October 1983.

Pruitt BA Jr: Postburn Impairment of Neutrophil Function. International Surgical Group, Nashville, TN, 14 October 1983.

Pruitt, BA Jr: Film Narration: Burn Wound Management; Invited Discussant: Cine Clinic Session - The Early Surgical Management of the Major Burn. Discussant of Four Papers at Surgical Forum Session "Endocrinology and Tissue Repair". Clinical Congress of the American College of Surgeons, Atlanta, GA, 16 October 1983.

Strome DR: Mechanism of Reduced Lipolytic Response in Rat Adipocytes Following Thermal Injury. The American College of Surgeons 1983 Clinical Congress, Atlanta, GA, 16-21 October 1983.

McManus AT: Effective Topical Chemotherapy of Burn Wound Sepsis with Chlorhexidine Disphosphanilate (Westwood WP-973). The 23th Annual Meeting of the Interscience Conference on Antimicrobial Agents and Chemotherapy, Washington, D.C., 24-26 October 1983.

PRESENTATIONS

1 October 1983 - 30 September 1984

Robertson KE: Emergency Management of Thermal Injuries. 2CF-7 Class, Aviators, Academy of Health Sciences, Fort Sam Houston, TX, 25 October 1983.

Pruitt BA Jr: Burn Patient Rehabilitation. Department of Physical Medicine and Rehabilitation, University of Texas Health Science Center, San Antonio, TX, 26 October 1983.

Vaughan GM: Thyroid Cancer, Hypercalcemia. BAMC Endocrinology Residents, Fort Sam Houston, TX, 27 October 1983.

Hoffman BE: Splinting Theory and Techniques. 91L Advanced Course, BAMC, Fort Sam Houston, TX, 28 October and 1-3 November 1983.

Bush, K: Physical Therapy in the Treatment of Burns. Association of Life Insurance Medical Directors of America, 92nd Annual Meeting, San Antonio, TX, 2 November 1983.

Cross, PJ: Psychosocial Aspects of Thermal Injuries. Introduction to Hospital Ministry and Pastoral Care Course, BAMC, Fort Sam Houston, TX, 2 November 1983.

Hoffman BE: Role of Occupational Therapists in the Treatment of Thermally Injured Patients. Association of Life Insurance Medical Directors of America, 92nd Annual Meeting, San Antonio, TX, 2 November 1983.

Mason AD Jr: The Epidemiology of Burn Injury. Association of Life Insurance Medical Directors of America, 92nd Annual Meeting, San Antonio, TX, 2 November 1983.

McManus WF: The Hospital Care of Burn Patients. Association of Life Insurance Medical Directors of America, 92nd Annual Meeting, San Antonio, TX, 2 November 1983.

Pruitt BA Jr: Burn Injury Triage and Transportation of Burn Patients. Association of Life Insurance Medical Directors of America, 92nd Annual Meeting, San Antonio, TX, 2 November 1983.

Pruitt BA Jr: Burn Prevention. Association of Life Insurance Medical Directors of America, 92nd Annual Meeting, San Antonio, TX, 2 November 1983.

Robertson KE: Initial Management of Thermal Injuries. Clinical Pastoral Education Group, BAMC, Fort Sam Houston, TX, 2 November 1983.

PRESENTATIONS

1 October 1983 - 30 September 1984

Robertson KE: Initial Management of Thermal Injuries. Physician's Assistants' Course, Academy of Health Sciences, Fort Sam Houston, TX, 4 November 1983.

Pruitt BA Jr: Current Management of the Severely Burned Patients. The 11th Annual Meeting of the Japanese Association for Acute Medicine, Osaka, Japan, 9-11 November 1983.

Pruitt BA Jr: Panel - The Hyperdynamic Septic State. The 11th Annual Meeting of the Japanese Association for Acute Medicine, Osaka, Japan, 9-11 November 1983.

Pruitt, BA Jr: Unsolved Problems in Burn Care, Nippon Medical School, Department of Surgery, Tokyo, Japan, 9-11 November 1983.

Cross PJ: Psychosocial Aspects of Thermal Injuries. Chaplains assigned to Garrison and Tenant Units, Fort Sam Houston, TX, 10 November 1983.

Aulick LH: Metabolic, Cardiovascular and Thermoregulatory Adjustments to Thermal Injury. Minority Biomedical Research Support Seminar Series, Incarnate Word College, San Antonio, TX, 18 November 1983.

Robertson KE: Initial Management of Thermal Injuries, Inhalation Injury and Transportation of Thermal Injuries. Kelly AFB Emergency and Clinic Staff, San Antonio, TX, 18 November 1983.

Robertson, KE: Thermal, Chemical and Electrical Injury; Inhalation Injury and Pulmonary Complications; Wound Care; Physical and Occupational Therapy; Aeromedical Evacuation of the Thermally Injured Patient. 57th AES Scott AFB, IL, 20-22 November 1983.

Cross PJ: Psychosocial Aspects of Thermal Injuries. Continuing Education Seminar entitled "Care of the Thermally Injured Patient", Scott AFB, IL, 21 November 1983.

Hoffman BE: Hand Injuries: Splinting Theory and Principles. 65Bs, 91Js, BAMC, Fort Sam Houston, TX, 29 November 1983.

Robertson, KE: Initial Management and Air Evacuation of Thermal Injuries. Operating Room Nursing Course, BAMC, Fort Sam Houston, TX, 2 December 1983.

Valdez JS: Operative Interventions in the Thermally Injured. Operating Room Nursing Course, BAMC, Fort Sam Houston, TX, 2 December 1983.

PRESENTATIONS

1 October 1983 - 30 September 1984

Pruitt BA Jr: Current Management of Severely Burned Patients. Grady Hospital Grand Rounds, Emory University School of Medicine, Atlanta, GA, 3 December 1983.

Schlachta LM: Initial Management and Air Evacuation of Thermal Injuries. 2CF7 Course, Academy of Health Sciences, Fort Sam Houston, TX, 6 December 1983.

Robertson KE: Initial Management, Inhalation Injury, Air Evacuation, Wound Therapy and Psycho-Social Aspects of Thermal Injuries. University of North Carolina at Charlotte on behalf of Recruiting Command, 6 December 1983.

Hoffman BE: Role of Occupational Therapists in the Management of Thermally Injured Patients. 91L Students, Academy of Health Sciences, Fort Sam Houston, TX, 7 December 1983.

Robertson KE: Orientation to ISR and Burn Nursing. BAMC newly assigned RNs, Fort Sam Houston, TX, 7 December 1983.

Strome DR: Lipid Metabolism in Burn Injury. Minority Biomedical Research Support Seminar Series, Incarnate Word College, San Antonio, TX, 9 December 1983.

Pruitt BA Jr: Metabolic Responses and Nutritional Support of Burn Patients. International Society for Burn Injuries Annual Burn Seminar, Denver, CO, 8-10 December 1983.

Robertson KE: Initial Management, Inhalation Injury and Air Evacuation of Thermal Injuries, BAMC Short Intensive Care Unit Course for RNs, Fort Sam Houston, TX, 9 December 1983.

Brown W: Protein Metabolism in Burn Injury. Minority Biomedical Research Support Seminar Series, Incarnate Word College, San Antonio, TX, 13 January 1984.

Vaughan GM: The Pineal Gland. Minority Biomedical Research Support Seminar Series, Incarnate Word College, San Antonio, TX, 20 January 1984.

Pruitt, BA Jr: Fluid Therapy for Burn Patients. Surgical Physiology Conference, VA Hospital, San Antonio, TX, 21 January 1984.

Pruitt, BA Jr: Epidemiology, Triage and Pathophysiology of Thermal Injuries. OT/PT Conference Management of Burns in the Theater of Operations, US Army Institute of Surgical Research, Fort Sam Houston, TX, 23 January 1984.

PRESENTATIONS

1 October 1983 - 30 September 1984

Vaughan GM: Hormonal Changes Following Burns: An Overview with Consideration of the Pineal Gland. Symposium on the Immune

Consequences of Thermal and Traumatic Injury, International Society for Burn Injury, Snowbird, UT, 23 January 1984.

Burleson DG: Biochemical and Immunological Aspects of Sepsis in Burned Patients. Minority Biomedical Research Support Seminar Series, Incarnate Word College, San Antonio, TX, 27 January 1984.

Pruitt, BA Jr: The History of Burn Care. Portland Surgical Society, Portland, OR, 27 January 1984.

Pruitt, BA Jr: Pulmonary Complications of Burn Injury. Emanuel Hospital, Portland, OR, 27 January 1984.

Pruitt, BA Jr: Metabolic Response to Burn Injury. St. Vincent Hospital, Portland, OR, 27 January 1984.

Pruitt, BA Jr: Pathophysiology and Treatment of Burns. Uniformed Services University of the Health Sciences, Bethesda, MD, 3 February 1984.

Pruitt, BA Jr: Management of the Patient with Burn Injuries in the Community Hospital. University of Texas Health Science Center, San Antonio, TX, 14 February 1984.

Pruitt, BA Jr: The Metabolic Response to Burn Injury. MIEMSS Clinical Center, Baltimore, MD, 16-17 February 1984.

Shirani, KZ: Pharmacokinetics of Intravenous Gamma Globulin in Burn Patients. The 1984 Spring Meeting of the Trauma Group Burn Care Research for US Army Medical Research and Development Advisory Committee, San Antonio, TX, 12 March 1984.

Vaughan GM: The Effect of Injury on Pineal Function. The 1984 Spring Meeting of the Trauma Group Burn Care Research for US Army Medical Research and Development Advisory Committee, San Antonio, TX, 12 March 1984.

Pruitt, BA Jr: Fluid Resuscitation of the Burn Patient. South African Burn Congress, Johannesburg, South Africa, 16-25 March 1984.

Pruitt, BA Jr: Treatment and Monitoring of the Burn Wound. South African Burn Congress, Johannesburg, South Africa, 16-25 March 1984.

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1 October 1983 - 30 September 1984

Pruitt, BA Jr: The Metabolic Changes and Nutritional Support of the Extensively Burned Patient. South African Burn Congress, Johannesburg, South Africa, 16-25 March 1984.

Pruitt, BA Jr: Current Management of the Severely Burned Patient. Central New York Surgical Society, Syracuse, NY, 29 March 1984.

Pruitt, BA Jr: Effects of Burn Injury on Neutrophil Function. Central New York Surgical Society, Syracuse, NY, 29 March 1984.

Pruitt, BA Jr: The Modern Management of Burns. The Wellesley Hospital Clinical Day, Toronto, Canada, 4 April 1984.

Pruitt, BA Jr: Fluid Resuscitation of Severely Burned Patients. Department of Plastic Surgery, Wellesley Hospital Clinical Day, Toronto, Canada, 5 April 1983.

Pruitt BA Jr: The Care and Monitoring of the Burn Wound. Quarterly Meeting of the Plastic Surgery Section of the Ontario Medical Association, Toronto, Canada, 5 April 1984.

Pruitt, BA Jr: Plastic Grand Rounds. Department of Surgery, Wellesley Hospital, Toronto, Canada, 6 April 1984.

Lin KD: Plasma Alpha-2 Acute Phase Globulin and Zinc as Indicators of Burn Infection in Rats. Federation of American Societies for Experimental Biology Annual Meeting, St. Louis, MO, 11 April 1984.

Lin KD: Plasma Indicators of Infection in Burn Injury. University of Tennessee College of Medicine, Knoxville, TN, 11 April 1984.

Burleson, DG: Indicators of Infection in Burned Patients. American Burn Association Annual Meeting, San Francisco, CA, 10-12 April 1984.

Lehner, LM: Poster Session: A Completely Integrated Real Time Computer Burn Injury Information System. American Burn Association 16th Annual Meeting, San Francisco, CA, 11-14 April 1984.

Shirani, KZ: Replacement Therapy with Modified Immunoglobulin G (IgG) in Burn Patients: Preliminary Kinetic Studies (Abstract 144). American Burn Association 16th Annual Meeting, San Francisco, CA, 11-14 April 1984.

PRESENTATIONS

1 October 1983 - 30 September 1984

Pruitt, BA Jr: Moderator - Panel on Radiation Injury. Annual Meeting of the American Burn Association, San Francisco, CA, 14 April 1984.

Pruitt, BA Jr: The Diagnosis and Treatment of Infection in the Burn Patient. Annual Meeting of the British Burn Association, London, England, 25-29 April 1984.

Farrell, KJ: Management of Burns in the Combat Setting. The 4th Annual 121st ARCOM Medical Seminar in Gatlinburg, TN, 28-29 April 1984.

McManus AT: Microbial Colonization in a New Intensive Care Burn Unit. The 4th Annual Meeting of the Surgical Infection Society, Montreal, Canada, 1 May 1984.

Pruitt, BA Jr: Host and Microbial Factors Influencing Burn Patient Sepsis. Infectious Disease Conference University of Minnesota, Minneapolis, MN, 7 May 1984.

Lehner, LM: Symposium on Computers in Texas Medicine Today Completely Integrated RealTime Computer Clinical Information System - A Demonstration. Texas Medical Association 117th Annual Session, Ft. Worth, TX, 9-13 May 1984.

Pruitt BA Jr: Fluid Resuscitation of Severely Burned Patients. Department of Surgery School of Medicine, Health Sciences Center State University of New York at Stony Brook, Stony Brook, NY, 10-11 May 1984.

Pruitt, BA Jr: Phase II, Clinical Panel for the Military Blood Program 2004 Project. Military Blood Program Office, Washington, DC, 21-23 May 1983.

Farrell, KJ: Initial Resuscitation and Management of Patient with Major Thermal Injury. Annual Meeting of the Association of Osteopathic Surgeons, San Antonio, TX, June 1984.

McManus, WF: Burns. The 8th Medical Brigade, 4th Annual Medical Symposium, Brooklyn, NY, 2 June 1984.

Vaughan GM: Mental Status, T4, and Survival After Burn Injury. 7th International Congress of Endocrinology, Quebec City, Canada, 1-7 July 1984.

McManus AT: Disappearance of Endemic Methicillin Resistant Staphylococcus aureus: Association with Vancomycin Usage with Four-year Maintenance of Sensitivity. The 3rd International

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Symposium on Infection in the Immunocompromised Host, Toronto, Canada, 24-28 June 1984.

Vaughan, GM: Correlation of Syrian Hamster Serum Tetraiodothyronine (T4) and Triiodothyronine (T3) with Testes Weight in a Natural Winter Photoperiod. 7th International Congress of Endocrinology, Pineal '84 Satellite Symposium, Digby, Nova Scotia, 7-10 July 1984.

Vaughan GM: Pineal Function in Burns: Melatonin is not a Marker for General Sympathetic Activity. 7th International Congress of Endocrinology, Pineal '84 Satellite Symposium, Digby, Nova Scotia, 7-10 July 1984.

Pruitt, BA Jr: Fluid Resuscitation of the Extensively Burned Patient. Department of Surgery, Albany Medical College of Union University, Albany, NY, 9 August 1984.

Pruitt, BA Jr: Opportunistic Infections in Severely Injured Patients, Department of Surgery, Albany Medical College of Union University, Albany, NY, 9 August 1984.

Vaughan GM: Free Tetraiodothyronine (FT4) and Relative Pineal Involvement in Two Syrian Hamster Models of Low Serum Total T4. Third Colloquium of the European Pineal Study Group, Pecs, Hungary, 13-17 August 1984.

Pruitt, BA Jr: Diagnosis and Treatment of Opportunistic Infections in Injured Man. Madigan Army Medical Center, Tacoma, WA.

Pruitt, BA Jr: Fluid Resuscitation of Burn Patients. Madigan Army Medical Center, Tacoma, WA, 16-17 August 1984.

Pruitt, BA Jr: The Metabolic Effects of Injury. Madigan Army Medical Center, Tacoma, WA, 16-17 August 1984.

Vaughan GM: The Effects of Local and Systemic Infection on Oxygen Uptake and Core Temperature in the Burned Rat. The American Physiological Society Annual Meeting, Lexington, KY, 26-31 August 1983.

Pruitt, BA Jr: Fluid Resuscitation. Postgraduate Course on Emergency and Acute Care of Burn, Pacific Burn Institute, Sacramento, CA, 6-7 September 1984.

Pruitt, BA Jr: Nutrition and Metabolism in Burn Patients. Post graduate Course on Emergency and Acute Care of Burn, Institute, Sacramento, CA, 6-7 September 1984.

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ACUTE INGESTIONS OF THYROID HORMONES. Lawrence M. Lehrner and M.R. Weir. *Pediatrics* 73:313-317, 1984.

BURN MASS CASUALTY MANAGEMENT: Lessons Learned. William F. McManus. *Disaster Medicine* 1:2930, 1983.

CHANGES IN LYMPHOCYTE SUBPOPULATIONS AFTER BURN INJURY AND BURN INJURY WITH INFECTION. David G. Burleson, George M. Vaughan, and Arthur D. Mason, Jr. *Journal of Leukocyte Biology* 36:433-434, 1984.

CUSHING'S SYNDROME: In, Current Therapy. George M. Vaughan and T.J. Taylor, (ed, H.B. Conn), W.B. Saunders Co., Baltimore, MD, pp 472-477, 1983.

CURRENT APPROACH TO PREVENTION AND TREATMENT OF PSEUDOMONAS AERUGINOSA INFECTIONS IN BURNED, TRAUMATIZED, AND SURGICAL PATIENTS. Basil A. Pruitt, Jr., R.B. Lindberg, William F. McManus, and Arthur D. Mason, Jr. *Symposium on Pseudomonas Aeruginosa Infections. Reviews of Infectious Diseases* 5(5):S889-S897, November-December 1983.

CURRENT TREATMENT OF THE EXTENSIVELY BURNED PATIENT: In, Surgery Annual 1983. Basil A. Pruitt, Jr. and Cleon W. Goodwin, Jr., (ed L.M. Nyhus), Appleton-Century-Crofts, E. Norwalk, CT, Chapter 17, pp 331-364, 1983.

ENTERAL AND PARENTERAL NUTRITION. In, Manual of Clinical Nutrition. Cleon W. Goodwin, Jr. and D.W. Wilmore, (ed, R.H. Paige) Nutrition Publications, Inc., Washington, D.C., Chapter 31, pp 1-39, 1983.

EFFECT OF PLASMAPHERESIS IN THE CRITICALLY BURNED PATIENTS. Proceedings - Frontiers in Understanding Burn Injury. William F. McManus. National Institute of General Medicine Sciences. *Journal of Trauma* 24:5137-5138, 1984.

EFFECTS OF INJECTIONS AND/OR CHRONIC IMPLANTS OF MELATONIN AND 5-METHOXYTRYPTAMINE ON PLASMA THYROID HORMONES IN MALE AND FEMALE SYRIAN HAMSTERS. M.K. Vaughan, B.A. Richardson, L.J. Petterborg, A.P. Holtorf, George M. Vaughan T.H. Champney, and R.J. Reiter. *Neuroendocrinology* 33:56-61, 1984.

EXAMINATION OF NEUTROPHIL FUNCTION IN A RAT MODEL OF DECREASED HOST RESISTANCE FOLLOWING BURN TRAUMA. Albert T. McManus. *Reviews of Infectious Diseases* 5(Suppl 5):S898-S903, 1983.

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1 October 1983 - 30 September 1984

GRAPHICS SOFTWARE HELPING ARMY TREAT BURN VICTIMS. Lawrence M. Lehrner and David R. Strome. Computerworld, p 84, July 2, 1984.

HORMONES AND THE CONTROL OF METABOLISM. In, Surgical Nutrition. D.W. Wilmore, Louis H. Aulick, and R.A. Becker, (ed, J.F. Fisher), Little, Brown and Co, Boston, 1983.

HYPERMETABOLISM IN TRAUMA. In, Mammalian Thermogenesis. Louis H. Aulick and D.W. Wilmore, (eds, L. Girardier and M.J. Stock), Chapman and Hall, New York, NY, 1983.

IMPROVED CONTROL OF NON-INSULIN-DEPENDENT DIABETES MELLITUS BY COMBINED HALOFENATE AND CHLORPROPAMIDE THERAPY. R.A. Hurl, R.A. Wagner, S.T. Persellin, George Vaughan, D.J. Pittman, and B.A. Freidberg. Diabetes Care 7:19-24, 1984.

INAPPROPRIATE VASOPRESSIN SECRETION IN BURN PATIENTS. Khan Z. Shirani, George M. Vaughan, R.A. Hurl, Basil A. Pruitt, Jr., William E. McManus, R.J. Stallings, and Arthur J. Mason, Jr. Journal of Trauma 24:117-124, April 1984.

INCREASED SUSCEPTIBILITY TO INFECTION IN BURN PATIENTS WITH INJURY. R.W. Yurt, Albert T. McManus, Arthur J. Mason, Jr., and Basil A. Pruitt, Jr. Archives of Surgery 119:1-10, 1984.

INTERACTIONS OF THYROID HORMONE AND CORTISOL IN BURN PATIENTS. R.A. Hurl, George M. Vaughan, R.J. Stallings, Basil A. Pruitt, Jr., M.J. Tiesler, R.A. Pittman, Arthur J. Mason, Jr., and Basil A. Pruitt, Jr. Surgery, Gynecology and Obstetrics (Suppl 5):1119-1123, November-December 1984.

IS THERE A ROLE FOR PHARMACOLOGIC THERAPY IN THE TREATMENT OF BURN PATIENTS? A Review of the Literature. Journal of Trauma 24:125-130, April 1984.

MECHANISMS OF BURN INJURY. In, Handbook of Burn Care. R.A. Hurl, George M. Vaughan, R.J. Stallings, Basil A. Pruitt, Jr., M.J. Tiesler, R.A. Pittman, Arthur J. Mason, Jr., and Basil A. Pruitt, Jr. (eds, R.A. Hurl and George M. Vaughan), Little, Brown and Co, Boston, 1984.

PHARMACOLOGIC THERAPY IN BURN PATIENTS. In, Handbook of Burn Care. R.A. Hurl, George M. Vaughan, R.J. Stallings, Basil A. Pruitt, Jr., M.J. Tiesler, R.A. Pittman, Arthur J. Mason, Jr., and Basil A. Pruitt, Jr. (eds, R.A. Hurl and George M. Vaughan), Little, Brown and Co, Boston, 1984.

PHARMACOLOGIC THERAPY IN BURN PATIENTS. In, Handbook of Burn Care. R.A. Hurl, George M. Vaughan, R.J. Stallings, Basil A. Pruitt, Jr., M.J. Tiesler, R.A. Pittman, Arthur J. Mason, Jr., and Basil A. Pruitt, Jr. (eds, R.A. Hurl and George M. Vaughan), Little, Brown and Co, Boston, 1984.

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NEURAL AND CHINESE ACTIVITIES IN THE SPINAL CORD FROM THERMALLY INJURED RATS. D.E. Blask, David A. Birge, Cleon W. Goodwin, Jr., Arnold L. Vane, Jr., and Basil A. Pruitt, Jr. Journal of Neurological Sciences 61:101-111, September 1983.

POSTBURN ANALGESIA IN THE BURNED ILL INPATIENT. In, Postburn Analgesia in the Burned Ill Patient. Basil A. Pruitt, Jr., and Arnold L. Vane, Jr., Chapter 4, pp 63-64, 1983.

POSTBURN ANALGESIA IN THE BURNED ILL INPATIENT RELEASE INDUCED BY POSTBURN ANALGESIA. D.E. Blask, David A. Birge, Cleon W. Goodwin, Jr., Arnold L. Vane, Jr., and Basil A. Pruitt, Jr. Journal of Neurological Sciences 61:56-61, 1984.

POSTBURN ANALGESIA IN THE BURNED ILL INPATIENT. William F. Pruitt, Jr., and Arnold L. Vane, Jr., Chapter 4, pp 63-64, 1983.

POSTBURN ANALGESIA IN THE BURNED ILL INPATIENT. In, Postburn Analgesia in the Burned Ill Patient. Basil A. Pruitt, Jr., and Arnold L. Vane, Jr., Chapter 4, pp 63-64, 1983.

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